



Retinoid therapy of high-risk neuroblastoma

C. Patrick Reynolds^{a,*}, Katherine K. Matthay^b, Judith G. Villablanca^a, Barry J. Maurer^a

^a*Division of Hematology-Oncology, Children's Hospital of Los Angeles and The University of Southern California Keck School of Medicine, Los Angeles, CA 90054, USA*

^b*Department of Pediatrics, University of California San Francisco, School of Medicine, San Francisco, CA 94143 USA*

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Abstract

Retinoids are derivatives of vitamin A that include all trans-retinoic acid (ATRA), 13-cis-retinoic acid, (13-cis-RA), and fenretinide (4-HPR). High levels of either ATRA or 13-cis-RA can cause arrest of cell growth and morphological differentiation of human neuroblastoma cell lines, and phase I trials showed that higher and more sustained drug levels were obtained with 13-cis-RA relative to ATRA. A phase III randomized trial showed that high-dose, pulse therapy with 13-cis-RA given after completion of intensive chemoradiotherapy (with or without autologous bone marrow transplantation) significantly improved event-free survival in high-risk neuroblastoma. The cytotoxic retinoid 4-HPR achieved multi-log cell kills in neuroblastoma cell lines resistant to ATRA and 13-cis-RA, and a pediatric phase I trial has shown it to be well tolerated. Cytotoxicity of 4-HPR is mediated at least in part by increasing tumor cell ceramide levels and combining 4-HPR with ceramide modulators increased anti-tumor activity in pre-clinical models. Thus, further clinical trials of 4-HPR in neuroblastoma, and of 4-HPR in combination with ceramide modulators, are warranted.

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1. Introduction

Neuroblastoma has the highest rate of spontaneous regression of any human tumor and even metastatic neuroblastomas have been shown to spontaneously mature to a benign tumor known as ganglioneuroma. These clinical observations have stimulated studies of neuroblastoma differentiation in vitro. A variety of agents have been shown to induce neurite outgrowth of human neuroblastoma cell lines, including retinoic

acid, cyclic AMP elevating agent, and nerve growth factor [1].

2. Retinoic acid

One of the most potent differentiation inducers for human neuroblastoma in vitro is retinoic acid (RA) [2]. Treatment of both *MYCN* gene-amplified and non-amplified human neuroblastoma cell lines with all-trans-retinoic acid (ATRA) caused a marked decrease in *MYCN* RNA expression and arrest of cell proliferation [3,4]. In some cell lines, 10 days of ATRA treatment caused a prolonged growth arrest that persisted for > 60 days after drug removal and the

* Corresponding author. Division of Hematology-Oncology MS 57, Children's Hospital Los Angeles, 4650 Sunset Boulevard, Los Angeles, CA 90054-0700, USA. Tel.: +1-323-669-5646; fax: +1-323-664-9226/9455.

E-mail address: preynolds@chla.usc.edu (C.P. Reynolds).

growth arrest correlated with decreased MYCN protein expression [4]. The few cells that re-proliferate after the sustained growth arrest responded to re-treatment using 10 μM ATRA with decreased MYCN protein expression and arrested cell proliferation [4]. These data with selected neuroblastoma cell lines suggested that pulse therapy with RA might produce a sustained arrest of tumor cell proliferation in a sub-set of neuroblastoma patients.

3. RA receptors

The differentiation and growth arrest of malignant cells produced by RA are likely mediated by one or more of the two families of RA receptors (RARs; RA receptor (RAR) or retinoid X receptor (RXR)) which have been cloned and sequenced: RAR α , β , γ and RXR α , β , γ [5]. All belong to the steroid/thyroid hormone family of transcription factors and possess discrete DNA-binding and RA binding domains. As depicted in Fig. 1, RA binds to the RARs, causing conformational changes that promote binding to specific cis-acting DNA sequences, which regulate transcription of certain target genes [1]. A study of the RAR and RXR families of RARs in neuroblastoma showed that they were expressed in most neuroblastoma cell lines and primary tumors [6]. While RAR β was only expressed in 4 of 14 MYCN amplified cell lines, it could be induced by ATRA in most of these cell lines [6]. There was no correlation seen between resistance to RA and the level of RAR or RXR expression. However, higher expression of RAR β has been associated with good outcome in neuroblastoma and overexpression of that gene by transfection appears to increase the responsiveness of some neuroblastoma cell lines to RA [7]. Thus, while alterations in RAR or RXR do not appear to be a major mechanism by which neuroblastoma achieves RA resistance, higher levels of expression for these receptors may enhance sensitivity to retinoids.

4. 13-cis-RA

The in vitro work with RA prompted investigators to determine if retinoids could be effective against tumors in a clinical setting. In the mid-1980's the only

retinoid available for clinical use was 13-cis-retinoic acid (13-cis-RA), which had been shown to induce differentiation in promyelocytic leukemia and had been used in trials with some objective responses in both promyelocytic leukemia, myelodysplastic syndrome, cutaneous T-cell lymphoma (mycosis fungoides), and advanced squamous carcinoma of the skin [1]. In spite of the minimal anti-tumor activity of 13-cis-RA against established and progressing solid tumors, it was shown to be effective as a single agent in preventing second tumors in patients with head and neck carcinoma and preventing skin cancers in xeroderma pigmentosum patients. In the latter settings it is possible that 13-cis-RA is not just preventing malignant transformation, but may be active against already transformed cells when in a state of minimal disease (prior to clinical presentation) [1].

Anecdotal trials of 13-cis-RA in neuroblastoma showed responses of mass disease and marrow metastases, including a complete response with a 2 year remission in one patient [4]. Although some responses were seen in neuroblastoma patients with progressive disease in a Childrens Cancer Group (CCG) phase II trial of 13-cis-RA, the overall activity of 13-cis-RA against progressive disease was disappointing [8]. It should be pointed out that in the CCG phase II trial, 13-cis-RA was given at a dose of 100 mg/m²/day (the maximally tolerated dose of the drug in adult trials given once a day on a continuous basis). At that dose of 13-cis-RA, subsequent pharmacokinetic studies [9,10] demonstrated that drug levels obtained were below the 5–10 μM levels known to be effective against neuroblastoma in vitro [11]. The major toxicities of 13-cis-RA at the 100 mg/m²/day dose are dryness of skin, dryness of mucous membranes, conjunctivitis, and hypertriglyceridemia.

4.1. High dose, pulse, 13-cis-RA

For 13-cis-RA to be effective in patients it was likely that drug levels known to be effective in vitro against neuroblastoma (5–10 μM) would have to be achieved [11]. The limited experience in children with moderately high doses of 13-cis-RA [4] suggested that the mucocutaneous toxicity could be dose limiting and that the skin dryness and cheilitis were tolerable in the initial 2 weeks of therapy. It had been demonstrated that 10 day exposures to 10 μM RA

Retinoic Acid Receptors Regulate Transcription

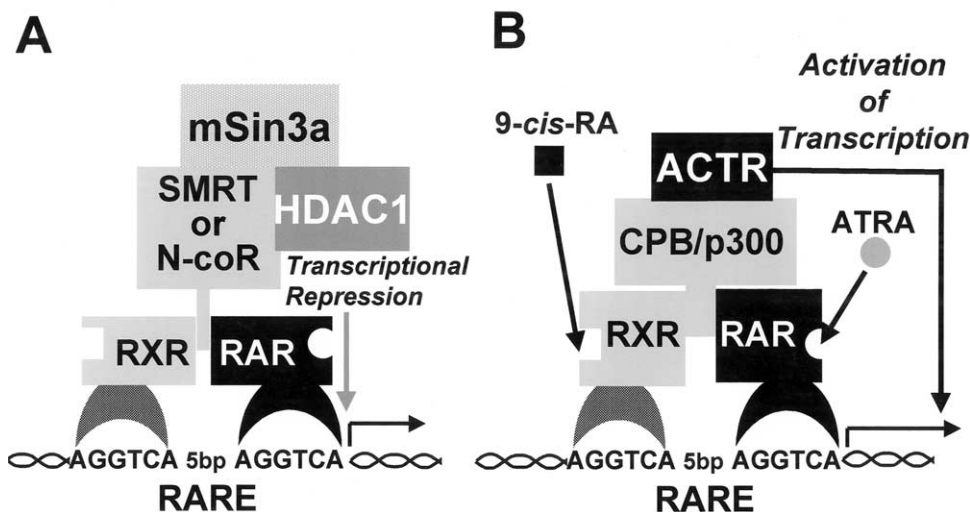


Fig. 1. The mechanism of action of retinoids is mediated via zinc-finger transcriptional regulators which function as heterodimers to regulate promoter activity of certain target genes. The RAR and RXR proteins bind to specific direct repeat DNA sequences (AGGTCA are separated by either 2 or 5 nucleotides) in gene promoters, known as RA response elements. (A) In the absence of ligand, the RAR/RXR heterodimers interact with nuclear co-repressors including N-CoR and SMRT, which in turn bind to a common adapter protein mSin3 that complexes to proteins with histone deacetylase activity to repress transcription. (B) RA binds to the RAR portion of the complex causing a conformational change in the RAR and RXR proteins which releases the co-repressor complex and facilitates binding of 9-cis-RA to the RXR protein (the latter enhances the activation response). The transcriptional co-regulator CBP/p300 then binds to the receptor complex and recruits the coactivator protein ACTR, which contains histone acetyltransferase activity, that promotes transcription [1].

could produce prolonged arrest of neuroblastoma cell proliferation *in vitro* [4], and it was predicted that levels obtainable in patients would be between 5 and 10 μM if therapy was given in 2 week courses alternating with 2 weeks for mucocutaneous recovery. Therefore, model studies were conducted with a human neuroblastoma cell line that showed sustained growth arrest and down-regulation of MYCN expression were achieved *in vitro* with sequential 2 week courses of 5 μM 13-cis-RA [11]. Based on the *in vitro* model studies, a phase I trial was undertaken to dose-escalate 13-cis-RA given in 2 week courses, with the goal being to obtain drug levels $>5 \mu\text{M}$ in patients who had recently completed myeloablative therapy supported by bone marrow transplantation (BMT). Using the intermittent schedule, 13-cis-RA was dose-escalated to a maximally tolerated dose (MTD) of 160 mg/m²/day in post-BMT patients, with the dose-limiting toxicity being hypercalcemia [9]. Peak drug levels at the MTD for 13-cis-RA were 7 μM [9,10], exceeding the target level of 5 μM , and as trough levels were found to be 4 μM , and drug was

given every 12 h, 13-cis-RA levels in patients closely approximated the *in vitro* model system. Complete responses were seen in the phase I trial in four of the ten patients with measurable disease after myeloablative therapy and bone marrow transplantation, and two of those four patients entered a prolonged remission (>2 years) [9]. The latter observation suggested that high-dose, pulse 13-cis-RA might delay or prevent tumor recurrence in high-risk neuroblastoma patients, if given to patients in a setting of minimal residual disease after completion of myeloablative therapy.

4.2. 13-cis-RA vs. ATRA

ATRA was used in treating acute promyelocytic leukemia (APL) with excellent results and little toxicity [12]. Although 13-cis-RA has never been compared directly to ATRA in APL, most investigators felt that ATRA was superior. Studies *in vitro* of APL cells and cell lines showed that ATRA was more effective than 13-cis-RA when tested at equal

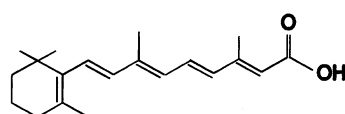
concentrations in the range from 0.1 to 1 μM [13]. Such a comparison was based on clinical trials of ATRA given continuously at the usual 45 $\text{mg}/\text{m}^2/\text{day}$ dose, which showed peak drug levels obtained were in the range of 0.1–1 μM for ATRA [14]. However, drug levels obtained in the post-BMT phase I trial of 13-cis-RA were considerably higher (4–7 μM) [9, 10]. Moreover, ATRA rapidly induces an increase in its own metabolism, such that peak levels and drug half-life significantly decrease after a few days of therapy [14]. Drug levels are further reduced in children, where pseudotumor cerebri was the dose-limiting toxicity for ATRA, limiting the MTD to 60 mg/m^2 [14]. The differences in pharmacokinetic properties of 13-cis-RA and ATRA are summarized in Fig. 2. These differences include higher peak levels and a much longer half life for 13-cis-RA (5 h compared to <45 min for ATRA), and the observation that 13-cis-RA levels remained consistent throughout the course of treatment [10], which is in contrast to the marked decrease of drug levels seen after several days of therapy with ATRA [15].

Because of minimal activity of 13-cis-RA in binding to RA receptors, it had been assumed by some investigators that 13-cis-RA would be less

potent than ATRA. However, studies comparing the activity against neuroblastoma cell lines of 13-cis-RA and ATRA at clinically achievable drug levels demonstrated 13-cis-RA to be superior to ATRA in terms of induction of morphological differentiation and growth arrest, and 13-cis-RA caused down-regulation of MYCN gene expression [11]. These data, and the documentation of anti-neuroblastoma activity for 13-cis-RA in patients [4,9,16], suggest that either 13-cis-RA acts via mechanisms that are independent of RA receptors, or that (more likely) 13-cis-RA serves as a pro-drug for ATRA, resulting in delivery of higher levels of ATRA inside tumor cells then are achievable in vivo with direct ATRA treatment. Given that induction of differentiation and growth arrest in neuroblastoma cell lines by retinoids is dose-dependent, and a sustained effect requires exposure to optimal levels of the retinoid over at least several days, 13-cis-RA may be superior to ATRA for treatment of neuroblastoma [11].

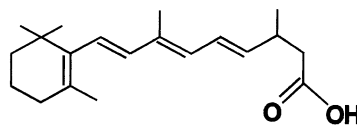
4.3. 13-cis-RA after completion of chemotherapy for high-risk neuroblastoma

CCG-3891 was a phase III trial that was



trans-retinoic acid

- Natural retinoid
- Clinically effective in APL
- MTD = 60 $\text{mg}/\text{m}^2/\text{day}$ (continuous)
- MTD = 90 $\text{mg}/\text{m}^2/\text{day}$ (intermittent)
- Peak levels = 0.62 to 1 μM
- T 1/2 < 1 hour
- AUC $\mu\text{M} \cdot \text{hour}$ = 1.1 to 1.5
- Peak levels and AUC $\downarrow\downarrow$ during treatment



13-cis-retinoic acid

- Synthetic retinoid
- Clinically effective in NBL
- MTD = 100 $\text{mg}/\text{m}^2/\text{day}$ (continuous)
- MTD = 160 $\text{mg}/\text{m}^2/\text{day}$ (intermittent)
- Peak levels = 7.4 μM
- T 1/2 = 5 hours
- AUC $\mu\text{M} \cdot \text{hour}$ = 75
- Peak levels and AUC steady during treatment

Fig. 2. Structures of 13-cis-RA and ATRA and a summary of the pharmacokinetic properties of these two retinoids in pediatric patients [9,10,14, 15].

conducted from January of 1991 to April of 1996 and that entered a total of 560 patients, 539 who were eligible after review [16]. Patients received an induction chemotherapy regimen using cyclophosphamide, doxorubicin, cisplatin, and etoposide, during which marrow harvest and purging, and surgical resection, was accomplished. Patients were initially randomized to either myeloablative therapy employing melphalan, carboplatin, etoposide, and total body irradiation and supported by autologous bone marrow transplantation (ABMT), or to three cycles of intensive non-myeloablative therapy utilizing cisplatin, etoposide, doxorubicin, and ifosfamide/mesna. A second randomization assigned patients who completed either myeloablative or non-myeloablative consolidation therapy to either no further therapy or to receive 13-cis-RA at 160 mg/m²/day (divided and given bid) for 2 weeks each month over a 6 month period. Patients who had documented active tumor by biopsy at the end of consolidation were non-randomly assigned to receive 13-cis-RA.

There were 190 patients randomized to consolidation chemotherapy and 189 to myeloablative therapy/ABMT, and subsequently 130 were randomized to receive 13-cis-RA, while 128 patients were randomized to no further therapy. There were 37 patients non-randomly assigned to 13-cis-RA for proven residual tumor and 24 patients who refused the second randomization, four of whom chose to receive 13-cis-RA. The first randomization showed that ABMT achieved a significantly higher event free survival (EFS) from time of first randomization of $34 \pm 4\%$ compared to $22 \pm 4\%$ for those randomized to consolidation chemotherapy ($P = 0.034$). The 3-year EFS (intent to treat analysis) from the time of second randomization for patients randomized to 13-cis-RA was $46 \pm 6\%$, significantly better than the 3-year EFS of $29 \pm 5\%$ for those randomized to no further therapy ($P = 0.027$). The effect of 13-cis-RA was most pronounced in a setting of truly minimal residual disease, as subset analysis showed the most significant effect for 13-cis-RA in stage IV patients was for those who achieved initial complete remission. The positive benefit of 13-cis-RA for those patients with minimal residual disease was not seen for children who were non-randomly assigned to 13-

cis-RA for histologically proven residual disease, as this latter group showed a 3-year EFS of $12 \pm 6\%$.

Because of the two randomizations in the CCG-3891 study, one can examine the apparent EFS for the four different treatment groups created, although small group size of each of these four treatment groups limits statistical power and the two different randomization time-points precludes a formal analysis. Treatment with 13-cis-RA appeared to be beneficial both for patients who received either ABMT or non-myeloablative chemotherapy. The three-year EFS from time of second randomization in patients undergoing both randomizations was higher for ABMT and 13-cis-RA ($55 \pm 10\%$), compared to ABMT alone ($41 \pm 10\%$; $P = 0.28$). The 3-year EFS for chemotherapy and 13-cis-RA was $33 \pm 7\%$, compared to chemotherapy alone ($19 \pm 7\%$; $P = 0.17$). It is likely that myeloablative therapy was most effective in reducing disease burden prior to 13-cis-RA therapy. The outcome for patients treated with both ABMT and 13-cis-RA, taken together with the poor survival of patients who had documented active disease at the time of beginning 13-cis-RA therapy, emphasizes that the optimal application for 13-cis-RA is in a setting of minimal residual disease [16].

In 1989 the European Neuroblastoma Study Group (ENSG) initiated a randomized trial of 13-cis-RA vs. no further therapy in children with advanced neuroblastoma who achieved remission after high-dose therapy [17]. When this trial was initiated, the dose escalation results with high-dose, pulse 13-cis-RA [9] (see above) had not yet been published, so patients randomized to 13-cis-RA on the ENSG study were given a single daily dose of 0.75 mg/kg (22.5 mg/m²/day continuously for 4 years or until relapse. Approximately 175 children were entered into the study with 88 patients randomized to receive 13-cis-RA (3 year EFS = 37%) and 87 patients randomized to placebo (3 year EFS = 42%). No advantage in EFS was shown in this trial for children randomized to receive low dose, continuous 13-cis-RA. These results contrast with the improved survival seen with high-dose, pulse 13-cis-RA [16] (see above) and indicate the need for utilizing adequate dose levels and optimal dosing schedules to achieve pharmacologically efficacious drug levels when employing retinoids as anti-cancer agents.

5. Fenretinide

A synthetic retinoid made in the late 1960's, N-(4-hydroxyphenyl) retinamide or fenretinide (4-HPR) has been reported to inhibit the growth of neuroblastoma cell lines in vitro with 1–10 μM concentrations in a dose dependent manner [18] and 4-HPR was highly active against retinoic-acid resistant neuroblastoma cell lines at 5–10 μM drug levels [19]. In contrast to 13-cis-RA and ATRA, 4-HPR does not induce maturational changes, but is cytotoxic, causing both apoptosis and necrosis [20]. Toxicity of 4-HPR in chemoprevention clinical trials has been minimal and no hematologic toxicity has been reported, with the major clinical toxicity of 4-HPR being decreased night vision, due to decreased plasma retinol levels [1]. Fig. 3 shows the structure of 4-HPR and summarizes the characteristics of the drug.

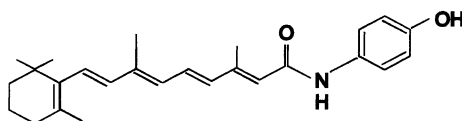
Clinical data indicate that many neuroblastomas are resistant, or develop resistance during therapy, to 13-cis-RA [16]. As 4-HPR has been shown to achieve multi-log cytotoxicity in neuroblastoma cell lines resistant to ATRA and 13-cis-RA [19], if suitable 4-HPR plasma levels can be achieved clinically with tolerable toxicity, 4-HPR could be effective against 13-cis-RA-resistant neuroblastomas. Resistance to

13-cis-RA in neuroblastoma cell lines appears to involve selection for increased expression of MYCN or c-myc, and such RA-resistant neuroblastoma cell lines are collaterally hypersensitive to 4-HPR [19]. Thus, sequential use of 13-cis-RA, followed by 4-HPR, could be an especially effective approach to treating minimal residual disease in neuroblastoma patients after myeloablative therapy.

5.1. Mechanism of action for 4-HPR

4-HPR appears to act by different pathways than those mediated by retinoid receptors. Studies in breast cancer cell lines have demonstrated that 4-HPR has a low binding affinity for nuclear RARs compared with trans-RA [21], and only minimally activates the RAR element and RXR response elements [22]. The mechanism by which 4-HPR achieves anti-tumor cytotoxicity is not completely understood, but current data indicate that more than a single mechanism is involved. In human leukemic cell lines, 4-HPR cytotoxicity may be suppressed by the activation of protein kinase C, and inhibited by antioxidants [23]. Generation of reactive oxygen species in the cytotoxicity achieved by 4-HPR has been implicated in cell lines from leukemia [24], cervical carcinoma [25],

Fenretinide



N-(4-hydroxyphenyl) retinamide = fenretinide (4-HPR)

Characteristics

- Synthetic retinoid
- Active in leukemia, neuroblastoma, and carcinoma cell lines
- Low systemic toxicity
- Chemoprevention clinical trials promising, phase I therapeutic trials achieving higher drug levels

Mechanism of Action

- Cytotoxicity independent of retinoid receptors
- Active against retinoic acid-resistant cell lines
- Generates reactive oxygen species (ROS) and ceramide
- p53-independent cytotoxicity
- Kills by apoptosis and necrosis

Fig. 3. Structure of N-(4-hydroxyphenyl) retinamide or 4-HPR and a summary of the currently known characteristics of the drug.

and neuroblastoma [20]. Cell death still occurs in leukemic cell lines that overexpress bcl-2, however the onset is delayed [23]. Thus, it is likely that 4-HPR effects multiple pathways involved in cell death, and it appears to do this preferentially in malignant cells.

Recent studies have shown that 4-HPR stimulated large increases of ceramide in neuroblastoma cell lines, which is likely one of the mechanisms by which anti-tumor cytotoxicity is achieved with 4-HPR [20]. A dose and time-dependent increase in ceramide was observed in neuroblastoma cell lines [20], including CHLA-90, which is a highly alkylator and etoposide-resistant cell line established after myeloablative therapy [26]. Future clinical trials may employ 4-HPR in combination with agents that modulate ceramide metabolism so as to increase the anti-tumor activity of 4-HPR. An example of the latter approach is to combine 4-HPR with agents that inhibit glucosylceramide synthase/1-O-acylceramide synthase or sphingosine kinase, or use of safinol (L-threo-dihydrosphingosine) [27]. Such agents, which modulate ceramide metabolism and/or action, can significantly increase 4-HPR anti-tumor activity at levels that are minimally toxic to normal fibroblasts or bone marrow myeloid progenitors (CFU-GM) [27].

5.2. 4-HPR clinical trials

Recently, phase I clinical trials of high-dose oral 4-HPR in adult and pediatric solid tumors have been completed. In pediatrics, the MTD of oral 4-HPR given for 7 days, every 3 weeks, was 2475 mg/m²/day, which achieved 4-HPR plasma levels of 6–10 μM with minimal systemic toxicity. [28]. Thus, the phase I trial achieved 4-HPR levels that were highly effective against 13-cis-RA-resistant neuroblastoma cell lines [19]. Due to the poor bioavailability and large capsule size of the current 4-HPR oral formulation (the latter is particularly a problem with young children), an intravenous formulation and a new oral formulation are in development.

6. Conclusions

In vitro studies led to clinical trials that have defined a dose of 13-cis-RA which was tolerable in patients after myeloablative therapy, and a phase III

trial that showed post-consolidation therapy with high-dose, pulse 13-cis-RA improved EFS for patients with high-risk neuroblastoma. However, there are still many high-risk neuroblastoma patients who have tumors that do not respond to 13-cis-RA, even when 13-cis-RA is used in a state of complete response to prior therapy. Given the significant benefit derived from post-myeloablative therapy with 13-cis-RA, it is logical to consider developing additional therapies in the post-myeloablative period which may be effective against tumor cells that persist after myeloablative therapy and 13-cis-RA. One such approach may be to use the cytotoxic retinoid fenretinide, which can achieve multi-log cell kills against neuroblastoma cell lines resistant to 13-cis-RA, especially when combined with modulators of ceramide metabolism. Randomized clinical trials will be needed to determine if new approaches to treating minimal residual disease in high-risk neuroblastoma patients can achieve a higher EFS than does the use of aggressive induction chemotherapy, myeloablative therapy, and subsequent therapy for minimal residual disease with 13-cis-RA.

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References

- [1] C.P. Reynolds, R.S. Lemons, Retinoid therapy of childhood cancer, *Hematol. Oncol. Clin. North. Am.* 15 (2001) 867–910.
- [2] N. Sidell, A. Altman, M.R. Haussler, R.C. Seeger, Effects of retinoic acid (RA) on the growth and phenotypic expression of several human neuroblastoma cell lines, *Exp. Cell Res.* 148 (1983) 21–30.
- [3] C.J. Thiele, C.P. Reynolds, M.A. Israel, Decreased expression of N-myc precedes retinoic acid-induced morphological differentiation of human neuroblastoma, *Nature* 313 (1985) 404–406.
- [4] C.P. Reynolds, D.J. Kane, P.A. Einhorn, K.K. Matthay, V.L. Crouse, J.R. Wilbur, S.B. Shurin, R.C. Seeger, Response of neuroblastoma to retinoic acid in vitro and in vivo, *Prog. Clin. Biol. Res.* 366 (1991) 203–211.
- [5] E. Linney, Retinoic acid receptors: transcription factors

- modulating gene regulation, development, and differentiation, *Curr. Top. Dev. Biol.* 27 (1992) 309–350.
- [6] C. Li, P.A. Einhorn, C.P. Reynolds, Expression of retinoic acid receptors alpha, beta, and gamma in human neuroblastoma cell lines, *Prog. Clin. Biol. Res.* 385 (1994) 221–227.
- [7] B. Cheung, J.E. Hocker, S.A. Smith, M.D. Norris, M. Haber, G.M. Marshall, Favorable prognostic significance of high-level retinoic acid receptor beta expression in neuroblastoma mediated by effects on cell cycle regulation, *Oncogene* 17 (1998) 751–759.
- [8] J.Z. Finklestein, M.D. Krailo, C. Lenarsky, S. Ladisch, G.K. Blair, C.P. Reynolds, A.L. Sitarz, G.D. Hammond, 13-cis-retinoic acid (NSC 122758) in the treatment of children with metastatic neuroblastoma unresponsive to conventional chemotherapy: report from the Childrens Cancer Study Group, *Med. Pediatr. Oncol.* 20 (1992) 307–311.
- [9] J.G. Villablanca, A.A. Khan, V.I. Avramis, R.C. Seeger, K.K. Matthay, N.K. Ramsay, C.P. Reynolds, Phase I trial of 13-cis-retinoic acid in children with neuroblastoma following bone marrow transplantation, *J. Clin. Oncol.* 13 (1995) 894–901.
- [10] A.A. Khan, J.G. Villablanca, C.P. Reynolds, V.I. Avramis, Pharmacokinetic studies of 13-cis-retinoic acid in pediatric patients with neuroblastoma following bone marrow transplantation, *Cancer Chemother. Pharmacol.* 39 (1996) 34–41.
- [11] C.P. Reynolds, P.F. Schindler, D.M. Jones, J.L. Gentile, R.T. Proffitt, P.A. Einhorn, Comparison of 13-cis-retinoic acid to trans-retinoic acid using human neuroblastoma cell lines, *Prog. Clin. Biol. Res.* 385 (1994) 237–244.
- [12] R.P.J. Warrell, S.R. Frankel, W.H.J. Miller, D.A. Scheinberg, L.M. Itri, W.N. Hittelman, R. Vyas, M. Andreeff, A. Tafuri, A. Jakubowski, Differentiation therapy of acute promyelocytic leukemia with tretinoin (all-trans-retinoic acid), *N. Engl. J. Med.* 324 (1991) 1385–1393.
- [13] C. Chomienne, P. Ballerini, N. Balitrand, M.T. Daniel, P. Fenaux, S. Castaigne, L. Degos, All-trans retinoic acid in acute promyelocytic leukemias: II, in vitro studies: structure-function relationship, *Blood* 76 (1990) 1710–1717.
- [14] M.A. Smith, D.R. Parkinson, B.D. Cheson, M.A. Friedman, Retinoids in cancer therapy, *J. Clin. Oncol.* 10 (1992) 839–864.
- [15] M.A. Smith, P.C. Adamson, F.M. Balis, J. Feusner, L. Aronson, R.F. Murphy, M.E. Horowitz, G. Reaman, G.D. Hammond, R.M. Fenton, Phase I and pharmacokinetic evaluation of all-trans-retinoic acid in pediatric patients with cancer [see comments], *J. Clin. Oncol.* 10 (1992) 1666–1673.
- [16] K. Matthay, J.G. Villablanca, R.C. Seeger, D.O. Stram, R. Harris, N.K. Ramsay, P. Swift, H. Shimada, C.T. Black, G.M. Brodeur, R. Gerbing, C.P. Reynolds, Treatment of high risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation, and 13-cis-retinoic acid, *N. Engl. J. Med.* 341 (1999) 1165–1173.
- [17] J.A. Kohler, J. Imeson, C. Ellershaw, S.O. Lie, A randomized trial of 13-cis retinoic acid in children with advanced neuroblastoma after high-dose therapy, *Br. J. Cancer* 83 (2000) 1124–1127.
- [18] M. Ponzoni, P. Bocca, V. Chiesa, A. Decensi, V. Pistoia, L. Raffaghello, C. Rozzo, P.G. Montaldo, Differential effects of N-(4-hydroxyphenyl)retinamide and retinoic acid on neuroblastoma cells: apoptosis versus differentiation, *Cancer Res.* 55 (1995) 853–861.
- [19] C.P. Reynolds, Y. Wang, L.J. Melton, P.A. Einhorn, D.J. Slamon, B.J. Maurer, Retinoic-acid-resistant neuroblastoma cell lines show altered MYC regulation and high sensitivity to fenretinide, *Med. Pediatr. Oncol.* 35 (2000) 597–602.
- [20] B.J. Maurer, L.S. Metelitsa, R.C. Seeger, M.C. Cabot, C.P. Reynolds, N-(4-hydroxyphenyl)retinamide increases ceramide and reactive oxygen species and induces mixed apoptosis/necrosis in neuroblastoma cell lines, *J. Natl. Cancer Inst.* 91 (1999) 1138–1146.
- [21] B.P. Sani, Y.F. Shealy, D.L. Hill, N-(4-hydroxyphenyl)retinamide: interactions with retinoid-binding proteins/receptors, *Carcinogenesis* 16 (1995) 2531–2534.
- [22] M.S. Sheikh, Z.M. Shao, X.S. Li, J.V. Ordonez, B.A. Conley, S. Wu, M.I. Dawson, Q.X. Han, W.R. Chao, T. Quick, N-(4-hydroxyphenyl)retinamide (4-HPR)-mediated biological actions involve retinoid receptor-independent pathways in human breast carcinoma, *Carcinogenesis* 16 (1995) 2477–2486.
- [23] D. Delia, A. Aiello, F. Formelli, E. Fontanella, A. Costa, T. Miyashita, J.C. Reed, M.A. Pierotti, Regulation of apoptosis induced by the retinoid N-(4-hydroxyphenyl) retinamide and effect of deregulated bcl-2, *Blood* 85 (1995) 359–367.
- [24] D. Delia, A. Aiello, L. Meroni, M. Nicolini, J.C. Reed, M.A. Pierotti, Role of antioxidants and intracellular free radicals in retinamide-induced cell death, *Carcinogenesis* 18 (1997) 943–948.
- [25] N. Oridate, S. Suzuki, M. Higuchi, M.F. Mitchell, W.K. Hong, R. Lotan, Involvement of reactive oxygen species in N-(4-hydroxyphenyl)retinamide-induced apoptosis in cervical carcinoma cells, *J. Natl. Cancer Inst.* 89 (1997) 1191–1198.
- [26] N. Keshelava, R.C. Seeger, S. Groshen, C.P. Reynolds, Drug resistance patterns of human neuroblastoma cell lines derived from patients at different phases of therapy, *Cancer Res.* 58 (1998) 5396–5405.
- [27] B.J. Maurer, L. Melton, C. Billups, M.C. Cabot, C.P. Reynolds, Synergistic cytotoxicity in solid tumor cell lines between N-(4-hydroxyphenyl)retinamide and modulators of ceramide metabolism, *J. Natl. Cancer Inst.* 92 (2000) 1897.
- [28] J.G. Villablanca, M.M. Ames, J.M. Reid, P.G. Bagniewski, M. Krailo, C.P. Reynolds, Phase I trial of oral N-(4-hydroxyphenyl)retinamide (4-HPR) in children with resistant/recurrent solid tumors: A Childrens Cancer Group Study (CCG 09709), *Proc. Am. Soc. Clin. Oncol.* 21 (2002) 398a.