Mediterranean Journal of Hematology and Infectious Diseases

# 8th SYMPOSIUM ON ACUTE PROMYELOCYTIC LEUKEMIA

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Mediterranean Journal of Hematology and Infectious Diseases

# Abstract and Scientific Letters

## Conventional and New Drugs for Relapsed APL

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The introduction over the last decades of differentiating agents targeting the specific genetic aberration involved in pathogenesis, such as all-trans retinoic acid (ATRA) and arsenic trioxide (ATO), has turned acute promyelocytic leukemia (APL) into the most curable acute leukemia with almost 90% long-term survivors.[1] However, 5-20% of patients still relapsed after an initial remission.[1] Current guidelines of treatment in relapsed APL indicated the use of standard chemotherapy for early relapses after initial ATO-based treatment and, on the contrary, the use of ATO in case of no prior exposure, early relapse (<6 months) to anthracycline-based therapy or late relapse (>6 months) after ATO regimen.[2] In a large series of relapsed patients treated with ATO, the hematological CR rate was 86%, the complete molecular CR was 52%, the estimated early death rate was 7%, and the estimated overall survival (OS) ranged between 50 and 81% at 24 months.[3] A meta-analysis compared the effect of ATO as single agent versus ATO plus ATRA, showing an advantage of the combination in terms of CR, and molecular CR, without increasing early death rate.[4] Is still a matter of discussion the best choice as consolidation treatment in patients re-treated with ATO reinduction: a recent study showed an advantage in OS after 5 years of patients who received autologous stem cell transplant (90.3%) compared to ATO-based maintenance (58.6%).[5] ATO was combined with bortezomib in a phase II trial with a molecular CR rate of 86%: PML/RARa is cleared by this combination through p62-dependent autophagy pathway.[6] Oral ATO could be also a possible option: at a median follow-up of 94 months, out of 73 relapsed patients, 43 are still in CR2 with a 10-year leukemic free survival (LFS) of 56.8%.7 Among other treatments, in the past was reported the effectiveness of gentuzumab ozogamicin (GO): as single re-induction agent a molecular CR rate of 81% was reported.[8] Tamibarotene was also tested in patients relapsed after ATO induction: the ORR was 64%, but unfortunately the relapses were frequently reported with a median OS of only 9.5 months.[9] Few cases of relapsed patients were treated with gilteritinib or with venetoclax in combination with chemotherapy.[10,11] In conclusions, ATO is still the best option also for patients with extramedullary localizations. New drugs have been explored but further data are needed to draw final conclusions.

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## Genotypic and Phenotypic Features of APL-Like Acute Myeloid Leukemia

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Although Acute Promyelocytic Leukemia (APL) represents a unique clinico-biological entity, some of its distinctive features (e.g. pancytopenia, hemorrhagic diathesis, morphology with typical hyper-granular blasts, chemosensitivity to retinoids/arsenic derivatives) are not exclusive to this rare subtype of leukemia. In fact, all the modern classification systems require the demonstration of t(15;17)(q23;q21) and PML-RARA for a conclusive diagnosis of APL.[1] Accordingly, some cases showing a microgranular blast population are commonly defined "variant APL" if the presence of the typical genetic pattern is demonstrated. At variance, several AML cases with typical morphology and suggestive clinical presentation lack the typical genetic pattern and are defined "APL-like". The first APL-like case was defined by the presence of t(11;17)(q23;q21). Since then, other alternative fusion transcripts have been described, some of them involving retinoic acid receptor alpha (RARA), others involving different variants of the same receptor (e.g. RARG).[2] Nevertheless, the most frequent variant of APL-like leukemia is represented by NPM1 mutated AML.[3] In fact, roughly 30% of these patients may have APL-like morphology, APL-like immunophenotype (typically CD34 negative/HLA-DR negative)[4] and a different degree of sensitivity to retinoid/arsenic derivatives. In the talk we will consider how modern diagnostic tools allow us to timely identify these cases and address patients to a proper treatment course.

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## Is Disease Monitoring Still Necessary?

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The development of molecular monitoring of *PML::RARA* fusion transcripts by reverse transcription – quantitative polymerase chain reaction (RT-qPCR) was a major advance in the management of APL. Early studies showed that patients with persistence or re-emergence of disease related transcripts after completion of therapy reliably predicted haematological relapse. The advent of effective salvage therapy for relapsed disease in the form of Arsenic Trioxide (ATO) then paved the way for pre-emptive treatment of patients in haematological remission but with molecular failure, allowing them to avoid the risks of haemorrhage, thrombosis and differentiation syndrome that are commonly associated with treatment of frank relapse and may be fatal. The outcomes of patients treated at molecular relapse are excellent, and cure can be achieved in nearly all cases, with more recent data showing that consolidation autograft is not needed in most patients.

As well as improving outcomes for patients with APL, the development of molecular monitoring and molecular guided therapy served as a paradigm for the development of molecular monitoring in other subtypes of acute myeloid leukaemia, which is increasingly considered as standard of care, as well as paving the way for molecularly guided treatment, which is under active investigation.

Now, with contemporary treatment protocols incorporating frontline ATO, almost all patients presenting with low or intermediate risk disease are cured, and molecular monitoring has less of a role to play. A small proportion of patients in frontline ATO trials did not achieve molecular complete remission and their outcomes appear adverse, therefore molecular monitoring during treatment is still important. In the UK NCRI AML17 trial, no patient achieving molecular complete remission on treatment with ATO+ATRA +/- Gemtuzumab has yet relapsed, with similar results reported by other groups, therefore sequential monitoring after completion of treatment is no longer recommended.

Molecular monitoring still has an important role to play in high-risk disease, for which the standard of care remains ATRA + chemotherapy. Here, incidences of relapse in the range of 30-40% have been reported, therefore molecular monitoring remains mandatory in this group. Soon, frontline ATO based protocols are likely to be adopted for treatment of high-risk disease. The role of molecular monitoring in this setting is not yet completely clear because data on the incidence of relapse in this population are insufficiently mature. However, it appears that these patients may have a relapse risk of up to 10%. In the absence of robust data, sequential monitoring is recommended for patients with high-risk APL treated with frontline ATO-based combinations both during treatment and for at least two years thereafter, which appears to be the period during which most relapses occur.

## Classification and Risk Assessment of AML at Diagnosis

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In 2022, two classification systems for acute myeloid leukemia (AML) have been introduced, the International Consensus Classification (ICC) and the 5th edition of the WHO classification. Furthermore, the European LeukemiaNet recommendations (ELN) for AML was updated.[1,2,3] Both classifications and ELN recommendations have a major impact on the clinical management of AML patients. The ICC and ELN were two initiatives that were developed in parallel and in close collaboration. As a consequence, the 2022 ELN recommendations adopted the ICC. In the new ICC, considerable changes were made: First, changes with regard to the blast thresholds defining AML: AML with recurrent genetic abnormalities now all require at least 10% blasts. For the other categories there is an important change in the nomenclature; cases with 10-19% blasts are now designated "MDS/AML" and as a consequence the former designation of MDS-EB2 was eliminated, and cases with 20% blasts or more are categorized as AML. Major reasons for this new nomenclature were i) the term MDS/AML better reflects the genetic and clinical continuum of these cases and ii) this terminology should allow patients to be entered either on an MDS or an AML clinical trial, allowing access for these patients to more novel agents. Second, the ICC introduced new genetically defined entities: AML with mutated *TP53*, AML with myelodysplasia-related gene mutations (*ASXL1*, *BCOR*, *EZH2*, *RUNX1*, *SF3B1*, *SRSF2*, *STAG2*, *U2AF1*, and/or *ZRSR2*), and AML with myelodysplasia-related cytogenetic abnormalities[4,5] Third, with regard to the antecedent AML history, the new ICC classification now describes prior MDS or prior MDS/MPN and prior therapy as 'qualifiers' to the diagnosis, rather than as separate categories. As a consequence of the above, the former category of AML with myelodysplasia-related changes (AML-MRC) was eliminated. It is important to note that the new disease classification also impacted the updated ELN risk classification as well as the initial genetic work-up, in particular the rapid screening for actionable therapeutic targets such as *FLT3, IDH1/2* and *NPM1*.In this presentation, major changes and differences in the new classification systems will be discussed. Furthermore, changes with regard to the ELN risk stratification will be highlighted, and emerging genetic data on the genetic risk categorization in elderly patients not eligible for intensive chemotherapy will be presented.[6]

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## Treatment Complications in APL: Differentiation Syndrome and Others

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The development of targeted therapies such as All-trans retinoic acid (ATRA) and Arsenic Trioxide (ATO) revolutionized the management of Acute Promyelocytic Leukemia (APL). This approach, was very effective in inducting remissions and preventing relapses in standard-risk APL (patients with less then 10.000/mm$^3$ leukocytes at onset). Furthermore, when compared to the AIDA regimen (ATRA/Idarubicin), ATRA/ATO presented a significantly lower incidence of hematologic toxicity (Grade 3/4 neutropenia lasting > 15 days 46% vs 79% in the pivotal study by Lo-Coco et al.[1]

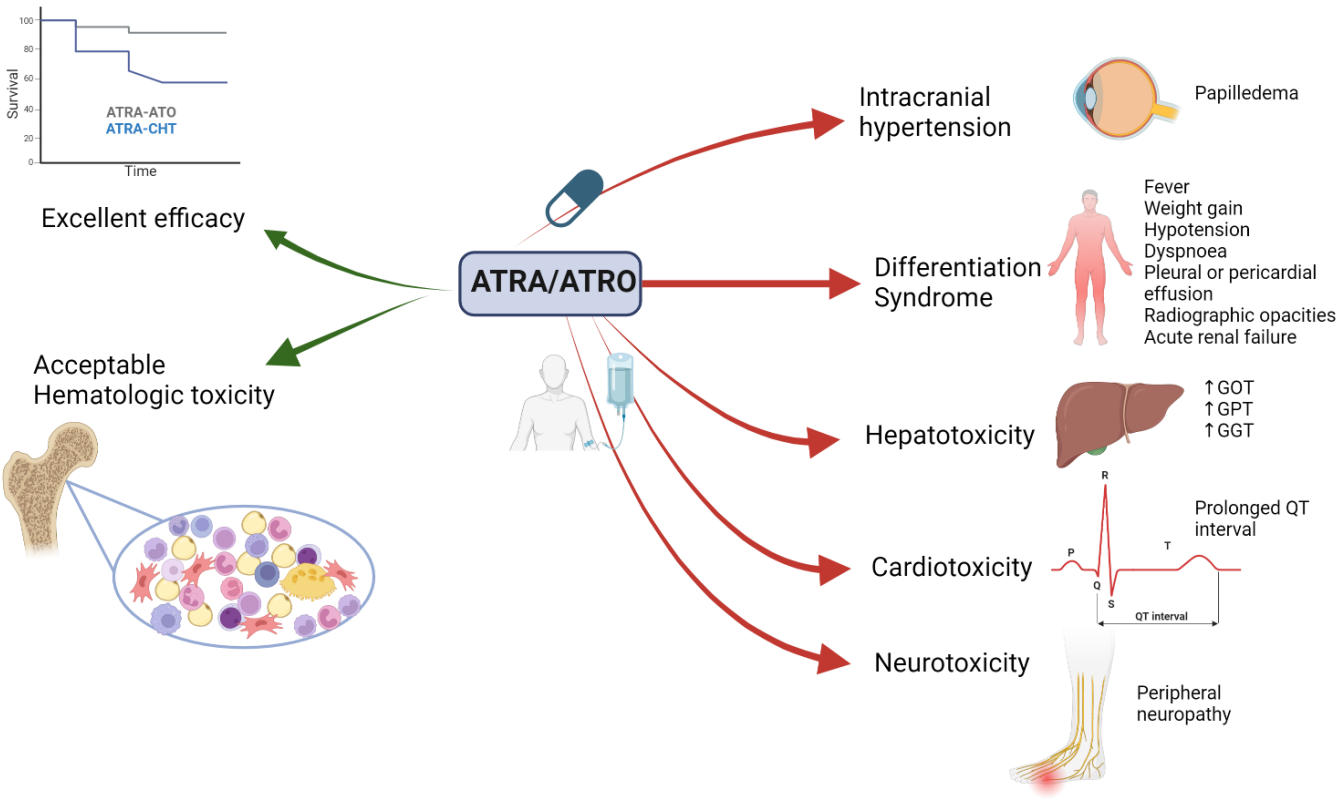
The inherent mechanisms of action of these 'smart drugs', able to induce differentiation and apoptosis in the atypical promyelocytes, are also responsible for the characteristic side effects.

One of the most common and dramatic is the differentiation syndrome (DS), whose diagnostic criteria, outlined by Montesinos et al.[2], are based on the detection of at least 3 clinical featuresincluding dyspnea, unexplained fever ≥38 C, weight gain greater than 5 kg, unexplained hypotension, acute renal failure and a chest radiography demonstrating pulmonary infiltrates and pleural or pericardial effusion. The onset of DS is usually observed at 10-14 days from the start of therapy and the incidence ranges from 10 to over 50% of cases.[1–3] The introduction of prophylactic steroid therapy and the early detection of the complication, though, reduced the DS-associated mortality to about 1-2%.[3]

ATO is also associated to other specific toxicities: an increase in hepatic markers is detectable in up to 90% of patients[4]. Nonetheless, grade 3/4 hepatotoxicity is observed in only half of the patients, recovering in all cases with temporary discontinuation of the drug.[1] Cardiotoxicity, in particular QT prolongation, is reported in 10-15% of patients[1,5], prompting accurate monitoring of electrolytes and ECG. Neuropathy occurs in 5-25% of patients; the main manifestation is peripheral neuropathy and usually is mild.[6] Residual symptoms, mostly non-severe, are observed in more than half of the cases.[6]

Notably, a very rare side effect of ATRA in pediatric population is intracranial hypertension (or pseudotumor cerebri), whose more characteristic manifestation is papilledema.[7]

In conclusion, ATRA/ATO is a powerful combination, whose side effects are mostly easily manageable and rarely life-threatening, making this chemo free approach the best option in most APL cases.

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# Australasian Protocols

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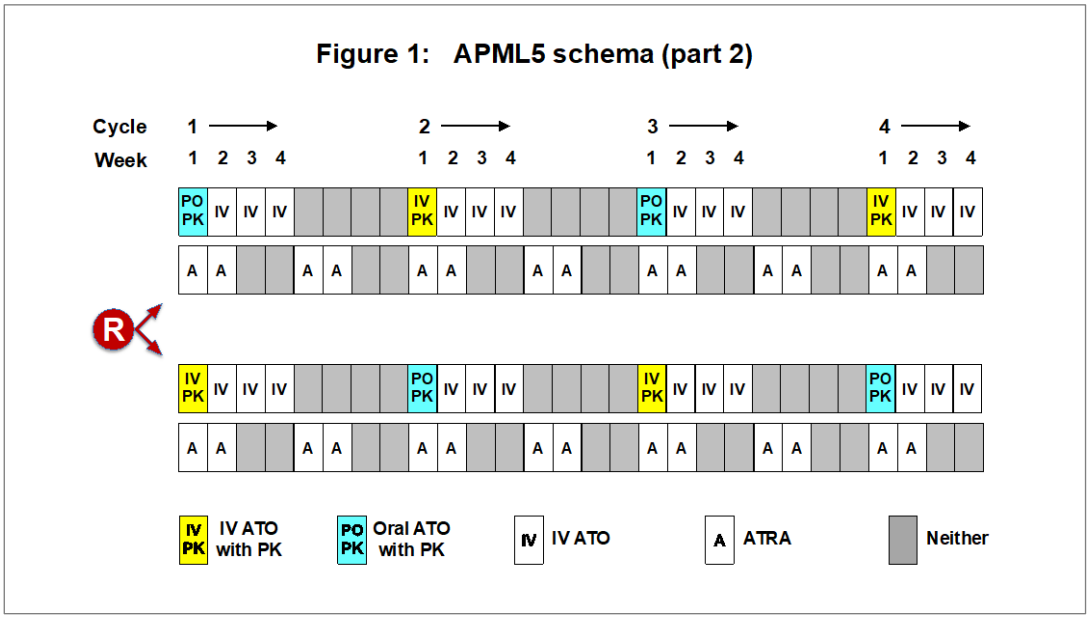
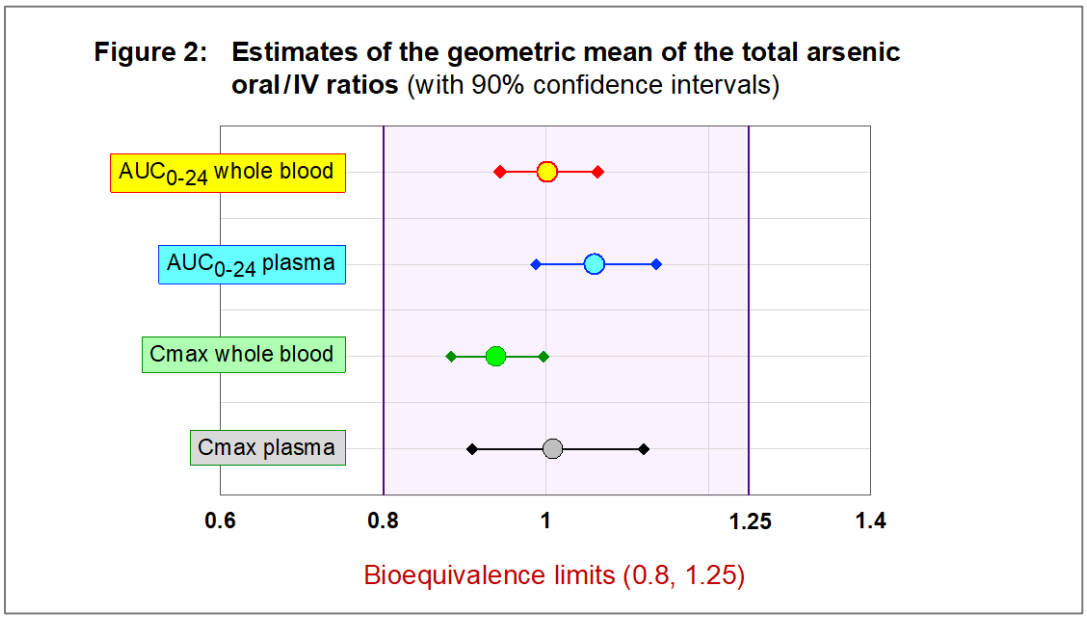
Over the last 25 years, the Australasian Leukaemia and Lymphoma Group (ALLG) has conducted 3 trials in previously untreated APL. APML3 (phase 2, n=101, 1997-2002) explored ATRA + intensive idarubicin. Although 4-year disease-free survival (DFS) was initially only 47%, it improved to 79% ($p<0.001$) with addition of triple maintenance (ATRA + 6MP + MTX).[1] FLT3 mutations (ITD and/or TKD) were the major determinant of overall survival (OS), 74% with mutations vs 96% without ($p=0.005$).
APML4 (phase 2, n=124, 2004-2009) combined intravenous (IV) arsenic trioxide (ATO) with ATRA + idarubicin, followed by 2 cycles of ATRA + ATO consolidation and triple maintenance.[2] Compared with APML3, clinical endpoints at 5 years were all significantly superior including DFS (95% vs 79%, $p=0.001$), event-free survival (EFS, 90% vs 72%, $p=0.002$) and OS (94% vs 83%, $p=0.02$).[3] DFS was not impacted by Sanz relapse risk (96% vs 95%, $p=0.87$), and APML4 abrogated the adverse OS impact of FLT3 mutations ($p=0.95$). ATO-based APML4 proved practice-changing in Australia and North America, particularly for high-risk APL (NCCN Clinical Practice Guidelines, AML v6.2023).
The randomised GIMEMA-AMLSG-SAL APL0406[4] and NCRI AML17[5] studies confirmed the superiority of ATO-based therapy, but a requirement for 2-hour infusions adversely impacts hospital resource utilisation and patient convenience. While oral arsenic theoretically offers economic and possibly safety advantages, its availability for clinical use is restricted to China and Hong Kong.
APML5 (phase 1, n=31, 2017-2020) was a 2-part bioavailability study of a novel oral arsenic formulation embedded within standard-of-care Lo Coco consolidation. The part 1 pilot study indicated 0.15mg/kg/d orally was suitable to assess bioavailability relative to IV ATO.[6] Part 2 evaluated oral and IV pharmacokinetics in a randomised crossover design (Figure 1). Total arsenic $AUC_{0\text{-}24}$ and $C_{max}$ confirmed bioequivalence in whole blood and plasma.[7] Estimates of the geometric mean of the oral/IV ratios approximated unity (Figure 2), and the 90% confidence intervals were within bioequivalence limits (0.80, 1.25). $AUC_{0\text{-}24}$ data for As(III), the active metabolite, supported bioequivalence. No safety signals were evident, particularly gastrointestinal or cardiac.
In conclusion, ALLG APL trials have contributed to (1) optimisation of curative APL treatment, and (2) clinical development of an Australian oral arsenic formulation with the potential to further improve management.

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## Chemo-Free Post-Induction Therapy for All APL Patients

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All-trans retinoic acid (ATRA) and arsenic trioxide (ATO) combination therapy has made acute promyelocytic leukemia (APL) highly curable, and the chemo-free concept has become a reality for almost all low-risk and partly high-risk patients.[1-3] Since leukocytosis is the major risk factor for early death (ED), complete exclusion of chemotherapy is hardly achievable during induction phase. So we raised a APL2018 trial aiming at the feasibility of chemo-free therapy in post-induction phase.

We conducted a randomized controlled trial APL2012 from 2012 to 2017.[4] In the APL2018 single-arm trial, we adjusted consolidation and maintenance therapy based on the ATO-group of APL2012 by totally removing chemotherapy from consolidation therapy for high-risk pts, and replacing ATO with oral arsenic Realgar-Indigo naturalis formula (RIF) in maintenance therapy, methotrexate was also removed.

A total of 323 patients were included in the APL2018 trial. 287 (88.9%) achieved complete remission (CR). The rate of early death was 11.1% (36/323). With a median follow-up of 29.2 months, the rate of 6-year overall survival (OS) for all patients was 86.3%, even-free survival (EFS) 83.8%, and disease-free survival (DFS) 94.3%. For low-, intermediate- and high-risk subgroups, the 6y OS were 95.6%, 89.4% and 73.5% ($P$<0.001), EFS 94.4%, 85.2% and 72.5%, respectively ($P$<0.001). The significant difference was mainly due to ED, with a rate of 2.5% (2/80), 9.3% (14/151) and 21.7% (20/92) among three risk groups ($P$<0.001). For DFS which excluded pts with early death, we found no significant difference among the three risk groups, with a 6y rate of 96.8%, 93.9% and 92.6%, respectively ($P$=0.663). 7 patients occurred relapses with a median time of 10.6 (range, 3.0 – 27.0) months from CR. Our results were consistent with the data of high-risk pts from clinical trials which just use ATRA and ATO as post-induction therapy, with a DFS of 89% (5y, n=52)[5] and 93.8% (2y, n=54),[6] respectively, and also those using ATRA+ATO+chemotherapy as post-induction therapy, with a DFS of 92% (2y, n=20)[7] and 93.2% (7y, n=120).[4] For low- and intermediate-risk patients, the results were consistent with those in most chemo-free trial, and also our previous APL2012 trial.

In summary, this trial confirmed the possibility of chemo-free post-induction therapy for APL pts at all risks. A completely chemo-free post-remission treatment including oral maintenance therapy will benefit more APL patients.

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# Current UK Protocols for Acute Promyelocytic Leukaemia (APL)

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The NCRI AML 17 trial randomized APL patients between standard chemotherapy with AIDA and the chemotherapy free combination of arsenic trioxide (ATO) plus ATRA in patients with either standard risk and high risk disease. An attenuated dosing schedule of ATO was used combining 60 days of induction with four blocks of consolidation. In addition Gemtuzumab was given for high risk APL. 235 patients were randomized if whom 57 (24%) had high risk APL. The trial closed in October 2013 with a median follow up of 60 months There was a significantly improved EFS benefit for ATO at 5 years of 93% versus 79% with AIDA ( 0.38 (0.19-0.77), p 0.007). No patient who achieved MRD negativity relapsed, however no overall survival benefit was seen primarily because of excellent results seen with salvage ATO at for AIDA treated patients. Following closure of AML17 ATO was not available until its approval by NICE in June 2018. In the interim, APL patients could be registered onto the AML 19 trial and treated with AIDA. 146 patients were registered, 25% of whom had high risk disease. ATO was only available for relapse. In AML19 overall survival at 5 years was 82% which was not as good as the 5 year survival in the AIDA arm of AML 17 (87% at 5 years).
In June 2018, ATO was approved by NICE for standard risk disease only. Both the GIMEMA-AMLSG – SAL and AML17 schedules were approved for reimbursement however the AML 17 schedule has been universally used in the UK because of familiarity, cost issues and the requirement for fewer outpatient visits. NICE also approved ATO for relapsed/refractory APL following first line treatment with AIDA. Refractory APL was not closely defined so has been variably interpreted to include persisting MRD positivity post AIDA. ATO was not approved for high risk disease which is treated with AIDA. In 2020 NICE issued temporary guidance for Covid precautions which allowed IV ATO consolidation for high risk APL in remission following an initial course of AIDA induction in order to minimise hospital admissions. Unfortunately, the outcome of these patients was not collected.
Future plans include a randomized comparison of oral versus IV ATO consolidation for patients in remission following initial IV ATO induction treatment. Funding opportunities for this trial are being explored.

# ATRA + Chemotherapy: Results of Clinical Studies in Childhood APL

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Childhood acute promyelocytic leukemia (APL) has customarily been treated on adult protocols and is currently the most curable subtype of acute myeloid leukemia (AML).[1,2] During the last 50 years, treatment has progressively evolved. The most important breakthrough came in 1988 when good results were observed in patients treated with the oral vitamin A derivate, all-trans retinoic acid (ATRA), an agent known to induce differentiation of leukemic cells. The introduction of ATRA represents the major turning point towards the curative treatment of APL. Subsequently, ATRA was combined with chemotherapy, particularly anthracyclines. In pediatric age, full ATRA dose (45mg/m$^2$) can cause the idiopathic intracranial hypertension called pseudotumor cerebri. Available data suggested that a half ATRA dose (25mg/m$^2$) could be as effective as the standard dose and such dose is the recommended standard for children.[3]
Since 1993, 3 consecutive specific disease-tailored protocols combining ATRA and anthracyclines have been carried out in Italy for pediatric APL; the first two included children and adults (GIMEMA/AIEOP AIDA0493, AIDA2000) and the last one was conducted in the framework of the International Consortium for Childhood APL (ICC-APL-01) for the treatment of children and adolescents (**Figure 1**). Results of these studies clearly demonstrated that induction combination ATRA-anthracyclines, and more precisely idarubicin, is highly effective in APL (**Table 1**).[3-5,7] ATRA extended consolidation (AIDA2000, ICC-APL-01) improved results in all patients. The presenting WBC significantly affected patients' outcome. Relapse risk groups based on WBC value led to design risk-adapted trials in which intensity of consolidation varied according to the risk-category (standard and high).[6] A relevant problem

linked to the use of high-dose anthracyclines, is the risk of cardiomyopathy. In the ICC-APL-01 trial, the reduced cumulative anthracycline dose combined with ATRA and high-dose cytarabine, did not affect patients' outcome[7]. Molecular bone marrow assessment for the PML–RARA fusion transcript, at the end of consolidation, provided more powerful predictor of relapse than presenting WBC.[8]

Recently, another differentiating agent, arsenic-trioxide (ATO), has been an additional step in the successful story of APL treatment; current studies with chemotherapy-free approaches (ATRA+ATO) are aimed at reducing therapy-related acute and long-term toxicities while maintaining high cure rates.

**Figure 1.** Italian Childhood APL: treatment history.

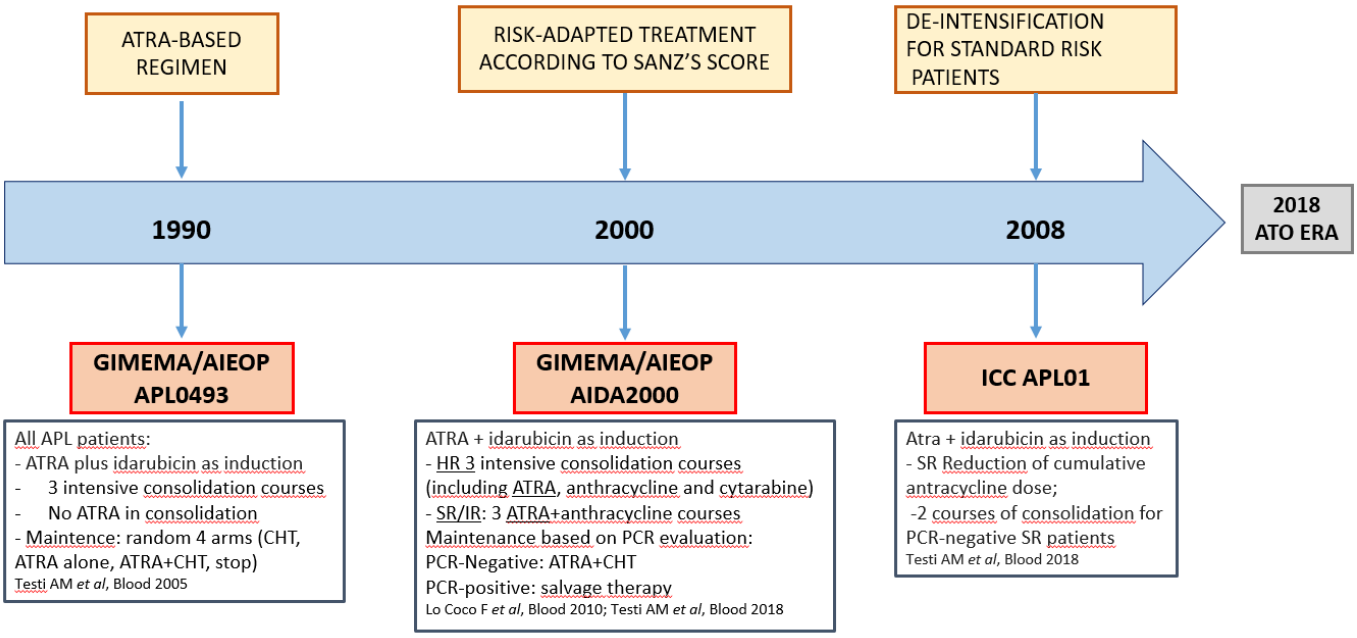


**Table 1.** APL protocols: pediatric patients' charcateristics and results

| Characteristics | GIMAMA/AIDA0493 | GIMEMA/AIDA2000 | ICC APL 01 |
|---|---|---|---|
| Number of patients | 124 | 127 | 258 |
| Median age (years) (range) | 11.6 (1.4-17.9) | 11.9 (1.1-18) | 10.3 (1.1-20.7) |
| Induction Therapy | ATRA+IDA | ATRA+IDA | ATRA+IDA |
| ATRA daily dose (mg/m$^2$) | 25 | 25 | 25 |
| Cumulative anthracycline dose (mg/m$^2$) | 650 | 650 Standard-Risk<br>650 High-Risk | 355 Standard-Risk<br>405 High-Risk |
| Risk-adapted trial | no | yes | yes |
| HCR*(%) | 96 | 96 | 97 |
| ID° rate (%) | 4 | 4 | 3 |
| N. Resistant | 0 | 0 | 0 |
| 5-year OS^ (%) | 89 | 93.7 | 94.6 |
| 5-year EFS^^ (%) | 76 | 84.9 | 79.9 |

*HCR: hematological complete remission; °ID: induction death: ^OS: overall survival; ^^EFS: event-free survival.

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# The Harmony APL Project

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Introduction: Acute promyelocytic leukemia (APL), is nowadays curable in 75-90% of patients using targeted agents [All-transretinoic acid (ATRA)/Arsenic Trioxide (ATO) or ATRA/chemotherapy]. Despite significant advances, many questions remain unanswered, such as the optimal setting of chemo-free regimens and the prevention of long-term relapses.
Methods: We analyzed a large cohort of newly diagnosed patients with APL included in the HARMONY Platform (derived from APL0406 and AML17 clinical trials, and Study Alliance Leukemia, Swedish Cooperative Group, HOVON database or AML study Group registries). After acquisition from the sources, data were harmonized and transformed using an Observational Medical Outcomes Partnership Common Data Model, and registered in the HARMONY Platform.
Results: Our study cohort included 1438 patients (pts) with APL, 721 males and 717 females, of a median age of 50.5y (range 16-94 y). Of these, 87 (6%) had a therapy-related APL.
Of 1309 pts, 562 were treated with ATRA-ATO and 747 with ATRA-Idarubicin (AIDA). According to Sanz risk score, 1117 pts (78.4%) had standard-risk APL and 308 (21.6%) high-risk. Early deaths (ED) occurred in 85 of 1438 pts (5.9%), mostly due to bleeding or infections, and were not associated with patient characteristics. The 10-year overall survival (OS) was 90% and 77% in ATRA-ATO vs AIDA-CHT cohorts, respectively ($p<0.001$), while event-free survival (EFS) was 86% and 67%, respectively ($p<0.001$). Age significantly correlated with OS and EFS (both $p<0.001$). Pts treated outside the clinical trial context had inferior outcomes when compared to clinical trials, in particular in ATRA-ATO cohort. The multivariate analysis for OS showed that age, Sanz-risk score, treatment type and treatment context (clinical trial vs non-clinical trial) were independent predictors of OS. The multivariate analysis for EFS showed an independent correlation with Sanz risk score, type of treatment and age.
Conclusions: The large APL Harmony database showed that elderly age and high Sanz risk-score were associated with decreased survival. However, relapse rates were similar, indicating a common biological substrate. The

ATRA/ATO chemo-free combination is confirmed as the best treatment option, prolonging survival up top 90% at 10 year f-up, and reducing the relapse probability, as compared to AIDA. Patients treated in the context of clinical trials had a higher rate of relapse, probably also due to closer and more accurate follow-up. Survival was significantly improved, probably due to both selection bias (fitness, stable clinical situation) and a successful ATRA/ATO salvage therapy in case of relapse following ATRA-CHT treatment. ED remains one of the major issues, occurring in at least 6% of patients at a median of 9 days after diagnosis, with no independent clinical predictors. These findings emphasize the key role of a prompt APL diagnosis, and of the control of bleeding and infectious complications to reduce the ED rates and further improve patients' outcome.

## APL Biology
## CO1

# Unraveling Coagulopathy in Acute Promyelocytic Leukemia: Insights from Single-Cell RNA Sequencing

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**Abstract.** *Background*: Acute promyelocytic leukemia (APL), a subtype of AML characterized by the t(15:17) chromosomal translocation, results in the accumulation of abnormal promyelocytes in the bone marrow and bloodstream. Despite being highly curable with targeted treatment, early death due to abnormal bleeding (coagulopathy) remains a significant challenge in APL management. However, the molecular mechanisms underlying coagulopathy in APL are poorly understood. In this study, we employed single-cell multiome sequencing (scRNA-seq and scATAC-seq) to elucidate the heterogeneity of abnormal promyelocytes, aiming to uncover the molecular pathogenesis of APL-coagulopathy.
*Methodology*: We collected abnormal promyelocytes from two APL patients: one experiencing severe bleeding (APL1) and the other with no bleeding (APL2) episodes. Additionally, CD34+ cells were obtained from the apheresis sample of a healthy bone marrow transplant donor. We isolated 11500 nuclei from these three samples and then used them to prepare scRNA and scATAC libraries using the Chromium X platform (10x Genomics). The libraries were sequenced on the Novaseq Illumina following 10x Genomics guidelines. The sequenced data underwent initial processing using the Cell Ranger ARC pipeline. Subsequently, scRNA-seq data were analyzed using the Seurat pipeline in R, while scATAC data analysis is ongoing. The scRNA-seq datasets were integrated, and standard analyses, including quality control, dimensionality reduction, and clustering, were performed to identify distinct cell populations within abnormal promyelocytes. Differential gene expression analysis was conducted among the clusters using the FindMarkers function and annotated using the Azimuth bone marrow reference dataset.
*Results*: Analysis of scRNA-seq data revealed substantial cellular and gene expression diversity within abnormal promyelocytes of APL1 and APL2. Seurat pipeline identified a distinct cell cluster in APL1 compared to control and APL2. A heatmap plot exhibited a unique gene expression pattern between APL1 and APL2, indicating diversity in gene expression. Cell type annotation revealed that the distinct cell population in APL1 exhibited a leukemic stem cell (LSC) signature, while the control group displayed a hematopoietic stem cell (HSC) signature. This distinction was confirmed by analyzing the unique expression of LSC marker genes (CD96, IL1RAP, and IL3RA) and HSC marker genes (CD34, CRHBP, NPR3). Notably, coagulopathy-related genes (ANXA2 and TF/F3) were exclusively upregulated in LSCs, implying the involvement of LSCs in coagulopathy. These LSCs also upregulated myeloperoxidase (MPO) and CD38 while diminishing CD34 expression, confirming the leukemic characteristics of abnormal promyelocytes. Pathway enrichment analysis indicated that LSCs were enriched in anti-apoptotic and pro-cell cycle pathways. This data suggests that these LSCs, with their proliferative advantage and coagulopathy-related gene expression, might induce a procoagulant state, initiating coagulopathy in APL.

*Conclusion*: Initial data suggests that coagulopathy in APL may be controlled by a specific subset of leukemic cells, rather than a general disruption of leukemic cells. A deeper understanding of these particular cells and their regulatory pathways, with a larger sample size, could lead to the revelation of potential therapeutic targets and regulatory networks for the management of APL-related coagulopathy.

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**APL Biology**
**CO2**

## In-Depth Analysis of the Pathophysiology of Differentiation Syndrome in Acute Promyelocytic Leukemia

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**Abstract.** Differentiation syndrome (DS) is a relatively common and potentially severe complication of patients with acute promyelocytic leukemia (APL) treated with all-trans retinoic acid (ATRA) and arsenic trioxide (ATO). Incidence of DS vary with treatment strategies, diagnostic criteria and prophylactic measures, reaching about 20% of cases during induction therapy. To date, little is known about the DS pathophysiology. While inducing the differentiation of promyelocytes to more mature myeloid forms, APL treatment also induces release of cytokines, triggering a systemic inflammatory response. Due to its life-threatening potential, a prompt recognition of this complication and steroids start is crucial to reduce DS-related mortality.
Among clinical factors associated with development of DS in 26 patients newly diagnosed with APL consecutively admitted to the “Tor Vergata University Hospital” between 2010-2022, we observed that a high transfusion burden during induction phase was associated with a higher rate of DS occurrence (p=0.033). This prompted us to explore whether specific markers could predict DS while shedding light onto its pathophysiology. To gain more insight into this, we studied at mRNA and cytokines level a retrospective cohort (rC) of 18 patients enrolled in the APL 0406 trial (8 of whom developed DS).
Whole transcriptomic profiling performed on the rC at the time of APL diagnosis did not clearly distinguish patients who subsequently developed DS as showed by principal component analysis (PCA). However, we identified a total of 93 Differentially Expressed Genes (DEGs), including 81 and 12 respectively up- and down-regulated when

comparing transcriptomics of patients developing or not DS. Furthermore, functional enrichment analysis performed by Reactome and GSEA showed a positive signal in "Hematopoietic stem cell differentiation", "GATA-1 targets", and "Heme metabolism" pathways.
To further substantiate these results, we prospectively collected, through a National collaboration, peripheral blood samples of 8 APL patients at the beginning of the induction therapy, at DS onset and its resolution (prospective cohort-pC). When looking at transcriptomic changes at the time of DS, compared to paired samples at diagnosis and DS resolution, we observed significant downregulation of several genes involved on erythroid maturation. This was also confirmed in patients not experiencing this complication. Finally, as DS is characterized by a hyper-inflammatory state, possibly linked to erythroid differentiation imbalance leading to anemia, we longitudinally studied cytokines profiles in patients with and without DS. Upregulation of IL-6, IL-1β and to a lesser extend of hepcidin levels was observed in association with DS.
In conclusions, patients with DS are characterized by a high transfusion burden at APL onset. This clinical observation is paralleled by transcriptomic features showing a deregulation of genes related to erythroid maturation already before treatment start, possibly identifying cases amenable to personalized prophylactic DS measures.

## APL treatment
## CO3

## Risk Stratification and Early Death Predictors in Acute Promyelocytic Leukemia: Insights from a Comprehensive Analysis of Sanz Risk Categories

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**Abstract.** *Background*: Early death (ED) is a primary cause of treatment failure in acute promyelocytic leukemia (APL). Despite arsenic trioxide (ATO) improving long-term outcomes, its impact on reducing ED is limited. This study examines ED risk factors across Sanz score-defined risk groups in APL patients.
*Objective*: This study aims to explore the risk factors for ED in newly diagnosed APL patients across different Sanz risk categories, all undergoing all-trans retinoic acid and ATO based treatment.
*Methods*: Our analysis consists of two cohorts: Cohort 1 from the APL2012 trial (NCT01987297) and Cohort 2 comprising patients treated at our center from February 2001 to June 2022, excluding APL2012 participants. We assessed clinical and laboratory parameters [age, sex, white blood cell (WBC) count, hemoglobin, platelet count, activated partial thromboplastin time (APTT), prothrombin time (PT), fibrinogen (Fg), alanine transaminase (ALT), aspartate aminotransferase (AST), serum creatinine, lactate dehydrogenase (LDH), and bone marrow (BM) blasts] to identify high-risk ED factors for each group using ROC curves, univariate, and multivariate logistic regression analyses.
*Results*: The study included 1411 patients (855 from APL2012 and 556 from non-APL2012). The overall ED rate (EDR) was 6.4%, with rates of 1.2%, 6.4%, and 10.4% in low, intermediate, and high-risk groups, respectively (P<0.001). Notably, patients over 60 years had a significantly higher EDR (19.8%) than younger patients (5.3%, P<0.001). The main ED cause was bleeding (58%), predominantly intracranial (81%).
Distinct clinical features were observed among low, intermediate, and high-risk APL patients. Prolonged PT was associated with ED in low-risk patients, while in intermediate-risk patients, factors like age, WBC count, APTT, PT, Fg, AST, and LDH were linked to ED. For high-risk patients, sex, age, WBC count, PT, Fg, AST, creatinine, and BM blasts were correlated with ED. Multivariate analysis revealed that no independent ED risk factors were identified in the low-risk group. For intermediate-risk patients, independent predictors of ED included age over 60, WBC count≥2.3×10^9/L, APTT≥30seconds, Fg≥1.1g/L, and LDH≥323U/L. In high-risk patients, age over 60, WBC count≥90×10^9/L, PT≥15seconds, AST≥32U/L, and creatinine≥68μmol/L were identified as independent risk factors (Table 1).
A novel risk scoring system was developed based on these factors. Each factor's odds ratio was normalized and converted into integer scores. Intermediate-risk patients had scores ranging from 0 to 9, while high-risk patients scored between 0 and 13 (Table 1). This scoring revealed significant risk stratification: intermediate-risk patients with 0-2 points (n=506) had an EDR of 2.8%, those with 3 points (n=99) had 11.1%, and 4-9 points (n=65) led to an EDR of 27.7%. In high-risk patients, scores of 0-6 points (n=258) corresponded to an EDR of 2.7%, 7-8 points (n=129) to 14.7%, and 9-13 points (n=26) indicated a very high EDR of 65.4%. These results highlight a particularly high-risk ED subgroup within both intermediate and high-risk Sanz groups.
*Conclusion*: Sanz risk stratification serves as an initial tool for identifying high ED risk patients. This study's findings enable more precise identification of high-risk ED groups by analyzing specific risk factors varying in impact across different Sanz risk groups. Tailored interventions based on these factors could potentially reduce mortality in APL patients.

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## APL Biology
## CO4

### The Oncogenic Δnp73 Isoform is Associated with Atra-Resistance in Apl

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**Abstract.** Treatment approaches for Acute Promyelocytic Leukemia (APL) have undergone significant advancements with the incorporation of all-trans-retinoic acid (ATRA) and arsenic trioxide (ATO). Yet, the exact molecular mechanisms contributing to the variability in treatment response remain unclear. In this context, p73 has been previously proposed as key protein related to prognostic in APL patients treated with ATRA+chemotherapy. p73 are composed of several isoforms that can be divided in two main groups of isoforms – TA, having the transactivation domain (TAD) and functions like p53, and ΔNp73, which by lacking the TAD, can bind to DNA but cannot activate the same target genes. Here, we studied the role of ΔNp73α and TAp73 isoforms in modulating the therapeutic efficacy of ATRA and ATO in APL. First, we evaluated the gene expression of TP73 isoforms in bone marrow (BM) mononuclear cells from 98 APL patients enrolled in the International Consortium of Acute Promyelocytic Leukemia (IC-APL), all treated with ATRA+chemotherapy. We found that the high ΔNp73 levels were associated with significantly lower overall survival rates (77.8%) compared to those with lower expression (96.6%). Next, using lentiviral based systems, we induced stable expression of ΔNp73α or TAp73 in NB4 (ATRA-sensitive) and NB4-R2 (ATRA-resistant) APL cell lines. We found that ΔNp73α overexpression (OE) increased the cell proliferation of APL cells. Next, we treated the APL cell lines with ATRA and ATRA+ATO to evaluate ΔNp73α and TAp73 as resistance inducers or sensitizers to these drugs; however, we did not observe differences in the apoptosis induction in comparison to the empty vector control. Since ATO is associated with activation of p53/p73 signaling pathway, we tested whether TAp73-OE would restore ATO sensitivity in ATO resistant APL models. TAp73α-OE in ATO resistant APL cells was able to revert the ATO resistance, suggesting the potential role of activation of TAp73 for ATO resistant cases. Also, ΔNp73α hindered the induction of ATRA-induced myeloid markers (CD11b, CD14, and CD15), suggesting an inhibitory role in differentiation processes. In this context, we evaluated the BMP4-ΔNp73-NANOG signaling cascade, identified as a positive inducer of stem cell-like phenotype in acute myeloid leukemia cells (Voeltzel et al., Cell Death Dis 2018). Our findings revealed increased expression of BMP4 and NANOG in ΔNp73α-OE cells following ATRA treatment, which was absent in non-treated cells, underscoring the significance of this signaling axis in APL response to ATRA. Finally, we performed in vivo experiments using a ΔNp73α- and TAp73α-OE model on murine APL blasts from the leukemic hCG-PML::RARA mice. Modified APL blasts were transplanted into NSG mice, and after two weeks of the transplant, mice were split in four groups of treatment (n=7/group), being treated for 21 days with ATRA (1.5 mg/Kg/day), ATO (5 mg/Kg/day), ATRA+ATO, and vehicles. Notably, ΔNp73α-OE in leukemic blasts led to a reduction of the ATRA-induced blast clearance in both BM and spleen. In conclusion, our study emphasizes the prognostic and therapeutic implications of ΔNp73α in APL. We observed that ΔNp73α -OE APL cells up-regulate NANOG and BMP4 in response to ATRA treatment, decreasing its efficacy, supporting the role of ΔNp73 expression in therapy response. This is particularly relevant in low and middle-income countries centers where ATO is not an available treatment option.

## APL Treatment
## CO5

### Long-Term Follow-Up of Patients with Acute Promyelocytic Leukemia: Insight from a Single-Center Cohort

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**Abstract.** The management of APL still faces unresolved challenges, including a high rate of early death (ED) and the management of relapses in high-risk cases. We conducted a retrospective analysis involving 220 APL patients (pts) diagnosed from March 1993 to May 2022, aiming to identify prognostic factors related to overall survival (OS) at diagnosis, compare outcomes among low and intermediate-risk pts receiving different treatment protocols, assess both OS and disease-free survival (DFS) based on risk and treatment, evaluate rates of ED and differentiation syndrome (DS), and examine second-line relapses and salvage therapies. At diagnosis, pts had a median age of 50 y (range: 19-85 y), with 49% being male. Median values of WBC and Plt count at diagnosis were 2.3 x 109/L (range: 0.4-286 x 109/L) and 29 x 109/L (range: 2-302 x 109/L), respectively. Pts were classified as low risk in 29% (n=64), intermediate risk in 45% (n=98), and high risk in 26% (n=58). CD34+ was more frequently associated with the

morphological variant (p<0.001), high risk (p<0.001), and elevated WBC count at onset (p<0.001). Conversely, CD15+ correlated with classic morphology (p=0.018), while CD56+ was associated with the bcr3 transcript (p=0.002).
Overall, 214 pts (97.2%) received induction therapy. CR was achieved in 97.4%, 100%, 100%, and 27% of pts treated with AIDA protocol, AIDA+Ara-C, ATRA+ATO, and ATRA monotherapy, respectively, with a median time to CR of 37.5 ± 20.05 days. DS occurred in 19.6% of pts, including 17% among AIDA-treated cohort, 57% of AIDA+Ara-C, 23% of ATRA+ATO, and 18% of ATRA monotherapy. Univariate analysis demonstrated a significant association between elevated WBC count at diagnosis and DS (p=0.003).
Sixteen ED (i.e., 0-30 days from diagnosis) were recorded, including 4 before induction and 12 during or after induction therapy. Median age was 65 y (range: 23-85), median WBC and Plt count were 15.3 x 109/L (range: 0.7-286 x 109/L) and 29 x 109/L (range: 14-116 x 109/L), respectively. The main causes of death were hemorrhagic or thrombotic complications 72.3% and infectious in 18.7%. Among the four patients who died before initiating treatment, three had cerebral hemorrhage and one had cerebral ischemia. Of the 12 patients who died during or after the induction therapy, six had cerebral hemorrhage, one had gastrointestinal bleeding, three had sepsis, and two had disseminated intravascular coagulopathy. Consolidation therapy was administered to 196 pts in CR: 34.1% received AIDA0493, 43.3% AIDA2000, and 19.8% the ATRA+ATO combination. Molecular complete response was achieved in 96.8% cases, and 142 pts proceeded to maintenance therapy. Overall, the 3-y and 5-y OS were 80.8% (95% CI, 78.1-83.5) and 79.1% (95% CI, 76.4-81.8), respectively. Considering only pts who completed induction and maintenance therapy, the 5-y OS were 82.1% (95% CI, 77.5-86.7) for the AIDA0493 cohort, 87.5% (95% CI, 84.4-91.1) for the AIDA2000 cohort, and 100% for the APL0406 cohort (p=0.044). Additionally, the DFS rates were 65.7% (95% CI, 60.4-70.9), 70% (95% CI, 65.8-75.2), and 95.1% (95% CI, 91.7-98.5) (p=0.016), respectively. Among low and intermediate-risk pts, age >70 years (p=0.027) and relapse (p<0.001) were significantly associated with reduced outcomes. This study contributes to the advancement of our understanding of APL treatment, underscoring the ongoing need for research to enhance outcomes and explore new therapeutic approaches and prognostic factors.

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## APL Treatment
## CO6

### Real-World Impact of First-Line Arsenic Trioxide on Early Death and Long-Term Outcomes of Patients with Acute Promyelocytic Leukemia in Portugal: A Multicentric Study from the Grupo Portugues de Leucemias Agudas

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**Abstract.** *Introduction*: Acute Promyelocytic Leukemia (APL) has evolved from the most rapidly fatal acute leukemia to the one with the highest cure rates. The chemotherapy-free combination of all-transretinoic acid (ATRA) plus arsenic trioxide (ATO) has dramatically further improved the outcomes of patients with non-high risk APL.
First-line ATO was adopted in Portugal as first-line in 2017, and it is unknown whether the outcomes of patients receiving ATRA plus ATO in Portugal reflect the reported excellent clinical trial results.
*Aims:* We aimed to evaluate the outcomes and toxicities of newly diagnosed APL patients treated with ATO plus ATRA in Portuguese academic hospitals, and to compare our real-world cohort results with those reported in the pre-ATO era in Portugal.
*Methods:* An observational, retrospective and multicentric study was conducted, including 5 University Teaching Hospitals in Portugal. All patients with genetically confirmed APL who were treated with first-line ATO plus ATRA were included, regardless of disease risk, between 2017 and 2023. Statistical analysis was performed in Stata (V13).

*Results:* A total of 113 patients were included in the study, with a median age of 50 years (ranging from 18 to 82 years), and 16.8% of patients were over 70 years old.
This cohort was composed mostly of non-high risk APL patients (94,7%), and only 6 patients with high-risk APL were treated with first-line ATO.
More than half of the patients first presented to a community or private hospital (59,3%) and were referred to one of the participating 5 university hospitals. The referral of these patients did not lengthen the time between diagnosis and treatment, with a median of 1 day (range of 0 to 2).
The 30-day early death (ED) rate was 4.4%, and intracranial bleeding was the dominant cause of ED.
The ED rate of this ATO-treated cohort is inferior to the ED rate reported by the Portuguese Group of Acute Leukemia in a real-world cohort treated with ATRA plus anthracyclines from 2010 to 2018, which in non-high risk patients was 11.4% (29 out of 254 patients).
Age was the sole statistically significant predictor of ED n this cohort (OR 1,1 [IC 95% 1,0–1,2], p=0,016).
Among the 108 patients who survived ATO plus ATRA induction, 100% achieved hematologic complete response.
Out of the 92 patients who completed the 4 consolidation courses with ATO plus ATRA, also 100% achieved negative measurable residual disease (as measured by real-time quantitative PCR).
The incidence of differentiation syndrome in the 99 patients who received corticosteroid prophylaxis (14.4%) was significantly inferior to 42.8% in the 14 patients who did not receive any (p=0.008).
With a median follow-up of 27.3 months, the median overall survival (OS) was not reached. The 2-year OS rate was 93.6%. There was only 1 case of APL relapse during follow-up.
*Discussion:* Our data demonstrates that ATO is also a very valuable tool in the treatment of high-risk APL, since 5 out of the 6 high-risk patients who were treated with ATO in our cohort achieved CR with negative MRD.
With first-line ATO treatment, the achievement of CR in all patients who survived induction and of negative MRD in all the patients who completed ATO consolidation courses show that ATO is highly effective in achieving deep APL remissions, leaving ED as the last obstacle to cure in APL.

## APL Biology
## P01

## Venetoclax Triggers Mitochondrial Mediated Apoptosis to Overcome Arsenic Resistance in Acute Promyelocytic Leukemia

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**Abstract.** *Background*: Development of resistance to arsenic trioxide (ATO) monotherapy can occur in a subset of patients with de novo and relapsed acute promyelocytic leukemia (APL) due to various factors, including altered mitochondrial metabolism possessing a significant challenge in the clinical management of APL. There is a need for drugs that can effectively deal with ATO resistance. Recent case reports suggest Venetoclax as a promising candidate for APL due to the elevated expression of Bcl2 in abnormal promyelocytes. In this study, we employed cellular and animal models of ATO resistant APL to assess the efficacy of Venetoclax in overcoming resistance to arsenic.
Materials & Methods: Human ATO-sensitive (NB4), ATO-resistant (NB4-AsR1;PML mutation A216V & UF1;PML unmutated) APL cell lines were kindly gifted by Late Prof Lo-Coo and Prof Mathews. Primary APL cells were obtained after informed consent (IEC/900535/2019). Proteomic profiling by high-resolution Liquid-Chromatography/Mass-Spectrometry (LC/MS), synergistic effects by Cell-titer Glo assay and Calcusyn program; mitochondrial structural alterations and ROS by Electron Microscopy (EM) and Flow Cytometry; apoptotic pathway by Immunoblotting; Orthotopic APL xenografts of ATO-resistance.
*Results*: The inhibitory concentration (IC50) of ATO in NB4, NB4-AsR1 & UF1 cells was 0.63, 2.7 & 2.4 μM, respectively. The LC/MS data revealed differential regulation of proteins involved in pathways of immune regulation, cell survival, signal transduction, and mitochondrial metabolism in ATO-resistant cells compared to sensitive counterparts. Immunoblotting analyses demonstrated that levels of phospho-STAT3 (pY705 and pS727), phospho-mTOR (pS2448), BCL2 and Beclin1 exhibited sustained stability following ATO treatment in ATOR cells. Notably,

upon treatment of resistant cells with Venetoclax, the levels of p-STAT3, p-mTOR were downregulated & Beclin1 were upregulated, with the exception of BCL2. ATO+Venetoclax showed remarkable synergy, decreasing cell proliferation and enhancing apoptosis in resistant cells. Consistent with our hypothesis ATO (1μM) + Venetoclax (0.1μM) significantly increases levels of mitoROS while ATO treatment in resistant cells failed to produce mitoROS. EM-based morphometric analyses of ATOR cells exhibit abnormal ultrastructure following Venetoclax treatment compared to ATO treatment. Flow cytometry-based JC1 assay revealed ROS-mediated mitochondrial depolarization in resistant cells treated with Venetoclax alone or combined with arsenic. Immunoblotting confirmed that ATOR cells were unable to undergo apoptosis as levels of pro-apoptotic (Cleaved caspase-3, cleaved-PARP, BAX), anti-apoptotic (Bcl2, Bcl-xL, XIAP) and PML-RARA proteins remains unchanged in ATOR cells except for a minor downregulation of Bcl2 & upregulation of cleaved caspase-3 in UF1 cells following ATO treatment. Strikingly, Venetoclax triggered PML-RARA degradation and apoptosis in ATO-resistant cells. Venetoclax+ATO-treated xenografts showed a remarkable reduction in hCD45+ marrow cells (mean 3.6%; range 1.6% to 4.8%) compared to ATO (mean 8.4%; range 6.0% to 12.3%) with a corresponding increase in apoptosis and levels of ROS and reduction in the transcript level of PML-RARA.
*Conclusion*: Our results indicate that Venetoclax+ATO cooperates in inducing apoptosis of ATO-resistant cells through mitochondrial stress-induced ROS production coupled with PML-RARA degradation.

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## APL Biology
## P02

## Nuclear Mir-223 Affects Chromatin Organization and Regulates Flotillin-1 Gene Expression in Myeloid Progenitors

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**Abstract.** Hematopoietic cell lineage-specification and fate are deeply regulated by epigenetic signals and microRNA (miRNA), the latest mainly mediating the post-transcriptional gene silencing of target mRNAs. However, recent studies indicate that miRNAs play also a role in the nucleus, where they can bind complementary DNA sequences at specific chromatin sites that induce epigenetic modifications silencing or activating gene promoters’ transcription. The nuclear activity of miRNAs paves the way for non-coding RNA function in somatic stem cell proliferation, lineage specification and differentiation. The alteration of miRNA nuclear function might induce neoplastic transformation.
To address this issue, we carried out chromatin immunoprecipitation-coupled deep sequencing (ChIP-Seq) in HL60 cells undergoing Retinoic Acid (RA)-induced granulocytic differentiation. By using anti-Cy5 antibodies we determined at the whole genome level the genomic sequences complementarily bound by Cy5-labeled double-stranded oligonucleotides, mimicking the activity of endogenous miR-223 (miR-223-Cy5), or by scramble Cy5-miRNA (Control). Moreover, we investigated the chromatin status at these genomic regions with antibodies recognizing the activating (H3K4me3) and/or or repressing (H3K27me3) histone marks. Among the complementary sequences bound by miR-223, we selected an evolutionary conserved region in the Flotillin-1 (FLOT1) gene promoter. We found that this FLOT1 region is bound by a complex comprising miR-223, RISC component AGO1 and trithorax (TrxG) protein RBBP5 and enriched in H3K4me3 marks during myeloid differentiation. Flotillin-1 is an essential component of lipid-rafts associated protein, whose role in hematopoiesis is still largely unknown. Flotillin-1 mRNA and protein levels are increased in primary human CD34+ hematopoietic progenitors undergoing granulo-monocytic differentiation and during RA-induced differentiation of HL60 and NB4 cell lines. In HL60 and NB4 cell lines, the silencing of miR-223 significantly impaired the RA-induced upregulation of FLOT1 levels and

granulocytopoiesis, whereas FLOT1 overexpression or silencing in myeloid progenitors, respectively enhanced or inhibited the expression of differentiation markers CD11b and CD14. We also found that FLOT1 overexpression drives CSF1R, a growth-factor receptor involved in myeloid differentiation, to Rab-4 endocytic vesicles, increasing receptor recycling to cell membrane after CSF1 stimulation. Consistently with the analysis of a large-scale AML datasets, FLOT1 mRNA expression is significantly downregulated in AML blasts compared to healthy bone marrow cells. The lowest expression of FLOT1 was identified in APLs. Data on FLOT1 mRNA and miR-223 expression from The Cancer Genome Atlas (TGCA) reported a consistency between their expression patterns and AML FAB classification; again, the lowest expression levels were identified in APL (M3). Overall, our findings suggest nuclear miR-223 as an epigenetic regulator of chromatin organization and of FLOT1 gene function during physiological and RA-induced myelopoiesis. Our observations are also consistent with the oncogenic role of aberrant expression of miR-223 and FLOT1 in AML and APL, thus opening novel insights into molecular mechanisms leading to leukemia and for the design of new intervention strategies.

## APL Biology
## P03

## Apl-Like Subset Within Npm1-Mutated Acute Myeloid Leukemia: A Distinct Phenotypic Signature Correlating with Early-Onset Vascular Complications

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**Abstract.** *Background*: Marked advancements in therapeutic management have been achieved in acute myeloid leukemia (AML). Nevertheless, the initial phase, including diagnostic workup, clinical stabilization, and induction delivery, remains critical as burdened by potential complications, including early vascular events. The assessment of hemorrhagic risk at AML onset is primarily directed at evaluating acute promyelocytic leukemia (APL); once excluded, the management is based on clinical picture, hemochrome/coagulation parameters but is independent from biological markers. A subtype of NPM1mut AML is characterized by a phenotypic profile resembling APL, thus defined APL-like, a signature featured by negativity for CD34 and HLA-DR, heterogenous CD117 expression and dim positivity for CD13 (Mason et al,AJH 2018;93,504).
*Aims*: The aim of the study was to investigate the APL-like subset as a potential predictor of susceptibility to early vascular events.
*Methods*: Pts diagnosed with NPM1mut AML according to standard criteria were recruited from the centres of Florence (n=150) and Rome (n=57) and classified for APL-like profile as specified above. Two control groups were studied: NPM1mut non-APL-like and NPM1 wild type (wt) bearing IDH1-2 mutations.
*Results*: From 2007 to 2023, 207 pts with NPM1mut AML were enrolled, of whom 47 (22.7%) APL-like (Table 1). APL-like pts were older (65 vs 57 y, P=.001); no difference emerged for baseline parameters. Early (within 30 days from diagnosis) vascular complications (n=8 bleeding events and n=5 thrombotic events) were significantly more frequent in APL-like (n=13, 28.9%) than non-APL-like (n=18, 11.6%, P=.009) group. Also, coagulopathy, as defined by INR ≥1.5 and/or fibrinogen (FBG) lower than 150 mg/dl, was significantly more frequent in APL-like (29.5%) than non-APL-like (15.3%, P=.046) pts. D-dimer (DD) and DD/FBG ratio were significantly higher in APL-like (median 38,165 ng/ml and 16.85) than in non-APL-like (2,258 ng/ml, P=.001, and 4.8, P=.003, respectively). Available molecular data showed an enrichment in IDH1 (32.1%) and IDH2 (50.0%) gene mutations in APL-like vs non-APL-like counterpart (12.2%, P=.001, and 18.6%, P=.000, respectively). We didn't observe a significance in the time of occurrence of vascular complications (7 days in APL-like vs 5 in non-APL like, P=.495). DD and DD/FBG ratio confirmed higher in APL-like than in a cohort of 17 NPM1wt IDH1/2mut pts, the latter group showing values of DD and DD/FBG ratio equal to 1,326 ng/ml (P=.013) and 1.88 (P=.001), respectively. The incidence of vascular

events and coagulopathy observed in APL-like pts showed higher trends with respect to NPM1wt IDH1/2mut group (5.9%, P=.087, and 6.3%, P=.086, respectively), non-significant likely due to limited numbers. These findings support a specific effect exerted by IDH1/2 mutation on coagulation profile only within NPM1mut context. In terms of outcomes, complete remission rate was similar in NPM1mut APL- and non-APL-like groups (81.9% vs 88.6%, respectively; P=.455), and there was no significant difference in overall survival, with 15.3 months for NPM1mut APL-like and 12.4 months for non-APL-like (P=.085).
*Conclusions*: Our findings suggest NPM1mut APL-like subset could be a biomarker of early vascular risk and thus support the adoption of disease-driven clinical measures, such as strict laboratory monitoring and more stringent thresholds for transfusion support, similarly to the current approach in APL.

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## APL Biology
## P04

### Phase Separation of PML/RARα Microspeckles Governs Transcriptional Dysregulation Through Genomic Rewiring of BRD4 in Acute Promyelocytic Leukemia

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**Abstract.** *Introduction*: Abnormal nuclear morphologies are frequently observed in cancer cells, which have been implicated in biological reactions such as gene regulation. However, the nature of altered nuclear structures, particularly molecular components, regulatory functions and oncogenic activity, has remained largely enigmatic and awaits characterization. In acute promyelocytic leukemia (APL), the PML/RARα fusion oncoprotein, which is produced by t(15;17), has been long observed for its ability in destroying PML nuclear bodies (NBs) and subsequently forming microspeckles. Despite being long observed, how PML/RARα-mediated microspeckles are formed and linked to APL leukemogenesis remains a mystery. To address this knowledge gap, we present a comprehensive analysis of the driving forces and constituent components of PML/RARα-assembled microspeckles and their functional implications in transcriptional dysregulation and leukemogenesis.
*Methods*: High-resolution imaging techniques, biophysical approaches, multi-omics strategies, and in-depth functional validation are used in this study. Biophysical strategies, such as FRAP, in vitro droplets formation assays, were utilized to unveil the biophysical nature of PML/RARα microspeckles. We employ immunofluorescence and RNA-scope to identify the spatial relationship between PML/RARα microspeckles and partner proteins as well as putative target genes. Co-IP and mass spectrometry are performed to identify interactome of PML/RARα. ChIP-seq is utilized to determine the genomic regions where PML/RARα microspeckles occur. ChIP-seq and RNA-seq is performed to evaluate the transcriptional output after perturbation of PML/RARα phase separation.
*Results*: We uncover the biophysical mechanism of liquid-liquid phase separation (LLPS) underlying the assembly of PML/RARα microspeckles and elucidate their role in APL leukemogenesis. Our findings reveal that PML/RARα co-assembles with BRD4 to form de novo phase-separated condensates, which distinguish them from PML nuclear bodies. PML/RARα and BRD4 co-assembled condensates exhibit preferential occupancy on super-enhancers and broad-promoters, targeting genes essential for APL leukemogenesis. Mechanically, PML/RARα incorporates BRD4 into nuclear condensates via its phase separation capacity, thereby facilitating BRD4 chromatin binding and redistribution. Importantly, blockage of BRD4 activity suppresses APL cell proliferation and induces apoptosis, thereby impairing PML/RARα-driven leukemogenesis. Finally, perturbation of LLPS depletes the chromatin co-occupancy of PML/RARα and BRD4 and attenuates their target gene activation, reinforcing the importance of LLPS in transcriptional dysregulation. In the newly diagnosed APL cohort, the abundance of PML/RARα and BRD4 co-assembled condensates is significantly positively related to the presenting white blood cell count and the mRNA expression level of PML/RARα and BRD4 co-regulated genes.
*Conclusions*: In this study, we have provided a comprehensive analysis of PML/RARα-assembled microspeckles, shedding light on their driving forces, constituent components, and regulatory function. We have also highlighted the role of PML/RARα phase separation in the pathogenesis of APL leukemogenesis. Therefore, our study provides valuable biophysical insights into the molecular basis for PML/RARα to exert its oncogenic activity.

**APL Biology**
**P05**

## Redefining Acute Promyelocytic Leukemia Identification by CRISPR-CAS System (Rapid-CRISPR)

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**Abstract.** While existing APL diagnostic methods demonstrate rapidity, sensitivity, and accuracy, none presently meet the criteria for a point-of-care assay suitable for diagnosis in an outpatient setting with minimal instrumentation. In this study, we propose to Redefine APL IDentification by CRISPR System (RAPID-CRISPR). This method provides LAMP-mediated CRISPR-Cas12a-based lateral flow assay as a proof-of-concept for PML-RARA detection. Setting, design & results: Biological materials from 48 APL patients, 36 non-APL leukemias (AML, CML, ALL) and 10 healthy individuals.
*Loop-Mediated Isothermal Amplification (LAMP) primers and CRISPR-RNA(CrRNA) design*: In addition to manually designed conventional LAMP primers (FP, FIP, RP, RIP), we modified FIP sequence to create a synthetic protospacer-adjacent motif (PAM) site (TTTV) for Cas12a recognition close to PML-RARA junction sequence for BCR1 and BCR2. Concerning BCR3, we found a natural PAM sequence close to PML-RARA junctional sequence. The CrRNA for BCR3 was designed using the junctional sequences of PML-RARA, while a single CrRNA was designed from RARA exon 3 for BCR1 and BCR2. Extracted cDNA from leukemic and healthy cells was subjected to LAMP reaction.
*1)Fluorescence-based readout*: After LAMP, fluorescence-based readout was performed as previously described ((Broughton et. al. Nat Biotechnol 2020). After formation of CrRNA-Cas12a complex, 5 µl of LAMP product was added to set at 37C for 15' followed by 500 nM of fluorescent reporter molecule (DNaseAlert, 5'HEX-TTATTATT-BHQ3') for Cas12a trans-cleavage reaction. The fluorescence signal was monitored for 30 minutes at 37C using Cytation 5 reader. To validate the assay, 94 clinical samples were analyzed, comprising 48 cases of PML-RARA positive APLs (bcr1 n=25, bcr2 n=5, bcr3 n=18). The assay displayed a four-fold increase ($p<0.0001$) in fluorescence intensity in APL (mean RFU=2233) when compared to non-APL (mean RFU=565) and healthy donors (mean RFU=265). The assay at a cut-off of 1092 RFU could rapidly (3 hours) and reliably discriminate APL patients from non-APL controls with 97.9% (89.1-99.8) sensitivity and 100% specificity (92.2-100). We found 100% concordance between the fluorescence assay and RQ-PCR performed using the primer/probe set developed within the Europe Against Cancer program. The blinded validation set comprising an independent cohort of 20 samples (10 APL and 10 non-APL) provided 90% sensitivity and 100% specificity. The assay demonstrated an analytical sensitivity, detecting PML-RARA transcript with a limit of detection (LOD) of 8 copies/µl).
*2)Lateral flow-based readout*: As an alternative to fluorescence-based readout, which requires an instrument, the Cas12a trans-cleavage reaction was allowed to proceed for 10 min at 37C, similarly as described above, except DNAase alert was substituted with lateral flow cleavage reporter (5'FAM-TTATTATT-BIO3'). A Milenia® HybriDetect lateral flow strip was subsequently immersed into the reaction tube. One band on the Control line signified a negative result, while either two bands on both the Control and Test lines or a single band on the Test line indicated a positive result.
*Conclusion*: With external validation and HUDSON-method-standardized RNA extraction, RAPID CRISPR holds promise as a point-of-care APL diagnostic. Its high sensitivity, accuracy, instrument-free operation, and <3hrs turnaround position it as an advanced and efficient tool to detect PML-RARA

https://www.medicalhosting.org/APL/Autori/Allegati/18-hasanFigure.jpg

**APL Biology**
**P06**

## The Potential of CIRCFN in Modulating PML-RARα Stability and Progression in Acute Promyelocytic Leukemia

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**Abstract.** The PML-RARα fusion protein is central to both the development and treatment of Acute Promyelocytic Leukemia (APL), with its functionality being significantly influenced by its stability. Recent studies have begun to uncover the layers of regulation impacting protein stability, particularly focusing on the role of non-coding RNAs. An intriguing area of research lies in exploring the potential role of circular RNAs (circRNAs), a covalently closed continuous loops known for their ability to bind proteins. It remains unknown whether these circRNAs can influence the degradation of the PML-RARα fusion protein. Our recent study employed RNA immunoprecipitation in conjunction with RNA sequencing (RNAseq) to investigate this hypothesis. This approach successfully identified a particular circular RNA, circFN, which exhibits a notable affinity for binding to the PML-RARα protein. Subsequent experiments demonstrated that manipulating circFN levels, whether through overexpression or knockdown, directly influences the stability of the PML-RARα fusion protein, rather than affecting its mRNA expression of the PML::RARA fusion gene. In a clinical context, our study observed a positive correlation between the expression of the PML::RARA fusion gene and circFN in APL patient samples. Additionally, in vitro experiments using the NB4 cell line, a model for APL, demonstrated that treatment with all-trans retinoic acid (ATRA) or the knockdown of the PML::RARA fusion gene leads to a significant decrease in circFN levels. The most striking implication of our findings is the suggestion of a positive feedback loop formed between circFN and PML-RARα. This loop appears to maintain the stability of the PML-RARα protein, thereby promoting the progression of APL. Understanding this feedback mechanism could provide new insights into the molecular pathology of APL and reveal novel therapeutic targets. In summary, the discovery of circFN's role in stabilizing PML-RARα underscores the importance of circRNAs in APLbiology and opens new possibilities for targeted therapies. The modulation of circFN levels could potentially serve as a novel therapeutic strategy in APL, either by destabilizing PML-RARα or by disrupting the feedback loop that promotes disease progression.

**APL Biology**
**P07**

## Elucidating the Oncogenic Role of RARg Rearrangements and Related Therapeutic Approaches in Acute Myeloid Leukemia Resembling Acute Promyelocytic Leukemia

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**Abstract.** Acute myeloid leukemia (AML) featuring retinoic acid receptor-gamma (RARG) rearrangements (RARG AML) exhibits morphological features resembling those of acute promyelocytic leukemia (APL) but is associated with drug resistance and poor clinical outcomes. However, the mechanisms underlying the role of RARG fusions in leukemogenesis remain elusive. Here, we report that RARG fusions disrupt myeloid differentiation and promote the proliferation and self-renewal of hematopoietic stem and progenitor cells (HSPCs), similar to the effects of the PML-RARA fusion gene in APL. Mechanistically, RARG fusions, akin to PML-RARA, exerted transrepressive effects on

PU.1 and CEBPE while also exhibiting transactivating effects on GFI1. Unlike PML-RARA, however, RARG fusions selectively upregulated BCL2 and ATF3 in HSPCs, driving the uncontrolled proliferation and disrupting the differentiation of RARG AML cells. Our in vivo data further demonstrated that overexpression of RARG fusions led to a preleukemic phenotype by inducing aberrant expansion of HSPCs but failed to induce oncogenic transformation. Given the notable prevalence of WT1 mutations among patients with RARG AML, we further utilized mice harboring Wt1-haploinsufficient hematopoietic cells and demonstrated that the cooccurrence of RARG fusions and heterozygous Wt1 loss induced fully penetrant AML. Detailed investigations revealed the activation of the MYC targets in leukemic mice but not in those with only RARG fusion overexpression or Wt1 loss. Additionally, leveraging the Connectivity Map resource and high-throughput screening (HTS), we successfully identified potential therapeutic options for RARG AML, including venetoclax, homoharringtonine, and daunorubicin. The efficacy of these compounds was further validated in leukemia transplantation mouse models and patient-derived xenograft models. Overall, our findings provide pivotal insights into the molecular mechanisms governed by RARG fusions and enhanced by WT1 loss in AML development and propose a rational therapeutic strategy for RARG AML.

## APL Biology
## P08

## Molecular and Genetic Characterization of Patients with Acute Promyelocytic Leukemia: Which is the Role of FLT3 Mutation? A Monocentric Experience.

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**Abstract.** Acute promyelocytic leukemia (APL) shows a typical pattern in genetic and molecular analysis. Few data exist on the role of other abnormalities, our study tried to characterize patients (plts) focusing on FLT3 and other genetic abnormalities found at diagnosis or during follow-up in the era of arsenic trioxide (ATO) plus all-trans retinoic acid (ATRA) treatment.
From our APL dataset we considered pts whose FLT3 status was known.
Thirty consecutive pts diagnosed as APL in our center from January 2015 were included in the study: 17 females and 13 males patients with a median age of 51 years (range 31-76), for the majority intermediate risk (19 pts, 63%). Twenty-five pts were treated with ATO plus ATRA (of whom only 3 need two doses of idarubicin in the induction phase) and 5 pts with ATRA plus chemotherapy. All pts achieved complete molecular remission (CMR) after a median time of 3 months (range 1-6). They maintained CMR at a median time of 46 months (range 3-97) except for 1 pt who relapsed many times and died after allogeneic stem cell transplantation. All but 2 pts (1 dead in progression and 1 dead for sepsis after surgery for bladder carcinoma) are alive and in CMR at a median follow-up of 46 months (range 3-108) without late sequelae effects.
We divided the population in 2 groups according to FLT3 status: 14 pts resulted positive (FLT3pos) and 16 negative (FLT3neg). From their comparison, FLT3pos pts showed higher white blood counts and lower platelet counts; we noted that all the 5 high risk pts were in FLT3pos subgroup, then, to better compare the impact of FLT3 on APL pts, we selected only pts at low or intermediate risk. We also did not consider into the analysis the 2 FLT3neg pts who received chemotherapy. Characteristics of 9 FLT3pos pts were compared to 14 FLT3neg pts, all at intermediate or low risk and treated with ATO plus ATRA, as shown in Table 1.
FLT3pos pts were mainly female (ratio F:M=2:1 versus 3:4 of the FLT3neg group), with higher white blood count (median 2.0x109/L vs 1.0x109/L), lower platelet counts (21x109/L vs 29x109/L) and hemoglobin (8.6 g/dL vs 9.6 g/dl). FLT3 positivity was also associated more frequently to bcr3 and not to bcr1 as seen in FLT3neg pts. Both subgroups showed additional trisomy 8 at genetic analysis: 1 FLT3pos case 46,XX,t(15;17)(q22;q12)[9]/46,XX,+8,-F,t(15;17)(q22;q12)[1] at diagnosis, rapidly disappeared after treatment; 2 FLT3neg cases: 47,XX,+8[8]/46,XX[12] disappeared after 5 months after the induction and 46,XY[1]/47,XY,t(15;17),-18,+8[4] disappeared after more than 1 year after the induction treatment even if in both the cases the molecular remission was reached within 4 months.
For the clinical point of view FLT3neg pts showed more frequently ATRA syndrome and needed temporary discontinuation ATRA and/or ATO, mainly for infective complications. Two thrombotic complications were registered in both groups; we found an higher incidence of these complications in FLT3pos pts who already showed higher median d-dimer and an higher DIC score: in the FLT3pos group 1 case of sovrahepatic veins thrombosis and

1 case of superficial femoral vein thrombosis, in the FLT3neg group 1 case of pulmonary embolism and 1 case of CVC-related thrombosis (in the same pt who showed mild cerebral hemorrhage).
These interesting data on clinical outcomes, correlations with genetic and molecular pattern, thrombotic complications, should be confirmed in a larger cohort to try to understand the role of FLT3 in APL.

https://www.medicalhosting.org/APL/Autori/Allegati/24-Table 1.docx

## APL Biology
## P09

## Is it Possible an Inverse Correlation Between Environmental Arsenic Concentration and Incidence of Acute Promyelocytic Leukemia?

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**Abstract.** *Background*: Acute promyelocytic leukemia (APL) has always shown tendency to time and geographical clustering with seasonal variations and areas characterized by a higher incidence of APL diagnoses.
In 2019 SIE congress we presented a poster on the distribution of APL cases in Basilicata over the last 15 years, with evidence of a non-homogeneous distribution among different areas of this region. In our study we had hypothesized that there might be an inverse correlation between incidence of APL and the concentration of Arsenic (As) detected in river waters by Regional Agency for the Environment (ARPAB).
Aim of this study is to verify the geographical location of all APL cases since 2005, year of start activity of Basilicata Cancer Registry to date and if ARPAB environmental determinations confirmed the concentrations of As detected in previous years.
*Results*: In the last four years our data confirm As concentration lower in the rivers of the southern Basilicata than in the northern with reproducible data among the different areas, except for a tendency towards an increase in As concentration in Metaponto area.
We confirmed how As concentration was lower where APL cases were more numerous. The study lends itself to many criticisms due to small number of cases, the distribution over a very long period of time and the difficulty in establishing if As concentration in river waters actually corresponds to the quantity of substance people actually come in contact with.
However, we assume that where As concentration is lower, PML/RARa clones are more likely to grow and develop clinical manifestations. It would be useful and not so complicated to carry out similar assessments in other Italian Centres.
Finally, to evaluate seasonal variations we evaluated the month of onset of all APL cases, even prior to 2005, and we verified out of 52 cases, 18 have been diagnosed from October to March and 34 from April to September. A study is ongoing to verify whether there is a periodicity of the concentration of environmental As in the different periods of the year.
We would like to resubmit these data to the APL scientific community to verify whether there is an interest to study the possible correlation between environmental arsenic concentration and the incidence of Acute Promyelocytic Leukemia. It could be possible to identify environmental detection methods other than those used in this study, or determinations on biological samples that would be representative of the arsenic accumulation in the organism, for example hair.

**APL Biology**
**P10**

## FLT3-ITD Mutation: Useful for Risk Stratification in Acute Promyelocytic Leukemia?

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**Abstract.** *Background*: Acute promyelocytic leukemia (APL) is a subtype of acute myeloid leukemia that has a distinctive molecular pathophysiology and clinical manifestations. It is cytogenetically characterized by reciprocal translocation of promyelocytic leukemia (PML) gene at chromosome 15 and the retinoic acid receptor alpha (RARα) gene at chromosome 17 leading to the termination of maturation at the promyelocyte stage. ATRA and ATO therapy has remarkably improved the outcome of APL patients. The long-term survival rate is now greater than 95%, yet refractory/relapsed disease is still seen in around 5% of patients. A high white blood cell (WBC) count (>10×109/L) is supposed to be the main factor associated with relapse. The Sanz score subdivides APL patients according to peripheral blood counts into three risk groups. The most suitable parameters for risk stratification in APL are still under debate. FLT3-ITD mutations have a significant incidence rate of about 12–38% in APL. The role of FLT3-ITD mutations in APL as a prognostic factor for long-term outcome has not yet been clarified, and the significance of these genetic alterations remains controversial. FLT3-ITD mutations have been associated with a variety of characteristics in APL including high WBC count, short bcr-3, or microgranular morphology.
*Purpose*: In this monocentric study, we investigated the impact of FLT3-ITD mutations on relapse-free survival (RFS) and overall survival (OS) of newly-diagnosed patients with APL treated with all-trans retinoic acid and arsenic trioxide.
*Methods*: The study was based on 24 newly-diagnosed APL patients (12 male and 12 female, median age 50 years, range 15 – 83 years) The FLT3-ITD mutation was assayed by PCR and gel electrophoresis analysis. Its impact on RFS and OS was investigated in patients with and without the mutations.
Results: The FLT3-ITD mutation rate in newly-diagnosed APL patients was 38% (9/24). The WBC count at diagnosis in patients with mutations was higher than that in patients without mutations and the FLT3-ITD mutation rate was significantly higher in the high-risk group than in the low/intermediate-risk group (P= 0.003). Patients with FLT3-ITD mutations had a significantly higher early death rate (33.3% vs 0%) than those lacking the mutation (P =0.017), all of them showed PML-RARA transcript bcr3 and a high WBC count. OS analysis showed a significant difference between the patients stratified by FLT3-ITD mutation status (P =0.0017) while survival outcome in terms of RFS did not differ significantly in our study.
*Conclusion*: Our study confirms that APL patients with FLT3-ITD mutations showed a higher WBC count than patients with FLT3 wild-type. Patients carrying mutations had a higher early death rate compared to those without mutations and reduced OS rates. Prospective trials should further investigate the clinical impact of the FLT3-ITD mutation aiming to evaluate whether this parameter might be included in risk stratification in APL.

**APL Biology**
**P11**

## WT1 Expression in APL: The Final Countdown

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**Abstract.** Wilms' tumor gene (WT1) is highly expressed in Acute Myeloid Leukemia (AML) and it is generally highest in Acute Promyelocytic Leukemia (APL). Although WT1 expression correlates with worse prognosis in AML, its prognostic role in APL is still controversial and literature data are lacking. Therefore, the aim of this study is to explore the impact of WT1 expression in APL and its prognostic significance for clinical endpoints.

From May 2008 to December 2023, a total of 33 consecutive patients (pts) (12 male and 21 female) with a median age of 51 years (range 24-81 ys) were diagnosed as having de novo APL in our Hematology Unit.
Among 33 pts, 15 (45,5%) received a combination of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) treatment, 11 (33,3%) pts were treated with Idarubicine (IDA) + ATRA, 5 (15,2%) pts received a combination of ATRA+ATO+IDA and 2 (6%) pts were treated with fludara based chemotherapy. Only 3 (9%) pts died, 1 after disease relapse and 2 of them during the induction cycle for lung infections and for a severe differentiation syndrome. Moreover, 31/31 pts were in complete morphologic remission after induction therapy. Clinical features at diagnosis are summarized in Table 1.
All pts were tested for PML-RARα and WT1 expression by quantitative reverse transcription polymerase chain reaction (RT-PCR) at diagnosis, after induction and after consolidation chemotherapies. The most frequent PML-RARα transcript type detected was the bcr 1 (21/33, 64%) and the median PML-RARα copies at diagnosis were 52 (11,6- 110). Median normalized WT1 copies were instead 13089 (4426-257579), a value statistically higher as compared to that observed in a series of AML cases tested during the same time frame (97 AML cases tested, median WT1 normalized copy value 3.254). After induction chemotherapy, median WT1 expression dropped to 53 copies (1-1001) and PML-RAR to 0,006 copies (0-52), with a slope of reduction of 2 logarithms for WT1 and more than 4 logarithms for PML-RARα transcripts (including, in the latter, several pts with undetectable copies).
When we tried to correlate the level of WT1 expression with clinical and biological features at diagnosis no statistically significant association was found with WBC, Hb, PTL, LDH, hypofibrinogenemia, D-Dimer or with other molecular alterations, such as FLT3. Furthermore, no correlation was found between the degree of overexpression of WT1 and time to complete remission and overall survival.
In conclusion, despite the limits of a small, yet homogenously monitored overtime, APL population we confirm that WT1 expression at diagnosis is higher than in other AML subtypes and that slower decreasing of WT1 copies after treatment is probably due to its double role as disease marker and marker of reconstitution of hemopoiesis. Furthermore, we confirm that WT1 expression does not have a prognostic role in APL, suggesting that it should not longer be tested.

https://www.medicalhosting.org/APL/Autori/Allegati/30-TABLE 1.pdf

## APL Biology
## P12

## Super-Enhancer-Associated Long Noncoding RNA LNC-SPI1U Participates In PU.1 Feedback Regulation by Interacting With HNRNPH1 And HNRNPF

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**Abstract.** Super-enhancers (SEs) are essential regulatory elements that orchestrate the precise transcriptional regulation of cell-type- and state-specific-gene expression during hematopoietic differentiation. The role of SEs on the regulation of long noncoding RNAs (lncRNAs) during myeloid differentiation remains poorly understood. In this study, we illustrated the landscape of SE-associated lncRNAs in acute promyelocytic leukemia (APL) by integrating 20 datasets of H3K27Ac ChIP-seq and 379 datasets of RNA-seq from the TCGA and Beat AML databases. We identified 44 lncRNAs that are located in close proximity to SEs, which may play a pivotal role in the pathogenesis APL. Among these, we designated the lncRNA RP11-750H9.5, which is found upstream of the SE region of the SPI1 gene encoding key hematopoietic transcription factor PU.1, as lnc-SPI1U. Our data found that lnc-SPI1U was involved in the regulatory loop of PU.1 during all-trans retinoic acid (ATRA)-induced differentiation. The lnc-SPI1U transcript and its genomic locus functionally play distinct roles in SPI1 gene expression. lnc-SPI1U inhibited cell differentiation and apoptosis while promoting cell proliferation during ATRA-induced myeloid differentiation. This effect was mediated by destabilizing SPI1 mRNA in concert with HNRNPH1 and HNRNPF interactions. Conversely, the genomic deletion at the lnc-SPI1U locus diminished SPI1 activation. Further analysis of transcriptional regulation

illustrated that PU.1 induced the expression of lnc-SPI1U by binding to its promoter and enhancer regions, and the induction of lnc-SPI1U by ATRA was dependent on PU.1. These findings suggest that lnc-SPI1U might function as a "brake" to modulate PU.1 level and participate in PU.1 feedback regulation, thereby accurately fine-tuning the expression of PU.1 at the optimal level for myeloid differentiation. However, the PML/RARα fusion protein, characteristic of APL, disrupted the lnc-SPI1U-SPI1 regulatory circuit by hijacking the lnc-SPI1U genomic region, leading to the inhibition of SPI1 activation. Our findings emphasize the significant role of SE-associated lncRNAs, such as lnc-SPI1U, in the gene regulatory networks involved in hematopoietic differentiation.

## APL Biology
## P13

### Acute Promyelocytic Leukemia: A New PML-RARa Fusion Transcript Identified by Flow Cytometry, Molecular and Cytogenetics Analysis

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**Abstract.** Acute promyelocytic leukemia (APL) is characterized by the PML-RARA fusion gene as a consequence of the t(15;17) (q22;q21) translocation. Depending on the PML breakpoint, three different PML/RARA transcript isoforms may be generated: long (bcr1), variant (bcr2), and short (bcr3), respectively.
Description: We describe a case of APL with atypical PML/RARA transcript, which was not detectable using standard molecular procedure. Differential detection of PML-RARA bcr1, bcr2, bcr3 fusion transcripts were analyzed using a LAMP technology based kit (DiaSorin Molecular) and no amplification was detected. In addition, a Real time PCR performed using HemaVision-28Q kit (DNA Diagnostic), detected no amplification. Given the strong clinical and morphological suspicion, we tested the sample for RT-PCR (Biomed protocol) using PML-A2 and RARA-B primers. Analysis of PCR product showed one specific band of 440 bp, a size that does not correspond to typical transcripts.
The immunophenotipic characterization of bone marrow aspirate showed 90% of abnormal mononuclear cells, 45% of which expressed CD34 and a myeloid phenotype (positive for CD117, CD13, CD33 and partial for CD2 and MPO). Apart from this population with the help of logical gate, we found a population that did not express CD34 and HLA DR and had cytometric characteristics of APL.
Morphological examination demonstrated a markedly hypercellular marrow with 40% myeloblasts and 30% abnormal promyelocytes of medium-sized, with bilobed nuclei and hypergranulated cytoplasm and rare Auer bodies.
The traditional cytogenetic analysis at the diagnosis, assayed by the R- banding, revealed the following karyotype: 46,XY,t(15;16;17)(q24;p13;q21)[18]/47,XY,+8,t(15;16;17)(q24;p13;q21)[2], as shown in fig.1.
Discussion: In this report, we described a novel atypical PML/RARA fusion transcript. Of note, standard molecular and immunophenotipic analysis was inconclusive, while morphology and clinical features were highly suggestive for APL.
By using different molecular test such as Lamp, technologies based Kit (DiaSorin Molecular) and a Real time PCR using HemaVision-28Q kit (DNA Diagnostic) no amplification was detected.
Then RT-PCR for PML/RARA detection was carried out using standard protocol (Biomed) and the analysis of sample by gel – electrophoresis showed an atypical size respect classic size of the typical isoform (bcr1, bcr2, bcr3).
In this case, it was necessary to carry out additional investigations for the identification of the PML-RARA isoform. For this reason, we used the Sanger sequencing approach, a methodology used to study gene regions associated with a defined phenotype, to confirm next generation sequencing (NGS) variants, to detect minor allele fractions up to 5%, or read contiguous sequences up to 1,000 bases.

The methodology used in this case report confirmed the sequence identified through the cytogenetic approach; in addition, it allowed establishing that the PML-RARA fusion gene sequence had kept the reading code unchanged. Conclusion: The final diagnosis made was APL and the patient started therapy with arsenic trioxide and all-trans-retinoic-acid. Nowadays he is in complete remission after 36 months after diagnosis. This case indicates that the use of different experimental approaches and different techniques are necessary to confirm the diagnosis of difficult cases of APL and is fundamental for minimal residual disease (MRD) monitoring.

https://www.medicalhosting.org/APL/Autori/Allegati/19-Figure 1 APL.pdf

## APL Biology
## P14

## Molecular Characterization of Acute Promyelocytic Leukemia in a Tertiary Care Cancer Center in India.

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**Abstract.** Acute promyelocytic leukemia (APL) accounts for 10-15% of all acute myeloid leukemia cases and is characterized by distinct clinical, morphological features and unique chromosomal anomaly. More than 95% of APL patients harbor a translocation between chromosomes 15 and 17, which fuses the RARA gene on chromosome 17 with the PML gene on chromosome 15. The fusion product, PML::RARA protein plays an important role in leukemogenesis. The PML::RARA protein has 3 isoforms which correlate with the unique presentation of the disease and can be subtyped by reverse transcriptase polymerase chain reaction (RT-PCR) and other assays. Depending on the location of breakpoints within the PML gene site, intron 6, exon 6 and intron 3, the respective PML::RARA transcript subtypes referred to as bcr1, bcr2 and bcr3 may be formed. They represent 55%, 5% and 40% of the cases respectively. A small proportion of patients harbor variant translocations that result in fusion of RARA to one of a number of alternative partner genes. Arsenic trioxide (ATO) and ATRA in combination with chemotherapy has revolutionized the therapy of acute promyelocytic leukemia by achieving complete remission rates more than 90% and long-term remission rates above 80%.
*Materials and Method*: This is a retrospective analysis of all the RT-PCR assays performed at our center for APL patients to detect PML::RARA fusion transcripts in diagnostic as well as follow-up sample over a 6 year duration (2018-2023). The RT-PCR assay was performed on RNA extracted from peripheral blood or bone marrow samples as per standard protocol. The PCR products were visualized on Ethidium bromide-stained agarose gel electrophoresis and subsequently the transcript isoforms were identified. Targeted Next generation sequencing (NGS) assay, Oncomine™ Myeloid Research Assay (ThermoFisher Scientific), was performed in cases which morphology favored APL but they were negative for t(15;17) and RT-PCR. The DNA and RNA sequencing was performed on GeneStudio S5 platforms.
*Result*: Over a 6 year duration, total of 301 RT-PCR assay were performed of which 80 assays were performed in diagnostic samples and 221 assays were performed in follow-up patients. In these diagnostic sample bcr-1 transcript was found in 54% (n=43) cases, bcr-2 transcript in 10% (n=8) and bcr-3 transcript was found in 35% (n=28) cases. Targeted NGS assay was performed in 6 cases which were negative for t(15;17) by FISH and RT-PCR assay to detect PML::RARA fusion protein. The NGS, RNA fusion analysis, highlighted one rare case of variant APL (STAT5B::RARA fusion), 3 cases of bcr3 and 2 cases of bcr1 PML::RARA isoforms. DNA mutation analysis was also performed for these cases and highlighted prevalence of FLT-ITD mutation (n=3 cases) in APL cases.
*Conclusion*: The diagnosis of APL is multifaceted and requires clinical suspicion, morphology, immunophenotyping and molecular diagnostics to work in tandem. Identification of the PML::RARA isoform in APL cases by RT-PCR has a remarkable impact into the management of APL and enables detection of residual disease during treatment and follow-up. However, in rare atypical variant cases with morphological evidence of APL, newer technologies like RNA based NGS can help in diagnosis. PML::RARA isoform detection by highly sensitive assays like digital PCR form basis of molecular MRD. At our center we perform molecular MRD analysis by RT-PCR assay and droplet digital PCR assay(228 assays for 95 patients).

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**APL Treatment**
**P15**

## Early Deahts in Acute Promeylocytic Leuekmia Patients: A Still Unmet Medical Need

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**Abstract.** *Background*: Acute promyelocytic leukemia (APL), is now curable in a high proportion of patients using targeted agents [All-transretinoic acid (ATRA)/Arsenic Trioxide (ATO) or ATRA combined with chemotherapy (ATRA+Idarubicin, AIDA-based)]. Despite significant advances, early death (ED), occurring within 30 days from the diagnosis, remains a clinical challenge and an unmet medical need, with rates as high as 20% even in recent reports. Several authors tried to identify clinical predictors and to formulate scores with variable results without, though, paradigm-shifting breakthroughs in APL management.
*Material and methods*: We analyzed a large cohort of newly diagnosed patients with APL included in the HARMONY Platform (derived from APL0406 and AML17 clinical trials, and Study Alliance Leukemia, Swedish AML Registry and AML study Group registries). After acquisition from the sources, data were harmonized and transformed using an Observational Medical Outcomes Partnership Common Data Model, and registered in the HARMONY Platform.
*Results*: Of 1533 patients included in the platform, 1438 met the quality requirement and were further analyzed. ED occurred in 85 patients (5.9%); in particular, 32 patients died before induction therapy start. Information on the treatment were available in 1309 patients. Of these, 563 received ATRA/ATO (43%) and 747 AIDA (57%). The rate of ED was 2.5% in ATRA/ATO arm (14 patients) and 5.2% in AIDA arm (39 patients). ED occurred at a median of 9 days from diagnosis (range 0-30 days), and data on the causes were available in 25 patients: the most frequent was bleeding (52%, 13 patients), followed by infections (44%, 11 patients) and differentiation syndrome (4%, 1 patient) (Figure 1a). At univariate analysis, treatment with AIDA ($p=0.022$), elderly age ($p<0.001$), high Sanz-risk score ($p<0.001$) and the non-clinical trial context ($p<0.001$) were significantly associated with ED (Figure 1b). However, at multivariable analysis, none of these variables maintained an independent value (Figure 1c).
*Conclusions*: Early death remains one of the major issues and an unmet clinical need in the management of APL, occurring in at least 6% of patients, with no identifiable clinical predictors. This finding emphasizes the key role of a prompt APL diagnosis and of the control of bleeding and infectious complications to impact on early unfavourable outcome of an otherwise highly curable disease. In the subsequent analysis, we aim to validate the promising, recently-published score by Österroos et al. (PMID: 35081688), based on age, white blood cells (WBC) and platelets. Furthermore, taking advantage of our large cohort of patients, we aim to investigate the role of WBC in predicting ED beyond the conventional 10.000/mm3 cut-off assessed by the Sanz-risk score.

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## APL Treatment
## P16

### Novel Insights into TTMV::RARA Fusion in Acute Promyelocytic Leukemia: Two Unique Case Reports with Successful Atra Treatment

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**Abstract.** Acute Promyelocytic Leukemia (APL), traditionally characterized by the PML::RARA fusion gene. However, recent studies have identified a novel fusion gene, TTMV::RARA, arising from the integration of torque teno mini virus (TTMV) into the RARA gene, marking a rare instance where a viral element is implicated in human cancer. To date, only five cases of TTMV::RARA fusion have been reported, leading to a limited understanding of its genetic complexity. Furthermore, the effectiveness of All-trans Retinoic Acid (ATRA) in treating this APL subtype has not been conclusively established. We present two pioneering case reports that expand our understanding of APL's molecular diversity and demonstrate the successful application of ATRA treatment.

The first case is a 4-year-old girl presenting with typical APL symptoms, but lacking known APL fusion genes. Whole-transcriptome sequencing revealed a 2974 bp RARA fusion transcript with a 371-nucleotide TTMV insertion. Despite initial resistance to conventional chemotherapies, she achieved complete remission (CR) after a treatment regimen involving ATRA, Venetoclax, and Homoharringtonine, highlighting the effectiveness of combining ATRA with other targeted therapies in this APL subtype.

The second case involves a 15-year-old boy, also presenting with typical APL symptoms but without established APL fusion genes. Laboratory findings included a high white blood cell count and a significant proliferation of abnormal promyelocytes. Whole transcript sequencing identified a 2354 bp TTMV::RARA fusion without retained RARA intron 2. Initial treatment with ATRA and arsenic trioxide (ATO) was followed by homoharringtonine and cytarabine, the CR was reached. This was closely followed by two additional cycles of ATRA and ATO, where he again achieved CR. Despite a subsequent recurrence, complete remission was once more attained through re-induction chemotherapies, supplemented with ATRA. This long-term, comprehensive approach resulted in sustained remission, underscoring the significance and effectiveness of personalized ATRA therapy in managing TTMV::RARA APL cases.

Earlier reported TTMV::RARA fusions included retained lengths of RARA intron 2 ranging from 13 to 38 nucleotides. Our findings of single or no nucleotide retention indicate a more intricate TTMV::RARA fusion than previously observed. The amino acid sequences of the RARA portion are predicted to remain unchanged, with the crucial ligand-binding domain (LBD) of RARA intact. This structure potentially explains the positive response to ATRA treatment.

In conclusion, these cases underscore the importance of comprehensive genetic analysis in identifying unique molecular subtypes of APL and highlight the challenges in diagnosing and treating these rare cases. The successful management of both patients with ATRA therapy emphasizes the need for tailored treatment approaches in APL. These reports not only expand the genetic landscape of APL but also reinforce the potential of ATRA as a key therapeutic agent, even in the rarest forms of the disease. Future research should focus on further understanding the molecular mechanisms behind TTMV::RARA fusion and exploring targeted treatment strategies for this and similar unique genetic contexts, with the aim of improving outcomes for patients with rare subtypes of leukemia.

**APL Treatment**
**P17**

## A Real Life Study of Activity of ATO Plus ATRA Regimen in Treatment of Acute Promyelocitic Leukemia.

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**Abstract.** *Background*: All-trans retinoic acid (ATRA) with Arsenic Trioxide (ATO) has become standard of care for low-intermediate risk acute promyelocitic leukemia. Pilot APL0406 and NCRI AML17 trials have shown high efficacy and reduced hematologic toxicity with ATO and ATRA. However real-life studies confirming activity of this regimen in real life setting are lacking and required.
*Methods*: APL cases from fuor experienced hematological institution, treated with ATO and ATRA, were retrospectively collected. Analysis included APL with low/intermediate Sanz risk at first diagnosis, or APL relapsed after ATRA plus chemotherapy treatment. Primary end points were Overall Survival (OS) and Event-Free Survival (EFS). Secondary end-points included analysis of quality of response, factor affecting survival and toxicity.
*Results*: From 2014 to 2019, 77 patients treated with ATO and ATRA protocol were identified. Median 5y-OS was 97.4%, and median EFS was 96.1%. Complete remission was achieved in all 77 patients (100%), with persistent molecular remission in all but on patient, where a molecular relapse was observed. Survival analysis didn't show statistically significant differences among age categories (under 60 years old vs over 60 years old ), risk stratification (low, intermediate, high) and frontline therapy vs salvage therapy. However epatoxicity and hyperleucocitosis was observed in 21% and 40% of patients respectively. QTc prolongation with needing for ATRA reduction was not observed. ATRA and ATO was associated with a good safety profile, with no treatment discontinuation.
*Conclusions*: Advances in the treatment of APL have changed the natural history from a highly fatal disease up to be definitely curable. Our real-life data confirm efficacy of ATO and ATRA regimen outside clinical trials. Furthermore, toxicity data show how this regimen could potentially be a curative strategy for all patients who are frail or unfit for age and comorbidities.

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**APL Treatment**
**P18**

## A Tricky and Unexpected Diagnosis Besides an Acute Promyelocytic Leukemia

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**Abstract.** A 46-year-old woman presented to the emergency department with neurological symptoms (headache, vertigo, nausea and vomiting), disseminated intravascular coagulopathy (DIC), pancytopenia and the presence of abnormal promyelocytes in blood smear. She had history of melanoma, surgically removed four years before and of breast carcinoma treated with surgery, chemotherapy and radiotherapy two years before. Both tumors reached the complete remission (CR).
The urgent diagnostic workup included cranial computed tomographic (CT)-scan showing a subarachnoid haemorrhage and bone marrow specimen revealing blasts and atypical promyelocytes, coherent with a diagnosis of acute promyelocytic leukemia (APL). Confirmation was obtained by FISH and Real Time-PCR (RT-PCR), detecting t(15;17) and PML-RARa rearrangement.

All-trans retinoic acid (ATRA) and arsenic trioxide (ATO) were promptly started, obtaining a rapid resolution of the DIC. However, due to neurological worsening (diplopia, dysmetria and visual hallucinations) additional exams were performed: cranial magnetic resonance imaging excluding a progression of intracranial hemorrhage; fundus oculi examination with high pressure sign suggesting a possible pseudotumor cerebri ATRA related or a leukemic meningeal involvement; lumbar puncture revealing atypical cells in cerebrospinal fluid (CSF), raising again concern about leptomeningeal leukemic involvement. However, cytofluorimetry did not evidence myeloid cells and FISH and RT-PCR were negative for APL features. Surprisingly, FISH analysis for PML-RARa showed multiple signals of both genes in the CSF cells, thus raising suspicion of the presence of tumour cells different from APL blasts. At the end of the induction patient obtained complete remission with persistence of PML-RARa fusion transcript. Despite the favourable hematologic course, the neurological picture progressively worsened. Suspecting meningeal metastasis from a hidden solid tumor, after consultation with Oncologists, total body CT scan and a positron emission tomography whole body scan were performed but all tests resulted negative. Anti-CNS antibody research, indicative of a paraneoplastic syndrome, also yielded negative results. Given that the prior melanoma was BRAF V600E mutated, a Q-RT-PCR analysis was performed on DNA extracted from CSF cells and the same mutation was detected. Eventually, we concluded for a leptomeningeal metastatic melanoma.
After oncological suggestion, we initiated targeted therapy with dabrafenib and trametinib while closely monitoring the PML-RARa fusion transcript. Patient conditions improved rapidly, and treatment was well tolerated, therefore, we proceeded with co-administering ATO-ATRA consolidation cycle. Unfortunately, two and a half months later, despite a good safety drugs profile, neurological symptoms recurred and patient deceased by melanoma progression 4 years from the solid tumour diagnosis, while in CR of APL.
The management of this case provides valuable insights: 1. Consider the possibility of tumour recurrence in patients with a history of cancer, even when in apparent remission. 2. Search for molecular alterations having by now the priority in the diagnostic work up of the majority of neoplastic diseases. 3. Actively collaborate in an interdisciplinary approach to navigate the diagnostic process and tailor a combined treatment strategy. 4. Demonstrate the safety and feasibility of the combination ATRA-ATO and Dabrafenib-Trametinib.

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## APL Treatment
## P19

## Complex Cytogenetic Profiles in Acute Promyelocytic Leukemia: Insights from Three Cases With IDER(17)(Q10)T(15;17)(Q24;Q21) and Additional Abnormalities

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**Abstract.** Acute promyelocytic leukemia (APL) represents a distinct subtype of acute myeloid leukemia, characterized by the chromosomal translocation t(15;17) leading to the PML::RARA fusion gene. This genetic mutation disrupts normal myeloid differentiation, pivotal in APL pathogenesis. APL's unique molecular pathophysiology has been a cornerstone in developing targeted therapies. The advent of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO) has revolutionized APL treatment, shifting the therapeutic landscape from chemotherapy to these targeted agents, significantly improving survival rates.
Chromosomal rearrangements in addition to t(15;17) have been observed in 25-40% of APL but it is yet unclear whether additional chromosomal abnormalities in APL directly influence the prognosis. Among the possible additional abnormalities, derivative isochromosome of the long arm 17 (ider17q) has been reported with frequencies far below 1%. Here, we describe three consecutive APL cases bearing ider(17)(q10)t(15;17)(q24;q21), in addition to the pathognomonic t(15;17). Despite the rarity of this cytogenetic abnormality, these patients were diagnosed following one another at our center between November 2022 and March 2023. All patients signed a written informed consent.
Patients were males aged between 32 and 63 years and were classified as either low or intermediate risk according to the Sanz criteria. Patient 1 exhibited a karyotype with derivative chromosomes 15 and ider17q in one subclone, and a complex karyotype with additional abnormalities including loss of one chromosome 6 and a translocation between chromosomes 3 and the other 6, in another subclone. Patient 2 presented the standard t(15;17) translocation

alongside additional chromosomal anomalies, including added material on chromosome 12 in one subclone and the presence of ider17q in another subclone. Patient 3 featured the classic t(15;17) translocation, in addition to derivative chromosome 15 and ider17q in a subclone. All karyotypes are summarized in Table 1. Abnormalities detected by conventional cytogenetics were all confirmed by FISH. The presence of ider17q resulted in the loss of one copy of TP53 in all patients. NGS analysis of the residual copy of TP53 is currently underway and results will be presented at the meeting.
All patients received ATRA and ATO according to Lo Coco et al (NEJM 2013) and none of the patients experienced differentiation syndrome. All three patients achieved complete hematologic remission at the end of induction and complete molecular remission at the end of consolidation one; however, Patient 2 required 50 days to achieve CR. These data do not suggest any specific prognostic impact for ider17q in patients receiving ATRA and ATO.
The role of additional chromosomal abnormalities in APL remains an area of active research. In this context, ider17q is a rare cytogenetic abnormality with limited reports in the literature. Here, we reported three consecutive APL cases with ider17q. Despite the loss of (at least) one copy of TP53, all patients achieved molecular remission at the end of consolidation with ATRA and ATO, suggesting no impact of such abnormality in patients treated with this regimen.

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## APL Treatment
## P20

## Bone Marrow Morphological Analysis of Acute Promeylocytic Leuekmia Patients Treated with Arsenic Trioxide

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**Abstract.** *Background*: Current treatment algorithm for standard-risk (white blood cells < 10.000/m3) acute promyelocytic leukaemia (APL) is based on the combination of all-trans retinoic acid (ATRA) and arsenic trioxide (ATO), which allows to achieve stable complete remission in the vast majority of patients. However, since this chemo-free treatment was approved only a decade ago, robust real-life data on long-term follow-up of patients are lacking. During the follow-up of APL patients treated at our centre with the ATRA-ATO combination, we noticed features of bone marrow morphological dysplasia in absence of cytopenia. This finding, which has not yet been described in literature, prompted us to perform a review of our case series to gain insights on this phenomenon.
*Material and methods*. This study enrolled 12 adult (≥18 years old) patients admitted to the Hematology Unit of the "Policlinico Universitario di Roma Tor Vergata" between October 2011 and July 2021 with newly diagnosed APL. Three patients were treated with AIDA regimen, nine with ATRA-ATO combination. For the purpose of this study, we performed a morphological revision of bone marrow blood smears at three time points: at diagnosis, at the end of consolidation and at +12 months after the end of consolidation or +12 month after the end of maintenance, in case of AIDA regimen.
In accordance to the indications provided by the World Health Organisation 2008 classification, we considered the following morphological parameters:
- Dyserythropoiesis: Multinuclearity, nuclear extrusions, hyperlobated nuclei, karyorrhexis, vacuolisation, megaloblastic appearance, punctate basophilia; nuclear bridges.
- Dysgranulopoiesis: Hypolobated nuclei (pseudo Pelger-Huet); irregular hyper-segmentation; Auer bodies; Döhle bodies; hypo/agranular cytoplasm; pseudo-Chediak-Higashi granules.
- Dysmegakaryocytopoiesis: Micromegakaryocytes; megakaryocytes with separate multiple nuclei; hypolobated or monolobated megakaryocytes.

To establish morphological dysplasia (cut-off ≥10% per maturation line), at least 200 granulocytes, 200 erythroid precursors, and 30 megakaryocytes were evaluated.
*Results*: During the follow-up, all patients were in continuous complete remission and no cytopenia was observed. In this preliminary analysis, we identified several morphological dysplastic features in all the cell lines at every time point, fostering the thesis of an ATO-induced dysplastic morphological dysplasia.
*Conclusions*: Our observations suggest the acquisition of dysplastic features in otherwise normal hematopoiesis in patients receiving the ATRA-ATO chemo-free regimen for APL. These findings, especially in concomitance with unrelated cytopenia, could induce the suspect of secondary myelodysplastic syndrome. Thus, in order to shed light on this phenomenon, in our subsequent analysis we aim to perform a systematic and comprehensive description of the morphological anomalies and to correlate specific dysplastic features to ATO administration (comparing morphological characteristics with the AIDA group of patients), demographic (age, gender) and clinical (development of differentiation syndrome during induction, comorbidities) characteristic of patients and laboratory data of the primary disease (PML-RARA isoform, FLT3 mutation, immunophenotype features).

## APL Treatment
## P21

## PLZF/RARA Unbalances Leukemic Cells Redox System Via NRF2, FOX3A and G6PD Functions

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**Abstract.** Acute promyelocytic leukemia (APL) is associated with the PLZF/RARa (ZBTB16-RARa) fusion gene, which disrupts the transcription of genes crucial for myeloid maturation; this rare APL variant is not responsive to ATRA-ATO therapy. Antioxidant activity plays a crucial role in normal hematopoiesis, with NRF2 (erythroid 2–related factor 2), a master regulator of cellular resistance to oxidative stress; G6PD (glucose-6-phosphate dehydrogenase), the rate-limiting enzyme in the pentosephosphate pathway and a major source of NADPH; FOXO3a, a member of the FOXO family of transcription factors. The study aims to elucidate the impact of PLZF/RARa on the antioxidant system in order to individuate new therapeutic targets. Primary blasts from PLZF/RARa+ APL patients, normal bone marrow cells (NBM) and a ZnSO4-inducible PLZF/RARa [U937-B412/control U937-MT] cell lines were employed for the investigation. Reactive oxygen species (ROS) levels were measured using MitoSOX-based assays; Western blot and qRT-PCR to evaluate expression of NRF2, and its target genes (HO-1, NQO-1); G6PD and FOXO3a. Localization of NRF2 and PLZF/RARa was assessed by immunofluorescence. Protein interactions were studied using co-immunoprecipitation and Duolink proximity assays (PLA). Results indicates that PLZF/RARa expression interferes with antioxidant balance, rising ROS levels in B412 cells, after the addition of 3mM Ascorbate (ASC, a ROS inductor) (B412, 8058,1±568,3724 vs MT, 5037,4±574,7364) and Rotenone-Antimycin (positive control) (B412, 38148±1756,453 vs MT,19588,5±2761,252) (Fig.1a). PLZF/RARa enhances NRF2 nuclear translocation (MT 6h, 0. 4±0. 2 vs B412 6h, 0. 9±0. 42) (p=0. 03) but downregulates the expression of NRF2 target genes HO-1 (MT 6h, 3. 6±0. 6 vs B412 6h, 1. 7±0. 1 (p=0. 002), and NQO1 (MT 6h,4. 9±1 vs B412 6h, 2. 8±0. 9) (p=0. 03), involving a functional interference. PLZF/RARa represses protein expression of FOXO3a, as evidenced by a significant decrease in protein levels (MT 6h, 1,55±0,07 vs B412 6h,0,35±0,07) (p=0,003) (Fig.1b), supported by decreased expression of its target gene SOD (MT 6h, 0,85±0,3 vs B412 6h, 1,49±0,1 (p=0.001). Immunofluorescence analysis reveals that PLZF/RARa co-localizes with NRF2. Co-immunoprecipitation and PLA assays confirmed a direct interaction between the two proteins. Primary blasts from t(11;17)-PLZF/RARa APL patients show a reduced expression of G6PD as compared to t(15;17) -PML-RARa APL, AML and NBM cells (APL PLZF/RARa n=5, 0. 6±0. 2); (vs APL PML-RARa n=10, 3. 7±2. 6, p=0. 02); (vs AMLs n=13, 2. 5±1. 8, p=0. 03); (vs NBM n=2, 1. 6±0. 8) (Fig.1c). Interestingly, primary blasts from an APL PLZF/RARa positive patient assayed for annexin and live/dead exclusion by flow cytometry showed a dose-dependent apoptotic response in the presence of ASC (Ctrl, 14%, ASC 1mM, 47%, ASC 3mM 99%), a potent pro-oxidant (Fig. 1d). Apoptosis assays demonstrate

a reduced antioxidant defense capacity in PLZF/RARa-positive AML cells. In conclusion alterations of the antioxidant system observed offer a promising therapeutic target in a rare but difficult to treat AML subtype.

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## APL Treatment
## P22

## A Case of Acute Promyelocytic Leukemia (APL) Arising as a Blastic Phase of Chronic Myeloid Leukemia (CML)

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**Abstract.** Promyelocytic blastic crisis of CML is an extremely rare event, and the cases reported in the literature are very limited in the TKI era.
We present a case of PML::RARα positive acute promyelocytic leukemia (APL) developing 12 months after initiation of Imatinib therapy for chronic phase CML.
A 68-year-old male with a history of CML diagnosed in August 2022 on treatment with Imatinib 400 mg/day presented with progressive thrombocytopenia after 12 months of TKI therapy. During Imatinib therapy, the monitoring of BRC::ABL1 transcript showed a 29% IS after 3 months of therapy and 1.4 % IS after 9 months. Imatinib was discontinued and blood counts were checked again a few days later, showing worsening of thrombocytopenia and leukocytosis. Laboratory investigations on the day of admission showed Hb: 12.1 g/dL; WBC 30.× 103 /ul, neutrophils: 13×103 /ul, monocytes: 13× 103 /ul, Platelets: 14 × 103/ul.
A peripheral blood smear showed 50% of abnormal hypergranular promyelocytes. Coagulation parameters showed typical APL-related (disseminated intravascular coagulation) DIC with hypofibrinogenemia. A Bone marrow (BM) study revealed 80% of abnormal promyelocytes (immunophenotype CD117/CD13/CD33/+; CD34/HLADr-). Chromosomal analysis revealed 46,XY, t(9;22)(q34;q11.2), t(15;17)(q24;q21). The quantity of BCR::ABL1 fusion transcript was 51%. Multiplex reverse transcriptase-PCR and interphase FISH detected both major BCR::ABL1 and PML::RARα fusion transcripts. The patient was diagnosed as having promyelocytic BP of CML and received induction as to high-risk APL with ATRA and idarubicin (AIDA2000). During induction, he developed atrial fibrillation after the 3rd Ida dose. After induction, he experienced incomplete hematologic recovery with prolonged cytopenias. Sixty days after induction therapy, the patient repeated a BM biopsy showed severe hypocellularity (<10%) and molecular studies showing a negative PML::RARα transcript and positive BCR::ABL1 (37% IS). In consideration of the high level of BCR::ABL1 transcript with the following Imatinib-resistant mutations: M244V, E255K, F359I, and I418V; the patient was switched to Dasatinib 100 mg/die and consolidation with ATO-ATRA was planned. However, the patient developed fever and pneumonia requiring antibiotic therapy. In addition, the patient presented with a gastric hemorrhage due to peptic ulcer and so was not able to undergo consolidation therapy. After one month of Dasatinib, BRC::ABL1 transcript showed only a mild reduction (16.4% IS), therefore, mutation analysis by NGS of BCR::ABL1 transcript showed the presence of several mutations (M244V, E225K, F359I, I418V,) including T315I (VAF 2.7%) (FIGURE 1). Therefore, Ponatinib was started at the dose of 30 mg daily, with a mild improvement of blood counts. Interestingly, a Retrospective analysis of PML::RARα mutation by quantitative PCR showed a subclone in the samples analyzed for BCR::ABL1 at 3 and 9 months during the chronic phase under Imatinib.
In conclusion, this case shows the importance of achieving MMR, to reduce the risk of developing Blastic Crisis. Our case, also shows the therapeutic dilemma when combining TKI and ATO due to possible cardiological toxicity (QTc prolongation, atrial fibrillation); the risk of developing prolonged cytopenia, infections, and bone marrow hypocellularity when using IDA+ATRA in high risk APL patients and poorly responsive to 1st generation Imatinib CML; because ATRA-ATO is not licensed for high-risk APL.

https://www.medicalhosting.org/APL/Autori/Allegati/31-NGS.jpg

## APL Treatment
## P23

### Arsenic Trioxide Neurotoxicity in Acute Promyelocytic Leukemia Patients: A Single Center Experience

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**Abstract.** Since 1998, standard-risk acute promyelocytic leukemia (APL) has been treated with a combination of arsenic trioxide (ATO) and transretinoic acid (ATRA). It was the first neoplasm curable without the use of chemotherapy. This therapeutic combination has become the standard of care for standard-risk APL. Over the years, reports of neurological ATO-toxicities have been described at variable times during therapy, often with a torpid course and a variable functional impairment depending on the affected area. Patients are known to experience anxiety (30%), depression (20%), dizziness (23%), fatigue (63%), headache (60%), insomnia (43%), pain (15%), paresthesia (33%) and rigors (38%). Neurological disorders related to ATO exposure are thought to derive from different molecular mechanisms such as cytotoxicity, increased reactive oxygen species, mitochondrial damage with uncoupling of oxidative phosphorylation, chromosomal aberrations, and DNA injury, resulting in increased neurons apoptosis and subsequent axonal degeneration. Published evidence are limited to few case reports. On the European database of suspected adverse events reports (Eudravigilance) less than 50 neurological side effects are related to ATO in APL patients. Here we report three cases of particularly disabling neurological toxicity during ATO therapy.
*Case 1*: A 59-year-old woman with a standard-risk APL in 2018 was treated with ATO plus ATRA. After 20 days, she suffered from incoercible vomiting and intestinal pseudo-obstruction. At the end of the induction course, she became lethargic, and she developed paraesthesia of limbs, with a "stocking-glove distribution". Neurologic examination showed weakness of limbs, muscle atrophy of the extremities and absence of tendon reflexes. Electromyography showed an axonal sensorimotor polyneuropathy and neuro counselling suggested Guillan-Barrè syndrome or pharmacological neurotoxicity. With thiamine and cobalamin supplement and physiotherapy, her neurological signs and symptoms gradually improved with a complete regression. Induction ATO phase was followed by maintenance with purinethol, methotrexate and ATRA.
*Case 2*: A 55-year-old female patient diagnosed in 2019 with a standard-risk APL, was treated with ATO plus ATRA. During the first consolidation course, she developed paraesthesia in her feet, with progressive extension to the hands and lower limbs in the subsequent consolidation courses. No ATO dose reduction was applied, and mild symptoms are still present after three years from the end of treatment.
*Case 3*: A 52-year-old woman with standard-risk APL relapsed after 10 years from her first line treatment with ATRA plus chemotherapy according to the AIDA protocol. At relapse, in 2020, she was treated with ATO plus ATRA. During the induction phase, she developed paraesthesia and peripheral neuropathy in the upper and lower limbs, associated with tremors in her fingers and toes. Brain MRI showed no lesions. Neurological events were managed with clonazepam and ATO dose reduction of up to 75%.
Based on our experience and on published data, neurological toxicities occurring during ATO treatment for APL can severely impact patients' quality of life. Early recognition and prompt management are crucial in a disease nowadays highly curable like the standard-risk APL.

## APL Treatment
## P24

### Second Molecular Relapse Associated with Central Nervous System Involvement in Patients with High Risk Acute Promyelocytic Leukemia

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Relapse occurs in approximately 5-,20% of acute promyelocytic leukemia (APL) patients, it is uncommon in patients with low-and intermediate-risk disease (<3%), but in the minority of patients, those with high-risk disease, the relapse rate is about 20%. Relapse at extramedullary sites occurs in approximately 3-5% of patients. We report a case of 46-year-old male patient with APL, PML/RAR□ □positive, BCR3 isoform, high risk, and central nervous system relapse. In May 2021, the patient was admitted to our hospital for anemia, thrombocytopenia, leukocytosis and bleeding, and he was diagnosed with APL. He was treated according to standard chemotherapy GIMEMA AIDA 2000: ATRA–idarubicin induction therapy followed by 3 cycles of consolidation therapy with ATRA plus chemotherapy. Prophylactic intrathecal methotrexate was performed, according to protocol, during every chemotherapy course. Patient achieved morphological response after induction and molecular complete response (MCR) after the third consolidation. During aplastic phase of this consolidation he presented Sars Cov2 infection, which caused a delay in maintenance beginning. In March 2022, maintenance therapy (ATRA + 6-mercaptopurine + methotrexate) started; two months later, we observed a slight increase of PML-RARA copies (0.0004%) in the bone marrow, and in June the molecular relapse (PML-RARA/ABL %: 0.0042) was diagnosed. In August 2022 he underwent ATRA-ATO-based salvage chemotherapy (according to GIMEMA APL 0406 protocol). In September 2022 he developed a second Sars Cov2 infection. He received other ATO-ATRA cycles from October 2022 to May 2023 . The bone marrow control during this period, showed few persisting PML-RARA copies, until March 2023, when he achieved the 2nd molecular remission, confirmed in successive controls. He received autologous stem cells mobilization chemotherapy with intermediate dose cytarabine, and in July 2023, underwent autologous HSCT. In October 2023, patient was readmitted because of severe headaches: a meningeal relapse was documented, despite MRI of the brain was normal. PML-RARAfusion transcript was documented in cerebral spinal fluid (CSF) as well as in bone marrow, which was persistently in complete hematological remission. He was treated according to ATRA+ATO therapy and weekly triple intrathecal therapy (ITT) with methotrexate, hydrocortisone, and cytarabine until complete clearance of blasts in the cerebrospinal fluid, obtained after 8 lumbar punctures, followed by 3 ITT treatments as consolidation. Patient's bone marrow and CSF were studied for PML-A216V mutation that confers resistance to ATO, and were negative. After this induction therapy, patient achieved complete molecular remission in bone marrow. This case shows two critical issues in the management of PML relapse: poor capacity of arsenic alone to prevent meningeal recurrences, and the importance of close monitoring of low PML-RARA positivity to predict relapse.

https://www.medicalhosting.org/APL/Autori/Allegati/25-PML-RARA%2016-01 (OT).pptx

## APL Treatment
## P25

## Leukocytosis During ATRA-ATO Induction Therapy in Low-Intermediate Risk APL: Incidence and Risk Factors

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The combination of arsenic trioxide and All-trans retinoic acid (ATRA-ATO) represents the standard front-line therapy for low/intermediate APL patients. Up to 50% of patients undergoing induction therapy with ATRA-ATO developes leukocytosis (WBC>10,000/ul) during induction therapy. However, few studies have explored the predisposing factors and kinetics of leukocytosis occurring during front-line therapy with ATRA-aTO.
Sixteen consecutive adult patients with low-intermediate risk APL treated with arsenic trioxide and all-trans retinoic acid between 2016-2023 at University Hospital SM Goretti, (Latina) were included in this study. All patients received ATRA-ATO as reported elsewhere (Lo-Coco, NEJM 2013) for induction and consolidation therapy. Leukocytosis developing during induction was defined as WBC >10x109/L.
Median age of our cohort was 58 (26-82). 11 patients were females and 5 were males. 8/16 patients were intermediate risk and 9 were low risk. During induction therapy, 10/16 (62%) patients developed leukocytosis (>10x109/L). Peak in leukocyte count was 26x109/L (11.7-50.6) and was reached at a median of 12.5 days from the start of ATRA-ATO

therapy (4-20 days). Hydroxyurea was started in all patients at a median dose of 2 g and was successful in all patients to control leukocyte counts. More patients with leukocytosis were classified as intermediate risk as compared to patients without leukocytosis (8/10 vs 1/6 ; p=0.03). Differentiations syndrome occurred in 2 patients and was suspect in other 2 cases who developed leukocytosis, while none of the patients without leukocytosis experienced DS (p=0.2). As compared to patients without leukocytosis, all the patients developing leukocytosis showed a progressive increase in LDH in parallel with leukocyte counts, while none of the patients without leukocytosis showed peak in LDH.(p<0.0001) Median time to peak in LDH was 11.5 days form start of ATRA-ATO, showing a almost equal kinetics as compared to leukocytes. Median LDH value at peak was 1498 (449-3000). Half of the patients experiencing leukocytosis developed lumbar pain often irradiated to the lower limb with negative radiologic evidence and poorly responsive to analgesics but responsive to steroids; this phenomenon was not observed in any of patients without leukocytosis (p=0.09). No difference was found in median age, Hb, Platelets, WBC, BMI distribution between patients with and without leukocytosis. However, patients developing leukocytosis showed significantly higher % of bone marrow blasts as compared to those not developing leukocytosis ( 90% vs 55% p=0.005 ).
Our study confirmed the high incidence of leukocytosis developing during therapy with ATRA-ATO, not correlated with differentiation syndrome and responsive to hydroxyurea. Intermediate risk APL and higher percentage of bone marrow blasts showed correlation with onset of leukocytosis. In addition rise in LDH paralleled in all cases that of leukocyte counts and severe lumbar pain was observed at leukocyte peak in a significant number of patients.

## APL Treatment
## P26

## A CASE REPORT OF PRES IN A HIGH-RISK APL PATIENT

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Posterior reversible encephalopathy syndrome (PRES) is a rare clinical syndrome characterized by reversible acute neurologic deterioration that can be caused by cytotoxic drugs. We describe here a case of methotrexate-induced PRES in an acute promyelocytic leukemia (APL) patient.
A 43-year-old woman affected by thalassodrepanocytosis treated with hydroxyurea was diagnosed with high-risk APL associated with thrombosis of the sigmoid sinus and the right internal jugular vein. At diagnosis, her hemoglobin (Hb) was 7.2 g/dL and hemoglobin S (HbS) was 13.9%. Her average pre-APL HbS was 60%.
According to the European Leukemia Net guidelines, the patient was treated with All-Trans Retinoic Acid (ATRA) and idarubicin induction therapy. She achieved cytofluorimetric complete response, but PML-RAR BCR1 transcripts were still detected. Therefore, she underwent consolidation therapy according to AIDA regimen and central nervous system prophylaxis (CNS) with intrathecal methotrexate (MTX).
Ten days after the second consolidation cycle, she complained of pain medication-unresponsive occipital headache, vomit, visual disturbances, and severe hypertension. Total Hb was 9.7 g/dL and HbS was 4.2%.
The brain MRI revealed focal subcortical areas with a hyperintense signal in T2-FLAIR and hypointense signal in T1 at the level of the parasagittal occipital region, mainly on the right, consistent with PRES (Fig. 1). ATRA administration was promptly interrupted and treatment with pain medication and anti-hypertensive drugs was started. Two days later, she had an episode of tonic-clonic seizures that resolved after midazolam administration. The electroencephalogram showed altered reactivity with bilateral slow wave abnormalities; thus, anti-epileptic medication was started. The follow-up brain MRI showed almost complete radiological resolution, with a new focal change in the right parietal parasagittal area.
After complete symptoms resolution, systemic treatment was resumed. To reduce the risk of PRES recurrence, in suspicion of PRES secondary to acute/subacute MTX neurotoxicity, after consultation with the neurologist, the last prophylactic intrathecal chemotherapy administration was omitted.
Currently, our patient is undergoing standard maintenance therapy, with no additional episodes of PRES.
PRES is probably due to vasogenic edema secondary to disordered cerebral autoregulation coupled with endothelial dysfunction.

MTX-induced neurotoxicity is well documented, with an incidence of 5% to 20%. It is probably due to inhibition of dihydrofolate reductase, leading to a decrease in myelin protein synthesis and DNA methylation and an increase in homocysteine levels, which directly damage the vascular endothelium. In addition, transfusion-dependent sickle cell disease (SCD) or SCD associated with risk factors such as arterial hypertension, renal failure or use of immunosuppressive drugs can also cause PRES, as described in case reports of pediatric and adult patients.
Although our patient has thalassodrepanocytosis, her disease was controlled during chemotherapy for APL, and she had no other risk factor. Nonetheless, her underlying disease and the need for frequent RBCT because of therapy-related anemia could have contributed to the development of PRES.
Furthermore, our decision to omit the last intra-thecal chemotherapy is also justified by lack of clear evidence for the need for CNS prophylaxis in high-risk APL.

https://www.medicalhosting.org/APL/Autori/Allegati/12-Fig 1.png

## APL Treatment
## P27

### Activated BCAT2 Serve as a Theraputic Target in High-Risk Acute Promyelocytic Leukemia

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The past few decades have witnessed unprecedented advances in the therapy of Acute promyelocytic leukemia (APL) with the introduction of arsenic trioxide (ATO) and all-trans retinoic acid (ATRA) as the standard therapeutic agents. However, improving the prognosis and disease-free survival (DFS) for APL patients classified in the high-risk group remain unsatisfactory. Therefore, there is a pressing need for finding more effective theraputic target to enhance treatment outcomes in the high-risk APL cohorts.
Reprogrammed cellular metabolism, especially amino acid metabolism, has been observed in various cancers and recognized as novel therapeutic targets. Aberrant activation of branched-chain amino acid (BCAA) metabolism owing to dysregulation of branched chain amino acid transaminase 1/2 (BCAT1/2), the rate-limiting enzyme of BCAA breakdown, has been implicated in glioblastoma, pancreatic cancer, non-small-cell lung carcinomas, leukemia and breast cancer. For many years, BCAT1 has been recognized and well-studied as the major isoform implicated in cancer growth, however, the role of BCAT2 remains elusive in tumorigenesis.
In this study, by profiling the expression of BCAT2 in AML cell lines and patients with different genetic features, we revealed a aberrant activation of BCAT2 in APL cohorts compared to other subtypes. Moreover, upon classifying APL primary samples into risk groups based on white blood count (WBC) count, RAS mutation, and APL9 score, we observed significantly higher BCAT2 expression in the high-risk group, particularly in those carrying RAS mutations.
Mechanistically, besides being stabilized by RAS mutation from ubiquitylation, we demonstrated in NB4 cell line that BCAT2 was directly activated by PML::RARa fusion through directly binding to cis-acting elements. Furthermore, pharmacological and genetic approaches BCAT2 inhibition resulted in significantly impaired leukemogenesis ability of APL cell lines in vitro.
In this study, we profiled the expression of BCAT2 in AML with different genetic features and based on results of pre-experiments, we found that BCAT2 may serve as a genetic vulnerability and a promising targeted therapeutic opportunity for high-risk APL patients.