THEMATIC REVIEW

How retinoic acid and arsenic transformed acute promyelocytic leukemia therapy

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This paper forms part of a special issue marking 35 Years Since the Discovery of the Retinoic Acid Receptor. The guest editors for this section were Simak Ali and Vincent Giguère.

Abstract

Acute promyelocytic leukemia (APL) is associated with severe coagulopathy leading to rapid morbidity and mortality if left untreated. The definitive diagnosis of APL is made by identifying a balanced reciprocal translocation between chromosomes 15 and 17. This t(15;17) results in a fusion transcript of promyelocytic leukemia (PML) and retinoic acid receptor alpha (RARA) genes and the expression of a functional PML/RARA protein. Detection of a fused PML/RARA genomic DNA sequence using fluorescence in situ hybridization (FISH) or by detection of the PML/RARA fusion transcript via reverse transcriptase polymerase chain reaction (RT-PCR) has revolutionized the diagnosis and monitoring of APL. Once confirmed, APL is cured in over 90% of cases, making it the most curable subtype of acute leukemia today. Patients with low-risk APL are successfully treated using a chemotherapy-free combination of all-trans retinoic acid and arsenic trioxide (ATO). In this review, we explore the work that has gone into the modern-day diagnosis and highly successful treatment of this once devastating leukemia.

Key Words
- oncology
- retinoic acid
- receptors
- transcription

Introduction

In 1957, Leif Hillstead first described a unique and deadly form of leukemia in three patients experiencing rapid decline and death ‘due to a severe bleeding tendency caused mainly by fibrinolysis’. He remarked that this should be called ‘acute promyelocytic leukemia’ (APL) because the peripheral blood resembled ‘the more chronic forms of leukemia, as it is dominated by promyelocytes and myelocytes with very few myeloblasts’ (Hillestad 2009). In 2022, this constellation of clinical and laboratory findings still constitutes a medical emergency. The hematologist-oncologist must swiftly make a morphologic diagnosis and initiate treatment using all-trans retinoic acid (ATRA) without delay until APL has been ruled in or out to avoid deadly complications in this highly curable form of leukemia.

The definitive diagnosis of APL is made by identifying a balanced reciprocal translocation between chromosomes 15 and 17. This t(15;17) results in a fusion transcript of the promyelocytic leukemia (PML) and retinoic acid receptor alpha (RARA) genes and the expression of a functional PML/RARA protein. The presence of t(15;17) can be confirmed in two ways: by detection of a fused PML/RARA genomic DNA sequence using fluorescence in situ hybridization or by detection of the PML/RARA fusion transcript via reverse transcriptase polymerase chain reaction (RT-PCR).
PML/RARA is present in greater than 95% of morphologically defined APLs (Mistry et al. 2003, Lo-Coco & Ammatuna 2006). APL corresponds to a subtype of leukemia known as M3 by French–American–British classification and represents around 10% of all leukemia cases (Jing 2004). Once confirmed, APL is cured in over 90% of cases, making it the most curable subtype of acute leukemia. The majority of deaths in APL patients are due to disease-related complications, rather than treatment failure (Coombs et al. 2015).

The treatment of APL depends on the risk stratification of the patient. Patients with low-risk APL, defined as a white blood cell count < 10,000 μL, are successfully treated using a chemotherapy-free combination of ATRA and arsenic trioxide (ATO). The work that has gone into the modern-day simplicity and elegance in diagnosis and highly successful treatment of APL spans many decades and continents. It is a testament to cross-disciplinary collaboration and the evolution in our understanding of molecular biology.

Early days of APL treatment

Success in the treatment of APL using chemotherapy began almost 20 years after it was first described by Hillstead (see Fig. 1 for timeline). Daunorubicin was first reported to have activity in leukemia in the late 1960s. In 1973, Bernard and colleagues in France first showed in a large study that daunorubicin induced complete remission (CR) in 50% of patients with APL, with a median duration of response of 26 months (Bernard et al. 1973). Daunorubicin is an anthracycline which intercalates DNA and stops the progression of topoisomerase II, preventing DNA replication. Anthracyclines remain a successful component of combined chemotherapy regimens for patients with hematological malignancy today. Indeed, combination chemotherapy remained the mainstay of therapy for APL until 1988, when Huang and colleagues in China first reported dramatic responses in APL patients to an oral therapy with ATRA, an active form of vitamin A (Huang et al. 1988).

The use of ATRA to treat patients with APL is grounded in pre-clinical research on cellular differentiation, which was first published over 10 years prior to its clinical use. Aberrant differentiation is a feature of cancer cells that was first investigated in the 1970s (Sachs 1978). Simultaneous disturbance in the signaling pathways required for cellular maturation and proliferation leads to an immature phenotype that is typical of most cancer cells. It was hypothesized that abnormalities involving genes in these pathways could explain de-differentiation of cancer cells. While chemotherapy successfully kills cancer cells by targeting mechanisms involved in DNA replication and stability that allow cancer to proliferate, it was thought that drugs could also be used to induce differentiation in cancer cells and overcome their oncogenic phenotype. In 1978, Sachs and colleagues first described how leukemia cells differentiate into normal granulocytes in vitro if exposed to certain drugs, including the chemotherapeutic agent cytarabine (Sachs 1978). Cytarabine impedes DNA synthesis by modifying the cytosine nucleoside and inhibiting the function of DNA polymerase. Continuous cytarabine infusion in combination with an anthracycline, known as the ‘7+3’ regimen, remains the standard induction chemotherapy for most non-APL subtypes of acute myelogenous leukemia.

In 1977, Rowley et al. first reported the t(15;17) in 3 patients with APL (Rowley et al. 1977). While it was suspected that chromosomal translocations can reorganize genes and result in fusion proteins which disrupt cellular growth, differentiation, self-renewal, and survival, it took over a decade before the role of retinoic acid (RA) in the treatment of APL was first elucidated. In 1980, Breitman and his colleagues first showed that RA induces promyelocyte terminal differentiation to granulocytes in cell culture in the HL-60 line, which shares some features of APL (Breitman et al. 1980, Imaiizumi & Breitman 2009). This in vitro response to RA was subsequently confirmed and extended in other laboratories and served as the basis for its clinical application (Chen et al. 1994).

How the RARA and RA ligand relate to hematopoiesis

In 1987, Giguere and colleagues and Petkovich and colleagues first reported the discovery of the retinoic acid receptor (RAR) (Giguere et al. 1987, Petkovich et al. 1987). This discovery provided molecular tools necessary to further the diagnosis and monitoring of APL. RARs are part of the nuclear receptor (NR) superfamily and can activate or repress the transcription of targeted genes. There are three major RARs: RARα, β, and γ. The structure of all RARs includes a ligand-independent activation function (AF-1), a DNA-binding domain, and a ligand-binding domain (see Fig. 2) (Geoffroy & de Thé 2020, Liquori et al. 2020). The ligand-binding domain includes the hormone-binding pocket, a dimerization domain, a binding site for coregulators, and a ligand-dependent activation function (AF-2) involved in transactivation of target genes upon hormone binding (Chambon 1996). Retinoid X receptors
(RXR) also exist in different isoforms and are obligatory heterodimerization partners for many NRs including RARs, thyroid hormone receptors, and vitamin D receptors (Evans & Mangelsdorf 2014).

RXR heterodimerizes with RARA, and together they bind DNA sequences known as retinoic acid response elements (RAREs), controlling the transcription of genes. The RARA/RXR heterodimer recognizes RAREs made of two (A/G)G(G/T)TCA motifs in the same orientation (direct repeat, DR) separated by two or five nucleotides (DR2 or DR5) (de Thé et al. 1990b). The presence of RAREs in the promoter sequence of the RARA gene fine-tunes RARA protein levels. RARA can also undergo post-transcriptional modifications further modulating its stability and structural conformation. Through recruitment of nuclear co-regulatory proteins, the RARA–RXR heterodimer can repress or activate RA-targeted gene transcription by forming co-activating or co-repressing protein complexes with histone acetyltransferases (HAT) and histone deacetylases (HDAC), respectively (Glass & Rosenfeld 2000, Evans & Mangelsdorf 2014). In the absence of RA, the RARA–RXR heterodimer associates with co-repressor protein complexes. However, RA binding to the ligand-binding domain triggers a conformational change in RARA that releases corepressors and recruits coactivators with HAT activities which open chromatin and attract the transcription machinery needed to transactivate target genes (Bourguet et al. 1995, Rochette-Egly et al. 1997). This results in hyperacetylation of histones at RARE sites, increasing accessibility of RARA-target genes and allowing for their transcription (Dilworth & Chambon 2001). At the same time, ligand-dependent activation triggers RARA catabolism through the ubiquitin/proteasome pathway. Overall, the RARA protein level is controlled by a negative feedback loop that involves both protein degradation and alterations to gene transcription.

RARA is the dominant RAR found in hematopoietic cells and is a bidirectional modulator of their differentiation. RA can block WNT signaling, directing hematopoietic stem cell (HSC) production from the hemogenic endothelium (Chanda et al. 2013). Maintenance of HSCs and their ability to self-renew in the bone marrow has been shown to require the inactivation of extracellular RA by surrounding stromal cells to inhibit differentiation in vitro (Ghiaur et al. 2013). Interestingly, RARA-deficient mice have a normal granulocyte population despite their inability to respond to RA. However, retinoid-deficient mice or those treated with an RAR antagonist have more immature granulocytes in their bone marrow, suggesting that unbound, RARA limits differentiation (Kastner et al. 2001). While modulation of the RARA pathway may control self-renewal and differentiation, these genomic mechanisms are not in themselves enough to lead to oncogenesis (Ablain & de Thé 2014). Physiological fluctuations in RA and their binding to RARA play an important part in modulating the transcriptional balance required for normal myeloid differentiation (Melnick & Licht 1999).

The discovery of PML–RARA fusion mRNA transcript and its therapeutic implications

In 1990, de Thé, Miller, Longo and colleagues first reported the presence of aberrant RARA messenger RNA (mRNA) transcripts using Northern blot analysis in the leukemic cells of three APL patients harboring t(15;17) (de Thé et al. 1990a, Longo et al. 1990, Miller et al. 1990). This...

In 1992, Miller and colleagues developed an RT-PCR assay to detect the \textit{PML}/\textit{RARA} fusion transcript, one of the methods still used today to molecularly confirm a diagnosis of APL. They identified the fusion \textit{PML}/\textit{RARA} mRNA transcript using Northern analysis and identified two isoforms of the fusion sequences corresponding to two different \textit{PML} breakpoints. They designed sets of primers that allowed detection of either isoform of the fusion transcript by RT-PCR and demonstrated excellent performance characteristics on clinical samples from APL patients (Miller \textit{et al.} 1992). In 1993, the \textit{PML}/\textit{RARA} RT-PCR was first used to monitor the response to treatment of APL patients receiving ATRA and detect the presence of minimal residual disease (Miller \textit{et al.} 1993). In this study, serially negative RT-PCR tests following therapy were associated with prolonged disease-free survival, whereas serially positive RT-PCR tests were associated with relapse. RT-PCR remained positive in nearly all patients treated with ATRA alone, whereas nearly all patients treated with ATRA and consolidative chemotherapy had a negative RT-PCR. Only three of the patients receiving consolidative chemotherapy relapsed whereas all patients on ATRA eventually relapsed. To this day, RT-PCR remains the standard of care to monitor response to therapy in APL and drives treatment decisions. These findings further suggested that ATRA alone is insufficient to induce deep and lasting therapeutic responses.

In addition, the use of ATRA alone can induce a differentiation syndrome (DS), clinically defined by weight gain and respiratory failure due to a cytokine storm released by a large burden of differentiating promyelocytes (Frankel \textit{et al.} 1992). DS is responsible for some of the early mortality associated with APL treatment. A balance must be reached between the release of cytokines and debulking the burden of leukemic APL cells. In 1995, Kanamaru and colleagues demonstrated that ATRA treatment administered simultaneously with traditional chemotherapy to reduce
DS led to a clinical response (CR) rate of 89% in 110 patients, in comparison to their previous study of chemotherapy alone in which the CR rate was 71% (Kanamaru et al. 1995). Thus, this became the standard of care treatment for APL until ATO entered the therapeutic landscape.

The addition of ATO to the therapeutic arsenal of APL

The medical use of arsonic dates to ancient Greece and Rome. The first recorded use of arsenic derivates was by Hippocrates in 400 BC for the treatment of ulcers. Dozens of oral, intravenous, and topical arsenic preparations have been developed and distributed by physicians throughout its history. Notably, British physician Thomas Fowler recommended the use of potassium arsenite for the treatment of fever in 1786. Over time, Fowler’s solution gained great renown. By the early 20th century, Fowler’s solution was used as a treatment for pernicious anemia, asthma, psoriasis, pemphigus, and eczema (Aronson 1994, Miller et al. 2002).

Additional experimentation with arsenic led Paul Ehrlich, German physician and father of chemotherapy, to discover salvarsan in 1910. Salvarsan was the standard therapy for syphilis for nearly 40 years prior to penicillin (Haller 1975). In the later 20th century, concern grew over the toxicity and carcinogenicity of arsenic with several studies linking Fowler’s solution to skin toxicities including keratosis and hyperpigmentation and cancer, particularly of the skin (Cuzick et al. 1982). Much further research has elucidated manageable toxicities and anti-cancer activity of ATO, particularly in APL (Miller et al. 2002). The biological effects of arsenic may be attributed to its ability to interact with proteins with a high cysteine content. Arsenic has been shown to encourage cellular alterations via numerous mechanisms including inhibiting angiogenesis, stimulating differentiation, inhibiting proliferation, and inducing apoptosis. Arsenic was therefore a molecule of interest in the treatment of cancers that particularly resonated in the era of differentiation theory.

In 1984, Zhang et al. first showed that ATO is clinically more effective than RA, curing up to 70% of newly diagnosed patients with APL as a single agent (Zhang 1984). In 1997, researchers at the Shanghai Institute showed a 90% remission rate in patients with relapsed APL treated with ATO (Shen et al. 1997). ATO can induce a CR in both RA-sensitive and RA-resistant clones in part due to its different mechanism of degradation of the PML–RARA transcript and restoration of PML function (Shao et al. 1998). The therapeutic activity of ATO may therefore be more effective because of how it eliminates the oncogenic driver (Mathews et al. 2010).

ATO was initially approved as a salvage therapy for relapsed APL but eventually made its way into frontline treatment. In 2004, clinical trials in China using ATRA and ATO in combination with chemotherapy showed similar CR rates of 90% with an earlier time to response and with an impressive increase in disease-free survival with no relapses occurring in the group which combined ATO and ATRA (Shen et al. 2004). In 2013, the frontline combination of ATO and ATRA in the treatment of low or intermediate-risk APL was established by Lo Coco and colleagues as a chemo-sparing regimen that improves disease-free survival over ATRA in combination with chemotherapy. In that study, all 77 patients achieved a CR in the ATRA-ATO group vs 75 of 79 patients in the ATRA-chemotherapy group. Two-year event-free survival rates were 97% in the ATRA-ATO group and 86% in the ATRA-chemotherapy group. Overall survival was also better with ATRA-ATO (Lo-Coco et al. 2013). The role of ATO is less established in the first-line treatment of high-risk APL. Chemotherapy with idarubicin, an anthracycline, is still used in combination with ATRA or ATRA and ATO in the treatment of high-risk APL in order to debulk the leukemic cell burden and mitigate DS (Sanz et al. 2019).

The PML–RARA fusion protein and how the RARA moiety drives oncogenesis

The RARA and PML genes are physically adjacent to each other in chromatin, which may explain why these two genes tend to fuse (Neves et al. 1999). Within the RARA gene, the breakpoints to make the fusion protein always map to genomic sequences lying upstream of the B-region of the RARA, leading all PML–RARA fusion proteins to contain both the DNA-binding and ligand-binding domain of RARA (see Fig. 2). The N-terminal PML sequence which joins the RARA gene is variable due to multiple breakpoints and alternative splicing. There are three breakpoint clusters (bcr) within the PML gene resulting in three isoforms of PML–RARA (Pandolfi et al. 1992). The long isoform (bcr1) is the predominant isoform found in 55% of adult APL cases (Slack et al. 1997). Alternative splicing of the RNA encoding the long isoform gives a medium isoform (bcr2) which is often co-expressed with the long isoform in the same patient. Finally, the short isoform (bcr3) is present in 45% of adult APL cases. Variations in structural motifs of the fusion protein do not alter the excellent prognosis of APL or response to therapy.
In the 1990s, the malignant phenotype of APL was described primarily as a disrupted regulation of gene transcription by the oncogenic fusion protein. PML–RARA alters the chromatin architecture by recruiting epigenetic-modifying factors such as HDAC and DNA methyltransferases in an aberrant fashion, leading to a block in cellular differentiation (Grignani et al. 1993, Di Croce et al. 2002). Physiological levels of RA do not alter the PML–RARA protein allowing propagation of the malignant cell. Ultimately, in the presence of pharmacological levels of ATRA, transcriptional activation in APL cells is restored by dissociation of co-repressor complexes from the aberrant protein conformation (see Fig. 3).

The aberrant protein structure of PML–RARA also allows for a broader range of transcriptional repression. The constitutive repressive properties of PML–RARA may be due to its dimerization through coiled-coil interactions between two PML moieties (Grignani et al. 1996, Lin & Evans 2000). This results in the formation of PML–RARA/RXR tetramers which allow for four DNA-binding surfaces to recognize promoter sequences beyond the canonical ones (Zhou et al. 2006). This binding to RXR was eventually found to be integral to APL leukemogenesis (Zhu et al. 2007). This unique property of the PML–RARA fusion protein plays a key role in APL leukemogenesis as it exerts transcriptional control over target genes other than those of RARA alone.

In addition to restoring the differentiation of promyelocytes, ATRA is eventually shown to degrade the PML–RARA transcript (Raelson et al. 1996, Zhu et al. 1999). While differentiation of the leukemic cells is noted with therapeutic doses of ATRA, it is not sufficient to cure leukemia. Interestingly, in patients receiving liposomal RA, which can deliver much higher intracellular concentrations, some cures have been achieved (Tsimberidou et al. 2006). While transcriptional activation can be achieved with a low concentration of ligand, catabolism of the NR requires prolonged exposure to high concentrations of ligand (Zhu et al. 1999). Degradation of PML–RARA requires activation of the hormone-sensitive AF-2 transactivating domain. In APL mice treated with retinoids who cannot trigger PML–RARA degradation via the AF-2 domain, the fusion protein is not degraded, and the disease remains (Ablain et al. 2013). These more recent studies of APL pathogenesis suggest that PML–RARA degradation is the key event that leads to the cure of APL, not cellular differentiation.

Figure 3
Classic model of the pathogenesis of the oncogenic fusion protein resulting from the t(15;17) chromosomal translocation of the promyelocytic leukemia (PML) and retinoic acid receptor alpha (RARα) genes. PML–RARA homodimers bind and repress RARA targets via recruitment of co-repressors leading to differentiation block and acute promyelocytic leukemia (APL). All-trans retinoic acid (ATRA) restores transcription activation of RARA targets and normal cellular differentiation. HAT, histone acetyltransferase; HDAC, histone deacetylase. Created with BioRender.com.
The role of PML in the nucleus and how the PML moiety drives oncogenesis

The PML gene encodes a nuclear protein with a characteristic cysteine-rich, zinc-finger motif. The PML protein exists in several isoforms and is part of the TRIM family of proteins. It contains a conserved RBCC motif: an N-terminal RING finger, a B1-box, a B2-box, and a C-terminal coiled-coil domain (see Fig. 2). The PML protein also contains a nuclear localization signal (NLS) and SUMO-interacting motif (SIM). Maintenance of the RBCC domain is needed for PML nuclear body formation. More recently, the B-box domains have been shown to facilitate the assembly of various oligomerizations of PML and cooperate with the RING and coiled-coil domains (Li et al. 2019).

PML is a key component of nuclear bodies, which are nuclear matrix-associated multiprotein complexes that play an important role in regulating essential cellular functions and maintaining the nuclear structure (de Thé et al. 1990a). The nuclear matrix serves as an anchor for nuclear functions including DNA replication and epigenetic silencing (Zhong et al. 2000a, Strudwick & Borden 2002, Lallemand-Breitenbach & de The 2010). Nuclear bodies are spherical structures formed by the aggregation of PML via coiled-coil interactions (Lallemand-Breitenbach & de The 2010). They are particularly important to the organization of chromatin within the nucleus and the recruitment of additional proteins to carry out nuclear functions. In healthy cells, PML nuclear bodies are modulated by stress including DNA damage, viral infection, and oxidative stress (Dellaire & Bazett-Jones 2004). PML has been implicated in the regulation of many cellular processes including proliferation, apoptosis, and self-renewal. These functions may be mediated by nuclear bodies which gather many proteins involved in the post-translation modification and degradation processes.

In particular, nuclear bodies are a site of small ubiquitin-related modification (SUMO)-ylation of proteins. Unlike ubiquitination, SUMOylation is a post-translational modification of proteins that modifies binding sites but does not necessarily lead to protein degradation. SUMOylation is necessary for the proper localization of PML to the nucleus. PML also requires SUMO-1 conjugation to properly form nuclear bodies and recruit other nuclear protein partners (Zhong et al. 2000b). Importantly, PML is the first protein described to be degraded by a process known as SUMO-dependent polyubiquitination which occurs in the RING finger (Duprez et al. 1999). This process is important to the therapeutic activity of ATO.

PML–RARA disrupts the localization of PML nuclear bodies. The wild-type PML gene localizes to the nucleus and produces a speckled pattern whereas the PML–RARA produces a micro punctate nuclear pattern or entirely cytoplasmic localization (Daniel et al. 1993). In 1993, using an APL-derived cell line, Daniel and colleagues showed that treatment with RA restores the wild-type PML pattern before the granulocytes mature (Daniel et al. 1993). By altering the nuclear structure, the malignant PML–RARA fusion disturbs the function of the PML gene. This phenomenon was later explained by a combination of PML–RARA degradation by RA and non-rearranged PML reforming nuclear bodies (Zhu et al. 1999).

Also, PML–RARA disturbs the oligomeric interfaces of PML and impairs its ability to form nuclear bodies and recruit its typical partner proteins. Loss of the integrity of the RBCC domain and the typical oligomerization patterns of PML are important to leukemogenesis and may contribute to the self-renewal capabilities of APL cells. In addition, PML–RARA disrupts the SUMOylation patterns of PML in the nucleus (Wang & Chen 2008, Brown 2009). In 2005, leukemic transformation of PML–RARA cells was linked to SUMOylation at K160 of the PML protein where a death domain-associated protein (DAXX) is recruited and results in the essential strong transcriptional repression of target genes, preventing cellular differentiation (Zhu et al. 2005). Interestingly, additional modification of the pathological post-translation SUMOylation helps explain some of the important contributions of ATO therapy.

Finally, TP53, a tumor suppressor gene, localizes to nuclear bodies and is directly inhibited by PML–RARA, allowing APL cells to evade TP53-dependent immune surveillance. Stress in the cellular environment can trigger TP53 activation in a normal cell leading to apoptosis (Guo et al. 2000). PML–RARA impairs TP3 acetylation and induces its degradation. This mechanism appears to be dependent on PML–RARA being in the presence of the remaining wild-type PML (Insginga et al. 2004). In a normal cell, PML is required for the acetylation of TP53, resulting in its activation (Pearson et al. 2000). The repressed activity of TP53 activity allows for continued self-renewal of the malignant APL cell. Overall, PML–RARA interferes with both nuclear body assembly and normal PML function as a tumor suppression gene. This likely explains the resistance to senescence and apoptosis seen in APL cells.

Variant APL

Over a dozen rare translocations involving RARA have been found in APL patients, representing less than 2% of cases.
Even more recently, rare fusions involving other RARs have been described (Conserva et al. 2019, Liquori et al. 2020). Therapy response in variant APL is characterized by inconsistent ATRA sensitivity and ATO resistance.

Further insights into APL pathogenesis and mechanisms of treatment resistance were gained via the study of the other rare RARA fusion partners. In 1993, Chen et al. described the variant translocation t(11;17) characterized by the fusion of a newly discovered novel zinc finger gene, promyelocytic leukemia zinc finger (PLZF), to RARA (Chen et al. 1993). In 1995, Licht et al. described the unique features of patients with this rare form of APL including aberrant expression of CD56, a less differentiated morphology with fine granules in the promyelocytes consistent with features intermediate between AML subtypes M2 and M3, and poor response to treatment with ATRA (Licht et al. 1995). The PLZF–RARA variant of APL accounts for 1% of all cases and represents roughly half of the described cases of variant APL to date.

Today, the PLZF gene is also known as the zinc finger and BTB domain containing 16 (BTB16) gene. It is a transcription factor involved in the self-renewal and differentiation of stem cells. PLZF directly represses the expression of target genes via zinc-finger motifs and shares some features with PML. Like PML, PLZF localizes to the nucleus; however, it does not localize to the same nuclear bodies. In addition, PLZF–RARA fusions do not co-localize with PML or de-localize PML from nuclear bodies, suggesting this disruption is not essential to the pathogenesis of APL (Koken et al. 1997). Interestingly, in an even rarer variant of APL, t(5;17), caused by RARA fusion with the NPM gene, PLZF is delocalized into a micro speckled pattern akin to PML–RARA, suggesting instead that the displacement of the PLZF gene may play a role in APL pathogenesis (Hummel et al. 1999). Like PML, PLZF can repress cell growth, potentially by binding to promoters and inhibiting the expression of cell cycle regulators such as MYC. Indeed, the down-regulation of PLZF is correlated with cell division. Interestingly, in vitro heterodimerization between PLZF–RARA and wild-type PLZF has been observed. The fusion protein may sequester PLZF from binding to its usual targets and carrying out its normal function as a tumor suppressor gene (Dong et al. 1996, Grignani et al. 1998).

There are a few proposed mechanisms to explain the relative insensitivity to RA of patients with PLZF–RARA variant APL. First, PLZF–RARA is able to repress target genes in a stronger and more stable way than PML–RARA (Guidez et al. 1998). In addition, the PLZF–RARA fusion protein represses wild-type RARA–RXR via its N-terminal region, known as the pox virus and zinc finger (POZ) domain. The POZ domain facilitates heterodimerization of PLZF–RARA with RXR (Dong et al. 1996, Licht 1996). Furthermore, the PLZF–RARA/RXR heterodimer binds to RAREs with very high affinity. In this way, PLZF–RARA may also sequester RXR away from its essential function as a RARA co-factor. Finally, like PML–RARA, PLZF–RARA is a negative inhibitor of wild-type RARA due predominantly to high affinity for the HDAC-containing co-repressor complex. However, while PML–RARA releases surrounding co-repressors in the presence of therapeutic ATRA, PLZF–RARA does not (Grignani et al. 1998, Guidez et al. 1998, Sirulnik et al. 2003). In this way, PLZF–RARA is a stronger repressor of gene transcription.

The transcriptional repression activity of PLZF–RARA is not sufficient to explain its pathogenesis in APL. RA treatment does elicit differentiation of blasts with this fusion, suggesting that these variant APL cells still maintain a capacity for self-renewal despite an unblocking of cellular maturation (Petti et al. 2002, Cassinat et al. 2006). The relative resistance to RA therapy may be explained by alteration to the function of the PLZF gene itself including MYC overexpression (Rice et al. 2009). In addition, unlike in other variant APLs and classical APL, the reciprocal RARA–PLZF fusion is required for full leukemogenesis and is not degraded by RA. In fact, the reciprocal transcript encoding RARA–PLZF instead activates gene transcription by activating cyclin A2 and supporting cellular renewal (Yeyati et al. 1999). In this way, t(11;17) variant APL is driven by two fusion oncogenes: RARA–PLZF activates cell cycle regulators and PLZF–RARA blocks cellular differentiation. The unpacking of this rare form of APL supported the concept of aberrant transcriptional repression as a contributing factor to the pathogenesis of APL and the importance of the partner gene of RARA to act as a tumor suppressor gene whose impairment contributes to self-renewal of the malignant cell.

**APL’s molecular landscape and genome-wide activity**

Today, therapeutic approaches in leukemia are determined through the lens of their molecular and cytogenetic signature. Co-operating gene mutations are found in patients with APL including FLT3, WTI, NRAS, KRAS, and MYC (Ibáñez et al. 2016). Many mutations related to relapse or resistance to therapy inhibit the direct binding of ATRA and ATO onto PML–RARA. The activation of the potent oncogene FLT3, a receptor tyrosine kinase, has been shown to impede the ATRA response in vitro. In vivo,
this resistance can be overcome by ATO and represents yet another example of the synergistic activity of ATRA and ATO (Esnault et al. 2019). Whole-genome sequencing studies have demonstrated that patients with APL present on average as few as three mutations in coding regions, with the only recurrent genetic abnormality associated with t(15;17) being FLT3-activating mutations. The PML–RARA fusion protein, therefore, serves as an oncogenic driver of APL (CGARN 2013).

While oligomerization of the fusion protein was classically considered to be crucial to its oncogenesis, newer studies confirmed RXR to be essential to a fully transformed APL cell, requiring a tetramerized PML–RARA fusion/RXR oncogenic complex (Zeisig et al. 2007). Indeed, silencing of RXR by short-hairpin RNA (shRNA) in transgenic mice was shown to suppress the development of APL and a 99% association between RXR and PML–RARA binding sites was detected genome-wide.

As 2010 approached, ChIP-seq and ChIP-on-ChIP finally made it possible to look at the genome-wide actions of PML–RARA and the epigenetic modifiers associated with its binding, shedding new light on its action (Saeed et al. 2011). First, genome-wide analysis of PML–RARA binding sites confirmed additional response elements including regions containing DR1, DR3, DR4, and other atypical motifs suggesting a major gain in DNA-binding capacity in the oncogenic fusion protein (Kamashev et al. 2004, Martens & Stunnenberg 2010, Martens et al. 2010). Furthermore, the fusion protein was found to be bound to RAR, RARB, and RARG genes themselves suggesting that the expression of these genes is modulated by the fusion protein. PML–RARA was found to interact with many other proteins, particularly transcription factors regulating hematopoiesis including GATA2, AP-1, RUNX1, RUNX3, and PU.1. The oncogenesis of PML–RARA includes exercising control of the hematopoietic process far beyond the reach of wild-type RARA.

PML–RARA acts by regulating wild-type ATRA–RARA expression and expanding the regulation of gene expression beyond classic target genes. More recent gene expression profiling shows that PML/RARA can upregulate gene expression and transactivate genes essential to APL pathogenesis. Building on the idea that PML–RARA can bind more sites than the canonical RAREs, Tan and colleagues recently showed that histone acetylation enhancement was significantly more abundant on PML–RARA activated genes and upregulated the transcription of super-enhancer associated PML–RARA regulated genes, including a target gene known as GFI1 (Tan et al. 2021). GFI1 is a transcriptional regulator with roles in hematopoiesis and leukemogenesis. Transactivation of GFI1 by PML–RARA and associated chromatin conformation regulation at the super-enhancer site results in APL. Mice transplanted with PML–RARA leukemic cells modified by shRNA targeting the GFI1 intronic enhancer region did not develop leukemia. Indeed, GFI1 and PML–RARA share many binding sites including those for myeloid TF such as CEBPA, ETS, and RUNX1 and coregulate lineage-specific gene expression. These more recent findings further challenge the traditional model of APL pathogenesis as being related solely to the repression of gene transcription necessary for myeloid differentiation.

**Summarizing the synergism between RA and ATO in the treatment of APL**

The primary therapeutic effect of promyelocytic differentiation in APL is thought to be from ATRA. However, ATO exerts a dose-dependent dual effect on APL cells in that at low concentrations it promotes cellular differentiation and at high concentrations it induces apoptosis (Chen et al. 1997). In addition, both ATRA and ATO contribute to the degradation of the PML–RARA protein (Nasr et al. 2008, de Thé & Chen 2010, de Thé et al. 2012). ATRA and ATO degrade the PML–RARA fusion via actions on the RARA and PML moieties, respectively. Resistance to ATRA and ATO is associated with genetic mutations with amino acid substitutions in either the RARA ligand-bind domain or the PML B-box domain (Tomita et al. 2013, Zhu et al. 2014). Overall, ATRA and ATO not only differentiate APL cells, but together trigger APL cell apoptosis, quickly restore the function of the nuclear matrix, and increase the SUMOylation, ubiquitination, and degradation of PML–RARA.

ATRA degrades the PML–RARA fusion protein via several mechanisms. First, ATRA binding to RARA simultaneously activates its catabolism through the binding of SUG1 component of the 19S proteasome leading to degradation through ubiquitination (Vom Baur et al. 1996, Thomas & Tyers 2000). Second, ATRA binding to RARA leads to caspase-mediated degradation (Nervi et al. 1998). Third, ATRA binding to RARA leads to the expression of the membrane-bound tumor-selective death ligand, known as tumor necrosis factor-related apoptosis-inducing ligand leading to apoptosis of the cell (Altucci et al. 2001). Fourth, ATRA binding restores the autophagy of the cell. Autophagy is a highly conserved degradation process by which intracellular compartments and materials are either recycled or removed. Autophagosomes, vesicles containing intracellular materials, fuse with
lysosomes for degradation. This process is regulated by autophagy-related genes (ATG). ATRA-induced cellular differentiation increases the expression of ATG and restores this housekeeping process (Moosavi & Djavaheri-Mergny 2019). Unfortunately, these mechanisms of PML–RARA degradation by ATRA are insufficient to completely eliminate the APL clone.

In a complementary way to ATRA, ATO binds to the PML CC-motif and B-box domain and is critical for the serial reactions of multimerization, SUMOylation, ubiquitination, and proteasomal degradation of the PML–RARA transcript. Given that ATO does not act on the NR or activate transcription, the differentiation of leukemic cells seen in the presence of ATO may in part be due to degradation of the PML–RARA transcript and restoration of normal RARA activity on gene promoters liberated from the malignant PML–RARA (Cassinat et al. 2017). Importantly, the ATO-triggered degradation process contributes to the differentiation of APL cells because of its action on the SUMOylated K160 on the PML gene stops the recruitment of the transcriptional repressing DAXX protein, releasing the cell from its differentiation block, while instead recruiting RING finger protein 4 which is a SUMO-dependent ubiquitin E3 ligase (Lallemand-Breitenbach et al. 2008, Tatham et al. 2008). Polyubiquitylated PML–RARA is then degraded by the 11S proteasome via the ubiquitin–proteasome pathway (Chen et al. 2011, Maroui et al. 2012). SUMOylation at the K65 and K160 in the presence of ATO is necessary to initiate SUMO-dependent ubiquitination.

Finally, ATO is a successful partner to ATRA in the treatment of APL because it acts independently on malignant APL cells and can also be potentiated by the presence of ATRA. First, ATO inhibits the migration of progeny PML bodies back to the nucleus after cell division. This limits the nuclear activities of both PML and PML–RARA in dividing leukemic cells ultimately severing the self-renewal advantages gained by the malignant fusion protein (Lång et al. 2012). Without this advantage, the malignant APL cell is no longer able to blunt the activity of TP53, restoring the senescent properties of PML. Next, ATO directly induces apoptosis of APL cells and downregulates SUMOylation to the already SUMOylated K160 on the PML gene.
the expression of the BCL2 oncogene (Chen et al. 1996). This mechanism occurs outside of the retinoic pathway and may explain how ATO overcomes ATRA resistance in APL. ATRA has been shown to potentiate ATO-induced apoptosis via pathways involving RXRA phosphorylation (Tarrade et al. 2005). In addition, ATRA and ATO have been shown to synergistically downregulate telomerase, causing telomere shortening and APL cell death (Tarkanyi et al. 2005). The productive relationship between ATRA and ATO leads to a broader ranging arsenal against the oncogenic properties of APL cells and is responsible for the high rates of remission and cure of this unique leukemia (see Fig. 4).

Conclusion
Understanding the evolution of our knowledge of APL takes us directly into the cytoplasm and nucleus of the promyelocyte. It is a dynamic place of steroid receptor signaling, protein repressor and activating complexes, ubiquitination, SUMOylation, and nuclear matrix regulation of proteins. Understanding this molecular world would not be possible without the international collaboration of scientists from Canada, China, France, the United States, Italy, and many other countries over several decades. The incredible legacy of their work has turned the deadliest form of leukemia into the most curable.

Declaration of interest
W H M has received honoraria from BMS, Merck, Roche, Novartis, GSK, Amgen, Mylan, EMD Serono and Sanofi. He has received consulting fees from BMS, Merck, Roche, Novartis, GSK, Amgen, Mylan, EMD Serono and Sanofi. He is a principal investigator who is participating in or has participated in clinical trials within the past two years sponsored by BMS, Novartis, GSK, Roche, AstraZeneca, Methy莲gene, MedImmune, Bayer, Amgen, Merck, Incyte Pfizer, Sanofi, Array, MiMic, Ocellaris Pharma, Astellas, Alkermes, Elexis and Genentech. He has received grants or contracts from Merck, CiHR, CRS, Terry Fox Research Institute, Samuel Waxman Cancer Research Foundation, CCSRI.

Funding
This work did not receive any specific grant from any funding agency in the public, commercial, or not-for-profit sector.

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Received in final form 14 September 2022
Accepted 16 September 2022
Accepted Manuscript published online 16 September 2022