# PML/ RARA Variants and their Role in Arsenic Trioxide Resistance in APL: A Scoping Review

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***Abstract.*** *Currently, the treatment of Acute Promyelocytic Leukemia (APL) is grounded on therapeutic regimens based on arsenic trioxide (ATO). However, mutations in the PML component of the PML/RARA oncoprotein are believed to be involved in the mechanism of resistance to this agent. We performed a scoping review to understand the role of variant PML/RARA fusion proteins in the mechanism of resistance in APL. Applying the defined criteria, we selected 10 studies, in which patients presented a picture of relapse and/or resistance after the administration of ATO, having been identified at least one PML mutation. We also reported that RARA is the most frequently mutated gene, although we also found mutations in genes related to other processes involved in cell differentiation. Briefly, this is a multifactorial mechanism and PML/RARA variants are important but not an obligatory condition for ATO resistance to occur.*

**Keywords.** Acute promyelocytic leukemia, arsenic trioxide, relapse, resistance, mutations

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# 1 Acute Promyelocytic Leukemia

Acute Promyelocytic Leukemia (APL) is a subtype of AML. The tumourigenesis is linked to the myeloid lineage precursor cells, occurring a clonal proliferation combined with a reduced ability of differentiation, leading to an accumulation of promyelocytes. Under the former FAB classification we categorized APL based on morphological and immunophenotypic features. The AML M3 variant is detected in 60 to 70% of the cases. Recently, it was adopted the World Health Organization Classification of Tumours of Haematopoietic and Lymphoid Tissues from 2016. This new system takes into account the clinical manifestations and, above all, the genetic aspects of each variant of the neoplasia. In despite of existing different possible translocations on the origin of APL, the reciprocal translocation between the retinoic acid receptor alpha gene (RARA) and the promyelocytic leukemia gene (PML) explains the pathogenesis in 95-98% of all cases. The t(15;17)(q24.1;q21.2);PML/RARA, identified in 1977 by Rowlley et al., is the biological hallmark of classic APL [1], [2].

The RARA protein belongs to a superfamily of nuclear receptors that act as nuclear transcription factors by binding to the oxidised form of vitamin A, i.e. retinoic acid, which is involved in cell differentiation. On the other hand, the PML protein contains a RING finger domain, which allows its organization in subnuclear macromolecular structures defined as nuclear bodies (PML NB). This gene is involved in several processes of tumour suppression and genomic stability, as well as in pathways related to apoptosis and senescence [2], [3]. In cells expressing PML/RARA, the classic model of pathogenesis states that this fusion prevents coactivator recruitment by exerting a dominant negative action on Retinoic Acid Response Elements (RARE) transcription, rendering them insensitive to the presence of physiological levels of retinoic acid [2], [4].

The blockade of myeloid lineage precursor cell differentiation leads to several clinical manifestations following pancytopenia. In particular secondary haemorrhages due to disseminated intravascular coagulation in combination with primary hyperfibrinolysis, characteristic of APL [5]. In order to attenuate the fatal outcome of this pathology it was applied the first therapeutic regimen consisting of anthracycline-based chemotherapy (QT). In the early 1990s the concept of differentiation therapy emerged, whereby Breitman et al. demonstrated that all-trans retinoic acid (ATRA) is able to induce myeloid leukemic cell differentiation *in vitro* [5]. The era of risk-adapted therapy has therefore been ushered in, based on evidence from a number of studies, such as GIMEMA AIDA0493 and PETHEMA LPA096. To overcome ATRA resistance, short and long-term toxicity, myelosuppression and consequent infections due to ATRA+QT, arsenic trioxide (ATO) was introduced in the late 1990s [2]. The GIMEMA-AMLSG-SAL APL0406 and UK NCRI AML17 studies confirmed the advantage of the combined ATRA+ATO regimen over ATRA+QT [2]. So, the most recent NCCN and European LeukemiaNet guidelines includes the targeted therapy of ATRA+ATO as the gold standard in APL treatment, aiming clinical remission [5], [6].

ATRA interacts with specific RARA domains triggering a PML/RARA conformational change, promoting the transcription of genes essential for differentiation. Furthermore, it contributes to protein degradation via proteases. ATO, in turn, establishes disulfide bonds with the rearranged PML through oxidation of cysteine residues (C212/213) at the B2 domain. This process promotes the covalent conjugation of SUMO modifiers to the PML lysine residues affecting the cellular localization of PML proteins, thereby promoting the rearrangement of PML NB. The substitution of zinc (RING finger) by arsenic induces conformational changes in PML that promotes polymer formation and interaction with the ubiquitin-conjugating enzyme 9 (UBC9),

forming high molecular weight chains. These chains recruit to the interior of PML NB a specific SUMO-dependent ubiquitin E3 ligase, RNF4 (Really interesting new gene (RING) Finger protein 4). Thus, PML/RARA is degraded via proteosome [7], [8].

In summary, ATO is now approved by the FDA (2000) and EMA (2002) for the treatment of APL [2]. However, approximately 5 to 10% of patients present relapse and probable resistance to ATRA and ATO. Some mutations in the rearranged PML of PML/RARA have been detected, which are believed to be involved in the resistance mechanism. Therefore, the following question is raised: What role do variant PML/RARA fusion proteins play in the mechanism of resistance to ATO in APL? [4].

## 2 Methodology

This review follows PRISMA-Scr guidelines. We looked at studies related to the human model published in the last decade, in which patients were diagnosed with APL and also had molecular relapse or resistance to ATO reported. It was imperative that these individuals would have been subjected to therapeutic regimes based on ATO or targeted therapy (ATRA+ATO). In addition, the molecular study of the rearranged PML gene would have been carried out throughout the follow-up, preferably with a comparative study between the molecular profile at diagnosis and at relapse.

We used PubMed (Medline) and Web of Science platforms with the following query: ("acute promyelocytic leukemia" OR "acute promyelocytic leukaemia" OR APL OR AML M3) AND ("arsenic trioxide") AND (relapse OR resistance OR mutation OR disease progression). Based on that strategy, 51 potential studies were identified. After a meticulous analysis, we included 10 studies that complied with all the stipulated criteria. We used the OCEBM LoE (Oxford Centre for Evidence-Based Medicine 2011 Levels of Evidence) to assess the level of evidence for each study.

## 3 Results

In the 10 studies included, a total population of 1070 cases diagnosed with APL was gathered. Approximately 345 of them relapsed. Thus, a PML-MT subpopulation was defined, consisting of cases with detected and identified mutations in the rearranged PML gene (representing about 13% of the relapses). The average age of the total population shows that the pathology falls within the young adult/adult age categories, ranging between 26 and 45 years, which is consistent with the statistical data in the literature [1].

In the PML-MT subpopulation (n=45) a total of 27 mutations were identified in the PML gene, 16 when not considering the repetitions: A216V, L218P, C213S, S214L, del(p.Asp219Glu), ins(p.Ser220Met), L218H, L211P, C213R, L217F, D219H, S221G, D241G, A216T, E224G and L218F. Note that only in 3 studies were detected mutations simultaneously in the PML-WT gene (not rearranged PML gene). On top of that, their identification was only available in 2 of the studies (A216T and A216V) [9]–[11]. The A216V mutation was reported most frequently, being identified in 60% of the studies [12]–[14].

In 80% of the analysed studies (n=8), genetic alterations other than PML mutations were also identified. According to the sources, RARA is the gene with the highest mutation rate, being mutated in 87.5% (n=7) of the 8 cases mentioned. RARA mutations were exclusively identified

in 4 of them. In other 3 studies, besides mutated RARA, other genetic alterations were identified. In the remaining study, besides the mutated PML only alterations in EMG genes were also reported (n=1) [15].

Only one study didn't provide information on the outcome of the patients [10]. In 20% of the studies no resistances were associated to the therapeutic regime applied. On the other hand, in the remaining 70% the following resistances were reported: probably acquired during treatment (10%, n=1); specifically to ATRA (10%, n=1); and to the administered therapy (50%, n=5). Regarding to the referred 50%, resistance specifically to ATO was identified in 3 studies. In one of those 3 studies, with a PML-MT subpopulation of 14 cases, 9 showed resistance to this agent. In the total n=14, 3 patients relapsed and another 6 died after two relapses, corresponding precisely to the 9 cases resistant to ATO. The remaining individuals in that same study achieved clinical remission (n=5), one of them after haematopoietic stem cell allotransplantation [14]. In the remaining 2 studies, resistance led to disease progression and death was reported in one of them [11], [13].

For the remaining sources of evidence where resistance was reported (40%, n=4), the outcomes were varied: in one of them, death of the individual was reported; in another, relapse after 3 months; and in a third study, only 2 individuals with resistance were identified. In the one clinical case where ATRA resistance was specifically reported, the individual also died [9], [12], [15], [16]. In the sources of evidence where no resistance was associated to therapy administration (20%, n=2), only one death was reported in one of the studies [17].

RARA and PML mutations have been reported and associated with changes in the mechanisms of action of ATO and ATRA on the oncogenic protein. Several examples of these mutations have been detected and identified in the PML-MT subpopulation. ATRA resistance is associated with mutations in the ligand binding domain (LBD) of RARA gene [12]. On the other hand, mutations in the PML gene are associated with resistance to ATO. As an example, evidence points out that mutations in codon 218 of the PML gene, described in 30% of the evidence sources considered, contribute to aberrant PML NB formation [13]. While A216V, identified in 60% of the sources, prevents the irreversible binding of ATO to cysteine residues. A216V was the first mutation identified in PML and is considered to be the most frequent [14]. Note that on 20% of the studies analysed, although mutations were detected in the rearranged PML gene, resistance to ATO wasn't reported [17].

Other genes frequently mutated in AML were analysed in some of the studies. It can be noted that most of them are genes whose functions are related to cell proliferation and differentiation. It was also found genes essential for the control of remodelling and methylation of chromatin, highlighting the importance of these processes in the physiological mechanism of retinoic acid. Mutations in TP53 were also reported, notice that this gene plays a crucial role as a tumour suppressor. Alterations in these genes negatively influence the response to treatment [2], [4], [10]–[12], [15].

In the studies comparing samples from the early stages of the disease with samples from advanced stages, it has been observed that the number of concomitant mutations per patient is higher in individuals with multiple relapses, indicating an accumulation of genetic alterations throughout the progression of the disease [2], [4]. Some of the sources of evidence used in this review described cases of evolution and selection of ATO-resistant subclones, verifying that the distinct subclones may decrease or vary with time and therapeutic intervention. Data from these studies suggest that subclones with a greater capacity to adapt and acquire new mutations may be selected over the course of treatment, giving them advantages in terms of self-renewal and

proliferation. Subclones with mutated PML may be present even before the start of treatment with ATO. They are sometimes present in such small numbers that they cannot be detected by conventional sequencing methods and become detectable only after clonal proliferation. More sensitive methods would add value to this type of analysis, such as NGS, which was used in one of the included studies [3], [4], [9], [11], [14].

## 4 Conclusion: PML/RARA variants and their role in resistance

The PML/RARA oncoprotein plays a central role in APL diagnosis and treatment, not only as a target for therapeutic agents but also as a biomarker for monitoring minimal residual disease, helping to define molecular relapse events [2]. Mutations in the B2 domain of PML gene are linked to ATO resistance, however, it was verified that some individuals expressing them did not present resistance to therapy. On the other hand, the development of resistance was observed in individuals who did not express them. The evidence suggests that there is no obligatory direct relation between mutated PML and resistance to ATO. Currently, there are different hypotheses that aim to explain the reduced or absent response to this therapeutic agent. Additional mutated genes and alterations in the tumoral microenvironment are also believed to be involved in the resistance mechanisms, which still remains unclear [3], [4], [17], [18].

In the future, it could be of great value a molecular study of a broader panel of genes at diagnosis [2]. In a nutshell, ATO resistance is now considered to be multifactorial, involving several mechanisms and events. So, mutations in PML/RARA play an important role but not as relevant as it was believed when they were initially detected [3], [4].

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