Potential role for all-*trans* retinoic acid in nonpromyelocytic acute myeloid leukemia

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Practice points

- All-*trans* retinoic acid (ATRA) has been well characterized to release the block in myeloid differentiation caused by the PML-RAR- α fusion protein, and is a critical component of therapy for this disease. Its efficacy in nonacute promyelocytic leukemia acute myelogenous leukemia (AML) has been less well studied.
- Multiple clinical trials in nonacute promyelocytic leukemia AML patients have incorporated ATRA or other retinoids with mixed results, but it may increase efficacy in some patient populations such as those with mutations in nucleophosmin 1, normal cytogenetics or *de novo* AML without prior therapy.
- Combining retinoids with epigenetic modifiers such as histone deacetylase inhibitors or hypomethylating agents may be a rational approach to treat myeloid neoplasms in which normal differentiation pathways and tumor suppressor genes are suppressed.
- ATRA in combination with FLT3 inhibitors has proven to be efficacious in mouse models of AML.

All-*trans* retinoic acid (ATRA) has been very successful in the subtype of acute myelogenous leukemia known as acute promyelocytic leukemia due to targeted reactivation of retinoic acid signaling. There has been great interest in applying this form of differentiation therapy to other cancers, and numerous clinical trials have been initiated. However, ATRA as monotherapy has thus far shown little benefit in nonacute promyelocytic leukemia acute myelogenous leukemia. Here, we review the literature on the use of ATRA in combination with chemotherapy, epigenetic modifying agents and targeted therapy, highlighting specific patient populations where the addition of ATRA to existing therapies may provide benefit. Furthermore, we discuss the impact of recent whole genome sequencing efforts in leading the design of rational combinatorial approaches.

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The term retinoid refers to vitamin A and all of its metabolites, whether synthetic or naturally occurring compounds. Retinoids play important roles in embryogenesis, vision, metabolism, inflammation and most importantly for the purposes of this review, cellular differentiation in hematopoiesis. The importance of retinoids for granulocytic differentiation was first elucidated in studies of acute promyelocytic leukemia (APL) which is most often defined by a chromosomal translocation which fuses the *RAR- α* gene on chromosome 17 to the *PML* gene on chromosome 15 [1]. This creates a dominant-negative retinoic acid receptor (RAR) that binds to promoters of retinoic acid (RA)-responsive target genes and functions as a corepressor, which in turn leads to a differentiation block at the promyelocytic stage [2–4]. There are also data that suggest that the

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PML–RAR- α fusion protein leads to increased cellular proliferation and decreased susceptibility to apoptosis [5]. All-*trans* retinoic acid (ATRA; **Figure 1**) can bind to RAR- α and dissociate the corepressor complex, allowing for coactivators to bind at these promoter regions. This leads to a release in the block of transcription of genes involved in differentiation, and the promyelocytes may then follow normal maturation to granulocytes [6,7]. A landmark study by Tallman *et al.* demonstrated that the addition of ATRA to standard induction chemotherapy with daunorubicin plus cytarabine significantly increased overall survival (OS) in patients with APL [8]. This targeted therapy truly changed the prognosis for newly diagnosed APL patients, changing it from one of the worst prognoses to a disease that can regularly be cured, and the current standard of care for low-risk APL does not include chemotherapy in the retinoic acid era [9].

Consequently, there has been great interest in applying this form of differentiation therapy to other cancers, including non-APL AML (henceforth referred to simply as AML), and numerous clinical trials have since been conducted. However, ATRA as monotherapy has thus far shown little benefit in AML. A small case series of four pediatric/young patients was reported in which the patients were originally diagnosed as APL based on morphology, but later on found to

have t(8;21) AML which can share very similar morphology. Each of these patients were treated with a maximum of 60 mg/day ATRA and did get into a remission, although these remissions were very short (2–12 weeks) and required treatment with standard of care AML therapy once the correct diagnosis was made [10]. Furthermore, there are limited data showing improvement in clinical series when ATRA was given in combination with chemotherapy, as will be discussed in further detail below.

Nevertheless, there may be specific AML patient populations that would benefit from the inclusion of ATRA in their treatment. Recent efforts in whole genome sequencing of leukemias have revealed a number of frequently recurring genetic alterations in AML [11]. These include the *NPM1* mutation, *FLT3/ITD*, *DNMT3A* mutation, *MLL* rearrangements, *WT1* and *IDH1/2* mutations, among others. Importantly, many of these genes have prognostic and/or predictive value [12]. Recent studies have attempted to devise combinations of ATRA with epigenetic therapy, targeted therapy and/or chemotherapy to treat specific patient populations.

In this article, we will review the literature on the use of retinoids in AML. We will first discuss their use in combination with chemotherapy, followed by epigenetic therapy and targeted therapy in AML. Finally, we will provide our perspective on future directions in which retinoids may be rationally combined with other therapies for increased antileukemic efficacy.

ATRA in combination with chemotherapy

In contrast to the use of ATRA in APL, which is now the standard of care due to its improvement in relapse-free survival rates compared with therapy of this disease without ATRA, studies of ATRA in combination with chemotherapy for AML have yielded more mixed results. A randomized trial was conducted in which 95 patients with either high-risk or refractory AML were treated with cytarabine and idarubicin and were randomized to also receive ATRA at 45 mg/m²/day, from day 1 to complete remission, or not. Please see **Table 1** for more detailed dosing of chemotherapy regimens for each trial discussed. From a safety perspective, no significant toxicities (including differentiation syndrome) were observed in the group which received ATRA, but unfortunately there were no differences seen in the rates of complete response (CR), disease-free survival or OS [13].

Figure 1. Chemical structure of therapeutic retinoid compounds.

Table 1. Summary of clinical trials using all-trans retinoic acid in nonacute promyelocytic leukemia acute myelogenous leukemia.

Study (year)	Study population, total number of patients in trial	ATRA dose, schedule	Additional therapeutic agents	Outcome	Ref.
ATRA trials in relapsed, refractory populations					
Belhabri <i>et al.</i> (2002); NCT00005863	Relapsed, refractory or high-risk AML, 20–80 years old, n = 95	45 mg/m ² /day, day 1 to hematologic recovery or day 28 if resistant	AraC 1 g/m ² /12 h days 1–6, IDA 12 mg/m ² /day, days 1–3	CR 55% with ATRA vs 58% without ATRA (p = NS)	[13]
Milligan <i>et al.</i> (2006)	Relapsed, refractory or high-risk AML, age 15+ years, n = 405	45 mg/m ² /day, day 1–90	Randomized to FLU 30 mg/m ² /day, days 1–5, AraC 2 g/m ² /day, days 1–5 or AraC 100 mg/m ² every 12 h days 1–10, daunorubicin 50 mg/m ² everyday, days 1, 3, 5, VP-16 100 mg/m ² /day, days 1–5. Also randomized $\pm$ G-CSF	CR 60% with ATRA vs 63% without ATRA (p = NS)	[14]
Estey <i>et al.</i> (1999)	Relapsed, refractory or high-risk AML or MDS, adults, median age 65 years, n = 215	45 mg/m ² /day, day 2–3 days after completion of chemo	FLU 30 mg/m ² /day, days 1–4, AraC 2 g/m ² /day, days 1–4, IDA 12 mg/m ² /day, days 2–4, G-CSF 200 mg/m ²	CR = 51%, no difference with ATRA	[15]
ATRA trials in newly diagnosed patients					
Burnett <i>et al.</i> (2010)	Newly diagnosed AML, <60 years of age, n = 1075	45 mg/m ² /day, days 1–60	Randomized to differing doses of chemotherapy, AraC 100–200 mg/m ² every over 12 h, days 1–8 or 1–10, daunorubicin 50 mg/m ² /day, days 1, 3, 5, thioguanine 100 mg/m ² every 12 h, days 1–8 or 1–10	CR = 68%, no difference with ATRA. OS 33% with ATRA and 30% without ATRA (p = NS)	[16]
Schlenk <i>et al.</i> (2004)	Newly diagnosed AML, $\geq$ 61 years old, n = 242	45 mg/m ² /day, days 3–5, 15 mg/m ² /day, days 6–28	IDA 12 mg/m ² /day, days 1 and 3, AraC 100 mg/m ² /day, days 1–5, VP-16 100 mg flat dose, days 1 and 3	CR 56% with ATRA vs 39% without induction phase	[17]
Bolaños-Meade <i>et al.</i> (2003)	Newly diagnosed AML, age 18+ years, median age 60 years, n = 63	45 mg/m ² /day, days 1–6, and consolidation	IDA 12 mg/m ² /day, days 2–4, AraC 2 g/m ² over 72 h, days 2–4, VP-16 300 mg/m ² over 72 h from day 9 to 11	Single-arm study, CR 60%	[18]
ATRA trials with nontraditional therapeutic agents					
Cimino <i>et al.</i> (2006)	High-risk or refractory AML, age 54–69 years, n = 8	45 mg/m ² /day, starting on day 14	VPA 10 mg/kg/day p.o., titrated to serum level >50 μ g/ml	Single-arm study, ORR 25%	[19]
Kuendgen <i>et al.</i> (2005)	MDS age 21–84 years, median age 67 years, n = 75	80 mg/m ² /day, days 1–7 of 14-day cycle	VPA titrated to reach serum concentrations between 50 and 100 μ g/ml	ORR 24%, RR 26% in VPA alone and 11% in VPA + ATRA, although populations differed	[20]
Wu <i>et al.</i> (2016)	High-risk AML or MDS, n = 31	20 mg/m ² /day, day 1–28 (6 cycles), maintenance	DAC 15 mg/m ² /day, days 1–5 of 28-day cycle	Single-arm study, ORR 58.1%, CR 22.6%	[21]
Thomas <i>et al.</i> (2015)	Refractory AML, age >60 years, n = 5	45 mg/m ² /day, daily	AZA 75 mg/day sc., days 1–7 of 28-day cycle, pioglitazone 45 mg/day	Single-arm study, 60% CR with residual cytopenias	[22]
Raffoux <i>et al.</i> (2010); NCT00339196	Relapsed, refractory or high-risk AML, high-risk MDS age 50–87 years, median age 72 years, n = 65	45 mg/m ² /day, days 8–28	AZA 75 mg/m ² /day, days 1–7, VPA 35–50 mg/day, days 1–7 of 28-day cycle	Single-arm study, 26% ORR and 22% CR	[23]
Lübbert <i>et al.</i> (2016)	Newly diagnosed age >60 years, n = 204	45 mg/m ² /day, days 6–28	DAC 20 mg/m ² days 1–5 of 28-day cycle, VPA dosed for target level 50–100 μ g/ml	Effect on ORR was 21.9% for ATRA and 13.5% with no ATRA	[24]
Ramsingh <i>et al.</i> (2014); NCT01161550	Relapsed, refractory AML, age 18+ years, median age 52 years, n = 11	45 mg/m ² /day, days 7–20	Midostaurin 25–50 mg p.o. b.i.d., cladribine 5 mg/m ² /day, days 1–6, AraC 2 g/m ² days 2–6, G-CSF 300 μ g sc., days 1–6	Single-arm study, 22% CR and 11% PR	[25]

AML: Acute myelogenous leukemia; AraC: Cytarabine; ATRA: All-trans retinoic acid; AZA: 5-azacytadine; CR: Complete response; DAC: Decitabine; DOXO: Doxorubicin; FLU: Fludarabine; G-CSF: Granulocyte-colony stimulating factor; IDA: Idarubicin; MDS: Myelodysplastic syndrome; ORR: Objective response rate; OS: Overall survival; p.o.: Per oral; PR: Partial response; sc.: Subcutaneous; VP-16: Etoposide; VPA: Valproic acid.

While the aforementioned induction regimen is a standard approach for AML, it was thought that perhaps ATRA would be more beneficial in a more aggressive induction regimen which includes other cytotoxic agents in addition to cytarabine plus anthracyclines. A trial designed to look at efficacy of fludarabine plus cytarabine versus cytarabine, daunorubicin and etoposide also asked a secondary question of whether CR rates could be boosted by the addition of either granulocyte-colony stimulating factor (G-CSF) or ATRA used as differentiating agents for AML patients with relapsed, refractory or high-risk disease. For those who were randomized to receive ATRA, it was dosed at 45 mg/m² starting on day 1 of treatment and given daily for a maximum of 90 days or until taken off protocol. Although the efficacy of ATRA was not designed as a primary outcome for the trial, the data did not support the addition of ATRA to either induction regimen as improving outcomes [14]. A similar study used fludarabine, cytarabine and idarubicin (FAI) for induction, and patients were randomized to receive ATRA (45 mg/m² daily in two divided doses) and/or G-CSF. Patients with white blood cell (WBC) count <10,000/μl started ATRA 2 days prior to chemotherapy (day 2), and those with higher WBC count started ATRA simultaneously with chemotherapy (day 1). Treatment with ATRA continued for 3 days after completion of FAI. Like the previous trial, eligibility criteria required patients to be at high risk either due to advanced age, history of myelodysplastic syndrome (MDS) or WBC counts, or failure of prior lines of treatment. Although there were no signals of harm in the ATRA arm, there was also no indication that it improved any measure of outcome [15]. Additional mutation and gene expression studies were performed to try to understand the role of RA signaling in AML. High levels of menin-gioma 1 (*MNI*) expression correlated with poor patient outcomes. They also went back to patient samples from the clinical trial and demonstrated that patients with low levels of *MNI* expression and who received ATRA had a significantly longer survival than patients with low *MNI* expression who did not receive ATRA, or those who had high *MNI* expression [26].

Each of the above trials included only high-risk patients who have a poor prognosis with any previously investigated therapy. It is therefore possible that ATRA may improve results in a younger patient population, or when used

as front-line therapy. In a trial done in the UK with 1075 patients younger than 60 years old with newly diagnosed AML, subjects were randomized to receive induction chemotherapy consisting of cytarabine, doxorubicin and thioguanine with or without ATRA (45 mg/m² from days 1 to 60). Two different doses of cytarabine were tested. Results were also examined to identify genetic mutations that may identify a subgroup that had benefit from the ATRA treatment. CR rate was 68% overall in this trial, and there was no subgroup which showed a benefit of ATRA in any metric [16].

There is a particularly pressing need for first-line treatment options in elderly patients, who may not tolerate standard intensive induction chemotherapy. In order to see if ATRA would have better efficacy in the upfront setting for these patients, a Phase III AMLSG trial (AMLHD98B) in previously untreated, elderly patients randomized subjects to receive induction chemotherapy of idarubicin, cytarabine and etoposide with or without ATRA. In the patients who were randomized to receive ATRA, the drug was started on day 3 of induction and given at 45 mg/m² for 3 days then the dose was reduced to 15 mg/m² through day 28. The response rate of 60% in the ATRA group was higher than the 43.5% observed in the standard induction arm ($p = 0.01$). The trial also demonstrated an OS advantage for the ATRA arm (11.3 vs 7 months) [17]. A retrospective analysis evaluated responses with respect to mutation status of *NPM1*, *CEBPA*, *FLT3* and *MLL* genes. They found that the benefit of adding ATRA to standard induction chemotherapy was limited to *NPM1*⁺ patients without *FLT3/ITD* mutations. This genotype correlated positively with achievement of CR and extended relapse-free and OS, while adverse cytogenetics and high WBC were negatively correlated with these outcomes. However, other studies have failed to identify *NPM1* mutations as a prognostic indicator of response to ATRA [16,27]. Thus, further research is warranted to improve the response rate to ATRA in this patient population.

Another trial included both young and old AML patients, as well as lower and high-risk cytogenetics [18]. This study consisted of one arm with patients induced with timed-sequential therapy comprised of cytarabine, idarubicin and etoposide and ATRA 45 mg/m² given on days 1–6, followed by consolidation chemotherapy with ATRA. As with other trials, there

was no increase in toxicity from the ATRA. The CR rate in this study was 60%, which was higher than the expected CR rate based on numerous prior studies, although it was a small study of 63 patients. Nonetheless, CR was significantly higher in lower-risk groups such as patients younger than 60 years old, those with nonadverse cytogenetics and *de novo* AML, lending some credence to the hypothesis that perhaps there are certain patient populations that may benefit from the inclusion of retinoids.

ATRA in combination with epigenetic agents

A number of molecular alterations noted in AML lead to epigenetic changes in chromatin structure and/or gene expression. Since retinoids induce release of corepressors and increase in expression of genes that are important for myeloid differentiation, several groups independently hypothesized that combining retinoids with epigenetic modifiers may lead to improved efficacy. Histone deacetylase inhibitors (HDACi) have been used in the treatment of MDS, a precursor lesion to AML and in AML patients deemed to be unfit for intensive induction chemotherapy. One of the first HDACi to be explored was valproic acid (VPA), which is more commonly known as an antiepileptic. Investigators hypothesized that the combination of VPA and ATRA would lead to improved differentiation of myeloid cells and better clearance of leukemic cells. The combination of these drugs led to increased expression of p21 in *ex vivo* treated AML samples and in turn, this led to cell cycle arrest and apoptosis [28]. Given these promising results, a pilot clinical study was designed to investigate the effects in patients. Eight high-risk AML patients who were unfit for induction chemotherapy were treated first with VPA to induce chromatin remodeling, followed by ATRA (45 mg/m² starting on day 14) to induce gene transcription and myeloid differentiation. Correlative assays performed on peripheral blood mononuclear cells from the treated patients demonstrated an increase in histone acetylation and myeloid differentiation, as predicted. Two patients showed hematologic improvement and five patients showed stable disease, but ultimately all patients progressed and succumbed within 6 months of enrollment. The study thus demonstrated that while the combination of drugs was having some biologic effect, it was insufficient to control the disease [19].

Due to these promising results, the combination was also tested in a larger trial of 75 patients with varying subtypes of MDS. Like the aforementioned trial, these patients received VPA first with later addition of ATRA (80 mg/m² per day on days 1–7, every other week) if they responded, although there were a small number of patients who received the combination upfront. Hematologic improvement occurred in a quarter of the patients following VPA treatment, but the addition of ATRA did not lead to any further improvement or increase the response rate. Interestingly, of the ten patients who showed an initial response after VPA and then worsened, four of them showed some improvement in blood counts after receiving ATRA. Overall, patients who responded in this trial tended to have less severe disease such as refractory anemia with ringed sideroblasts. Patients with higher-risk phenotypes such as refractory anemia with excess blasts had poorer response rates [20].

Another interesting area of exploration is the use of retinoids with hypomethylating agents. The prototypical drug in this class is 5-azacytidine, which is a cytosine nucleoside analog that inhibits DNA methyltransferases, and has been shown to reactivate the expression of tumor suppressor genes and induce differentiation in AML. Based on promising *in vitro* studies that demonstrated synergistic increased expression of the p16 tumor suppressor (encoded by the *CDKN2A* gene) in K562 cells treated with the combination of ATRA and decitabine (a derivative of 5-azacytidine), a clinical study was undertaken [29]. In this single-arm study, elderly patients felt to be unable to tolerate a more aggressive induction regimen, they were treated with decitabine plus ATRA (20 mg/m² daily) for six cycles, followed by ATRA maintenance treatment. The CR rate was 22.6%, and the median OS was 11.0 months [21]. A small pilot study in elderly patients with refractory AML was done using ATRA (45 mg/m² daily), 5-azacytidine and the peroxisome proliferator-activated receptor gamma ligand, pioglitazone, which is known to be a binding partner for retinoid X receptor. In this study of five patients, there were three CRs and one patient whose disease reverted to chronic myelomonocytic leukemia, which was a very promising result although follow-up was less than 1 year at the time of publication [22]. A Phase II clinical study which combined 5-azacytidine, VPA and ATRA (45 mg/m²/day, days

8–28) in high-risk AML/MDS patients resulted in OS of 12.4 months, and the response correlated with demethylation of genes involved in tumor suppression [23]. This study had only one arm, but a similar randomized trial was recently presented which evaluated the effects of adding VPA and/or ATRA (45 mg/m²/day, days 6–28) to decitabine in an elderly population of MDS patients, including a high proportion of poor-risk cytogenetics or antecedent hematologic disorders. Interim results demonstrate that addition of ATRA led to an increase in OS, but VPA did not provide any marginal benefit [24]. It is encouraging that this biologically targeted approach had a signal of efficacy and was well tolerated, warranting further study of ATRA and epigenetic modifiers.

MLL-rearrangement (*MLL-r*), in which balanced translocations involving chromosome 11q23 produce fusion genes with one of the multiple partner genes, most commonly t(4;11) (*MLL-AF4*) and t(9;11) (*MLL-AF9*), commonly occurs in acute lymphoblastic leukemia (ALL) and AML, and these patients typically respond poorly to conventional chemotherapy. *MLL-r* leads to hypermethylation of key promoter regions, including those of tumor suppressor genes such as *CDKN2A*, thus inactivating gene expression and contributing to leukemogenesis [30]. DNA hypomethylating agents thus may represent a strategy for overcoming the epigenetic changes associated with *MLL-r*. In one study, 5-azacytidine treatment promoted sensitivity to ATRA in *MLL-r* AML cell lines, leading to increased expression of myeloid maturation markers and decreased cell growth [30]. Similar results were reported in another study stating that the combination was highly active in *MLL-AF9* expressing cells in particular [31]. Investigators stated that inactivation of the RA pathway in AML is one hypothesis for why ATRA as monotherapy is ineffective. However, treatment with ATRA and 5-azacytidine restores the expression of C/EBP α and thus induces monocytic/granulocytic differentiation.

MLL-r AML may be sensitive to other epigenetic modifying agents as well, including HDACi. The HDACi trichostatin A combined with ATRA increased the killing of *MLL-AF9* cells [32]. This approach may also be applicable to AML patients harboring *AML1/ETO* translocations [33]. *AML1/ETO* represses RA signaling via an HDAC-dependent mechanism [33]. Again, trichostatin A was able to restore sensitivity to ATRA

resulting in growth arrest and increased differentiation. Recently, it was reported that *MLL-AF4* cells could be sensitized to ATRA when treated in combination with the lysine-specific demethylase 1 inhibitor, tranylcypromine [34]. Indeed, there is great interest in exploring the combination of ATRA and epigenetic modifying agents in AML, and a number of clinical trials are ongoing.

ATRA in combination with targeted agents

Through the implementation of whole genome sequencing, we have gained a better understanding of the identity and frequency of specific molecular alterations driving leukemogenesis. This information allows researchers to design therapeutic strategies to target these aberrant signaling pathways and improve the response rates in particular patient populations. *NPM1* mutations are present in approximately 30% of AML. These alterations lead to accumulation of mutant protein in the cytoplasm, and are thought to be one of the common founder lesions in AML [35,36]. As mentioned previously, AML patients with the *NPM1*⁺ *FLT3*/*WT* genotype in one study were found to benefit from the addition of ATRA to their therapy [37]. Expanding on this finding, a search was conducted for a response to other already available drugs, where it was discovered that *NPM1*-mutated AML cells were more sensitive to arsenic trioxide (ATO) [38]. Following up on this study, the biological mechanism for the response to the combination of ATRA and ATO in cell-based models of *NPM1*-mutated AML was explored [39]. Human AML cell lines and primary patient samples carrying *NPM1* gene mutations or wild-type *NPM1* were treated with ATO and/or ATRA at pharmacologically relevant concentrations. A significant induction of apoptosis was observed in *NPM1*-mutant cells when treated with the combination relative to single drug treatments. In this study, the effect of ATRA on AML cells was not restricted to the *NPM1*-mutant subset, but rather had a general antileukemic effect in terms of cell proliferation and colony-forming unit ability, and potentiated the selective activity of ATO against *NPM1*-mutant cells. The investigators postulate that this selectivity may be due to targeted degradation of the *NPM1*-mutant protein by ATO (and to a lesser extent, ATRA), a phenomenon that is in line with the degradation of PML–RAR- α by ATRA and ATO in APL. Furthermore, they provide preliminary evidence that this treatment

combination may sensitize *NPM1*-mutant cells to anthracycline-based chemotherapy.

Mutations in *FLT3* are another frequently occurring genetic alteration in AML, with *FLT3/ITD* present in approximately 20–25% of patients [40,41]. *FLT3* is a receptor tyrosine kinase that is expressed during normal hematopoietic development; however, constitutively activating mutations lead to increased proliferation, a block in differentiation and poor prognosis. Several preclinical studies have demonstrated synergy when *FLT3* tyrosine kinase inhibitors are combined with ATRA in *FLT3/ITD*⁺ cell lines and patient samples [42–44]. One study found increased targeting of leukemia stem cells with the treatment combination of ATRA and the multikinase inhibitor, sorafenib, utilizing mouse models of *FLT3*-mutant AML [44]. Members of the STAT family of proteins were implicated as targets for the combinatorial effects observed in *FLT3/ITD*⁺ cells due to their differential regulation of the antiapoptotic protein BCL6.

Together, these studies support the development of clinical trials combining ATRA with a *FLT3* tyrosine kinase inhibitor. The results of one such trial were recently reported [25]. In a Phase I study of midostaurin (recently awarded Breakthrough Therapy designation from the FDA for newly diagnosed *FLT3*-mutant AML) in combination with ATRA (15 mg/m² daily on days 7–20) and CLAG (cladribine, cytarabine and G-CSF) in relapsed/refractory AML, patients achieved a CR rate of 22% with an additional 11% of patients achieving CR with partial count recovery, and a median survival of 106 days. The lower than expected response rate was likely attributable to the highly pretreated population. Additionally, a small case report of three patients with concurrent *FLT3/ITD* and *NPM1* mutations treated with ATRA 45 mg/m² daily from days 1–21 of 28-day cycle and sorafenib yielded durable responses [45]. Therefore, optimization of the treatment regimen and identification of the target patient population warrant further study.

Within a smaller subset of AML patients, mutations in *IDH1/2* have been reported in 15% of AML cases [46]. *IDH* is a metabolic enzyme that converts isocitrate to α -ketoglutarate; however, mutations in *IDH1/2* result in aberrant production of the oncometabolite 2-hydroxyglutarate, leading to disruption of the myeloid differentiation pathway [47,48]. DNA and histone hypermethylation are observed, particularly in

genes of the RA pathway [49,50]. The presence of *IDH* mutations has also been shown to confer a poor prognosis, hence the need for more effective therapies. Interestingly, cell lines expressing the mutant form of *IDH1* (R132H) have a gene signature that is enriched in ATRA responsive genes [47]. Indeed, treatment with ATRA *in vitro* resulted in differentiation of *IDH*-mutant cell lines and primary AML blasts, with further *in vivo* evidence of differentiation and decreased tumor burden in mice. Thus, *IDH* status could be predictive of ATRA response in patients, and warrants further investigation. More recently, mutant *IDH1*- and 2-specific small molecule inhibitors have entered Phase I and II clinical trials, with an objective response rate of 41% with AG-221, a first-in-class inhibitor of *IDH2* [51]. In a Phase I study of the *IDH1* inhibitor, AG-120, the drug was well tolerated, and achieved an objective response rate of 36%, with a CR of 18% with durable responses of 5.6 months on average [52]. These early results are encouraging, and support the study of ATRA in combination with *IDH* inhibitors in the future.

Conclusion

The above trials demonstrate that while ATRA may be beneficial in some cases of AML, a specific patient population which is likely to receive a significant benefit has not yet been identified. One limiting factor common to all trials done to date has been the small number of patients included in trials of retinoids. In addition, AML is a very heterogeneous disease in terms of genetic mutations that makes numbers of comparable patients even smaller, and not all patients have genetic sequencing available for analysis. As the field develops a greater understanding of the genetic drivers and passenger mutations of AML, perhaps we can more clearly identify molecular mechanisms of oncogenesis which could be altered with differentiation therapy. Although the response rates were low in most trials, the fact that some patients benefitted from ATRA provides hope that with more study, retinoids could represent a viable, relatively non-toxic component of AML treatment.

Future perspective

One of the most exciting areas in cancer research today is to develop an understanding of how to use mutational analysis of tumors to predict therapeutic treatment options with the highest probability of success. In AML, several driver

and many passenger mutations have been identified, and the goal is to target these mutations as selectively as possible. While not everyone with APL is cured, targeting the differentiation block caused by PML-RAR- α with ATRA and ATO has been quite successful. The mutations which lead to oncogenesis in AML have not been as easily targeted; however, aberrant differentiation is likely still an important mechanism of disease, and targeting this with retinoids remains an attractive option, especially given the relatively low toxicity seen in clinical trials of AML patients. Results from the clinical trials discussed previously indicate that with better patient selection, there may be benefit to inclusion of retinoids in the leukemia treatment paradigm. As DNA/RNA sequencing becomes more cost efficient and widely available, it will be important to have this information for all patients and correlate which genetic mutations seem most responsive to retinoid therapy. The early results in small studies of *NPM1*- and *FLT3*-mutated patients seem particularly promising.

More intelligent design of retinoid compounds may help to improve their efficacy. CYP450 enzymes are highly expressed in the bone marrow microenvironment and have a dramatic effect on local concentrations of retinoids available at the site of disease [53,54]. Modifications of retinoids which lead to evasion of CYP enzymes and thus higher concentrations in the bone marrow are an active area of investigation. A promising compound known as IRX 5183 (also known

as NRX 195183) has been designed which is resistant to degradation by CYP enzymes and is being considered for an upcoming clinical trial in AML patients (Figure 1). There are other retinoid derivatives which have entered preclinical and/or clinical investigation, and some may have the added benefit of bypassing CYP metabolism, leading to greater drug efficacy [55–59]. ATRA is a pan-RAR agonist, activating signaling through RAR- α , β and γ , with varying downstream effects on self-renewal and differentiation [58,60–61]. Studies of more selective RAR- α agonists such as tamibarotene have been undertaken to target the prodifferentiation pathways associated with the receptor, with less off-target effects (Figure 1). The ultimate goal is to move such compounds to human clinical trials. Fortunately, in the next 5–10 years it is likely that factors including tumor genetic changes, patient selection, dosing and compound optimization will be addressed in order to fully realize the benefit of retinoid therapy in AML.

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Papers of special note have been highlighted as:
• of interest; •• of considerable interest

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