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Clinical Cancer Research

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Adaptive Dosing Approaches to the Individualization of 13-*Cis*-Retinoic Acid (Isotretinoin) Treatment for Children with High-Risk Neuroblastoma

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Abstract

Purpose: To investigate the feasibility of adaptive dosing and the impact of pharmacogenetic variation on 13-*cis*-retinoic acid (13-*cis*RA) disposition in high-risk patients with neuroblastoma.

Experimental Design: 13-*cis*RA (160 mg/m^2 or 5.33 mg/kg/d) was administered to 103 patients ages 21 years or less and plasma concentrations of 13-*cis*RA and 4-oxo-13-*cis*RA quantitated on day 14 of treatment. Seventy-one patients were recruited to a dose adjustment group, targeting a 13-*cis*RA C_{max} of 2 μmol/L, with dose increases of 25% to 50% implemented for patients with C_{max} values less than 2 μmol/L. A population pharmacokinetic model was applied and polymorphisms in relevant cytochrome P450 genes analyzed.

Results: 13-*cis*RA C_{max} values ranged from 0.42 to 11.2 μmol/L, with 34 of 103 (33%) patients failing to achieve a C_{max} more than 2 μmol/L. Dose increases carried out in 20 patients in the dose adjustment study group led to concentrations more than 2 μmol/L in 18 patients (90%). Eight of 11 (73%) patients less than 12 kg, receiving a dose of 5.33 mg/kg, failed to achieve a C_{max} of 2 μmol/L or more. Significantly, lower C_{max} values were observed for patients treated with 5.33 mg/kg versus 160 mg/m^2 (1.9 ± 1.2 *vs*. 3.1 ± 2.0 μmol/L; mean ± SD; $P = 0.023$). C_{max} was higher in patients who swallowed 13-*cis*RA capsules as compared with receiving the drug extracted from capsules (4.0 ± 2.2 *vs*. 2.6 ± 1.8 μmol/L; $P = 0.0012$). The target C_{max} was achieved by 93% (25/27) versus 55% (42/76) of patients in these 2 groups, respectively. No clear relationships were found between genetic variants and 13-*cis*RA pharmacokinetic parameters.

Conclusions: Dosing regimen and method of administration have a marked influence on 13-*cis*RA plasma concentrations. Body weight–based dosing should not be implemented for children less than 12 kg and pharmacologic data support higher doses for children unable to swallow 13-*cis*RA capsules. *Clin Cancer Res; 19(2); 469–79. ©2012 AACR.*

Introduction

Despite remarkable improvements in survival rates for childhood cancer over the past several decades, the treatment of children with high-risk neuroblastoma remains a major challenge. The retinoid drug 13-*cis*-retinoic acid (13-*cis*RA; isotretinoin) is now an established component of high-risk neuroblastoma treatment, currently being used as maintenance treatment in conjunction with the antibody therapy in the United States and Europe. The use of 13-*cis*RA in this setting is supported by the publication of favorable long-term follow-up data published from a Children's Cancer Group study (CCG-3891), showing improved survival rates in patients treated with 13-*cis*RA following autologous bone marrow transplantation (1, 2). However, despite its widespread use in neuroblastoma for the past decade, there remain a number of drawbacks to its clinical use.

Previous studies have indicated a significant level of interpatient variation in 13-*cis*RA plasma concentrations following standard dosing regimens, with many patients achieving potentially suboptimal drug exposures (3). In addition, concentrations of the major metabolite 4-oxo-13-*cis*RA were shown to accumulate to exceed those of the parent compound during the 14-day course of treatment in approximately 70% of the patients studied. As 4-oxo-RA metabolites have been shown to be less active than the

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Translational Relevance

Following the publication of encouraging data from clinical trials, the use of isotretinoin [13-*cis*-retinoic acid (13-*cis*RA)] alongside immunotherapy with anti-GD2 is now the established treatment for minimal residual disease in children with high-risk neuroblastoma. However, marked interpatient variability in 13-*cis*RA pharmacokinetics may lead to some children receiving subtherapeutic drug concentrations. In this study, we have shown the feasibility of adaptive 13-*cis*RA dosing, based on individual patient drug exposure, which markedly reduces the variability observed within this patient population. Results strongly indicate that reduced body weight–based dosing should not be implemented for children less than 12 kg and that higher doses may be beneficial for children unable to swallow 13-*cis*RA capsules, where the drug is extracted before administration. These data are significant in that these 2 patient groups represent a total of 74% of the studied patient population. While we strive to develop innovative therapies for children with poor prognosis tumor types such as high-risk neuroblastoma, it is essential that those treatments available are optimally used in all patients. The findings of the current study highlight the challenges faced in treating younger children and the need for appropriate pharmaceutical formulations of medicines for use in all paediatric patient populations.

parent retinoic acid in various tumor cell lines, this level of metabolism *in vivo* could lead to a diminished efficacy of 13-*cis*RA (4, 5). This may be particularly important given that the lowering of retinoid plasma levels due to induced metabolism has been linked with the development of resistance to all-*trans*-retinoic acid in patients with acute promyelocytic leukemia (6, 7).

The metabolism of 13-*cis*RA has previously been characterized *in vitro*, with cytochrome P450 enzymes (CYP) including 2C8, 3A7, 4A11, 1B1, 2B6, and 2C9 responsible for the generation of 13-*cis*RA metabolites including 4-oxo-13-*cis*RA (8, 9). The expression of many CYPs can vary markedly between individuals, potentially impacting on drug disposition and plasma concentrations of 13-*cis*RA observed in patients. While *in vitro* studies have indicated that the presence of CYP2C8.3 or CYP2C8.4 variants are unlikely to explain the high degree of observed interindividual variability in the pharmacokinetics and metabolism of 13-*cis*RA (10), this has not been explored in a clinical setting. In addition, it remains possible that other CYP polymorphisms could have a role to play. As well as CYP-mediated phase I metabolism, phase II glucuronidation of 13-*cis*RA has also recently been characterized, with human UDP-glucuronosyltransferases (UGT) 1A1, 1A3, 1A7, 1A8, and 1A9 shown to represent the major isoforms responsible for glucuronidation of both 13-*cis*RA and 4-oxo-13-*cis*RA *in vitro*, with a possible additional role for UGT2B7 in glucuronidation of the metabolite (11). It is therefore feasible that common polymorphisms reported in UGT genes could also impact on the pharmacokinetics of 13-*cis*RA.

While pharmacogenetic variation in key genes responsible for 13-*cis*RA metabolism may have a role to play in explaining the large interpatient variability in pharmacokinetics, there are also practical concerns regarding the administration of 13-*cis*RA to young patients. Because of the large size and number of 13-*cis*RA capsules required to obtain the specified dose, younger children are physically unable to take the drug unless the capsules are opened and the contents mixed with food before administration. This practice raises concerns regarding the actual dose of drug that these patients are receiving. These difficulties were highlighted in a recent case report, indicating that dose modification would be essential to ensure optimal therapy (12).

The current study was designed to investigate the feasibility of carrying out an adaptive dosing approach to 13-*cis*RA treatment, with dose modifications made following course 1 of treatment for patients achieving C_{max} values below a predefined minimum cutoff point. While the most appropriate therapeutic window for 13-*cis*RA exposure has yet to be established, the current approach was aimed to minimize the more than 10-fold variability in plasma concentrations previously observed with standard dosage regimens. Additional novel data were also generated relating to the pharmacokinetics and pharmacogenetics of 13-*cis*RA in a high-risk neuroblastoma patient population, providing insight into the potential impact of variation in key genes on 13-*cis*RA disposition.

Materials and Methods

Patient eligibility and details

Study protocols were approved by the UK Trent Multicentre Research Ethics Committee and written informed consent was obtained from patients or parents as appropriate. Patients less than 21 years of age who were receiving 13-*cis*RA as part of their standard clinical treatment for high-risk neuroblastoma were eligible to participate. The trial was registered through the appropriate clinical trials registries (ISRCTN37126758; ClinicalTrials.gov identifier: NCT00939965) before patient recruitment. All patients had a central venous catheter in place to allow for pharmacokinetic sampling. Age and weight together with 13-*cis*RA administration details were recorded for each patient. The most recent GFR, ALT, bilirubin, and creatinine measurements before 13-*cis*RA treatment were obtained from the patients' notes, in addition to baseline hemoglobin (Hb), white blood cell (WBC), and platelet counts. Details of concomitant medications being administered before and/or in combination with 13-*cis*RA were recorded.

13-*cis*RA treatment

Treatment with 13-*cis*RA (Roaccutane brand) was initiated between 80 and 120 days postmyeloablative and radiotherapy as part of a protocol for high-risk neuroblastoma. 13-*cis*RA was administered orally at a dose of 160 mg/m^2/d, or 5.33 mg/kg for children less than 12 kg, with each course consisting of 14 days of treatment followed by a 14-day break. A total of 6 courses were planned for all patients, during which toxicity was assessed by the National Cancer Institute Common Terminology Criteria of Adverse Events (CTCAE v3). For patients who were unable to swallow 13-*cis*RA capsules, each capsule was snipped with a pair of scissors and the contents carefully squeezed onto a spoon. Following the opening of all capsules, the extracted drug was mixed with food and ingested or mixed with an appropriate diluent and administered via a nasogastric tube. Patients were not fasted before administration. On each study day, administration of the studied dose of 13-*cis*RA was conducted in hospital and was fully documented by a trained research nurse.

For patients studied in the dose adjustment group, the dose of 13-*cis*RA administered on subsequent study courses was modified on the basis of plasma pharmacokinetics, following analysis of samples obtained on day 14 of the previous course. In the majority of cases, this was the first treatment course of 13-*cis*RA, although some patients were studied on later courses (Table 1). A dose increase of 25% (to 200 mg/m^2/d or 6.66 mg/kg/d if child <12 kg) was made for patients attaining 13-*cis*RA C_{max} values of 1.0 to 2.0 μmol/L and who experienced minimal or no toxicity (≤ CTCAE grade 2). A dose increase of 50% (to 240 mg/m^2/d or 8.0 mg/kg/d if child <12 kg) was implemented for patients attaining 13-*cis*RA C_{max} values less than 1.0 μmol/L and who experienced minimal or no toxicity. The dose was maintained at the standard dose (160 mg/m^2/d or 5.33 mg/kg/d if child <12 kg) for patients attaining 13-*cis*RA C_{max} values 2 μmol/L or more. For those patients where a dose adjustment was implemented, pharmacokinetics and toxicity were again monitored on the following course of treatment. Further dose adjustments were made on subsequent courses as appropriate, depending on 13-*cis*RA C_{max} values achieved at the higher dose, with the aim of achieving concentrations more than 2 μmol/L in all patients. Dose reductions were recommended for patients experiencing specific grade 3 or 4 CTCAE toxicities associated with 13-*cis*RA use as per standard treatment.

Blood sampling and analysis

A single 5 mL blood sample was taken from each patient before the first course of 13-*cis*RA treatment, transferred to an EDTA tube, and stored at −20°C for pharmacogenetic analysis. Blood samples for measurement of concentrations of 13-*cis*RA and metabolites were obtained from a central line before administration and at 1, 2, 4, and 6 hours postadministration. Samples were obtained on day 14 of the study treatment course following administration of the first dose of 13-*cis*RA on the particular study day. For patients who required a 13-*cis*RA dose increase, samples for pharmacokinetic analysis were also obtained as detailed above on day 14 of treatment at the higher dose and on one additional course of treatment at the individualized dose. These additional samples were collected to confirm the

Table 1. Patient characteristics and 13-*cis*RA treatment

Characteristic	No. of patients (%)
Evaluable patients	103
Age, y	
0−1	10 (10)
2−3	37 (36)
4−5	29 (28)
6–10	20 (19)
11+	7 (7)
Sex	
Male	64 (62)
Female	39 (38)
Ethnicity	
White British	83 (80)
White Other	4 (4)
Pakistani	3 (3)
Asian Other	5 (5)
Black Caribbean	1 (1)
Black African	2 (2)
Black Other	2 (2)
Any Mixed Background	2 (2)
Other	1 (1)
BW (kg)	
Median	15.9
Range	7.1–48.9
BSA (m^2)	
Median	0.70
Range	0.35–1.5
13-*cis*RA dose level	
160 mg/m^2 (≥12 kg)	92 (89)
5.33 mg/kg (<12 kg)	11 (11)
Method of 13-*cis*RA administration	
Capsules swallowed	27 (26)
Drug extracted and mixed with food	53 (52)
Drug extracted and administered via NGT	23 (22)
Pharmacokinetic data collected	103 (100)
Course 1	60 (58)
Course 2	38 (37)
Course 3	24 (23)
Course 4	13 (13)
Course 5	3 (3)
Course 6	6 (6)
Pharmacogenetic sample obtained	73 (71)
Dose adjustment group	
Total number	71
Dose increase following 1 course of treatment	13
Further dose increases on additional course(s)	7
25% dose increase	14
>25% dose increase	6

Abbreviations: BW, body weight; BSA, body surface area; NGT, nasogastric tube.

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consistent attainment of C_{max} values more than 2 μmol/L on more than one course of treatment. Blood samples (5 mL) were collected in heparinized tubes and centrifuged at 1,200 × *g* for 10 minutes at 4°C. Plasma was separated and frozen at −20°C before analysis using a high-performance liquid chromatography assay, with a limit of quantitation of 0.02 μg/mL for all retinoids. This analytical assay allowed for individual quantification of 13-*cis*RA and the metabolite 4-oxo-13-*cis*RA as previously described (3). All blood and plasma samples were wrapped in aluminum foil to protect them from light and sample handling was carried out in dim light. The assay was validated for linearity, reproducibility, and stability of the analytes according to standard practice (13).

Pharmacogenetics

DNA was extracted from whole blood using a Qiagen QIAamp DNA Blood Maxi Kit or purified from lymphocytes using a Qiagen QIAamp DNA Mini Kit. All kits were used according to the manufacturer's instructions. DNA was quantified using a NanoDrop ND-1000 UV-Vis Spectrophotometer (Wilmington) and stored at −20°C before pharmacogenetic analysis. Genotyping for CYP2C8*3, CYP2C8*4, CYP3A5*3, CYP3A7*1C, CYP3A7*2, and UGT2B7*2 alleles was conducted with the use of TaqMan probes. For completeness, both single-nucleotide polymorphisms (SNP; K139R and R399K) contributing to the CYP2C8*3 genotype were analyzed and, as expected, were found to be in complete linkage disequilibrium. For the CYP2C8*3 (R139K), CYP2C8*3 (K399R), CYP2C8*4, CYP3A5*3, CYP3A7*2, and UGT2B7*2 alleles, primers and TaqMan probes were designed by Applied Biosystems (TaqMan Assays-by-Design, Applied Biosystems). For the CYP3A7*1C allele, primers and TaqMan probes were custom designed and synthesized by Applied Biosystems. The TA indel variant of UGT1A1 was studied by fragment analysis.

Pharmacokinetics

A population pharmacokinetic model was fitted to all 13-*cis*RA data obtained from the first available course of treatment based on a model previously reported (3). As patients in the current report were studied on day 14 of treatment, as opposed to day 1, the model was modified to allow for nonzero concentrations at the time of 13-*cis*RA administration. In summary, a one-compartment model with modified zero-order absorption and an absorption lag time was used. The model assumes that the appearance of drug in a dose compartment is described by a zero-order process over a fixed duration (D1). Absorption into a central observation compartment was described by a first-order process with rate parameter K_a. Non-zero concentrations at the time of dosing were modeled by a steady-state infusion dose into the observation compartment, ending at time 0, and having an unknown rate. The unknown rate, R2, was modeled. All pharmacokinetic parameters were allowed to vary across the population and, in addition, covariance parameters were included for clearance and volume of distribution; and for K_a, ALAG, and D1. Noncompartmental, trapezoidal estimates of AUC_{0-6h} were determined using Stata/SE (StataCorp. 2009. Stata Statistical Software: Release 11.2: StataCorp LP.). Relationships between covariates including gender, age, weight, body surface area, GFR and baseline ALT, bilirubin and creatinine levels, and 13-*cis*RA pharmacokinetics were assessed by visual examination of plots against empirical Bayes estimates of pharmacokinetic parameters.

Statistical analysis

For the analysis of pharmacogenetic data, overall differences between groups were assessed with the Mann–Whitney and Kruskal–Wallis tests using GraphPad Prism version 5.0 software (GraphPad Software, Inc.). The Mann–Whitney test was used to determine differences between 13-*cis*RA C_{max} values in patients receiving different dosing regimens and methods of drug administration. Analysis of linkage disequilibrium was conducted using Fisher exact test (2-sided) for general contingency tables with SPSS version 15.0 software (SPSS Inc.). Statistical significance was given for $P < 0.05$.

Results

Patient characteristics and treatment

A total of 103 children with high-risk neuroblastoma were recruited over a period of 7.5 years between August 2004 and January 2012. Of these 103 patients, 71 were recruited to the 13-*cis*RA dose adjustment study group. The additional 32 patients were studied on a single cycle of 13-*cis*RA treatment and contributed to the population pharmacokinetic model and pharmacogenetic data analysis. The overall study population had a median age of 4.3 years (range 0.8–20.5) and included 64 male and 39 female patients. Patient characteristics for the 103 evaluable patients are given in Table 1. 13-*cis*RA was extracted from capsules and administered with food in 53 patients and by nasogastric tube in 23 patients. For those patients extracting 13-*cis*RA from capsules and administering the drug with food, yoghurt, ice cream, or milk was used; drug extracted from capsules and administered by nasogastric tube was mixed with olive oil or milk. The remaining 27 patients were able to swallow the 13-*cis*RA capsules.

Pharmacokinetics

The population pharmacokinetic model provided an appropriate fit to the data. Supplementary Fig. S1 shows observed 13-*cis*RA plasma concentrations together with individual predictions from 4 patients chosen to represent the diversity of response. Mean population pharmacokinetic parameters were: apparent clearance 0.24 L/min (14.5 L/h); apparent volume of distribution 63 L; absorption lag time 20 minutes; zero-order duration 62 minutes; absorption rate (K_a) 0.026 L/min, and steady-state infusion rate, R2 0.15 L/min. There was large interindividual variability associated with all of these parameters, in particular the parameters representing the absorption process, as shown in Table 2. Noncompartmental, trapezoidal estimates of AUC_{0-6h} ranged from 1.9 to 33.9 μmol/L.h, with a median

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Table 2. 13-*cis*RA population pharmacokinetic parameters

	Mean	95% bootstrap confidence interval for mean	Coefficient of variation (%)	95% confidence bootstrap interval for CV
CL/F (L/min)	0.24	(0.21–0.27)	45	(34–56)
V/F (L)	63	(51–77)	64	(49–78)
KA (L/min)	0.026	(0.015–0.033)	227	(183–263)
ALAG (min)	20	(13–29)	100	(72–132)
D1 (min)	62	(38–68)	137	(123–176)
R2 (L/min)	0.15	(0.13–0.19)	72	(55–84)

Abbreviations: CL/F, apparent clearance; V/F, apparent volume of distribution; KA, absorption rate; ALAG, absorption lag time; D1, absorption duration; R2, rate of unknown steady-state infusion dose into central compartment, ending at time 0.

value of 9.7 μmol/L.h. Covariates including gender, age, weight, body surface area, GFR and baseline ALT, bilirubin, and creatinine levels were not observed to have a significant effect on 13-*cis*RA pharmacokinetics. In addition, concurrent administration of other medications had no impact on variability in 13-*cis*RA pharmacokinetic parameters. A positive linear relationship was observed between 13-*cis*RA C_{max} and AUC_{0-6h} on study day 14 ($r^2 = 0.8418$), supporting the use of the C_{max} value for individualization of 13-*cis*RA dose (Supplementary Fig. S2).

Oxidative metabolism

Extensive accumulation of 4-oxo-13-*cis*RA occurred in all patients, with peak plasma concentrations higher than those of 13-*cis*RA on day 14 of treatment in 64 of 96 (67%) patients for whom data were available. C_{max} values for the 4-oxo-13-*cis*RA metabolite ranged from 0.48 to 14.3 μmol/L as compared with a concentration range of 0.40 to 11.2 μmol/L for 13-*cis*RA. Comparable 4-oxo-13-*cis*RA levels on day 14 of treatment were observed in subsequent courses where studied. No other retinoic acid metabolites were detected in plasma samples of patients receiving 13-*cis*RA.

Pharmacogenetics

The impact of pharmacogenetic variation on 13-*cis*RA pharmacokinetics was investigated in a total of 73 patients, for whom both pharmacogenetic and pharmacokinetic data were available. Six SNPs were analyzed in 4 genes of putative relevance for 13-*cis*RA disposition. The allele frequencies for CYP2C8*3, CYP2C8*4, CYP3A5*3, CYP3A7*1C, CYP3A7*2, and UGT2B7*2 were 8.9%, 2.8%, 91.8%, 6.2%, 6.2%, and 49.3%, respectively. Four different UGT1A1 promoter $(TA)_n$ genotypes were identified because of the presence of $(TA)_5$, $(TA)_6$, and $(TA)_7$ repeats. Of the 73 samples evaluated, 25 (34%) were homozygous for the 6/6 genotype (UGT1A1*1), 42 (58%) were heterozygous for the 6/7 genotype (UGT1A1*1/*28), and 4 (5%) were homozygous for the 7/7 genotype (UGT1A1*28). The remaining 2 patients were homozygous for the rare 5/5 genotype (UGT1A1*36). The frequencies reported for all polymorphisms were in accordance with those observed previously in Caucasian populations (14–17) and were consistent with Hardy–Weinberg equilibrium. Relationships between day 14 13-*cis*RA AUC_{0-6h}, day 14 4-oxo-13-*cis*RA C_{max}, and ratio of 13-*cis*RA C_{max}/4-oxo-13-*cis*RA C_{max} and the studied genetic variants were investigated. No statistically significant relationships were found between any of the genetic variants and 13-*cis*RA AUC_{0-6h} or ratio of 13-*cis*RA C_{max}/4-oxo-13-*cis*RA C_{max}. Significant differences in day 14 4-oxo-13-*cis*RA C_{max} were observed for the CYP2C8*4 and CYP3A7*1C polymorphisms ($P = 0.037$ and $P = 0.043$, respectively). Relationships between genotype for CYP2C8*3, CYP2C8*4, CYP3A5*3, CYP3A7*1C, CYP3A7*2 and UGT2B7*2, and day 14 4-oxo-13-*cis*RA C_{max} values are shown in Fig. 1.

13-*cis*RA dose adjustment

A total of 71 patients were recruited to the 13-*cis*RA dose adjustment group, with doses of 13-*cis*RA administered on course 2 of treatment based on plasma pharmacokinetics on course 1. Within this group, 13-*cis*RA C_{max} values ranged from 0.42 to 11.2 μmol/L, with a total of 24 of 71 (34%) patients failing to achieve a target C_{max} of 2μmol/L or more on course 1 of treatment. Dose increases and additional pharmacokinetic studies were carried out in 20 of these 24 patients, with no additional pharmacokinetic data obtained for the additional 4 patients due to loss of central line access or disease relapse. A dose increase of 25% was implemented in the 14 patients attaining 13-*cis*RA C_{max} values of 1.0 to 2.0 μmol/L on treatment course 1, with a 50% dose increase implemented in the 6 patients attaining 13-*cis*RA C_{max} values less than 1.0 μmol/L. On course 2, C_{max} values of 2μmol/L or more were achieved in 12 (60%) patients. A further 6 patients (30%) achieved the target C_{max} following further 25% dose increases on course 3 (4 patients) or course 4 (2 patients). The remaining 2 patients did not achieve the target C_{max} despite several dose increases. C_{max} values obtained on course 1 following the protocol-based dose and at the individualized dose are shown in Fig. 2 for all patients where dose adjustments were carried out. Pharmacokinetic samples were obtained on an additional course of treatment at the individualized dose in a total of 12

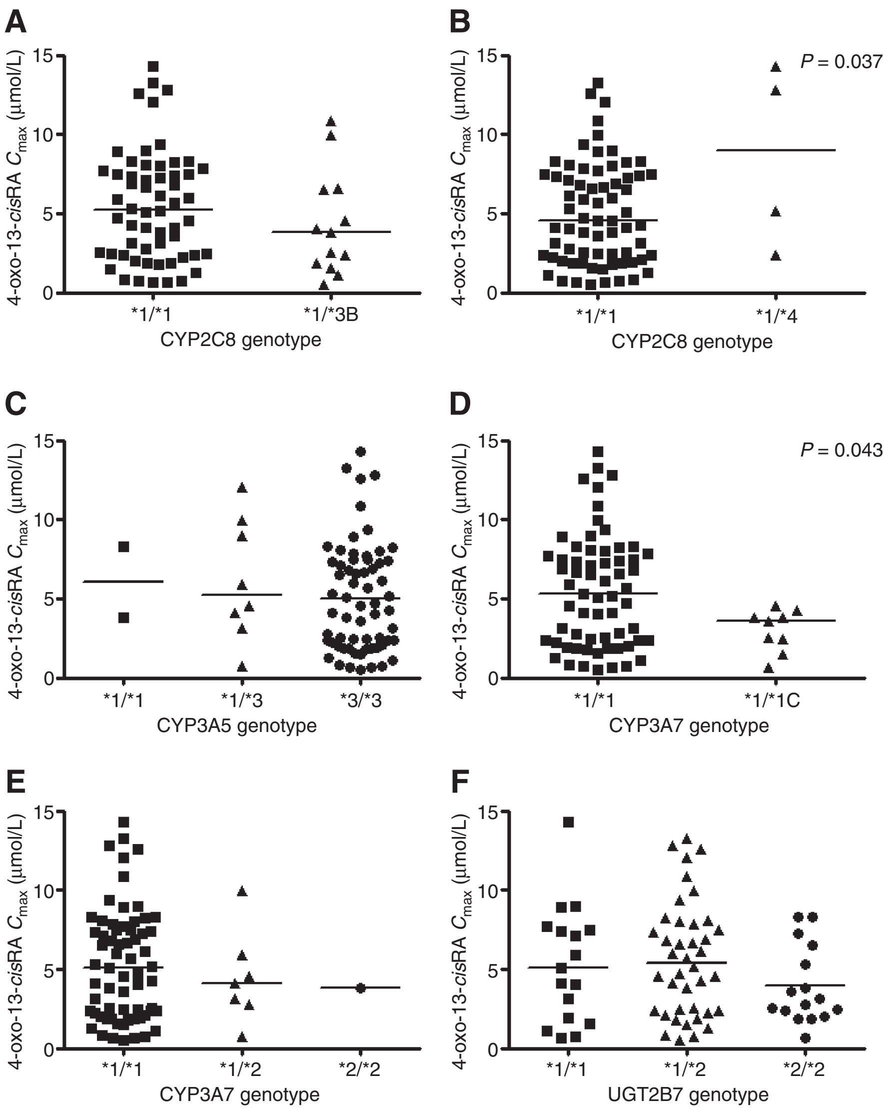

Figure 1. Effect of CYP2C8*3 (A), CYP2C8*4 (B), CYP3A5*3 (C), CYP3A7*1C (D), CYP3A7*2 (E), and UGT2B7*2 (F) genotypes on peak plasma concentrations of 4-oxo-13-*cis*RA on day 14 of treatment with 13-*cis*RA in 73 patients with high-risk neuroblastoma.

patients, to confirm the plasma concentrations achieved at the increased dose. Eleven of 12 patients maintained the C_{max} above 2 µmol/L, with values ranging from 1.75 to 5.94 µmol/L.

Effect of body weight–based 13-*cis*RA dosing in children <12 kg

The overall patient cohort included a total of 11 patients less than 12 kg who received a 13-*cis*RA dose of 5.33 mg/kg, with 8 (73%) of these patients failing to achieve the target C_{max} of 2 µmol/L or more. Six of these 8 patients were studied as part of the dose adjustment cohort and all attained the target C_{max} on course 2 or 3 of treatment at an increased dose. Table 3 shows the initial protocol-based doses and final individualized 13-*cis*RA doses administered to patients less than 12 kg recruited to the dose adjustment study cohort. A dose level of 5.33 mg/kg was equivalent to a daily dose of 100 to 122 mg/m^2 in these younger patients, representing 24% to 38% dose reductions as compared with the standard dose of 160 mg/m^2. After dose adjustment to the target C_{max}, final individualized doses were equivalent to 109 to 167 mg/m^2. A significant difference in C_{max} values of 3.1 ± 2.0 µmol/L versus 1.9 ± 1.2 µmol/L (mean ± SD; $P = 0.0228$) was observed in patients more than or equal to 12 kg receiving a dose of 160 mg/m^2 ($n = 92$) as compared with patients less than 12 kg receiving a dose of 5.33 mg/kg ($n = 11$), respectively (Fig. 3A).

Effect of method of 13-*cis*RA administration

All 14 patients within the dose adjustment study cohort who swallowed 13-*cis*RA capsules achieved the target C_{max}, as compared with 21 of 39 (54%) patients when the drug was extracted and mixed with food or 12 of 18 patients (67%) where the extracted material was administered via

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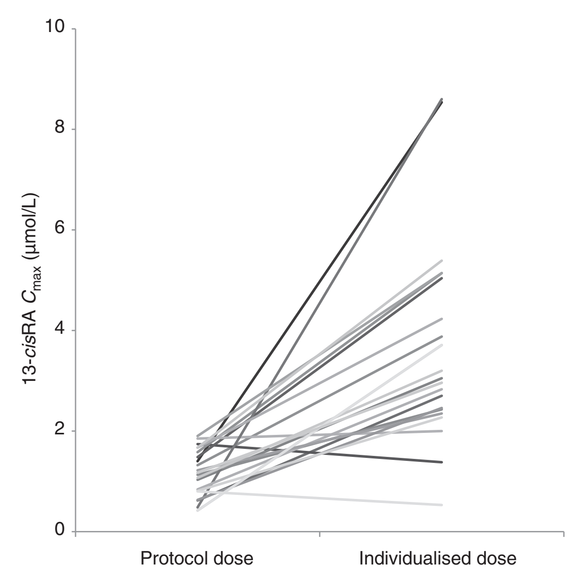

Figure 2. Peak plasma concentrations (C_{max}) of 13-*cis*RA observed with protocol-based dosing and following dose increases to identify an individualized dose for all patients with initial C_{max} values less than 2 μmol/L ($n = 20$).

nasogastric tube. Considering all patients for whom pharmacokinetic data were obtained, the target C_{max} was achieved by 93% (25/27) of patients who swallowed capsules as compared with 55% (42/76) of patients unable to swallow the capsules. A significantly higher C_{max} value of 4.0 ± 2.2 μmol/L was observed in patients who swallowed capsules as compared with 2.6 ± 1.8 μmol/L in patients who required the drug to be extracted before administration (mean ± SD; $P = 0.0012$; Fig. 3B). Comparable results were seen if the dataset was restricted to include only those patients who received a dose of 160 mg/m^2 (C_{max} values of 4.0 ± 2.8 vs. 2.2 ± 1.9 μmol/L in patients who swallowed capsules vs. drug extraction; $P = 0.006$). These data were supported by mean trough plasma levels, determined immediately before the dose administered on day 14 of treatment, which were also higher for patients who swallowed capsules (1.14 μmol/L vs. 0.71 μmol/L). For patients where the drug was extracted before administration, C_{max} values tended to be higher if the drug was administered via nasogastric tube following extraction, as opposed to being mixed with food (C_{max} values of 3.4 ± 2.4 μmol/L and 2.3 ± 1.4 μmol/L, respectively), although this difference did not reach statistical significance. For patients who had the drug mixed with food, no relationships were observed between the type of food used for administration and 13-*cis*RA C_{max}, although numbers of patients were small in some cases. Of interest, one patient who required the drug to be extracted and mixed with food on course 1 but then swallowed the capsules on course 2, achieved a 3-fold higher C_{max} on course 2 of treatment (5.4 vs. 1.7 μmol/L).

13-*cis*RA levels and toxicity

Treatment was reasonably well tolerated, although 25 of 103 (24%) patients had persistent grade 3/4 hematologic toxicity following previous myeloablative therapy. The most common grade 3/4 toxicities experienced on courses where pharmacokinetic studies were carried out were infection in 11 of 103 (11%) patients, elevated ALT in 5 of 103 (5%) patients, and nausea and vomiting in 3 of 103 (3%) patients. Although patients commonly experienced some form of mild skin toxicity, only 5 of 103 (5%) patients experienced CTC grade 3/4 skin toxicity or cheilitis. Importantly, no patients reported grade 3/4 hypercalcaemia, a dose-limiting toxicity previously reported in a phase I study

Table 3. Initial protocol-based doses (5.33 mg/kg) and final individualized doses required to achieve 13-*cis*RA C_{max} values more than 2 μmol/L observed for patients less than 12 kg treated as part of the dose adjustment patient cohort

			Daily dose				C_{max} (μmol/L)	
Patient No.	BW	BSA	Initial (mg)	Initial (mg/m^2)	Individualized (mg)	Individualized (mg/m^2)	Initial dose	Individualized dose
1	11.8	0.56	60	107	100	167	1.1	3.4
2	11.6	0.55	60	109	n/a	n/a	3.2	n/a
8	10.9	0.53	60	113	80	143	1.4	8.5
11	7.6	0.38	40	105	50	119	1.3	3.9
17	8.2	0.4	40	100	70	159	1.1	2.4
23	11.9	0.53	60	113	80	151	1.5	5.0
29	10.9	0.49	60	122	80	163	1.8	2.0
48	10.7	0.51	60	118	n/a	n/a	4.8	n/a
50	10.7	0.52	60	115	n/a	n/a	2.1	n/a

NOTE: No grade 3/4 toxicity was reported in these patients at either the initial or individualized dose levels.
Abbreviations: BW, body weight (kg); BSA, body surface area (m^2); n/a, no adjustment.

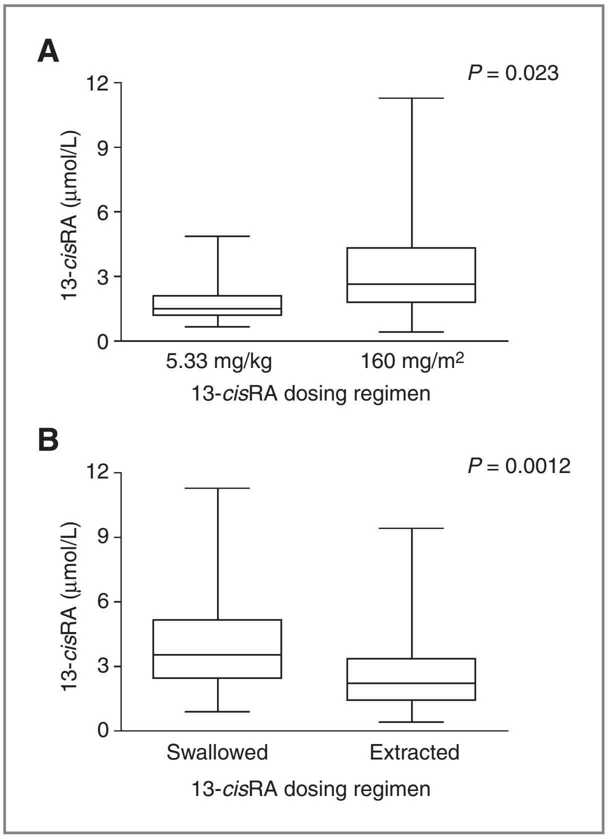

Figure 3. Peak plasma concentrations (C_{max}) of 13-*cis*RA achieved in patients more than 12 kg treated on a 160 mg/m^2 dosing regimen ($n = 92$) as compared with those weighing less than 12 kg treated on a 5.33 mg/kg dosing regimen ($n = 11$) (A) and in patients who swallowed 13-*cis*RA capsules ($n = 27$) as compared with those patients where the drug was extracted from capsules ($n = 76$) (B).

in patients with high-risk neuroblastoma (18). There was no evidence to suggest that any of the toxicities observed were linked to the pharmacokinetics of 13-*cis*RA or its metabolite. In patients studied on the 13-*cis*RA dose adjustment cohort, no relationship was observed between 13-*cis*RA C_{max} or 4-oxo-13-*cis*RA C_{max} and incidence of toxicity at higher individualized 13-*cis*RA doses. However, one patient who received several 13-*cis*RA dose adjustments, with the dose increasing from 160 mg/m^2 to 290 mg/m^2, experienced skin toxicity and behavioral changes which negated further dose increases.

Discussion

Despite the proven clinical benefits of using 13-*cis*RA following high-dose myeloablative chemotherapy for the treatment of high-risk neuroblastoma, a significant number of patients still suffer relapse within 5 years of retinoid treatment (2). While this may be related to factors such as tumor biology in some cases, the previously reported high degree of variability in the pharmacokinetics and metabolism of 13-*cis*RA would suggest that further improvements based on individualization of dosing or schedules may be feasible. There are a number of factors relating to the clinical pharmacology of 13-*cis*RA which, taken together, provide a strong case for the benefit of a therapeutic monitoring approach to ensure that uniform plasma concentrations are achieved in all patients: (i) low dose, continuous use of 13-*cis*RA has previously been shown to provide limited or no clinical benefit in patients with neuroblastoma (19, 20), suggesting that dose intensity and therefore plasma concentrations of drug are important determinants of efficacy; (ii) the current 13-*cis*RA dosing regimen of 160 mg/m^2/d results in marked variation in plasma concentrations between patients, but limited intrapatient variability between treatment courses (3); (iii) as 13-*cis*RA is given as repeated cycles, patients may be exposed to subtherapeutic concentrations of drug for the entire 6-month treatment period. As a clearly defined therapeutic window for 13-*cis*RA exposure has yet to be established, the current approach was designed very much as a feasibility study to minimize the marked variability in plasma concentrations previously observed with standard protocol-based dosage regimens. The minimum C_{max} value of 2 μmol/L being targeted in the current study was supported by published preclinical and clinical data. While it can be difficult to compare between *in vitro* and *in vivo* studies, preclinical studies in neuroblastoma cell lines have shown that 13-*cis*RA concentrations of 2 to 10 μmol/L are required for growth arrest and effects on retinoid biologic response markers (21, 22). A phase I study of 13-*cis*RA in patients with neuroblastoma, which determined the maximum tolerated dose (MTD) of 160 mg/m^2, reported mean serum levels between 4.1 ± 2.7 and 7.2 ± 5.3 μmol/L, with a marked increase in grade 3/4 clinical toxicity observed at concentrations more than 10 μmol/L (23). Targeting peak plasma concentrations between 2 to 10 μmol/L, therefore, would seem an appropriate therapeutic window for 13-*cis*RA in this patient population. However, it should be noted that the current trial represents a feasibility study to reduce the variability in 13-*cis*RA exposure between patients, as opposed to a study designed to define the most appropriate target therapeutic window.

Pharmacokinetic data generated in the current study were analyzed using a modified one-compartment, zero-order absorption model combined with an absorption lag time, a model previously shown to be the most appropriate approach for 13-*cis*RA in a comparable clinical setting (3). The model was further developed to allow for nonzero concentrations at the time of 13-*cis*RA administration on day 14 of treatment, providing a good fit to the data. Population pharmacokinetic model parameters from 103 patients studied were comparable to those generated in the previous study, reporting preliminary results from a limited dataset (3). As reported in this previous study, conventional dosing of 13-*cis*RA at 160 mg/m^2 (5.33 mg/kg in children <12 kg) was associated with significant interpatient variation in 13-*cis*RA pharmacokinetics, with more than 20-fold variability in 13-*cis*RA C_{max} and AUC. As 13-*cis*RA treatment approaches are based on body weight or surface area-based dosing, it is clearly a concern that neither of these covariates were observed to have a significant effect on 13-*cis*RA pharmacokinetics in the current study.

On the basis of findings from both *in vitro* and *in vivo* studies, there is a good rationale for hypothesizing that drug metabolism plays a key role in influencing 13-*cis*RA pharmacokinetics following drug administration. A number of commonly expressed CYP enzymes responsible for the metabolism of 13-*cis*RA have been characterized. The current study investigated the extent of metabolism of 13-*cis*RA in a relatively large pediatric neuroblastoma patient cohort. The potential influence on the pharmacokinetic profile of 13-*cis*RA of common SNPs affecting enzymes responsible for 13-*cis*RA metabolism was explored. Although statistically significant differences in day 14 4-oxo-13-cisRA C_{max} values were observed for CYP2C8*4 and CYP3A7*1C polymorphisms, overall these studies failed to show any clear impact of pharmacogenetics in determining peak plasma concentrations of 13-*cis*RA. Of particular note, functionally relevant SNPs in CYP and UGT enzymes studied did not seem to significantly impact on the ratio of parent drug to metabolite, a parameter which should be unaffected by confounding variables such as 13-*cis*RA dose level and/or method of administration. However, bearing in mind the overall patient numbers and relatively small numbers of patients in certain genotype groups, these findings should be seen as preliminary data which may help to guide future research in this area.

The results obtained in the current study highlight a number of important factors relating to the administration of 13-*cis*RA. These include both the appropriateness of dosing based on body weight for smaller patients as well as problems relating to the lack of availability of an appropriate 13-*cis*RA drug formulation. A 13-*cis*RA dose level of 5.33 mg/kg is currently recommended for children <12 kg, which represents a significant number of patients with neuroblastoma. As compared with a dose level of 160 mg/m^2, a dose of 5.33 mg/kg administered to children less than 12 kg in the current study equated to 13-*cis*RA dose reductions of 24% to 38%. A total of 11 of 103 (11%) patients received this reduced dose level, with C_{max} values below 2 μmol/L observed in 73% of these patients and a significantly lower mean 13-*cis*RA C_{max} achieved relative to children receiving 160 mg/m^2. Dose increases of 25% or 50% implemented in patients receiving an initial dose of 5.33 mg/kg resulted in the achievement of plasma concentrations of more than 2 μmol/L in all cases, with the final individualized doses approximately equivalent to the standard surface area-based dose of 160 mg/m^2. These dose increases were well tolerated in all patients. These data would strongly suggest that a 13-*cis*RA dosage regimen of 5.33 mg/kg should not be implemented for children below 12 kg. These findings have implications beyond the dosing of neuroblastoma patients with 13-*cis*RA, with dosing based on body weight used for the vast majority of anticancer drugs used in paediatric oncology in infants and very young children. In addition to the implicit dose reduction that is often seen when shifting from body surface area to body weight–based dosing, additional dose reductions may also be recommended for patients below specified cutoff points, for example, an age of 6 months or 1 year, or a body weight of 10 or 12 kg (24). Although the implementation of variable cutoff points and dose reductions may be based on sound evidence for certain anticancer drugs, in many cases, the scientific rationale behind the dosing regimens used is limited. The current study data would suggest that further studies are warranted to consider whether more rational approaches to dosing in infant patients should be established for other chemotherapeutics.

Again related to the fact that children diagnosed with high-risk neuroblastoma are commonly aged between 1 and 5 years, the administration of 13-*cis*RA capsules can represent a considerable practical problem. The current study very much highlights this issue, with only 27 of 103 (26%) patients able to swallow the capsules. This cohort included a small number of patients who chewed and swallowed the capsules as opposed to swallowing capsules whole. For the remainder of patients, 13-*cis*RA was extracted from the capsules and either mixed with food or administered via nasogastric tube. Target C_{max} values were achieved by 93% of patients who swallowed capsules as compared with 55% of patients unable to swallow capsules. Bearing in mind the potential loss of drug during handling, it is unsurprising that mean C_{max} values achieved in these patients were lower than in those patients able to swallow the capsules (2.6 ± 1.8 vs. 4.0 ± 2.2 μmol/L; $P = 0.0012$). These data were supported by trough levels determined immediately before the dose administered on day 14 of treatment, which were also higher for patients who swallowed capsules (1.14 vs. 0.71 μmol/L). While these data clearly point to the method of administration as being a major factor influencing 13-*cis*RA plasma concentrations, it should be noted that these patients were generally the younger patients recruited to the study. As such, it can not be excluded that other factors, such as differences in drug absorption, may also have a role to play. It is unclear whether or not this administration problem was an issue for younger children recruited to the phase I study of 13-*cis*RA in patients with neuroblastoma, which reported mean serum levels between 4.1 ± 2.7 and 7.2 ± 5.3 μmol/L at the MTD of 160 mg/m^2 (23). While plasma concentrations observed in the current study are generally in agreement with these previously reported levels, the 13-*cis*RA C_{max} range of 0.4 to 11.2 μmol/L includes a relatively large number of patients who achieved plasma concentrations clearly below the minimum concentrations reported in the phase I trial.

It is also important to consider the potential impact of compliance on the results obtained. When a family is told that their child is not receiving a sufficient dose of drug, it is a natural reaction for the family to make increased efforts to maximize extraction from the capsule. It is therefore almost inevitable that a more thorough and meticulous approach to administering the drug will occur on the following course of treatment at the increased dose level. Indeed, factors relating to drug compliance may go some way to explaining comparatively large increases in 13-*cis*RA peak plasma concentrations observed in some patients following a 25% or 50% dose increase. If this is the case, then it is reassuring that

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once an individualized dose level has been determined for a particular patient, confirmatory plasma levels obtained on an additional course of treatment show that C_{max} values more than 2 μmol/L are being consistently achieved at the increased dose level. On a related note, it is also possible that variability is likely to be higher when 13-*cis*RA is administered to patients at home, as compared with under the supervision of a trained research nurse on a pharmacokinetic study day.

The current study shows the feasibility of 13-*cis*RA dose individualization based on C_{max} values achieved in individual patients, with marked reduction in interpatient pharmacokinetic variability and 13-*cis*RA exposures observed following dose modifications. These data strongly indicate that a standard 13-*cis*RA dosing regimen of 160 mg/m^2 is valid for all patients, with no pharmacologic rational for implementation of reduced dosing in children less than 12 kg. In addition, a 25% dose increase to 200 mg/m^2 is recommended for children more than 12 kg who are unable to swallow 13-*cis*RA capsules, when the drug is extracted from the capsules and mixed with food or administered by nasogastric tube. These amended dosing guidelines are likely to provide a more uniform exposure to 13-*cis*RA across the patient population as a whole, thus allaying concerns of pediatric oncologists that potentially subtherapeutic plasma concentrations may be achieved in some patients due to formulation and compliance issues. While we anticipate that these approaches may benefit patients receiving 13-*cis*RA in the short-term, the findings of the current study emphasize a clear need for the availability of an appropriate oral formulation of this drug to facilitate more accurate dosing in children with high-risk neuroblastoma.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): G.J. Veal, J. Errington, S. Rowbotham, N.A. Illingworth
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): G.J. Veal, S. Rowbotham, N.A. Illingworth, M. Cole, A. Boddy
Writing, review, and/or revision of the manuscript: G.J. Veal, M. Cole, A. K. Daly, A.D.J. Pearson, A. Boddy
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): G.J. Veal, J. Errington, G. Malik
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