CYP3A5 genotype and its impact on vincristine PK and development of neuropathy in Kenyan children with cancer

Jodi L Skiles[1,2], ChienWei Chiang[1], Claire H Li[1,] Steve Martin[1], Ellen L Smith[3], Gilbert Olbara[2], David R Jones[4], Terry A Vik[1], Saskia Mostert[5], Floor Abbink[5], Gertjan J Kaspers[5], Lang Li[1], Festus Njuguna[2,3], Tammy J Sajdyk[1], Jamie L Renbarger[1]

[1] Indiana University School of Medicine, Department of Pediatrics; [2] Moi University College of Health Sciences, School of Medicine, Department of Child Health and Paediatrics, Eldoret, Kenya; [3]University of Michigan School of Nursing; [4]Indiana University School of Medicine, Department of Medicine; [5]Pediatric Oncology-Hematology and Doctor2Doctor program, VU University Medical Center, Amsterdam, Netherlands

Corresponding Author: Jodi Skiles, MD, MS

Department of Pediatrics

705 Riley Hospital Drive, RI 2606

Indianapolis, IN 46202

317-274-8806 (office)

317-278-7856 (fax)

jskiles@iu.edu

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AMPATH	Academic Model for Providing Access To Healthcare
AUC	Area Under the Curve
BSA	Body Surface Area
CV	Coefficient of Variation
DBS	Dried Blood Spot
HPLC-MS/MS	High Performance Liquid Chromatography-Mass Spectroscopy/Mass Spectroscopy
HIV	Human Immunodeficiency Virus
MAPT	Microtubule-associated Protein Tau
MDR1	Multi-Drug Resistance 1
NCI-CTCAE	National Cancer Institute Common Terminology Criteria for Adverse Events
PK	Pharmacokinetics

SNP	Single Nucleotide Polymorphism
TNS©-	Total Neuropathy Score
VCR	Vincristine
VIPN	Vincristine-induced Peripheral Neuropathy

Abbreviations

Abstract

Background: Vincristine is a critical part of treatment in pediatric malignancies and is associated with dose-dependent peripheral neuropathy (VIPN). Our previous findings show vincristine metabolism is regulated by the *CYP3A5* gene. Individuals who are low *CYP3A5* expressers metabolize vincristine slower and experience more severe VIPN as compared to high expressers. Preliminary observations suggest that Caucasians experience more severe VIPN as compared to non-Caucasians.

Procedure: Kenyan children with cancer who were undergoing treatment including vincristine were recruited for a prospective cohort study. Patients received IV vincristine 2mg/m^2/dose with a maximum dose of 2.5mg as part of standard treatment protocols. Vincristine PK sampling was collected via dried blood spot cards and genotyping was conducted for common functional variants in *CYP3A5, MDR1, and MAPT*. VIPN was assessed using five neuropathy tools.

Results: The majority of subjects (91%) were *CYP3A5* high-expresser genotype. *CYP3A5* low-expresser genotype subjects had a significantly higher dose- and BSA-normalized AUC than *CYP3A5* high-expresser genotype subjects (0.28 ±0.15 hr*m^2/L vs. 0.15 ± 0.011 hr*m^2/L, p=0.027). Regardless of which assessment tool was utilized, minimal neuropathy was detected in this cohort. There was no difference in the presence or severity of neuropathy assessed between *CYP3A5* high- and low-expresser genotype groups.

Conclusion: Genetic factors are associated with vincristine PK. Due to the minimal neuropathy observed in this cohort, there was no demonstrable association between genetic factors or vincristine PK with development of VIPN. Further studies are needed to determine the role of genetic factors in optimizing dosing of vincristine for maximal benefit.

Introduction

In resource-limited settings, access to chemotherapeutic agents is confined to a few integral therapies that are available, affordable, and well tolerated. Vincristine is a mainstay of therapy in such settings due to its lack of associated myelosuppression and is utilized in the treatment of over half of all pediatric malignancies; however, it is associated with highly variable and cumulative dose-dependent peripheral neuropathy.[1] Despite its broad use and utility across a variety of environments, little is known regarding vincristine PK and optimal dosing in relation to genetic factors and observed toxicity. Thus, current dosing strategies for vincristine are largely empiric.[2]

Studies previously conducted by our group in the U.S. have shown that patients who are high-expresser genotype for cytochrome P450 (CYP) 3A5 metabolize vincristine more

efficiently.[3] Additionally, African American children are more likely to be *CYP 3A5* high-expresser genotype and are less likely to develop VIPN.[4] Based on these findings, it is reasonable to hypothesize that *CYP3A5* genotype, vincristine PK, and neurotoxicity (VIPN) may substantially different in Africa. The primary aims of this study are to 1) describe the *CYP3A5* genotype and vincristine PK in Kenyan children with cancer, and 2) assess the presence and severity of VIPN in Kenyan children with cancer. The exploratory aims are the investigation of the association of *CYP3A5* genotype and vincristine PK with development of neuropathy in the subset of patients with evaluable VIPN.

Methods

Setting

Kenya is a low-income country in sub-Saharan Africa. This study was carried out at Moi Teaching and Referral Hospital in Western Kenya in collaboration with AMPATH (Academic Model for Providing Access To Healthcare) Oncology Institute. Available treatment options include surgery and chemotherapy. Radiotherapy is not currently locally available.

Study Design

This study was approved by the Moi University Institutional Review Ethics Committee and the Indiana University School of Medicine Institutional Review Board. Patients were recruited prospectively from June 2011 to August 2013 and provided written informed consent and assent (children ≥7 years) for participation in the study. Children aged 1 to 18 years being treated for any type of cancer in which vincristine was part of routine therapy

were eligible to participate. Patients with HIV or underlying baseline neuropathy were excluded. Patients were treated based on standard institutional treatment protocols, in which the vincristine dose is 2mg/m^2/dose with a maximum dose of 2.5mg administered via peripheral venous access by IV push. Subjects were evaluated for development of neuropathy while they were receiving vincristine as part of their routine anti-cancer treatment. All patients were followed longitudinally from the time of enrollment until completion of cancer therapy, death from disease, toxicity, or abandonment of care (defined as failure to sustain treatment during 4 or more successive weeks).[5] Genotyping, limited PK sampling, and detailed neuropathy assessments were conducted on each subject as outlined below.

Vincristine Confirmation: Given that counterfeit pharmaceuticals have been noted to be problematic in Sub-Saharan Africa, the vincristine dispensed at this treatment center was tested for active vinca alkaloid by High Performance Liquid Chromatography-Mass Spectroscopy/Mass Spectroscopy (HPLC-MS/MS). All vials tested had active vincristine present and, on average, had 43.4 ± 15.7% *more* active drug than standard U.S. vincristine. No vials were found to have less vincristine than U.S. standards.

Genotyping: DNA sampling for Single Nucelotide Polymorphism (SNP) analysis was obtained using Oragene saliva kits. DNA was extracted and selectively genotyped using an intermediate throughput OpenArray® genotyping platform. Genotyping for CYP3A5*3, *6, and *7 (the most common functionally significant polymorphisms) was performed using Taqman Real-Time polymerase chain reaction assays.[6] Other SNP analyses were

performed on candidate genes in the vinca alkaloid pathway, including polymorphisms in *MDR1* and *microtubule-associated protein tau (MAPT).*

***Pharmacokinetic Analysis*:** Limited plasma PK sampling was collected on all patients. Up to six samples were collected at the following intervals post-vincristine dose: 30 minutes, 60 minutes, then daily as long as the patient remained in the hospital. Samples were by collected by finger stick onto Whatman Protein Saver human dried blood spot collection paper. Dried blood spot (DBS) cards were stored at room temperature in sealed light-protective bags with desiccant and humidity sensor detector cards until transport back to U.S. for analysis. Stability at room temperature was evaluated and confirmed. Vincristine (VCR) was quantified on Whatman protein saver 903 DBS cards using vinorelbine as the internal standard. DBS samples, n=5 punches of 6 mm diameter, were diluted with water and precipitated with acetonitrile. Chromatographic analysis was performed using an Agilent 1290 series HPLC coupled with a PAL HTC-xt Leap autosampler. All compounds were monitored using an ABSciex 5500 QTRAP triple-quadrupole mass spectrometer equipped with electrospray ionization probe in positive mode. Mass spectrometry settings for the m/z of the parent and daughter ions for VCR and vinorelbine were 413.2/392.2 and 390.1/122.1, respectively. The intra-day and inter-day accuracy and precision (% coefficient of variation, % CV) estimates for VCR at four different concentrations were >80% and <20% respectively. Accuracy and precision were also evaluated at a hematocrit of 30, 45, and 60. VCR accuracy was >80% at all hematocrits tested. The lower limit of quantification for VCR was 0.06 ng/mL. VCR concentration was further normalized by the dose for the follow-up association analysis.

Neurotoxicity Evaluation: The current standard VIPN measurement approach in national cooperative group trials is to utilize the National Cancer Institute Common Terminology Criteria for Adverse Events, version 4.0 (NCI-CTCAE). However, this tool lacks sensitivity to detect subtle subclinical peripheral neuropathy due to its coarse scoring criteria.[7] Therefore, VIPN phenotype was assessed at the time of each vincristine administration using five different neuropathy assessment tools including NCI-CTCAE, Balis Pediatric Scale of Peripheral Neuropathy, Faces Pain Scale, Pediatric Neuropathic Pain Scale, and Total Neuropathy Score (TNS©). All instruments are available as appendices and the most sensitive tool, the TNS©, is summarized in Table 2.[8] Because the TNS was the most sensitive tool for detecting neuropathy, this tool was utilized for statistical analysis when comparing *CYP3A5* high- and low-expressers. Each instrument was back- and forward-translated into Swahili. Serial assessments were conducted while patients were receiving vincristine.

Population Pharmacokinetics (PK) Modeling and Statistical Data Analysis:

The dose and BSA normalized vincristine was fitted into a one-compartment model using NONMEM. The associations between the clearance and demographic and genetic variables were initially tested using either first order or conditional first order approximation algorithms in the NONMEM population PK modeling, but they failed to converge. We decide to use a two-step strategy instead. At step one, a population one-compartment PK model was fitted to the data with only subject specific clearance parameter. Then these subject specific PK clearances were transformed into subject specific AUCs, before the second step association analysis was performed between dose normalized AUC for vincristine and the demographic variables (age, gender, height, weight, and BSA) and genetic variables (*CYP3A5, MDR1*, and *MAPT)*. The second step analysis was analyzed in linear regression using R package

(lm). SNPs in *CYP3A5, MDR1*, and *MAPT* were coded by dominant, recessive, and gene dose models. Haplotypes were estimated by the R package, haplo.stats. Maximum NCI-CTCAE scale score and maximum TNS© score during treatment were used as the neuropathy phenotype. All the non-assessable evaluations were discarded. The associations between neuropathy score and genetics and demographic variables are analyzed similarly using R package lm. Means and standard deviations are reported for the continuous variables, and frequencies are reported for the categorical variables. The association between the genetics and time to the first TNS score $\geq$ 2 is analyzed using the Cox proportional hazard model. In this analysis, patients who abandoned treatment or died prior to completion of therapy are considered lost to follow-up. In addition, we also compared maximum TNS scores between two groups of patients: patients who completed treatment and patients who were lost to follow-up.

Results

Seventy-eight patients were recruited and all subjects enrolled in the study. Nine patients (11.5%) abandoned treatment prior to completion of therapy. Demographic characteristics are summarized in Table 1. The ethnic background for patients in this study was reflective of the normal population of patients treated at AMPATH Oncology Institute.[9,10]

Genotyping: Seventy-one of the 78 subjects (91%) are *CYP3A5* high-expresser genotype (homozygous or heterozygous for the *1 allele, which are phenotypically identical). There were no statistically significant differences in *CYP3A5, MDR1* or *MAPT* genotypes across the demographic variables in this cohort.

Vincristine Pharmacokinetics: Plasma vincristine concentrations obtained from dried blood spots demonstrated that *CYP3A5* low-expresser genotype subjects had a significantly higher dose and BSA normalized AUC than *CYP3A5* high-expresser genotype subjects (0.28 ±0.15 hr*m^2/L vs. 0.15 ± 0.011 hr*m^2/L, p=0.027, Figure 1). The average number of vincristine measurements among 77 patients is 3.4 with a standard deviation of 1.0.

Vincristine-Induced Peripheral Neuropathy: A cohort of 78 subjects completed a median of 10.2 weeks of vincristine-containing therapy. They were followed prospectively for a median time of 6.5 weeks from the time of study enrollment and received a median cumulative vincristine dose of 8.5mg/m^2. A total of 1,166 complete neuropathy assessments were conducted and the median score of all assessments was 0. Using the NCI-CTCAE scale for neuropathies (motor, sensory, and autonomic), 57 of 72 evaluable subjects (79.2%) experienced no detectable neuropathy and only 2 of 72 subjects (2.8%) experienced Grade 2 motor neuropathy. No patients experienced Grade 2 sensory neuropathy, $\geq$ Grade 3 motor or sensory neuropathy. Using the much more sensitive TNS©, only 46 subjects were able to be evaluated due to developmental inability of younger children to complete the full assessment (118 neuropathy assessments on 36 subjects were excluded). Of the 46 evaluable subjects, 13 (28.3%) experienced no detectable neuropathy and only 2 of 46 subjects (4.3%) experienced clinically significant neuropathy[11] with a total neuropathy score of ≥ 5 (scale range 0-28, max score in any subject = 8). Further neuropathy assessments were conducted using the Balis scale and Pediatric Neuropathic Pain Scale. Cumulative results are summarized in Table 3. Regardless of which assessment tool was used, very minimal neuropathy was detected in this cohort. There was no difference in the presence or severity of neuropathy assessed via TNS between *CYP3A5* high- and low-expresser

genotype groups, as well as between *MDR1* and *MAPT* haplotypes. The *CYP3A5* genotype is not associated with the time to the first TNS score $\geq$ 2 ($p = 0.73$). Likewise, there was no statistically significant association between vincristine AUC and development of neuropathy. No subjects required vincristine dose reduction for the development of VIPN. There was no statistically significant difference in presence or severity of neuropathy between subjects who completed treatment and patients who were lost to follow-up ($p = 0.56$).

Discussion

Our results demonstrate that the majority of Kenyan children (91%) have a *CYP3A5* high-expresser genotype and that those with *CYP3A5* high-expresser genotype have 58% less vincristine exposure (dose- and BSA-normalized vincristine AUC) compared to Kenyan children with *CYP3A5* low-expresser genotype. Furthermore, Kenyan children experience negligible clinically significant VIPN[11] compared to U.S. children despite receiving *at least* 33% more vincristine at baseline due to protocol-based dosing differences between the U.S. and European-derived protocols utilized in Kenya.[4,12] Interestingly, the genotype and toxicity phenotype findings in this cohort of Kenyan children are drastically different than a cohort of n=148 U.S. (primarily Caucasian) children with ALL. In our Kenyan cohort of predominantly *CYP3A5* high-expresser genotype, only 4.3% developed clinically significant neuropathy as defined by a TNS score ≥5. By contrast, the U.S. cohort in which only 14% were *CYP3A5* high-expresser genotype, 64% developed clinically significant neuropathy using the same assessment tool (data not yet published; personal communication with Dr. Jamie Renbarger).

After ruling out counterfeit vincristine as a possible explanation for the negligible VIPN observed in this cohort, we hypothesize that the minimal toxicity observed in Kenyan children is due in part to lower vincristine exposure (dose-normalized and BSA-normalized vincristine AUC) compared to U.S. children. There are two significant challenges in optimizing vincristine exposure in children and adults: 1) there is currently no consensus on what constitutes an appropriate therapeutic vincristine AUC, and 2) there are significant differences in the methodology of vincristine PK measurement. To this end, it is really only currently possible to compare vincristine dose- and BSA-normalized AUCs within a given cohort of subjects. Because PK specimens in this study were collected by dried blood spot samples for feasibility in this low-resource setting, it is not possible to directly compare vincristine dose-normalized AUC from Kenyan subjects to that of U.S. subjects whose PK specimens were obtained from plasma samples. One potential reason for the suspected differential vincristine exposure is increased expression of the drug-metabolizing enzyme CYP3A5, resulting in significantly lower dose- and BSA-normalized vincristine AUC than is observed in subjects with *CYP3A5* low-expresser genotype. Despite this finding, there was no demonstrated association between vincristine exposure and the presence or severity of VIPN within our cohort of Kenyan children. This finding is most likely secondary to the very low incidence of clinically significant VIPN in this population, making a strong association between genotype and toxicity phenotype difficult to demonstrate despite the use of the extensively validated and exceptionally sensitive Total Neuropathy Score (TNS©).[7,8,11,13-20]

Previous studies evaluating genotype and toxicity phenotype have documented conflicting results. Egbelakin et al demonstrated that *CYP3A5* genotype is associated with vincristine exposure and toxicity phenotype in a cohort of U.S. (primarily Caucasian) subjects.[4] Several other publications have reported no association between *CYP3A5*

genotype and development of VIPN;[21,22] however, the assessment tools utilized for evaluating neuropathy in those studies (NCI CTCAE and the Movement Assessment Battery for Children) were suboptimal. The NCI CTCAE has been demonstrated to have a significant floor-effect that lacks adequate sensitivity to detect slight changes in neuropathy.[7] The Movement Assessment Battery for Children assesses motor function only, which is likely confounded by steroid myopathy in children being treated for leukemia. Ultimately, although we hypothesize that *CYP3A5* genotype and toxicity phenotype are linked, it is possible that there are also other genetic factors or pharmacokinetic factors which have not yet been elucidated that may make Kenyan children less susceptible to development of VIPN. Despite evaluating for associations between *MAPT* and *MDR1* genotype and vincristine exposure, no statistically significant associations were apparent in this cohort.

Interestingly, a recently published genome-wide association study of n=222 U.S. subjects demonstrated that a SNP in the promoter region of *CEP72*, which encodes a protein involved in microtubule formation, had a significant association with development of vincristine neuropathy. Furthermore, the frequency of the *CEP72* risk allele (T) differed by ancestry with a lower frequency in patients with African ancestry. [23] A genome-wide association study is being planned in this cohort of patients to further evaluate possible genetic variants, including *CEP72* that may contribute to the lack of neuropathy observed in Kenyan children.

Vincristine is one of the core chemotherapeutic agents in the treatment of over half of all treatment regimens for both adults and children. On one hand, it is exceptional to have identified a population of children who may not be as susceptible to development of neuropathy and who, therefore, experience less treatment-related morbidity. On the other hand, the question remains what effect, if any, this finding has on disease response and

treatment outcomes. One recently published study in a U.S. cohort demonstrated that faster vincristine metabolism was associated with a five times increased risk of disease relapse.[24] Because the children enrolled in this study have only recently completed treatment, it is too early to determine whether *CYP3A5* genotype has an impact on disease outcomes in this cohort. Disease response to treatment, event-free survival, and overall survival outcomes in this cohort of Kenyan children will be evaluated for an association with *CYP3A5* genotype when all subjects have been off-treatment for a minimum of 1 year. Despite this, the significantly lower vincristine dose normalized AUC of Kenyan children who are *CYP3A5* high-expresser genotype leads us to hypothesize that the majority of Kenyan children may be receiving subtherapeutic vincristine dosing and could possibly tolerate and potentially benefit from vincristine dose escalation to achieve more optimal vincristine exposure.

Kenya, like many low-income countries, lacks the resources and infrastructure to provide the basic supportive care measures that are inherent in most high-income settings.[25-27] Consequently, complications of myelosuppression are a major cause of treatment-related morbidity and mortality in low and middle-income countries.[25,28] Because of this, several international cooperative oncology groups are now advocating for utilization of reduced intensity chemotherapy protocols in an effort to avoid unacceptable treatment-related morbidity and mortality.[25-30] Since vincristine is one of the few chemotherapeutic agents that does not cause myelosuppression, it is possible that carefully monitored dose escalation of vincristine may allow for safer de-escalation of other myelosuppressive therapies and improved disease outcomes for this vulnerable population of children with limited treatment options.

Findings of this study must be interpreted with caution due to the small sample size of this cohort with a lack of significant variation in the studied genotype. Additionally, in this

study, vincristine was administered by peripheral intravenous access, thereby introducing the possibility of extravasation of vincristine resulting in skewed pharmacokinetic data. When extravasation was clinically suspected (pain at infusion site, tissue necrosis following infusion), pharmacokinetic samples were repeated with the next administered vincristine dose. Furthermore, patients in this study were enrolled at any point in their therapy and followed longitudinally as long as the patient was available for follow-up. Because many children in Kenya die of treatment-related complications early in therapy or abandon care secondary to financial constraints, conducting ongoing longitudinal assessments is difficult in this setting, which is a large contributor to the low average number of neuropathy assessments per child. Since VIPN is believed to be a cumulative dose-dependent phenomenon, having children enrolled at any point in therapy allows for neuropathy evaluation across a variety of different time points in treatment, thereby increasing the likelihood of detecting neuropathy if it, in fact, exists. Despite this approach, negligible neuropathy was observed even at later time points in treatment when higher cumulative doses of vincristine had been administered. Lastly, *CYP3A5* low-expressers had limited PK samples available for analysis beyond 24 hours. Using only the one-compartment model precludes differentiation of the metabolism process from the distribution process. Hence, the clearance is a combination of metabolism and distribution. Utilizing a longer sampling window for low-expressers, we anticipate a better power to investigate the associations between vincristine metabolism and CYP3A genetic and/or other PK genetics.

Because we hypothesize that Kenyan children are likely to be *CYP3A5* high-expressers who could possibly tolerate and potentially benefit from more therapeutic vincristine exposure, future studies will be aimed at vincristine dose escalation in Kenyan children paired with frequent detailed neuropathy assessments to establish the maximum

tolerated dose in this population. Additionally, more extensive genotyping or GWAS analysis may be beneficial in Kenyan children to establish other potential genetic changes that contribute to variability in vincristine metabolism and/or susceptibility to toxicity. Once the maximum tolerated dose of vincristine is established for this population, we will aim to validate our findings and potentially establish other biomarkers of vincristine exposure and possibly subsequent toxicity in addition to *CYP3A5* genotype.

Conflict of Interest Statement

The authors whose names are listed on the title page certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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Legends:

Table 1 Title: Patient Demographics and CYP 3A5 Genotyping

Table Legend:

-Homozygous variant = *3, 6, or 7/*3, 6, or 7

-Heterozyous = *1/*3, 6, or 7

-Homozygous wild type = *1/*1

Table 2. TNS© Scoring (Reprinted with Permission from Smith, et al, 2013)[8]

Table 3 Title: Neuropathy assessment cumulative descriptive statistics

Table Legend:

Abbreviations: CTC, Common Terminology Criteria for Adverse Events; TNS©, Total Neuropathy Score

[a] reflects the total number of completed neuropathy or pain assessments

Fig 1: Vincristine pharmacokinetic profile of CYP 3A5 low-expresser versus high-expresser genotype groups. The blue curve is the population-average PK model for CYP3A5 high-expressers, and the red curve is the population-average PK model for CYP3A5 low-expressers. Dotted lines reflect 95% confidence intervals. Vincristine dose normalized area under the curve (AUC) of CYP3A5 high-expresser genotype = 0.28 ±0.15 hr*m^2/L. AUC of CYP3A5 low-expresser genotype = 0.15 ± 0.011 hr*m^2/L, p=0.027.

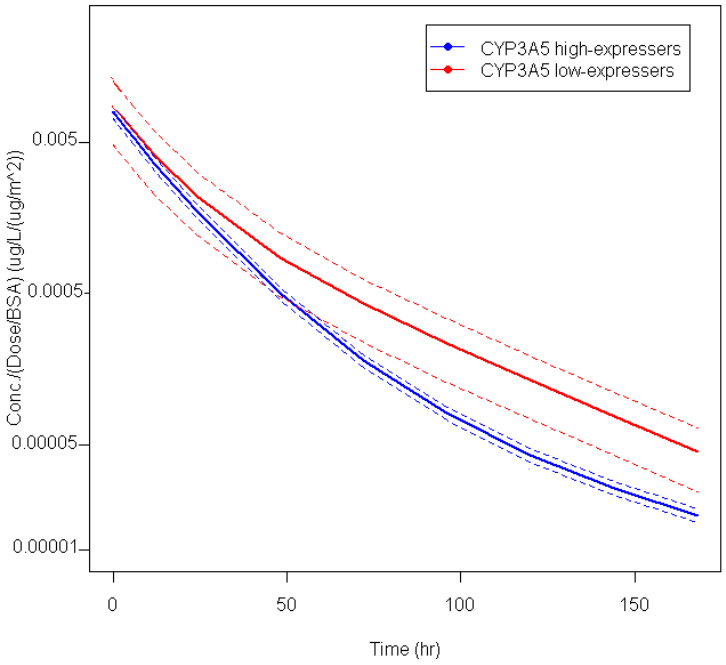

Table 1: Patient Demographics and CYP 3A5 Genotyping

Demographic Variables	**CYP 3A5 Genotype**			**p-value**
	Homozygous Variant (low expresser) (n = 7)	**Heterozygous** (n = 50)	**Homozygous Wild Type (high expresser)** (n = 21)	
Age (years), mean (SD)	6.14 (5.21)	6.54 (3.98)	6.10 (4.60)	0.9114*
Sex -Male, n (%) -Female, n (%)	2 (4.8) 5 (13.9)	28 (66.7) 22 (61.1)	12 (28.6) 9 (25.0)	0.4620#
Body Surface Area (m^2), mean (SD)	0.81 (0.40)	0.81 (0.26)	0.75 (0.30)	0.7744*
Height (cm), mean (SD)	113.06 (31.40)	113.55 (23.90)	108.34 (26.03)	0.7253*

Weight (kg), mean (SD)	21.56 (15.49)	21.01 (9.57)	19.42 (11.66)	0.8280*
Tribe -Luhya, n (%) -Kalenjin, n (%) Other, n (%)	3 (10.7) 3 (10.7) 1 (4.6)	18 (64.3) 17 (60.7) 15 (68.2)	7 (25.0) 8 (28.6) 6 (27.3)	0.9575#
Oncology Diagnosis -Solid Tumor, n (%) -Leukemia/Lymphoma, n (%)	3 (8.8) 4 (9.1)	21 (61.8) 29 (65.9)	10 (29.4) 11 (25.0)	0.9367#

*ANOVA and #Fisher's exact test.

Table 2. TNS© Scoring (Reprinted with Permission from Smith, et al, 2013) 8

	0	1	2	3	4
Worst Subjective Symptom[a,b] (Tingling, Numbness, Neuropathic Pain)	None	Limited to fingers or toes	Extension to ankle/wrist	Extension to knee/elbow	Above knees/elbows or functionally disabling
Temperature Sensibility[a]	Normal	Reduced in fingers/toes	Reduced to wrist/ankle	Reduced to elbow/ knee	Reduced above elbow/knee
Vibration Sensibility[a]	Normal	Reduced in fingers/toes	Reduced to wrist/ ankle	Reduced to elbow/ knee	Reduced above elbow/knee
Strength[a,c]	Normal	Mild weakness, but can overcome	Moderate weakness, can overcome gravity but	Severe weakness, cannot overcome	Paralysis

		resistance	not resistance	gravity	
Tendon Reflexes [a]	Normal	Ankle reflex reduced	Ankle reflex absent	Ankle reflex absent/ others reduced	All reflexes absent
Autonomic/Constipation	Normal	Requiring stool softeners or dietary modification	Requiring laxatives	Obstipation requiring enemas or manual evacuation	Life threatening consequences (e.g. toxic megacolon; obstruction) including death
Laryngeal/Hoarseness	Normal voice/ cry	Mild or intermittent hoarseness	Persistent hoarseness, but able to vocalize; may have mild to moderate edema	Whispered speech, not able to vocalize; may have marked edema	Marked dyspnea/stridor requiring tracheostomy or intubation

Abbreviations: TNS© = Total Neuropathy Score A; [a]Original TNS item.(Chaudhry, Rowinsky, Sartorius, Donehower, & Cornblath, 1994; Cornblath et al., 1999); [b]Score is based on the worst of the three symptoms; [c]Toe extension/flexion, ankle dorsiflexion, hip flexion, hand grip, thumb abduction, wrist extension, arm abduction; score is based on the weakest muscle grouo

Table 3: Neuropathy assessment cumulative descriptive statistics

Grading Scale	Number of evaluable	Number of Assessments per patien	Possible Range of Scores	Actual Range of Scores	Percentage of 0 scores	Median Score	Clinically significant neuropathy	Mean Score ± SD

	subjects	t (Mean ± SD)					present (%)	
Balis Motor	72	2.63 ± 1.74	0-4	0-3	96.3	0	1.4 (Grade ≥ 2)	0.08±0.41
Balis Sensory	72	2.63 ± 1.74	0-4	0-2	94.7	0	1.4 (Grade ≥ 2)	0.06±0.26
CTC Motor	72	2.63 ± 1.74	0-5	0-2	94.7	0	2.8 (Grade ≥ 2)	0.07±0.33
CTC sensory	72	2.63 ± 1.74	0-5	0-1	95.8	0	0 (Grade ≥ 2)	0.04±0.20
Pediatric Neuropathic Pain Scale	78	3.17 ± 1.85	0-25	0-4	94.3	0	1.3 (Total score ≥ 4)	0.12±0.52
TNS© Total score A	46	3.11 ± 1.93	0-28	0-8	53.5	0	4.3 (Total score ≥ 5)	1.09±1.44

Abbreviations: CTC, Common Terminology Criteria for Adverse Events; TNS©-PV, Total Neuropathy Score – Pediatric Vincristine

[a] reflects the total number of completed neuropathy or pain assessments