Where to Next with Retinoids for Cancer Therapy?

Malcolm A. Smith and Barry Anderson
Pediatric Section, Cancer Therapy Evaluation Program, National Cancer Institute, Bethesda, Maryland 20892

The report by Adamson and colleagues describes a Phase 1 trial in children of 9-cis-RA\(^2\) (1). This report follows by nearly 10 years the report of the pediatric Phase 1 evaluation of ATRA and by more than a decade the initial reports that ATRA induced high remission rates in patients with APL. The authors conclude their report by noting the continuing challenge of identifying the important retinoids and the optimal way to integrate retinoids into multiagent treatment regimens. In addressing this challenge, we first briefly summarize those malignancies for which retinoids have definitively shown therapeutic benefit, and then we discuss clinical research opportunities for retinoids and how these can be prioritized.

Three retinoids have proven oncological utility: ATRA, a naturally occurring retinoid that binds to and activates RAR\(\alpha\), \(\beta\), and \(\gamma\); 13-cis-RA which likely acts as an ATRA prodrug; and bexarotene (Targretin, LGD1069), which selectively binds and activates RXR\(\alpha\), \(\beta\), and \(\gamma\). Best defined among the oncological indications of retinoids is the use of ATRA for APL. The basis for the dramatic efficacy of ATRA against APL is the ability of pharmacological doses of ATRA to overcome the repression of signaling caused by the PML-RAR\(\alpha\) fusion protein at physiological ATRA concentrations. Restoration of signaling leads to differentiation of APL cells and then to postmaturation apoptosis (2). A series of randomized clinical trials have defined the benefit for combining ATRA with chemotherapy during induction therapy and also the utility of using ATRA as maintenance therapy (3–5).

Bexarotene is approved by the FDA for the treatment of cutaneous manifestations of cutaneous T-cell lymphoma in patients whose disease is refractory to at least one prior systemic therapy (6). Consistent with the RXR specificity of bexarotene, ATRA toxicities such as cheilitis and headache appear to be less prominent in patients receiving bexarotene (7, 8).

13-cis-RA improves outcome for children with advanced stage neuroblastoma when given at high doses after intensive consolidation chemotherapy (9). For many years, it has been known that exposure of neuroblastoma cell lines to ATRA, 13-cis-RA, and 9-cis-RA causes decreased tumor cell proliferation and morphological differentiation. A Phase 2 trial of single agent 13-cis-RA demonstrated minimal antitumor response in children with recurrent or progressive neuroblastoma receiving a dose of 100 mg/m\textsuperscript{2}/day (10). However, complete responses were seen among children with residual tumor in the bone marrow who were treated in a Phase 1 trial evaluating higher doses of 13-cis-RA given in two-week pulses (maximum tolerated dose, 160 mg/m\textsuperscript{2}/day; Ref. 11). Subsequently, improved event-free survival for children with advanced stage neuroblastoma was demonstrated in a Phase 3 study of patients randomized after completion of cytotoxic therapy to receive 6 months of maintenance treatment using 13-cis-RA (160 mg/m\textsuperscript{2}/day) on the 2-week pulsed schedule (9). The clinical importance of 13-cis-RA dosage for antitumor effectiveness has been suggested by the lack of 13-cis-RA benefit among children with advanced neuroblastoma randomized to receive a lower daily dose (0.75 mg/kg/day or 30 mg/m\textsuperscript{2}/day) after high-dose chemotherapy and autologous stem cell transplant (12, 13).

What criteria ought to be used in selecting among these and other retinoids for future clinical evaluation? To the extent that the primary goal of treatment is cure and not palliation, an important criterion is the ability of the agent to kill tumor cells or to enhance tumor cell killing when combined with other anticancer drugs. Given this criterion, a group of retinoids of particular interest are the apoptogenic retinoids, as exemplified by fenretinide [\(N\)-(4-hydroxyphenyl) retinamide] and CD437/6-[3-(1-adamantyl)-4-hydroxyphenyl]-2-naphthalene carboxylic acid. Fenretinide at micromolar concentrations is able to induce apoptosis in a number of different types of cancer cells, including neuroblastoma (14, 15), non-small cell lung cancer (16), cutaneous squamous cell carcinoma (17), and others. Although fenretinide appears to be a potent transactivator with RAR\(\gamma\) and a moderate activator with RAR\(\alpha\), but is able to induce apoptosis by mechanisms independent of RAR activation (16, 17).

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CD437, like fenretinide, induces apoptosis in a variety of cancer cell types. CD437 selectively binds to and transactivates RAR\(\beta\), but is able to induce apoptosis by mechanisms independent of RAR transcriptional activation (27, 28). In lung and prostate cancer cell lines, an apoptosis-inducing CD437 ana-
logue induces expression and mitochondrial translocation of transcription factor TR3/Nur77 and induces inner mitochondrial membrane depolarization and mitochondrial release of cytochrome c (29, 30). CD437 can also up-regulate expression of a number of apoptosis-related genes, including Killer/DR5, Bax, and Fas (28, 31, 32). This interesting compound has not been evaluated in the clinical setting.

Concerning the use of retinoids in multiagent combinations, of particular interest is the use of retinoids in combination with HDAC inhibitors. The biological basis for this combination is the ability of non-ligated RARs to recruit HDAC complexes leading to transcriptional repression of target genes (33). For APL, resistance to ATRA can in some cases be overcome by combination therapy using ATRA with a HDAC inhibitor (34, 35). For some non-promyelocytic cases of acute myelogenous leukemia, the poor sensitivity to retinoid-induced differentiation may reflect HDAC-dependent repression of retinoid signaling pathways, and HDAC inhibition can restore ATRA-induced differentiation (36). For neuroblastoma, combination treatment (either *in vitro* or *in vivo*) of neuroblastoma cells with ATRA and a HDAC inhibitor leads to greater antitumor activity than that obtained with either single agent (37, 38). Clinical evaluations of retinoids with HDAC inhibitors have to date been limited to the combination of ATRA with phenylbutyrate (35), but this strategy warrants continued consideration given the availability of new HDAC inhibitors in clinical evaluation [e.g., MS-275, suberoylanilide hydroxamic acid (SAHA), and CI-994].

The effective use of ATRA with conventional cytotoxic agents for APL (see above) provides proof-of-principle for the potential utility of combining retinoids with cytotoxic agents. Combinations of retinoids with conventional cytotoxic agents have shown promise in some preclinical models, including combinations of 9-cis-RA with cisplatin (39) and combinations of fenretinide with either cisplatin or etoposide (15, 40, 41). The supra-additive activity of fenretinide in combination with cytotoxic agents appears to be associated with the ability of fenretinide to induce ROS (15).

Other retinoid combinations of potential utility include retinoids plus receptor tyrosine kinase inhibitors (42) and combinations of fenretinide with modulators of ceramide metabolism (24). The ability of CD437 to up-regulate DR4 and DR5 death receptor expression in some cancer cell lines suggests the possible utility for the combination of CD437 with TRAIL, should these compounds enter clinical evaluation (32).

There are no clear roadmaps for prioritizing which retinoid or combination strategy should be put forward for clinical evaluation. Possible characteristics arguing for prioritization of a retinoid, alone or in combination, include: (a) the biological plausibility for anticancer activity based on retinoid-induced modifications of critical survival and apoptosis signaling pathways for the cancer under study; and (b) the reproducibility of *in vitro* and *in vivo* antitumor activity in multiple cell lines (xenografts) at concentrations of agent(s) that are achievable in patients. As an example of the application of the latter criterion, the data reported by Adamson argue against prioritizing 9-cis-RA for neuroblastoma (1), because the 9-cis-RA serum levels achievable in young children are well below the 1-μM levels associated with growth inhibition in preclinical studies (43). Too often, preclinical models provide insufficient insight into the best use of an agent to achieve clinical benefit. Nonetheless, decisions concerning prioritizing clinical research opportunities must be made based on the best available information, however imperfect that information is. To the extent possible, it will be important to determine whether the signaling pathways identified as important in preclinical models are indeed modulated in the clinical setting. This closing of the loop between preclinical and clinical observations allows confirmation (or refutation) of hypotheses concerning critical factors associated with retinoid antitumor activity, thereby permitting more rational applications of retinoids in oncological practice.

**REFERENCES**


