Gota V, Chinnaswamy G, Vora T, Rath S, Yadav A, Gurjar M, Veal G, Kurkure P.
Pharmacokinetics and pharmacogenetics of 13-*cis* retinoic acid in Indian high-risk neuroblastoma patients.
***Cancer Chemotherapy and Pharmacology* 2016, 78(4), 763-768.**

Pharmacokinetics and pharmacogenetics of 13-cis retinoic acid in Indian high-risk neuroblastoma patients

Vikram Gota[1], Girish Chinnaswamy[2], Tushar Vora[2], Sanhita Rath[1], Akanksha Yadav[1], Murari Gurjar[1], Gareth Veal[3], Purna Kurkure[2]

[1]Department of Clinical Pharmacology, ACTREC, Tata Memorial Center, Kharghar, Navi Mumbai 410210

[2]Department of Pediatric Oncology, Tata Memorial Hospital, Parel, Mumbai 410210 India

[3] Northern Institute for Cancer Research, Newcastle University, Newcastle upon Tyne, NE2 4HH, UK

Address for correspondence:

Dr. Vikram Gota
Scientific Officer 'F' and Associate Professor
Dept. of Clinical Pharmacology, ACTREC, TMC
Kharghar, Sector '22'
Navi Mumbai – 410210, India.
Telephone: +91-22-2740 5130
Fax: +91 22 2740 5061
Email: vgota76@gmail.com

Funding: The study was funded by the Terry Fox Foundation through the Tata Memorial Centre Intramural Grant (Grant No. 716 dated December 21, 2011)

Abstract

Purpose

To compare the pharmacokinetics of 13-cis retinoic acid (13-cisRA) between Indian and UK neuroblastoma patients receiving comparable treatment, alongside measures of toxicity and response.

Methods

13-cisRA (160mg/m^2/day) was administered to 36 patients ≤16 years in two divided doses. Plasma 13-cisRA concentrations were determined on days 1 and 14 of cycles 1 and 4 of treatment. Area under the plasma concentration-time curve ($AUC_{0\text{-}6h}$) was estimated using non-compartment modeling. Patients were genotyped for UGT2B7, CYP3A5*3, CYP3A7*2 and *2,*3 and *4 variants of CYP2C8.

Results

Marked inter-patient variability in 13-cisRA pharmacokinetics was observed. There was a trend towards a higher $AUC_{0\text{-}6h}$ on day 1 *versus* day 14 for both treatment cycles studied. Children who swallowed 13-cisRA capsules (n=18) achieved higher $AUC_{0\text{-}6h}$ values compared to those who could not (n=16) (Mean AUC 21.53 vs. 9.35 μM.h, $P<0.05$). Patients who were event free at one year tended to have higher $AUC_{0\text{-}6h}$ on C1D1 compared to those patients who progressed, although this did not reach significance with the number of patients studied ($P=0.08$). Similarly, patients who achieved a 13-cisRA C_{max} of ≥2 μM on C1D1 tended to have higher median EFS compared to those who did not (17.0 vs. 8.1 months). UGT2B7, CYP2C8*2/*3/*4 or CYP3A5*3 genotype did not have any effect on 13-cisRA pharmacokinetics.

Conclusions

Method of administration markedly affects 13-cisRA pharmacokinetics in Indian neuroblastoma patients, supporting similar findings in UK patients. An appropriate oral liquid formulation of 13-cisRA that can be administered to all children with neuroblastoma is urgently needed on an international level.

Keywords

13-cis retinoic acid, Neuroblastoma, Pharmacokinetics, Pharmacogenetics, Drug formulation

Introduction

The retinol derivative 13-cis retinoic acid (13-cisRA) is now an established component of the treatment of high-risk neuroblastoma [1]. Low-dose 13-cisRA previously showed limited clinical benefit in patients with recurrent disease. Administration of 13-cisRA at a higher dose of 160 mg/m^2/day and use of an intermittent regimen in a Children's Cancer Group phase III randomized trial showed significant improvement in 3-year event-free survival (EFS) [2]. Thus, dose intensity and corresponding drug exposure in patients treated with 13-cisRA may represent important determinants of drug efficacy in this scenario [3]. There is potential for optimizing retinoid treatment through adaptive dosing approaches in a neuroblastoma clinical setting, owing to the observed large degree of interpatient variability in drug disposition and narrow therapeutic window for toxicity versus efficacy [4, 5].

While 13-cisRA is an established component for treatment of high risk neuroblastoma, its administration in pediatric patients may cause altered or reduced exposure in patients who are unable to swallow the capsules. This is particularly an issue in a disease such as neuroblastoma where the majority of patients being treated are less than 5 years of age. In these patients the contents are routinely extracted and mixed with ingestible food or drink, as previously described in UK patient populations [1, 5].

Retinoids are also known to undergo autoinduction, a phenomenon associated with disease relapse in acute promyelocytic leukemia patients treated with all-trans-retinoic acid (ATRA). Similar autoinduction has also been described for 13-cisRA which might necessitate an adaptive dosing approach in neuroblastoma patients [5, 6]. Drug metabolism studies have shown that 13-cisRA is a substrate for CYP2C8 and UDP glucuronyltransferase and therefore genetic

polymorphisms of genes coding these enzymes may have an effect on the disposition and plasma concentration of 13-cisRA [5, 7, 8].

The current study was designed to evaluate the pharmacokinetics of 13-cisRA, establish its correlation with treatment outcomes and study the impact of certain genetic polymorphisms on 13-cisRA disposition in an Indian patient population.

Materials and Methods

Patients and setting

Study protocols were approved by the Institutional Review Board (IRB) and written informed consent was obtained from patients and their parents / guardians as appropriate. Patients up to 16 years of age who were receiving 13-cisRA as part of standard clinical treatment for high-risk neuroblastoma at our centre were eligible to participate in the study. The standard treatment comprised of induction chemotherapy (cisplatin, carboplatin, vincristine, etoposide and cyclophosphamide) followed by surgery, autologous bone marrow transplantation and radiotherapy, prior to starting 13-cisRA. Demographic data including age, weight and height were recorded for each patient. The baseline Hb, WBC, platelet counts and the most recent ALT, serum transaminases, bilirubin and creatinine were documented prior to 13-cisRA treatment. Details of 13-cisRA administration and concomitant medications being administered prior to and/or in combination with 13-cisRA were recorded.

Patients with progressive disease after consolidation regimen, those not likely to show compliance to the study medication, those who consumed grapefruit or grapefruit products less than one week before sample collection for pharmacokinetic analysis or those receiving any medication which is a known CYP2C8 enzyme inducer or inhibitor (including omeprazole, montelukast, trimethoprim and rifampicin) less than 14 days prior to participation in the study, were excluded from the trial.

Treatment regimen for pediatric patients

13-cisRA (Isotroin 10/20mg soft gel capsules, Cipla, India) was administered orally at a dose of 160 mg/m^2/day in two divided doses for 14 days every 28 days for 6 cycles. For patients who were unable to swallow 13-cisRA capsules, each capsule was punctured and the contents were mixed with water. All patients and/or parents were adequately trained on drug administration, especially those who required the capsules to be punctured and administered. The child's parent/guardian would observe the clinical research coordinator (CRC) prepare the morning dose of 13-cisRA on day 1 of cycle 1. The evening dose would be prepared and administered by the parent/guardian under the CRC's supervision. Subsequent doses were administered at home by the parent/guardian until the next hospital visit for pharmacokinetic sampling. Administration of 13-cisRA on the days of pharmacokinetic sampling was performed in hospital under supervision.

Blood sampling and analysis

Pharmacokinetic blood samples were collected on day 1 and 14 of cycle 1 and 4 at 0, 1, 2, 4 and 6 hours after the morning dose. Blood samples (4 ml) were collected in heparinised tubes and centrifuged at 1,200 g for 10 min at 4°C. Plasma was separated and frozen at -20°C, before analysis using a high performance liquid chromatography (HPLC) assay. All blood and plasma samples were stored protected from light and all sample handling was carried out in dim light. The assay was validated with regard to linearity, reproducibility and stability of the analytes according to bioanalytical method validation guidelines of the FDA [9]. The assay was also cross validated against the method employed at Newcastle University, where several large clinical trials have been conducted and published [1,5], by analyzing three blinded spiked samples in the low, mid and high range of the 13-cisRA linearity curve.

A HPLC system consisting of a Dionex UHPLC Ultimate model 3000 (Dionex, USA) connected to a UV/Visible absorbance detector (Dionex) was used for sample analysis. Chromatographic separations were performed using a reverse-phase HPLC column [C18 (5 μm, 250 × 4.6 mm i.d., Merck, USA)]. 13-cisRA was detected by the UV-vis absorbance at 360 nm. The mobile phase used was composed of 40% acetonitrile, 17% sodium dihydrogen orthophosphate and 43% methanol at a flow rate of 1.7 mL/min. The injection volume was 20 μL and the column was maintained at 40°C.

Toxicity assessment

Toxicity was assessed in all patients who received at least a single dose of the drug. Adverse effects were graded as per the CTCEA version 3.00. All patients underwent routine biochemistry tests and blood count assessment before the start of each cycle. Any lab abnormalities were documented in addition to any side effects or dose interruptions experienced in the previous cycle.

Pharmacogenetics

A 3 ml blood was collected in an EDTA tube at the beginning of treatment for pharmacogenetic analysis. DNA was extracted from leucocytes using a QIAamp DNA Blood Mini Kit (QIAGEN) and quantified using a NanoDrop ND-1000 UV-Vis Spectrophotometer. The frequency of allelic variations for *2,*3 and *4 variants of CYP2C8 were investigated by polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method. After PCR amplification, 5 μl of the diluted PCR product (1:10) was digested with appropriate restriction enzymes before electrophoresis using 3% agarose gel. Gene polymorphisms for UGT2B7, CYP3A5*3 and

CYP3A7*2 were assessed using TaqMan® probes. The primers and TaqMan® probes were designed by Applied Biosystems (TaqMan® Assays-by-Design, Applied Biosystems).

Statistical analysis

Pharmacokinetic parameters including C_{max}, AUC, T_{max} and half-life were analyzed descriptively using WinNonlin version 6.0. 13-cisRA AUC values determined on C1D1 *versus* C1D14, and in patients who swallowed capsules *versus* those patients where capsules were punctured on C1D1, were compared using paired and unpaired t-tests, respectively. The median event free survival (EFS) was estimated from Kaplan-Meier plots and survival was compared using the log-rank test. Linear regression analysis was performed to assess significant covariates which may affect AUC.

Results

Demographic information

Thirty five children with high risk neuroblastoma including 26 males and 9 females, with a median age of 5 years (range 1-13), were enrolled over a four year period between April, 2010 and May, 2014. A summary of the demographic data of all patients studied is shown in Table 1. A flowchart depicting the process of patient screening, enrolment and evaluation is shown in Online Resource 1. Eighteen patients were able to swallow the capsules. For sixteen patients, the capsules had to be punctured and the contents mixed with a teaspoonful of water for administration.

Pharmacokinetics and Toxicity

A marked level of inter-patient variability in 13-cisRA pharmacokinetics was observed (CV, C1D1 $AUC_{0\text{-}6h}$ = 121%). There was a trend towards a higher $AUC_{0\text{-}6h}$ on day 1 as compared to day 14 in both treatment cycles studied. Pharmacokinetic parameters for all patients are shown in Table 2. 13-cisRA clearance (Cl) ranged from 7.68-14.03 L/hr, which is comparable with a mean value of 14.4 L/hr reported by Veal *et al* on day 14 of cycle 1 [5]. The volume of distribution (Vd) ranged from 41.8-174.8 L, comparable on all occasions but C1D14 with that reported by Veal *et al*. A notable feature of pharmacokinetics observed in the Indian cohort of patient is the extremely high coefficient of variation (>100%) for Cl and Vd, particularly in the first cycle of treatment. Children who were able to swallow the 13-cisRA capsules (n=18) achieved higher $AUC_{0\text{-}6h}$ values compared to those who could not (n=16), the difference being statistically significant (21.53±5.05 vs. 9.35±2.78 µM.h; $P<0.05$) (Fig. 1). Additional covariates including

serum albumin, creatinine, weight, gender and age were not found to have any significant effect on exposure to the drug.

Twenty four out of 36 patients (67%) in Cycle 1, reported Grade 1 toxicity such as vomiting (n=2), dryness of skin and mild itching (n=10), chelitis and stomatitis (n=7), and skin lesions (n=7). Severe grade 2 chelitis (n=3) and skin lesions (n=3) were also observed in Cycle 1. In Cycle 4, 16 out of 19 patients (84%) were affected by Grade 1 toxicity such as vomiting (n=1), dryness of skin, peeling and mild itching (n=13), chelitis (n=5) and skin lesions (n=3). Two patients reported Grade 2 toxicity such as severe chelitis and peeling of skin all over the body associated with itching. Only 1 patient reported Grade 3 toxicity of extensive skin lesions with severe itching. It is interesting to note that this patient exhibited the highest AUC and C_{max} on all courses of treatment studied. However, no clear correlation was observed between drug exposure and toxicity.

Pharmacogenetics

Pharmacogenetic associations were evaluated in 22 patients, for whom concomitant pharmacokinetic data were available. Six SNPs were analyzed in four genes of putative relevance for 13-cisRA disposition. None of the patients carried CYP2C8*2/*3/*4 or CYP3A5*3 variants. Eleven out of 22 children were heterozygous for CYP3A7*2 allele. When these data were combined with data for the selected SNPs from 72 patients recruited to the recently published UK study [5], no significant association was found between the various genotypes and C_{max} for C1D14 (Fig. 2).

Survival Data

The median event free survival (EFS) was 8 months. Patients who were event free at one year tended to have a higher 13-cisRA $AUC_{0\text{-}6h}$ on C1D1 compared to those who progressed or died (23.3±8.79 vs. 9.00±3.86μM.h) However, the difference was not statistically significant. Patients who achieved a maximum plasma concentration ≥2 μM on C1D1 had a trend towards a higher median EFS compared to those who did not (16.98 vs 8.05 months). Similarly this difference was not statistically significant(Online Resource 2).

Discussion

Recent data have been published relating to the potential clinical importance of 13-cisRA pharmacokinetic variability in neuroblastoma patients, but only in European and US patient populations [1, 5, 6]. The current study was designed to address how the published data compared to results obtained from an Indian neuroblastoma population. Cross-validation of the assays used to analyse clinical samples in India and the UK was carried out to ensure the integrity of the data obtained. It was observed that the estimates of clearance and volume of distribution were comparable between UK and Indian neuroblastoma populations, except for markedly higher variability observed in Indian patients as compared to the UK cohort. In spite of the marked variability in pharmacokinetic parameters, none of the known covariates affected drug exposure in the linear regression analysis, suggesting that multiple factors govern the disposition of 13-cisRA.

A number of cytochrome P450 (CYP) enzymes have been identified as playing a role in the metabolism of 13-cisRA, with CYP2C8 identified as the most important in terms of activity and level of expression in the liver [7, 8]. In the Caucasian population, the allele frequencies for CYP2C8*3, CYP2C8*4, CYP3A5*3, and UGT2B7*2 were 8.9%, 2.8%, 91.8% and 49.3%, respectively [5, 8]. Genetic polymorphisms for CYP2C8*2/*3/*4 or CYP3A5*3 variants were not detected in this study. 50% of our patient population were heterozygous for CYP3A7*2 allele, as opposed to an allele frequency of 6.2% in the Caucasian population, as reported in the UK study. Although the occurrence of polymorphisms is variable amongst the populations, no significant impact was observed on the metabolism or exposure of 13-cisRA when these data were combined. From the current and published findings, it suggests that genetic variation in the CYP and UGT genes investigated does not play a significant role in influencing the

pharmacokinetics of 13-cisRA in a neuroblastoma patient setting. While a larger study should be carried out, taking into account genetic differences that may be prevalent between Caucasian and Asian populations, the data presented are supportive of previously published findings in this area [5].

Although the current study involves a relatively limited number of patients, it clearly shows that the level of interpatient pharmacokinetic variability previously observed following 13-cisRA treatment in UK and US studies is replicated in an Indian patient population. Incidentally, our findings suggest a potential association between drug exposure and EFS. However, confirmatory studies should be undertaken to validate these findings due to the small number of patients studied.

With a median age of 4 years, route of 13-cisRA dose administration in a neuroblastoma setting poses a serious practical challenge as the majority of patients are unable to swallow the capsules. This results in lower plasma retinoid concentrations, often below the proposed therapeutic threshold of 2 μM [5]. These children are likely to benefit from a higher 13-cisRA dose. The data from Veal *et al* also support similar dose modifications in children who are unable to swallow 13-cisRA capsules [5]. More importantly, it highlights the need for a paediatric friendly liquid formulation of 13-cisRA to achieve optimal levels of the drug in plasma [3]. In a recent review, Bauters et al have discussed the practical implications for the administration of 13-cis retinoic acid in children [10]. The EMEA also acknowledges the need for an appropriate formulation of 13-cisRA that is suitable for children, since the large capsules of 13-cisRA are difficult for small children to swallow [11]. Our data confirm that this is a global problem and not one relevant only to Western patient populations.

In addition, the mean AUC of 13-cisRA was approximately 30% lower on day14 of treatment as opposed to day 1 in both cycles. This difference however was not statistically significant. While this trend might point towards the phenomenon of auto-induction, determination of the 4-oxo-13-cisRA metabolite concentration in patient samples would have been more conclusive. Interestingly, Veal *et al* reported accumulation of the 4-oxo metabolite between days 1 and 14 of treatment but did not observe a corresponding decrease in the plasma concentrations of the parent drug [1]. However, both 13-cisRA and its 4-oxo metabolite are reported to be equipotent at inhibiting human neuroblastoma cell lines *in vitro* [12], and therefore the phenomenon of auto-induction may be less clinically relevant for 13-cisRA.

To conclude, high inter-patient variability was observed in 13-cisRA pharmacokinetics. Further, it could be a candidate for therapeutic drug monitoring practices if the suggested exposure-effect relationship is established in a confirmatory trial. Children who cannot swallow capsules risk reduced drug exposure which could affect treatment outcomes. These data highlight that administration of this drug is a worldwide problem and needs to be addressed. The findings can also be contrasted to a drug such as vincristine, where markedly different pharmacokinetics have been shown between UK Wilms tumour patients and comparable patients studied in Malawi [13]. Clearly therefore it will be challenging to draw conclusions concerning potential differences in the clinical pharmacology of a wide spectrum of different anticancer drugs, between developing countries and the US and Europe, without conducting well designed studies. It is important that such studies include the cross-validation of appropriate assays in order to accurately compare data generated in different laboratories and that collaborative pharmacogenetic studies are carried out to obtain the patient numbers required for the generation of clinically meaningful data.

Acknowledgements

We thank the Terry Fox Foundation for providing financial support through the Tata Memorial Centre intramural grant. The authors are also grateful to UICC for the ICRETT fellowship offered to Dr. Vikram Gota which facilitated his training in HPLC at Northern Institute of Cancer Research. The authors are also thankful to Ms. Julie Errington for imparting this training.

Conflict of interest

Disclosures: None

REFERENCES

1. Veal GJ, Cole M, Errington J, Pearson AD, Foot AB, Whyman G, Boddy AV (2007) Pharmacokinetics and metabolism of 13-cis-retinoic acid (13-cisRA) in children with high-risk neuroblastoma - a study of the United Kingdom Children's Cancer Study Group. Br J Cancer 96(3): 424-31
2. Matthay KK, Villablanca JG, Seeger RC, StramDO, Harris RE, Ramsay NK, Swift P, Shimada H, Black CT, Brodeur GM, Gerbing RB, Reynolds CP (1999) Treatment of high-risk neuroblastoma with intensive chemotherapy, radiotherapy, autologous bone marrow transplantation and 13-cisretinoic acid. New Engl J Med 341: 1165–1173
3. Matthay KK, Reynolds CP (2000) Is there a role for retinoids to treat minimal residual disease in neuroblastoma? Brit J Cancer 83: 1121–1123
4. Veal G, Rowbotham S, Boddy A (2007) Pharmacokinetics and pharmacogenetics of 13-cis-retinoic acid in the treatment of neuroblastoma. Therapie 62(2):91-3.
5. Veal GJ, Errington J, Rowbotham S, Illingworth NA, Malik G, Cole M, Daly AK, Pearson AD, Boddy AV (2013) Adaptive dosing approaches to the individualization of 13-cisRA in treatment for children with high risk neuroblastoma. Clin Cancer Res 19(2): 469-479. doi: 10.1158/1078-0432.CCR-12-2225
6. Khan AA, Villablanca JG, Reynolds CP, Avramis VI (1999) Pharmacokinetic studies of 13-cis-retinoic acid in pediatric patients with neuroblastoma following bone marrow transplantation. Cancer Chemother Pharmacol 39: 34–41
7. Marill J, Capron CC, Idres N, Chabot GG (2002) Human cytochrome P450s involved in the metabolism of 9-cis- and 13-cis-retinoic acids. Biochem Pharmacol 63: 933-43
8. Rowbotham SE, Illingworth NA, Daly AK, Veal GJ, Boddy AV (2010) Role of UDP-glucuronosyltransferase isoforms in 13-cis retinoic acid metabolism in humans. Drug Metab Dispos 38(7):1211-7. doi: 10.1124/dmd.109.031625
9. Guidance for Industry: Bioanalytical Method Validation. www.fda.gov/downloads/Drugs/ GuidanceComplianceRegulatoryInformation/ Guidances/UCM070107.pdf (Accessed Sept 2015)
10. Bauters TG, Laureys G, Van de Velde V, Benoit Y, Robays H (2011) Practical implications for the administration of 13-cis retinoic acid in pediatric oncology. Int J Clin Pharm 33:597-8. doi: 10.1007/s11096-011-9519-9

11. EMEA Evaluation of Medicines for Human Use: Overview of comments received on list of paediatric needs oncology I (cytotoxic therapy). http://www.ema.europa.eu/docs/en_GB/document_library/Other/2009/10/WC500004052.pdf (assessed June 2016)
12. Sonawane P,Cho HE, Tagde A, Verlekar D, Yu AL, Reynolds CP, Kang MH (2014) Metabolic characteristics of 13-cis-retinoic acid (isotretinoin) and anti-tumour activity of the 13-cis-retinoic acid metabolite 4-oxo-13-cis-retinoic acid in neuroblastoma. BJP 171: 5330-44. doi: 10.1111/bph.12846
13. Israels T, Damen CW, Cole M, van Geloven N, Boddy AV, Caron HN, Beijnen JH, Molyneux EM, Veal GJ (2010) Malnourished Malawian patients presenting with large Wilms tumours have a decreased vincristine clearance rate. Eur J Cancer 46:1841-7. doi: 10.1016/j.ejca.2010.03.002

Table 1: Demographic Data

Age (years)	**No. of Patients (%)**
<3	6 (17)
3-5	15 (43)
6-10	10 (29)
11-15	4 (11)
Sex	
Male	26 (74)
Female	9 (26)
Weight (kg)	
Median	14.75
Range	7-47
BSA (m^2)	
Median	0.67
Range	0.47-1.3
Method of 13-cisRA administration:	
Capsules swallowed	18
Drug extracted and mixed with food	16
Pharmacokinetic data collected	
Cycle 1 day 1	33
Cycle 1 day 14	28
Cycle 4 day 11	19
Cycle 4 day 14	18

Table 2: Pharmacokinetic parameters of 13-cis retinoic acid

Parameter	Course 1	N	Mean	Std Dev	Course 4	N	Mean	Std Dev
AUC (µM.h)	1	33	14.9	18.1	1	19	19.7	18.8
	14	28	10.3	12.2	14	18	13.8	13.6
VZ_F (L)	1	33	70.64	85.14	1	19	43.4	2.2
	14	28	174.8	243	14	18	41.8	11.9
T_{max} (hr)	1	33	3.4	2.0	1	19	3.68	1.63
	14	28	3.07	2.17	14	18	3.2	1.6
C_{max} (µM)	1	33	4.57	5.10	1	19	5.9	5.4
	14	28	3.57	4.4	14	18	4.2	4.5
Cl_F (L/hr)	1	33	7.68	7.48	1	19	9.2	11.2
	14	28	11.2	13.04	14	18	14.03	0.52

Abbreviations: AUC, Area under curve; V_d, Volume of distribution; T_{max}, Time to achieve maximum concentration; C_{max}, Maximum concentration; Cl_F, Clearance.

Figure Legends:

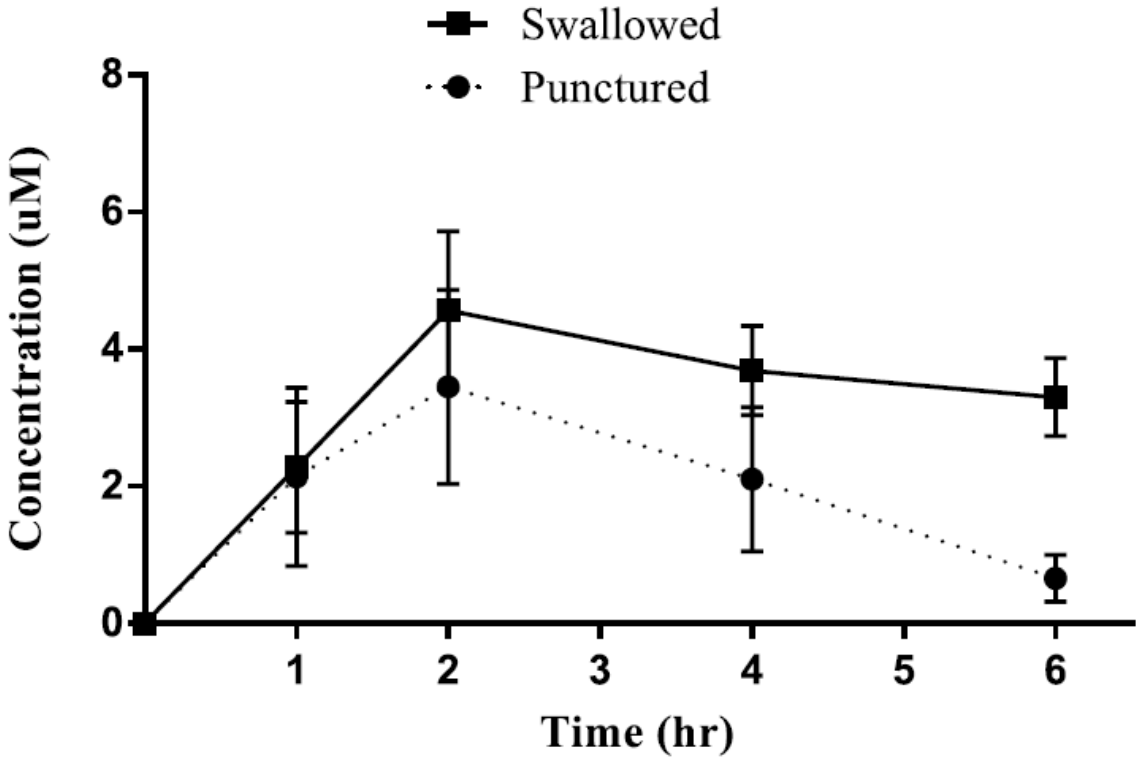
Fig. 1 Plasma concentrations of 13-cisRA observed on day 1 of course 1 of treatment in patients who swallowed the capsules versus those patients for whom the contents were extracted and administered with water. The difference in exposure (AUC) was statistically significant (21.53±5.05 vs. 9.35±2.78 µM.h, $P<0.05$)

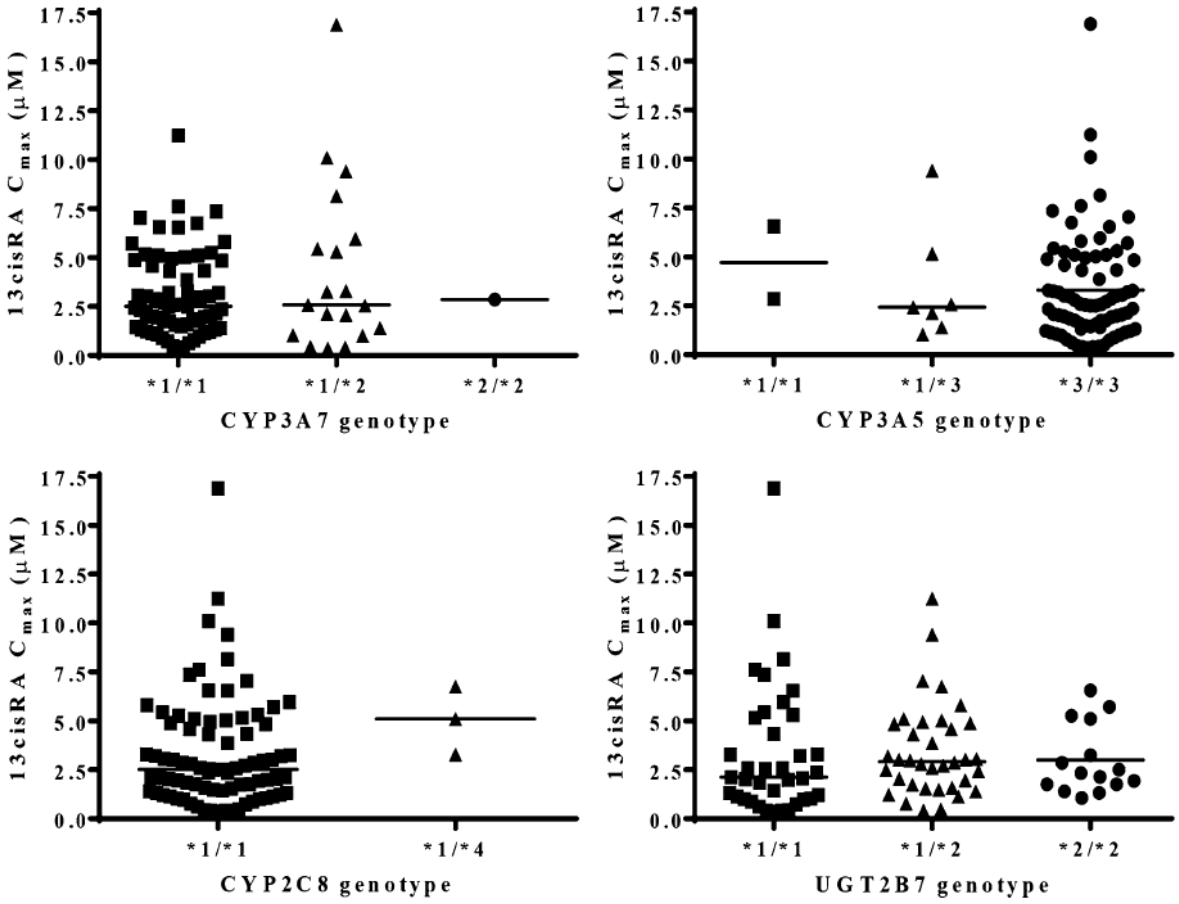
Fig. 2 Combined data from UK and Indian patients showing association between various genotypes and 13-cisRA C_{max} on C1D14. No statistically significant difference was observed between the different genotypes studied (N=94)

Electronic Supplementary Materials

Online Resource 1: Flowchart showing number of patients screened, enrolled and evaluated for pharmacogenetic and pharmacokinetic outcomes

Online Resource 2: Patients who achieved maximum 13-cisRA plasma concentration >2 µMon C1D1 of treatment had higher median EFS compared to those whose C_{max} was <2 µM(16.98 vs 8.05 months, log rank P=0.15)


Swallowed
Punctured
Concentration (uM)
0
2
4
6
8
1
2
3
4
5
6
Time (hr)


13cisRA Cmax (μM)
17.5
15.0
12.5
10.0
7.5
5.0
2.5
0.0
*1/*1
*1/*2
*2/*2
CYP3A7 genotype
*1/*1
*1/*3
*3/*3
CYP3A5 genotype
*1/*1
*1/*4
CYP2C8 genotype
*1/*1
*1/*2
*2/*2
UGT2B7 genotype