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HIV Integrase Inhibitor Pharmacogenetics and Clinical Outcomes: An Exploratory Association Study

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by

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ABSTRACT

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As HIV is now primarily a chronic condition, treatment is given life-long with changes as necessitated by alterations in tolerability and efficacy. Thus, personalized medicine may be useful in the prevention of unnecessary drug exposure and avoidable side effects. Three of the four currently available HIV integrase strand transfer inhibitors (INSTIs), raltegravir, elvitegravir, and dolutegravir, are widely utilized antiretrovirals in the USA and exhibit variations in outcomes among subjects. To interrogate differences among subjects receiving these drugs, we investigated the association of several single nucleotide polymorphisms (SNPs) with drug exposure, clinical outcomes, and subject-reported adverse events. HIV+ adults (≥18 years old) receiving an INSTI regimen were recruited (n=88). Subject genotypes were evaluated using an iPLEX PGx Panel. Genetic variations within our population, underwent multiple regression with covariates [age, sex, BMI, regimen duration, and baseline variables (as required) along with specific regimen in the comprehensive group] to detect significant (p<0.05) associations with concentration and selected clinical data. Additionally, multiple logistic regression, with the previous covariates, tested for association with binary traits including central nervous system-related (abnormal dream, anxiety, fatigue, headache, and insomnia) and gastrointestinal-related (diarrhea and nausea) adverse events. With a median age of 52.5 years (IQR 45.7-57.2) being predominately Caucasian (88.6%) and male (86.4%), we found an association (p=0.028) between abnormal dream occurrence and specific INSTI regimen with the raltegravir grouping presenting a higher frequency. This exploratory study also discovered

several SNP-outcome associations when using INSTIs. Although these SNPs were found to have a role in predicting segments of adverse effect profiles, the clinical significance of these findings remains to be determined. Larger studies will be needed to confirm these exploratory findings with functional studies to understand pathogeneses. In conclusion, the associations found in this study strengthen the need for further assessment, within the HIV+ population, of factors contributing to unfavorable subject outcomes.

DEDICATION

For my parents,

James and Bobie Murrell,

who have supported me through over two decades of education.

Soli Deo Gloria

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CHAPTER 1

INTRODUCTION

After nearly four decades, human immunodeficiency virus (HIV) remains a high priority in biomedical research with thousands of new infections occurring each day adding to the upward of 36 million HIV positive individuals the world over ("HIV Data and Statistics," 2017). Current drug treatments are highly effective in reducing plasma HIV viral load; however, several factors may interact to alter the efficacy and tolerability of antiretrovirals (Gandhi et al., 2012; Hewitt, 2002). Because HIV positive individuals are living relatively longer and healthier lives due to improved treatment, the frequency and severity of side effects may increase with age-related physiological changes and the increased probability of comorbidities (Dumond et al., 2013). In addition, variations in the genetic make-up of an individual may also alter the behavior of some drugs, resulting in differences in efficacy and toxicity (Wyatt, Pettit, & Harirforoosh, 2012). Compounded with the current absence of a cure, treatment regimens must be continued lifelong. Thus, the availability of safe and continually effective treatment options is an increasing concern to the HIV health care provider.

HIV: Overview

Transmission

HIV is typically contracted sexually, parenterally, or vertically (Shaw & Hunter, 2012). Sexual contact involving the exchange of bodily fluids, such as semen or vaginal secretions, possesses an increased probability of infection. Parenteral transmission, occurring through the sharing of virally contaminated needles, is common among those who abuse intravenous drugs. Vertical transmission occurs when the virus travels from mother to child through contact with

maternal blood at birth, via breast feeding, or *in utero*. Other transmission avenues that are possible, but less probable include accidental needle sticks, mucocutaneous exposure, or contaminated blood/tissue transplants (DiPiro, 2011; Longo, 2012).

Replication

As a single-stranded RNA virus (two copies per retrovirus), HIV must enter a host cell to replicate (Metifiot, Marchand, & Pommier, 2013). Infection occurs when HIV interacts with CD4 receptors present on a host cell, primarily $CD4^+$ T-cells, then fuses with the cell. The viral coat is removed inside the cell revealing the viral RNA, which is reverse transcribed by a viral enzyme, reverse transcriptase (RT), to complementary DNA (cDNA). Viral double-stranded DNA (dsDNA), created from cDNA via host polymerases, is processed then translocated into the cellular nucleus prior to integration into the host genome via HIV integrase (IN). This integration creates a provirus which evades host immune responses through latency (Lampiris, 2012); however, upon activation, the viral genome is expressed, leading to protein translation and processing by viral proteases. Viral RNA, enzymes, and coat are then organized into mature viruses, which bud from the host cell (Metifiot et al., 2013). Disease progression and antiretroviral therapy efficacy may be determined using HIV RNA concentration in plasma and $CD4^+$ cell count as biomarkers (DeJesus et al., 2012; Sax et al., 2012; Zolopa et al., 2013).

Antiretroviral Therapy

Currently, there is no effective vaccine against or method of cure for HIV infection; however, numerous antiretroviral medicines have been devised to combat the progression of HIV infection into acquired immune deficiency syndrome (AIDS). Because no drug has proven to be exceedingly effective individually and the HIV genome is capable of rapidly developing drug resistance, the use of multiple drug classes each addressing a different aspect of HIV infection

and replication is preferred. Thus highly active antiretroviral therapy (HAART), consisting of one or more members of the following drug classes: protease inhibitor (PI), nucleoside and nucleos(t)ide reverse transcriptase inhibitor (NRTI) or non-nucleoside reverse transcriptase inhibitor (NNRTI), entry inhibitor, and/or integrase strand-transfer inhibitor (INSTI), has become routine in the treatment of HIV (Metifiot, Marchand, Maddali, & Pommier, 2010; Olin, Spooner, & Klibanov, 2012; Pavlos & Phillips, 2012; Zanger & Klein, 2013). Strict compliance with medication regimens is required to avoid viral mutations which can render individual or even classes of antiretrovirals ineffective. This study focused on three of the four currently available INSTIs which were and remain frontline regimens (Tsiang et al., 2016). Bictegravir was not included due to the lack of usage and clinical experience at the commencement of this observational study.

Integrase Strand Transfer Inhibitors (INSTIs)

Mechanism of Action

Following reverse transcription of viral RNA by reverse transcriptase and synthesis of dsDNA by cellular enzymes, viral IN recognizes the newly synthesized dsDNA and performs a function known as 3'-processing (Dayam et al., 2008). Two bases, G and T, are removed from both 3' ends of the viral dsDNA then the pre-integration complex (PIC), consisting of processed dsDNA, IN, and other necessary cofactors, moves to the nucleus. The dsDNA is initially integrated into the host DNA through IN then completed via host DNA repair enzymes (Liao, Marchand, Burke, Pommier, & Nicklaus, 2010).

IN consists of three subunits, an N-terminal domain, a catalytic domain, and a C-terminal domain. The DNA binding function of the catalytic domain is targeted by INSTIs (Lampiris, 2012). Mg^{2+} ions are believed to be essential for the catalytic capabilities of IN as well as the

formation of the PIC through dsDNA binding (Liao et al., 2010). INSTIs chelate the Mg^{2+} ions at the active catalytic site of IN, preventing 3'-processing and dsDNA covalent binding (Pommier & Marchand, 2012). Without the interaction between IN and dsDNA, viral DNA is unable to integrate into the host genome and replication is not possible (Correll & Klibanov, 2008).

Raltegravir

Merck introduced raltegravir, formerly MK-0518, as the first FDA-approved INSTI in October 2007 under the brand name of Isentress for adults then (December 2011) approved for use in pediatric subjects (Hajimahdi & Zarghi, 2016; Traynor, 2007). Discovered while searching for a HCV polymerase inhibitor, raltegravir is a derivative of dihydroxypyrimidine carboxamide (Hajimahdi & Zarghi, 2016).

Although primarily administered twice daily as a film-coated 400 mg tablet, single 800 or 1200 mg doses have also been examined (Cahn et al., 2017; Eron et al., 2011). The 1200 mg dose was recently approved by the FDA ("Isentress Prescribing Information," 2017). Administration is not dependent upon the presence of food and pharmacokinetic boosting is not necessary. In the fasted state, the time to reach maximum plasma concentration, or the C_{max}, (T_{max}) is reached in 3 hours with a half-life of nearly 9 hours (Brainard, Wenning, Stone, Wagner, & Iwamoto, 2011); however, raltegravir pharmacokinetics have been shown to be variable within and between subjects (Rizk et al., 2012). Protein binding appears to be approximately 83% with 51% of the drug being excreted unchanged in feces ("Isentress Prescribing Information," 2017). Raltegravir undergoes glucuronidation via uridine diphosphate glucuronosyltransferase (UGT) 1A1 (UGT1A1). Raltegravir is dosed with various antiretroviral backbone regimens, such as tenofovir disoproxil fumarate/emtricitabine or tenofovir alafenamide/emtricitabine.

Several clinical studies have shown the utility of raltegravir. The double-blind STARTMRK trial has demonstrated raltegravir efficacy (over five years) and superiority over efavirenz/emtricitabine/tenofovir disoproxil fumarate (Lennox et al., 2009; Rockstroh et al., 2011). The proportion of virally suppressed individuals with HIV RNA < 50 copies/ml at week 240 was 71% vs. 61% for raltegravir and efavirenz regimens, respectively. Although the QDMRK study, in which groups received the tenofovir disoproxil fumarate/emtricitabine background, showed that raltegravir (800 mg; once-daily) did not reach non-inferiority compared to raltegravir (400; twice-daily) at 48 weeks, over 83% of the once-daily group achieved viral suppression (Eron et al., 2011). Several reviews have provided further detail on each of the studies concerning INSTIs (Raffi & Wainberg, 2012).

Elvitegravir

Elvitegravir, formally known as JTK-303 and GS-9137, is a hydroxyquinolone (quinolone-3-car1boxylic acid derivative) which interferes with HIV viral integration (Correll & Klibanov, 2008; Hajimahdi & Zarghi, 2016). Although discovered by Japan Tobacco, Gilead Sciences currently produces two treatment options, Stribild and its younger sibling Genvoya, which were approved for use in the United States by the FDA in August 2012 and November 2015, respectively ("Genvoya Prescribing Information," 2017). Both combination regimens contain elvitegravir, cobicistat, and emtricitabine; while differing in the tenofovir prodrug (D. E. Murrell, Harirforoosh, S, 2016; Sax et al., 2015). Elvitegravir has also been approved for independent administration as Vitekta by the FDA and European Commission.

Elvitegravir is administered orally in tablet form with the presence of food playing a significant role in bioavailability (Lampiris, 2012; Olin et al., 2012). When co-formulated in Stribild, elvitegravir peak drug concentrations are achieved within 4 hours post dose and

absorption is elevated when administered with food (light meal, increased 34% vs fasting; high fat meal, increased 87% vs. fasting). Plasma protein binding is high for elvitegravir (98-99%) and nearly 95% of the drug is excreted in feces (Ramanathan, Mathias, German, & Kearney, 2011).

Phase I metabolism of elvitegravir is performed by cytochrome P450 (CYP) 3A4 (CYP3A4); the drug can also undergo glucuronidation by UGT1A1/3 (Adams, Greener, & Kashuba, 2012; Olin et al., 2012; Ramanathan, Kakuda, Mack, West, & Kearney, 2008). As an inducer of CYP3A4 and CYP2C9, which diminishes the half-life of substrates metabolized by these enzymes, elvitegravir has a relatively short half-life of 3 hrs (Adams et al., 2012). Rather than increasing dosage to achieve appropriate systemic exposure, elvitegravir is administered with a pharmaco-enhancer which decreases drug metabolism (Olin et al., 2012).

Originally paired with ritonavir, elvitegravir is now partnered with a more precise inhibitor, cobicistat. Co-formulation with cobicistat, which triples the half-life of elvitegravir to 9 hrs, is beneficial in helping to prevent the development of drug resistance (Adams et al., 2012). Also as elvitegravir is not altered by most NRTIs, co-formulation with emtricitabine and tenofovir disoproxil fumarate is possible (Correll & Klibanov, 2008). Elvitegravir/cobicistat/tenofovir disoproxil fumarate/emtricitabine has been documented to be non-inferior to efavirenz/tenofovir disoproxil fumarate/emtricitabine and to atazanavir (a PI)/ritonavir+/tenofovir disoproxil fumarate/emtricitabine as evaluated by the proportion of formerly drug-naïve patients demonstrating viral load suppression to below 50 copies RNA/ml after 48 weeks of treatment (DeJesus et al., 2012; Sax et al., 2012).

Dolutegravir

ViiV Healthcare, a joint venture between GlaxoSmithKline and Pfizer, developed dolutegravir, a tricyclic carbamoyl pyridine, and introduced the drug as Tivicay (50 mg DTG) in August 2013 (Ballantyne & Perry, 2013; Hajimahdi & Zarghi, 2016). One year later, Triumeq, a combination of dolutegravir (50 mg), abacavir (600 mg), and lamivudine (300 mg), also received FDA approval (Gohil, 2014). Due to the inclusion of abacavir, this single tablet regimen is only available for HLA-B*5701 negative individuals (Greig & Deeks, 2015).

Dolutegravir was thought to be an improvement upon raltegravir, in terms of dosing schedule (prior to the 1200 mg once-daily dose) ("Isentress Prescribing Information," 2017), and elvitegravir, in terms of boosting (Molina et al., 2015). Although food does not seem to have a clinically significant effect on drug absorption, meal fat content (low, moderate, or high) has been shown to increase the area under the plasma concentration time curve (33%, 41%, and 66%, respectively compared to fasting) ("Tivicay Prescribing Information," 2017). Apparent volume of distribution was determined to be approximately 17.4 L with protein binding of nearly 99% when administered as Triumeq (Greig & Deeks, 2015). Dolutegravir is principally metabolized by UGT1A1; however, CYP3A enzymes produce a minor metabolite as well (Castellino et al., 2013). A half-life of 14 hours has been seen with dolutegravir (Min et al., 2010). Unchanged drug is excreted 53% in the feces with very little (<1%) in the urine; however, 31% of metabolized dolutegravir is found in the urine ("Tivicay Prescribing Information," 2017). Dolutegravir was also found to distribute into the cerebrospinal fluid (Greig & Deeks, 2015).

A meta-analysis of four randomized controlled trials comprised of treatment-naïve individuals performed by Jiang *et al*. found that dolutegravir regimens were superior to efavirenz (an NNRTI) and raltegravir based regiments in terms of safety and efficacy (Jiang et al., 2016).

Nausea and headache were the most frequent adverse events associated with dolutegravir. When dosed with dual NRTI regimens (abacavir/lamivudine or tenofovir disoproxil fumarate/emtricitabine) in the SINGLE study, dolutegravir was shown to be superior to efavirenz/tenofovir disoproxil fumarate/emtricitabine at 48 weeks of therapy (88% vs 81%, respective viral suppression) (Walmsley et al., 2013). Similar results were observed at 96 weeks (80% vs 72%, respectively) and at 144 weeks (71% vs 63%, respectively) (Greig & Deeks, 2015). In the FLAMINGO study, dolutegravir was compared to a PI and pharmacokinetic booster combination, darunavir/ritonavir, (both dosed with NRTIs) for viral suppression at week 48 (90% vs 83%, respectively) and week 96 (80% vs 68%, respectively) (Molina et al., 2015). Non-inferiority and superiority was conferred at 96 weeks. In the SPRING-2 study (a randomized double-blind double dummy study), dolutegravir+NRTI was deemed non-inferior to bid 400 mg raltegravir+NRTI at 48 weeks (88% vs 85% respective viral suppression) and showed comparable safety and tolerability at 96 weeks (81% vs 76%) (Raffi, Jaeger, et al., 2013; Raffi, Rachlis, et al., 2013). The SAILING study also determined that dolutegravir was not only non-inferior to raltegravir, but superior as well with 71% viral suppression opposed to 64% in the comparator group following 48 weeks of treatment (Cahn et al., 2013).

Pharmacogenetics

Pharmaceutical decision-making consists of several parameters; however, a relatively recent addition to the puzzle is the utilization of pharmacogenetics, which is the subsection of genetics dealing with pharmacokinetic and pharmacodynamic outcomes (Pouget, Shams, Tiwari, & Muller, 2014). As such, metabolic enzymes have been shown to exhibit alteration when genetic polymorphisms are present (Elens et al., 2013; Okubo et al., 2013; Wang, Guo, Wrighton, Cooke, & Sadee, 2011). Recent data suggests that CYP3A4 shows reduced activity

and/or expression with the presence of the CYP3A4*22 allele. Although one study did not find a correlation with activity (Garcia-Martin et al., 2002), another study reported a polymorphism, CYP3A4*1B, correlating with an increase in CYP3A4 activity (Klein & Zanger, 2013). The presence of CYP3A5 polymorphism has also been suggested to relate to the metabolism of CYP3A4 substrates (Wang & Sadee, 2012); thus CYP3A5*3 (rs776746) which yields a null phenotype may be important to dolutegravir and elvitegravir metabolism (Elens et al., 2013). CYP2D6 also has a large number of polymorphisms which influence enzyme activity (Khlifi, Messaoud, Rebai, & Hamza-Chaffai, 2013). In a study by Ritchie *et al.*, a polymorphism in the ABCB1 gene, which encodes for p-glycoprotein (P-gp), was suggested to influence toxicity of a P-gp substrate such as dolutegravir (Ritchie et al., 2006; "Tivicay Prescribing Information," 2017). In a recent study by D'Avolio *et al.* (D'Avolio et al., 2014), a SNP (rs4149056) in organic anion transport protein (OATP) 1B1, which interacts with cobicistat, was found to correlate with changes in ritonavir plasma concentrations.

Specific Aims

Because polymorphisms in drug metabolizing enzymes, transporters, and/or receptors can influence drug pharmacokinetics and thereby alter drug properties, we conducted an exploratory pharmacogenetic analysis of INSTI regimens consisting of numerous SNPs included on the iPLEX PGx ProPanel. We hypothesized that particular drug outcomes will be influenced by pharmacogenetics. This hypothesis is proposed based on the following observations. First, raltegravir and dolutegravir are primarily metabolized by UGT1A1 (Arab-Alameddine et al., 2012; Castellino et al., 2013). Second, elvitegravir is metabolized by CYP3A4 (Olin et al., 2012). Third, the expression of most enzymes is modulated by nuclear receptors (Coleman & Wiley InterScience (Online service), 2010), which like the expression of many drug

metabolizing enzymes and transporters, are influenced by genetic polymorphism (Michaud et al., 2012). The genetic variability in the expression of enzymes and transporters may produce alterations in drug pharmacokinetics, and consequently drug effects (Wyatt et al., 2012). The following specific aims were designed to test our hypothesis: evaluate drug exposure in HIV-1 patients, document genetic polymorphisms, collect clinical outcomes, and perform association analyses.

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CHAPTER 2

EXPLORATORY GENETIC ASSOCIATION OF DRUG EXPOSURE AND SELECTED TOLERABILITY OUTCOMES OF HIV INTEGRASE INHIBITORS

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Conflict of Interest
The authors declared no conflict of interest.

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Abstract

Integrase strand transfer inhibitors (INSTIs) have become integral in HIV treatment with close monitoring of continued efficacy and tolerability. This exploratory study evaluated polymorphism influence on drug exposure and tolerability. HIV+ adults (≥18yrs) receiving INSTI-based regimens were recruited (n=88) and genotyped with an iPLEX PGx Panel. Genetic variants within our population, underwent multiple regression with covariates [age, sex, BMI, regimen (comprehensive group), regimen duration, and baseline variables (as required)] to detect significant ($p<0.05$) association of concentration data and selected clinical data. With a median age of 52.5 years (IQR 45.7-57.2) being predominately Caucasian (88.6%) and male (86.4%), this exploratory study discovered that dolutegravir trough concentration was influenced by selected single nucleotide polymorphisms (SNPs). In addition, several associations were identified between variables and SNPs, when using INSTIs; however, clinical significance is unknown. These exploratory findings require confirmation in larger studies which may also investigate interaction mechanisms.

Introduction

Over the past decade, HIV integrase strand transfer inhibitors (INSTIs) have moved to the frontlines of antiretroviral therapy (1). Regarding three of the four approved INSTIs (dolutegravir, elvitegravir, and raltegravir), each is frequently efficacious; however, variability in regimen tolerability may be of concern(2). Dolutegravir was thought to be an improvement upon raltegravir, in terms of reduced dosing frequency (prior to once-daily 1200 mg raltegravir) (3), and elvitegravir, in terms of boosting necessity (4); however, elevated drug concentrations can be problematic (5). In the case of elvitegravir, the drug concentration at the end of the dosing interval at steady-state (C_{trough}) seems related to outcomes (6, 7). The pharmacokinetics of raltegravir have been shown to have intra- and inter-subject variation which may influence drug outcomes (8). Thus, drug exposure play an important role in the use of these regimens.

Concurrent with the rise of the INSTIs, the field of personalized medicine has also gained traction in the clinical realm. One method of informing pharmaceutical decision-making is the integration of pharmacogenetics, the interaction of genetic information with drug pharmacokinetics and outcomes. Recent data suggests that cytochrome P450 3A4 (CYP3A4), a metabolizing enzyme of many drugs, including dolutegravir and elvitegravir, shows reduced activity and/or expression with the presence of the CYP3A4*22 allele (rs35599367) (9-11). Although a different study discovered such a correlation with activity (12), a further study reported a polymorphism, CYP3A4*1B (rs2740574), correlated with an increase in CYP3A4 activity (13). The presence of CYP3A5 polymorphism has also been suggested to relate to the metabolism of CYP3A4 substrates, such as elvitegravir, (14); thus SNPs such as CYP3A5*3 (rs776746) which yield a null phenotype may partially reduce metabolism (9). Drug transporters, such as the ATP-binding cassette transporter B1 (ABCB1), also known as p-glycoprotein, have

been suggested to influence toxicity of substrates (15). Dolutegravir and raltegravir, p-glycoprotein substrates, may be adversely affected by changes in p-glycoprotein (16, 17). In this study, we conducted an exploratory pharmacogenetic analysis of INSTI regimens, consisting of numerous SNPs included on the iPLEX PGx Panel v1.0, to understand the influence of genetic polymorphism on overall drug exposure and clinical tolerability.

Results

Subject Demographics

All HIV+ individuals receiving care at the East Tennessee State University (ETSU) Center of Excellence (COE) for HIV/AIDS Care (n=341) were screened for this study. Overall demographic characteristics, along with stratification by INSTI, are presented in Table 1. Of the eligible subjects (n=216), eighty-eight HIV+ individuals (86.4% male) with a median age of 52.5 years were recruited. The primarily non-Hispanic Caucasian population presented with a mean BMI of 26.2. Only 3 of the 88 subjects reported a missed dose within the two weeks prior to sample collection. The majority (85/86) of subjects were virally suppressed (<20 RNA copies/mL) or had a low-level viremia (below 60 RNA copies/mL) at or near sample collection; while viral load data was not available for two subjects.

Table 1: Subject demographics

	Dolutegravir (n=42)	Elvitegravir (n=23)	Raltegravir (n=23)	Total (n=88)
Age in years, median (IQR)	53.0 (42.7 - 58.5)	50.0 (42.0 - 54.5)	53.0 (50.0 - 58.5)	52.5 (45.7 – 57.2)
Male, count (%)	37 (88.1%)	20 (87.0%)	19 (82.6%)	76 (86.4%)
Mean Body Mass Index (SD)	24.7 ± 4.8	29.1 ± 6.6	26.0 ± 5.1	26.2 ± 5.63
Race or ethnic group (All that apply)				
Black	5	3	0	8
White	36	19	23	78
Other	2	1	3	6
Regimen Duration (weeks)	66.9 ± 39.0	80.8 ± 56.3	162.0 ± 77.3	95.4 ± 68.2

Pharmacogenetic Analysis

All samples had a call rate of ≥97%. The genotyping efficiency was greater than 95% for all, but three SNPs (rs5030865, 77.7%; rs28371706, 57.4%; and rs1065411, 83.0%) which showed low yield were not included in analysis. Of the remaining 175 SNPs on the panel, 86 were polymorphic within this population. SNPs were further excluded based on low minor allelic frequency below 1%.

Drug Exposure

Mean dolutegravir C_{trough} (n=23) was determined to be 764.13±401.66 ng/mL. When stratified by genotype, the concentrations were associated with five SNPs as revealed in Table 2.Two *CYP2D6* SNPs (rs1065852 and rs3892097) were shown to increase dolutegravir concentration along with rs7294 in *VKORC1*. Meanwhile, rs4149056 and rs8192709 were associated with decreases in dolutegravir concentration. Two of the associated SNPs, rs1065852 and rs3892097, were in linkage disequilibrium (LD) (r^2=0.850). Although the C_{trough} of elvitegravir (n=15; 263.84±146.92 ng/mL) and raltegravir (n=6; 567.16±307.19 ng/mL) were determined, no SNPs showed association with elvitegravir or raltegravir concentration following multiple regression.

Table 2: SNPs significantly associated with drug concentration

Drug	Gene	SNP	Chromosome	BP[a]	A1[b]	MAF[c]	HWE[d]	Beta (95% CI)[e]	*p*-value
Dolutegravir	***CYP2D6***	rs1065852	22	42526694	T	0.190	1.000	430.80 (149.40 — 712.20)	0.008
	SLCOB1	rs4149056	12	21331549	C	0.095	1.000	-508.20 (-840.80 — -175.50)	0.009
	CYP2D6	rs3892097	22	42524947	A	0.167	1.000	407.60 (102.50 — 712.60)	0.019
	CYP2B6	rs8192709	19	41497274	T	0.060	1.000	-785.80 (-1422.00 — -149.80)	0.028
	VKORC1	rs7294	16	31102321	A	0.452	0.984	230.30 (41.33 — 419.20)	0.030

[a]Physical position (bp); [b]Minor allele; [c]Minor Allele Frequency; [d]p-value for Hardy-Weinberg equilibrium test; [e]Beta adjusted for covariates (sex, age, BMI, and regimen duration)

Hepatic Parameters

No group had mean alkaline phosphatase (ALP) levels outside the normal range of 30-125 U/L (18) (comprehensive group, 73.25±42.94 U/L; dolutegravir group, 69.94±24.30 U/L; elvitegravir group, 85.39±72.57 U/L; and raltegravir group, 66.73±24.37 U/L). The difference in mean ALP among the regimens was not significant (p=0.277). Table 3 shows that two SNPs (rs2273697 and rs737865) reached a positive significant association with ALP levels across all regimens. No SNPs reached multiple regression significance in the dolutegravir group. Seven SNPs, two of which (rs9934438 and rs9923231) being in LD (r^2=1), were associated with ALP levels in elvitegravir-receiving individuals. The addition of minor alleles revealed increases of ALP in six SNPs; while minor alleles in rs7294 were negatively associated with ALP level. The raltegravir group had two SNPs (rs1045642 and rs17708472) yielded positive association with ALP levels.

Table 3: SNPs significantly associated with ALP levels

Drug	Gene	SNP	Chromosome	BP[a]	A1[b]	MAF[c]	HWE[d]	Beta (95% CI)[e]	*p*-value
All	***ABCC2***	rs2273697	10	101563815	A	0.199	0.165	16.39 (2.75 — 30.03)	0.021
	COMT	rs737865	22	19930121	C	0.301	0.406	17.27 (4.90 — 29.64)	0.008
Elvitegravir	***CYP2C8***	rs1058930	10	96818119	G	0.043	1.000	228.60 (151.90 — 305.20)	<0.001
	VKORC1	rs9934438	16	31104878	A	0.261	0.933	72.94 (25.48 — 120.40)	0.008
	VKORC1	rs9923231	16	31107689	T	0.261	0.933	72.94 (25.48 — 120.40)	0.008
	ABCC2	rs2273697	10	101563815	A	0.217	0.501	62.42 (18.29 — 106.50)	0.014
	COMT	rs737865	22	19930121	C	0.196	0.329	69.59 (21.03 — 118.20)	0.013
	VKORC1	rs7294	16	31102321	A	0.413	0.799	-55.92 (-106.70 — -5.17)	0.046
	CYP2C9	rs1799853	10	96702047	T	0.109	1.000	91.44 (7.08 — 175.80)	0.050
Raltegravir	***ABCB1***	rs1045642	7	87138645	T	0.478	0.166	17.49 (3.21 — 31.78)	0.032
	VKORC1	rs1770847	16	31105353	A	0.239	1.000	16.53 (2.90 — 30.15)	0.033

[a]Physical position (bp); [b]Minor allele; [c]Minor Allele Frequency; [d]p-value for Hardy-Weinberg equilibrium test; [e]Beta for ALP levels adjusted for covariates [sex, age, BMI, integrase inhibitor (in All group), integrase inhibitor duration, and baseline ALP]

The elvitegravir group (32.48±28.25 U/L) had a mean alanine aminotransferase (ALT) level in the upper normal range (0-40 U/L) (18); while the other groups, comprehensive, dolutegravir, and raltegravir, showed 26.81±20.05 U/L, 24.44±16.41 U/L, and 25.32±15.18 U/L, respectively. Mean ALT among the regimens did not show significant differences (p=0.285). Two SNPs (rs2282143 and rs1048943) were determined to have a positive association with ALT levels (Table 4) when all regimens were combined. There were no associated SNPs after dolutegravir stratification. Five SNPs, four (rs9934438, rs9923231, rs2282143, and rs1048943) with a positive beta and one (rs7294) with a negative beta, were associated with ALT in terms of elvitegravir-receiving patients. As previously noted, rs9934438 and rs9923231 were in LD along with rs2282143 and rs1048943 (r^2=1). Additionally, raltegravir-grouped samples presented two associated SNPs, one negative (rs165599) and one positive (rs34059508) when analyzed for ALT levels.

Table 4: SNPs significantly associated with ALT levels

Drug	Gene	SNP	Chromosome	BP[a]	A1[b]	MAF[c]	HWE[d]	Beta (95% CI)[e]	*p*-value
ALL	***SLC22A1***	rs2282143	6	160557643	T	0.028	0.113	29.77 (17.01 — 42.53)	<0.001
	CYP1A1	rs1048943	15	75012985	G	0.045	0.306	19.01 (7.41 — 30.61)	0.002
Elvitegravir	***VKORC1***	rs9934438	16	31104878	A	0.261	0.933	28.97 (11.68 — 46.25)	0.005
	VKORC1	rs9923231	16	31107689	T	0.261	0.933	28.97 (11.68 — 46.25)	0.005
	VKORC1	rs7294	16	31102321	A	0.413	0.799	-24.96 (-40.60 — -9.32)	0.006
	SLC22A1	rs2282143	6	160557643	T	0.065	0.133	25.39 (6.00 — 44.77)	0.021
	CYP1A1	rs1048943	15	75012985	G	0.065	0.133	25.39 (6.00 — 44.77)	0.021
Raltegravir	***COMT***	rs165599	22	19956781	G	0.283	1.000	-10.65 (-18.17 — -3.13)	0.014
	SLC22A1	rs34059508	6	160575837	A	0.065	1.000	18.34 (4.50 — 32.17)	0.020

[a]Physical position (bp); [b]Minor allele; [c]Minor Allele Frequency; [d]p-value for Hardy-Weinberg equilibrium test; [e]Beta for ALT levels adjusted for covariates [sex, age, BMI, integrase inhibitor (in All group), integrase inhibitor duration, and baseline ALT levels]

Mean aspartate aminotransferase (AST) levels (comprehensive group, 24.79±17.24 U/L; dolutegravir group, 23.49±10.38 U/L; elvitegravir group, 29.48±29.19 U/L; and raltegravir group, 22.32±8.48 U/L) were each within the high end of the 3-44 U/L normal range (18). No significant difference (p=0.307) was detected among the AST levels between the regimens. The four AST level-SNP associations (rs9934438, rs9923231, rs2273697, and rs4680), each being positive, across regimens are found in Table 5. The *VKORC1* SNPs (rs934438 and rs9923231) were found to be in complete LD (r^2=1). Dolutegravir grouping did not yield association between SNPs and AST values. AST levels were associated SNPs within four genes (*VKORC1, CYP2C8, ABCC2,* and *CYP2E1*) across elvitegravir regimens with only two SNPs in LD (r^2=1). Raltegravir AST levels were deemed to be associated with three SNPs (rs2231142 and rs9282861 being positive and rs4244285 being negative).

Table 5: SNPs significantly associated with AST levels

Drug	Gene	SNP	Chromosome	BP[a]	A1[b]	MAF[c]	HWE[d]	Beta (95% CI)[e]	*p*-value
ALL	***VKORC1***	rs9934438	16	31104878	A	0.318	0.723	8.43 (2.86 — 14.00)	0.004
	VKORC1	rs9923231	16	31107689	T	0.318	0.723	8.43 (2.86 — 14.00)	0.004
	ABCC2	rs2273697	10	101563815	A	0.199	0.165	7.42 (1.44 — 13.39)	0.017
	COMT	rs4680	22	19951271	G	0.455	0.534	6.19 (0.82 — 11.55)	0.027
Elvitegravir	***VKORC1***	rs9934438	16	31104878	A	0.261	0.933	33.24 (16.68 — 49.80)	0.001
	VKORC1	rs9923231	16	31107689	T	0.261	0.933	33.24 (16.68 — 49.80)	0.001
	CYP2C8	rs1058930	10	96818119	G	0.043	1.000	82.01 (44.06 — 120.00)	0.001
	ABCC2	rs2273697	10	101563815	A	0.217	0.501	30.12 (13.76 — 46.47)	0.002
	VKORC1	rs7294	16	31102321	A	0.413	0.799	-28.99 (-47.70 — -10.28)	0.008
	CYP2E1	rs2070673	10	135340567	A	0.239	1.000	-29.60 (-56.83 — -2.36)	0.049
Raltegravir	***ABCG2***	rs2231142	4	89052323	A	0.109	0.429	8.53 (1.95 — 15.12)	0.025
	CYP2C19	rs4244285	10	96541616	A	0.174	1.000	-6.39 (-11.75 — -1.03)	0.036
	SULT1A1	rs9282861	16	28617514	A	0.304	0.619	6.20 (0.94 — 11.46)	0.038

[a]Physical position (bp); [b]Minor allele; [c]Minor Allele Frequency; [d]p-value for Hardy-Weinberg equilibrium test; [e]Beta for AST levels adjusted for covariates [sex, age, BMI, integrase inhibitor (in All group), integrase inhibitor duration, and baseline AST levels]

Renal Parameters

All mean blood urea nitrogen (BUN) levels (comprehensive group, 15.10±5.11 mg/dL; dolutegravir group, 14.66±4.55 mg/dL; elvitegravir group, 15.00±4.32 mg/dL; and raltegravir group, 16.05±6.74 mg/dL) were discovered as normal (8-21 mg/dL) (18). As mean BUN among the regimens was similar, no significant changes (p=0.592) were found. Numerous SNPs were associated with BUN levels (Table 6) in the comprehensive group; while no associations were found with dolutegravir. The top two SNPs in the comprehensive group rs4149117 and rs7311358 were found to be in LD (r^2=1); the third (rs1799930) and fourth (rs1041983) SNPs were highly related (r^2=0.882) as well; the four SNPs in *SLC15A2* were also in high LD (rs1143671 vs rs2293616/rs2257212, r^2=1; rs1143671 vs rs1143672, r^2=0.978); and the VKORC1 gene SNPs as mentioned in the AST section earlier. Elvitegravir associations were found between BUN levels and six SNPs. Several SNPs were discovered to be significant in association with raltegravir-dosed subject BUN levels. The LD was similar to the elvitegravir group with rs2293616 vs rs1143671/rs2257212 yielding r^2=1 and rs2293616 vs rs1143672 showing r^2=0.912; however, the *UGT2B7* SNPs (rs7662029 and rs7668258) in this group were also in LD (r^2=1).

Table 6: SNPs significantly associated with BUN levels

Drug	Gene	SNP	Chromosome	BP[a]	A1[b]	MAF[c]	HWE[d]	Beta (95% CI)[e]	*p*-value
ALL	***SLCO1B3***	rs7311358	12	21015760	G	0.205	0.014	1.65 (0.43 — 2.86)	0.010
	NAT2	rs1799930	8	18258103	A	0.335	0.192	-1.21 (-2.30 — -0.12)	0.033
	NAT2	rs1041983	8	18257795	T	0.364	0.169	-1.16 (-2.23 — -0.10)	0.036
	CYP2D6	rs1080985	22	42528382	G	0.216	0.337	2.00 (0.53 — 3.46)	0.009
	VKORC1	rs7294	16	31102321	A	0.455	0.502	1.62 (0.41 — 2.84)	0.011
	COMT	rs4680	22	19951271	G	0.455	0.534	-1.51 (-2.64 — -0.37)	0.011
	SLC15A2	rs2293616	3	121641693	C	0.483	0.966	1.29 (0.09 — 2.48)	0.039
	SLC15A2	rs2257212	3	121643804	G	0.483	0.966	1.29 (0.09 — 2.48)	0.039
	SLC15A2	rs1143671	3	121647286	C	0.483	0.966	1.29 (0.09 — 2.48)	0.039
	SLC15A2	rs1143672	3	121648168	G	0.477	0.804	1.29 (0.09 — 2.48)	0.039
	VKORC1	rs9934438	16	31104878	A	0.318	0.723	-1.27 (-2.48 — -0.06)	0.044
	VKORC1	rs9923231	16	31107689	T	0.318	0.723	-1.27 (-2.48 — -0.06)	0.044

[a]Physical position (bp); [b]Minor allele; [c]Minor Allele Frequency; [d]p-value for Hardy-Weinberg equilibrium test; [e]Beta for BUN levels adjusted for covariates [sex, age, BMI, integrase inhibitor, integrase inhibitor duration, and baseline BUN levels]

Table 6: SNPs significantly associated with BUN levels (continued)

Drug	Gene	SNP	Chromosome	BP[a]	A1[b]	MAF[c]	HWE[d]	Beta (95% CI)[e]	*p*-value
Elvitegravir	***CYP2D6***	rs1080985	22	42528382	G	0.217	0.575	4.48 (1.58 — 7.38)	0.008
	COMT	rs4680	22	19951271	G	0.391	0.329	-3.35 (-5.23 — -1.47)	0.003
	CYP2C9	rs28371685	10	94981224	T	0.022	1.000	8.84 (1.74 — 15.93)	0.027
	COMT	rs737865	22	19930121	C	0.196	0.329	-3.02 (-5.57 — -0.45)	0.035
	VKORC1	rs7294	16	31102321	A	0.413	0.799	3.85 (0.47 — 7.23)	0.040
	CYP2C8	rs1058930	10	96818119	G	0.043	1.000	-7.10 (-13.50 — -0.70)	0.045
Raltegravir	***CYP1A1***	rs1048943	15	75012985	G	0.022	1.000	10.52 (1.35 — 19.69)	0.044
	SLC15A2	rs1143672	3	121648168	G	0.370	1.000	3.75 (0.54 — 6.95)	0.041
	SLC15A2	rs2293616	3	121641693	C	0.391	1.000	3.75 (0.54 — 6.95)	0.041
	SLC15A2	rs2257212	3	121643804	G	0.391	1.000	3.75 (0.54 — 6.95)	0.041
	SLC15A2	rs1143671	3	121647286	C	0.391	1.000	3.75 (0.54 — 6.95)	0.041
	UGT2B15	rs1902023	4	69418747	T	0.435	0.049	-3.82 (-6.64 — -0.99)	0.021
	UGT2B7	rs7662029	4	69961912	A	0.413	0.799	3.42 (0.74 — 6.10)	0.028
	UGT2B7	rs7668258	4	69962078	T	0.413	0.799	3.42 (0.74 — 6.10)	0.028

[a]Physical position (bp); [b]Minor allele; [c]Minor Allele Frequency; [d]p-value for Hardy-Weinberg equilibrium test; [e]Beta for BUN levels adjusted for covariates [sex, age, BMI, integrase inhibitor, integrase inhibitor duration, and baseline BUN levels]

All estimated glomerular filtration rates (eGFR) were under the normal range of greater than 90 mL/min/1.73 m^2 (comprehensive group, 63.45±16.54 mL/min/1.73 m^2; dolutegravir group, 61.88±15.97 mL/min/1.73 m^2; elvitegravir group, 63.43±15.73 mL/min/1.73 m^2; and raltegravir group, 66.41±18.67 mL/min/1.73 m^2). Regimen differences in mean eGFR were not significant (p=0.590). Exploration of eGFR uncovered three SNPs (Table 7) that presented significant positive association across regimens (rs4986989, rs34130495, and rs3213619). Significance was not found concerning eGFR levels in dolutegravir dosing. In elvitegravir group analysis, five different SNPs were associated following multiple regression; however, rs28371686 and rs28399454 as well as rs4149117 and rs7311358 were in strong LD (r^2=1). Each SNP, apart from rs717620, was associated with a decrease of eGFR with each minor allele. Evaluation of the raltegravir group identified no SNPs that were associated with eGFR.

Table 7: SNPs significantly associated with eGFR

Drug	Gene	SNP	Chromosome	BP[a]	A1[b]	MAF[c]	HWE[d]	Beta (95% CI)[e]	*p*-value
ALL	***NAT1***	rs4986989	8	18222008	T	0.023	1.000	14.81 (2.65 — 26.97)	0.019
	SLC22A1	rs34130495	6	160560824	A	0.017	1.000	13.01 (0.83 — 25.19)	0.039
	ABCB1	rs3213619	7	87230193	C	0.028	1.000	10.20 (0.27 — 20.12)	0.048
Elvitegravir	***CYP2C9***	rs28371686	10	96741058	G	0.022	1.000	-20.67 (-36.33 — -5.01)	0.019
	CYP2A6	rs28399454	19	41351267	A	0.022	1.000	-20.67 (-36.33 — -5.01)	0.019
	SLCO1B3	rs4149117	12	21011480	T	0.283	0.069	-5.08 (-9.27 — -0.89)	0.029
	SLCO1B3	rs7311358	12	21015760	G	0.283	0.069	-5.08 (-9.27 — -0.89)	0.029
	ABCC2	rs717620	10	101542578	A	0.130	1.000	7.74 (0.83 — 14.64)	0.041

[a]Physical position (bp); [b]Minor allele; [c]Minor Allele Frequency; [d]p-value for Hardy-Weinberg equilibrium test; [e]Beta for eGFR adjusted for covariates [integrase inhibitor (in All group), integrase inhibitor duration, and baseline eGFR]

Discussion

The purpose of this study was to examine the effects of genetics, adjusted for individual patient characteristics, on systemic INSTI exposure along with hepatic and renal tolerability. We found that in this population of primarily Caucasian male subjects that the majority were virally suppressed and tolerating their respective regimens (Table 1).

The concentration of dolutegravir we found was below the geometric means previously reported as 1070 ng/mL and 1500 ng/mL for dolutegravir (19) and dolutegravir/abacavir/lamivudine (20), respectively. Based on reported geometric mean elvitegravir concentrations of 490 ng/mL, in the formulation containing tenofovir disoproxil fumarate (21), and 290 ng/mL, in the tenofovir alafenamide formulation (22), our mean concentration was somewhat less. Our average raltegravir C_{trough} concentration of 567.16 ng/mL was much higher than the geometric mean 142 nM (approximately 68.52 ng/mL) previously reported (23). The reductions seen in our study may have several explanations ranging from subject reported dosing to differences in food intake (6, 24).

None of the SNP-associations that we found with dolutegravir have been reported elsewhere (Table 2). Additionally, none of these genes are known to interact with dolutegravir. Concerning *ABCB1* SNP (rs1045642), an absence of correlation has been reported with dolutegravir plasma concentration (25) and this supports the absence of the association in seen in our study. The high variability within dolutegravir concentrations coupled with small sample size and distribution of genotypes necessitate confirmation, but the presence of associations may serve as a road map to future discovery. These SNP-associations would be very important to confirm as the effects are rather large (Table 2).

The absence of any associations in the elvitegravir and raltegravir groups may be a function of further reduced sample size through inability to detect drug concentrations. Alternatively, the reason could be a property of the selected SNPs. UGT1A1*28/*28 (AA in rs8175347) has been shown to yield higher raltegravir in blood (26); UGT1A1*28 has produced a mild reduction in elvitegravir clearance (27); and homozygous UGT1A1*28 has also been shown to increase dolutegravir exposure (28). This SNP; however, was not included on the panel. Also some of the included SNPs, 1128503, rs2032582, rs1045642, and rs2231142, have previously been found to not change raltegravir C_{trough}, but they did find alterations in peak concentrations (25). We would not have seen this effect as we only took C_{trough}.

In this study, no regimen grouping of subjects showed a mean ALP level outside the normal range which suggests that these associations may not be clinically significant unless other factors are involved. In a previous study by Tebas *et al.*, dolutegravir, when co-administered with abacavir and lamivudine, was shown to increase bone-specific alkaline phosphatase by a 50% change from baseline following 144 weeks of administration (29). Additionally, when switching from an efavirenz-based regimen to a raltegravir-based regimen, serum ALP was significantly decreased in the raltegravir group compared to an efavirenz group at 24 weeks (30). In our study, the alkaline phosphatase was nonspecific; however, an increase in the bone-specific form would elevate the nonspecific form. It seems that none of the SNPs, which showed significance in this study, have been previously reported to influence ALP levels (Table 3). Although many showed high variability, most, apart from rs7294, found that adding a minor allele would tend to increase ALP levels. The genes identified with ALP levels in this study include transporters (ABCC2) and metabolizing enzymes (CYP2C8) (31). Thus, these changes may occur through drug remaining in hepatocytes rather than being pumped out.

ALT levels were not significantly altered among treatment groups. None of the ALT-SNP associations (Table 4) we presented have been previously reported to the best of our knowledge. A study conducted in a Japanese population did find that female subjects with homozygous A alleles in rs4680 had lower odds ratios, when using logistic regression, of having elevated ALT levels compared to both homozygous G alleles and heterozygous individuals with a similar trend was found in males aged 45-54 (32). We did not see an association with rs4680 in any of our groups for ALT levels. In our study, the transporter SLC22A1 appeared frequently which may indicate, as noted earlier with ALP, that drug accumulation may play a role in these associations.

Although none of the mean AST levels were above normal, the elvitegravir group had a very high variation. This variation may have inflated the betas for the multiple linear regression results at least for the comprehensive and the elvitegravir groups. Otherwise none of the presented SNPs (Table 5) have been reported elsewhere in relation to AST levels. As suggested with ALP and ALT, drug accumulation or lack thereof may explain the minor alterations in hepatic function enzymes. Overall, these regimens appear to be relatively well tolerated in terms of hepatic outcomes. However, some of the polymorphisms may have a large effect on respective marker levels. Thus, these may need to be monitored closely in certain patients.

In regard to renal effects, many SNP associations were found with BUN levels (Table 6); however, most effects were minor. There were a large number of SNPs in LD within these groupings which lowers the number of useful polymorphisms; however, these SNPs have not been previously identified as relating to BUN. Even if these SNPs associations are confirmed, the likelihood of clinical significance is small unless other problem factors are present.

Mean eGFR was lower than normal across all groups; however, this is common in those receiving antiretrovirals. While eGFR did not seem to be related to dolutegravir or raltegravir alone, the three SNPs in the comprehensive group seemed to improve renal function (Table 7). Meanwhile, the associated SNPs found in elvitegravir each lowered eGFR. These may need to be examined more closely to protect against renal insufficiency. As all of the elvitegravir groups contained a form of tenofovir, especially tenofovir disoproxil fumarate, as well as cobicistat, these lower values may be a function of renal damage or creatinine clearance changes (7, 33). In addition, alterations in the movement of drugs in renal tubular cells may have contributed to the associations identified.

The strong LD of certain SNPs in this population would allow for the use of one SNP to cover the presence of both if r^2 equals 1, which may be useful in the reduction of redundant genotyping. In future studies which seek to analyze these exploratory associations, SNPs which show LD may need to undergo haplotype analysis to further understand associations.

This study had a few limitations. The first being the low sample size, in terms of regimen group size and the occurrence of different alleles, which may have influenced study outcomes. Another limitation is the relatively homogenous population, predominately Caucasians which may prevent analysis of SNPs that occur more frequently in different races; however, population specific SNPs may be examined in greater numbers.

In conclusion, we determined that several SNPs found on the iPLEX panel were significantly associated with various patient outcomes. The large number of previously unreported associations may be due to the absence of clinical significance which may have precluded publication. More studies are needed, preferably in a larger population to determine the clinical applicability of our findings.

Methods

Subject recruitment

All personnel, involved in patient contact and/or private health information use, received the necessary training through the Collaborative Institutional Training Initiative program and various other programs for ETSU/Veterans Affairs (VA) Medical Campus Institutional Review Board (IRB) approval. Recruitment for this observational study was conducted between July 2015 and February 2017. Those individuals, being HIV-1 positive, who met the inclusion criteria (≥18 years old, non-pregnant, and receiving an INSTI regimen) were contacted by phone. Interested subjects were advised of a requisite regimen dosing schedule which would allow for the capture of the C_{trough} required for this study without influencing antiretroviral efficacy. Informed consent was obtained in the presence of at least one investigator and witness. A review of subject electronic health records was also performed. Subjects were compensated with a gift card, with documentation of receipt, at the completion of study participation.

Dolutegravir regimens consisted of the single tablet regimen of dolutegravir (50 mg)/abacavir (600 mg)/lamivudine (300 mg), dolutegravir (50 mg) plus tenofovir disoproxil fumarate (300 mg)/emtricitabine (200 mg), or dolutegravir (50 mg) plus tenofovir alafenamide (25 mg)/emtricitabine (200 mg). Subjects on an elvitegravir regimen received one of two once-a-day forms [elvitegravir (150 mg)/cobicistat (150 mg)/tenofovir disoproxil fumarate (300 mg)/emtricitabine (200 mg) or elvitegravir (150 mg)/cobicistat (150 mg)/tenofovir alafenamide (10 mg)/emtricitabine (200 mg)]. Raltegravir was given twice-a-day concurrently with once-a-day tenofovir disoproxil fumarate (300 mg)/emtricitabine (200 mg) or tenofovir alafenamide (25 mg)/emtricitabine (200 mg). A form of the tenofovir/emtricitabine background was used in over

67% over subjects. Other antiretrovirals, such as protease inhibitors, were included in some patients. Background regimens were not included in analysis.

Clinical Tolerability Analysis

Baseline values for hepatic and renal parameters were taken at the closest available point prior to starting the regimen of interest; while current parameters were taken from the closest possible point relative to sample collection. Sample collection and previous dosing times were recorded in the questionnaire. Estimated glomerular filtration rate (eGFR) was calculated using the 2009 Chronic Kidney Disease-Epidemiology Collaboration (CKD-EPI) based upon plasma creatinine. Comparison between regimens was conducted using one-way ANOVA with SPSS 25.

Drug Exposure Analysis

The C_{trough} measurement has been used in previous studies with success and is less invasive than traditional inpatient pharmacokinetic sampling (34, 35). Trained phlebotomists collected whole blood sample (20 mL) at the end of respective regimen dosing intervals (24 hours for dolutegravir and elvitegravir as opposed to 12 hours for raltegravir). Plasma, for pharmacokinetic analysis, and remaining cells, for genetic testing, were separated then placed at -80 °C until analysis.

Samples were analyzed using an LC-MS method developed by Simiele *et al.* with modifications (36). Briefly, a standard curve ranging from 10 ng/mL to 1,500 ng/mL was created for drugs of interest from respective stock solutions in a 50:50 ratio of acetonitrile and water, using blank human plasma (Innovative Research Inc Novi, MI). Verapamil, a drug which no subject was concurrently receiving, was used as the internal standard in acetonitrile and 1% formic acid in water (80:20). Pharmacokinetic samples (1 mL) underwent pH viral inactivation

over a period of 1 hr at ambient temperature (23 °C) at a pH of 4, achieved using the addition of 100 µL of 1M HCL (37). One hundred microliters of internal standard were added then samples were vortex mixed. Sample (200 µL) and 0.1% formic acid in acetonitrile (600 µL) were aspirated in Ostro pass-through sample preparation 96-well plates (Waters Corporation, Milford, MA). Collected samples underwent direct chromatography with a Waters X-Select HSS T3 column (150 x 4.6 mm, 3.5 micron) and a gradient of 0.1% formic acid in water (A) and 0.1% formic acid in acetonitrile (B) (5-100 %). Mass spectrometric detection was achieved using direct MS/MS channels for each drug, specific to their $[M+H]^+$ ion. All solvents used for LC-MS/MS analysis were of LC-MS Grade from Honeywell-Burdick and Jackson (Muskegon, MI). Stock chemicals were purchased from Chemscene (Monmouth Junction, NJ).

Pharmacogenetic Analysis

A Sequenom iPLEX® ADME PGx Panel v1.0 (Sequenom, San Diego, CA) comprised of assays for numerous genetic areas of interest, was used to evaluate the genetic profile of each subject. DNA extraction and genotyping were performed at the Vanderbilt Technologies for Advanced Genomics (VANTAGE) according to manufacturer specifications. Briefly, following extraction from whole blood (Autopure LS, QIAGEN, Hilden, Germany), DNA was amplified via PCR then free nucleotides were dephosphorylated. The iPLEX Gold reaction, being the addition of a primer to the site of interest which is then extended by one nucleotide based on genotype, was conducted. A MassARRAY® Analyzer returned subject alleles and the MassARRAY Typer was used to determine genotype call rates. Hardy-Weinberg equilibrium (HWE), minor allele frequency (MAF), and pairwise linkage disequilibrium (LD) statistics (r^2) were assessed using HAPLOVIEW software (38).

SNPs are represented with the more frequent allele in the specific sampling being identified as the reference; while the less frequent allele was identified as the minor allele (reference allele>minor allele). Subject genotypes were analyzed using the additive genetic association model. As such the dosage of minor allele was considered to have an additive effect for example homogenous major alleles were coded as 1, heterogenous alleles as 2, and homogenous minor alleles as 3. Then, to test for association with quantitative traits, multiple linear regression, with the inclusion of covariates [age, sex, BMI, regimen (in the across regimen group), regimen duration, and baseline variables (as required)], were performed by PLINK v1.07 to obtain the regression coefficient and *p*-value (39). Correction for multiple testing was not conducted due to the exploratory nature of this study (40). Subject information, following de-identification, was uploaded into the ETSU version of REDCap (41).

Study Highlights

o What is the current knowledge on the topic?

Pharmacogenetics play an important role in selected outcomes of drug dosing.

o What question did this study address?

This study sought to explore the relationship of selected single nucleotide polymorphisms (SNPs) with drug exposure as well as respective patient hepatic and renal effects in subjects receiving HIV integrase strand transfer inhibitors (INSTIs).

o What does this study add to our knowledge?

Several SNPs were identified that had previously been unrelated to clinical variables in INSTIs. This exploratory study seeks to slightly expand the frame through which researchers are looking to promote new lines of inquiry.

o How might this change clinical pharmacology or translational science?

The associations found in this study may spark interest in otherwise uncritically explored genomic areas. If these results are supported and expanded in larger trials, new suggestions and/or precautions for INSTI dosing may be developed.

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CHAPTER 3

ASSOCIATION OF PHARMACOGENETICS AND HIV INTEGRASE INHIBITOR ADVERSE EVENTS: AN EXPLORATORY STUDY

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Abstract

Dolutegravir, elvitegravir, and raltegravir, are widely utilized HIV integrase strand transfer inhibitors (INSTIs). As side effect occurrence varies among patients receiving these drugs, we investigated the role of several single nucleotide polymorphisms (SNPs) in subject-reported adverse events. SNPs underwent multiple logistic regression for association ($p<0.05$) with binary traits including central nervous system-related (abnormal dream, anxiety, fatigue, headache, and insomnia) and gastrointestinal-related (diarrhea and nausea) adverse events adjusted for age, sex, BMI, and regimen duration along with specific regimen in the comprehensive group (included all patients). HIV+ adults (≥18 years old) receiving an INSTI were recruited (n=88). Abnormal dream occurrence was found to be associated ($p=0.028$) with regimen-received. Additionally, several SNPs were found to be associated with adverse event profiles primarily in the comprehensive group. In conclusion, the associations found in this study strengthen the need for further assessment, within the HIV positive population, of factors contributing to unfavorable patient outcomes.

Introduction

After nearly four decades, the human immunodeficiency virus (HIV) remains a high priority in scientific research with thousands of new infections occurring each day adding to the upward of 36 million HIV positive individuals worldwide (1). Current antiretroviral therapy is highly effective in reducing plasma HIV viral load; however, several factors may impact the efficacy and tolerability of antiretrovirals (2-4). Of the four currently available integrase strand transfer inhibitors (INSTIs), clinical experience is greatest with dolutegravir, elvitegravir, and raltegravir (5). Nausea and headache are the most frequent adverse events associated with dolutegravir (6) with neuropsychiatric adverse events in general having been reported as reasons for changing regimens (7). Meanwhile, elvitegravir and raltegravir also show central nervous system (CNS) as well as gastrointestinal (GI) adverse events (8-10).

While many factors contribute to the patient outcomes, variations in the genetic make-up of an individual may also alter the behavior of some drugs resulting in differences in both efficacy and toxicity (11). For example, reduction in the activity and/or expression of metabolic enzymes or transporters may greatly influence drug pharmacokinetics and thereby alter patient outcomes (12-14). As adverse effect profiles often play an important role in the selection and maintenance of antiretroviral therapy, we sought to identify associations between the occurrence of CNS and GI adverse events with single nucleotide polymorphisms (SNPs) in an exploratory cohort of patients receiving currently available INSTIs.

Results

Eighty-eight HIV positive adults (comprehensive group), differentiated by INSTI (regardless of nucleoside backbone), dolutegravir group (n=42, 88.1% male), elvitegravir group (n=23, 87.0% male), or raltegravir group (n=23, 82.6% male) were recruited. Further

demographic information has been previously reported by our group (15). Adverse event occurrence stratified by genotype, in significant associations, are shown in supplemental tables.

Central Nervous System Adverse Events

Abnormal Dream Occurrence. With a significant association (p=0.028) between regimen and adverse event occurrence, abnormal dreams were reported more frequently in the raltegravir group (30.4%) compared with either the elvitegravir (4.3%) or the dolutegravir group (9.5%). One SNP (rs1143672) was determined to have a protective (decreased occurrence likelihood) association with abnormal dreams in the comprehensive group (Table 1). Dolutegravir and elvitegravir stratification yielded no significant SNPs; while one SNP (rs1128503) was associated with decreased abnormal dream occurrence in the raltegravir group when minor alleles were present.

Table 1: SNPs significantly associated with CNS adverse event occurrence

CNS adverse event	Drug	Gene	SNP	Chromosome	BP[a]	A1[b]	MAF[c]	HWE[d]	OR (95% CI)[e]	*p*-value
Abnormal Dreams	**ALL**	***SLC15A2***	rs1143672	3	121648168	G	0.477	0.804	0.31 (0.11 — 0.93)	0.037
	Raltegravir	***ABCB1***	rs1128503	7	87179601	T	0.370	0.644	0.07 (0.01 — 0.98)	0.049
Anxiety	**ALL**	***SLC15A2***	rs1143672	3	121648168	G	0.477	0.804	0.45 (0.22 — 0.92)	0.028
		VKORC1	rs9934438	16	31104878	A	0.318	0.723	2.08 (1.01 — 4.30)	0.048
		VKORC1	rs9923231	16	31107689	T	0.318	0.723	2.08 (1.01 — 4.30)	0.048
Fatigue	**ALL**	***ABCB1***	rs1128503	7	87179601	T	0.358	0.764	0.33 (0.15 — 0.76)	0.009
		CYP2E1	rs2070673	10	135340567	A	0.210	1.000	2.62 (1.14 — 6.01)	0.023
		UGT2B15	rs1902023	4	69418747	T	0.494	0.181	2.08 (1.10 — 3.93)	0.025
	Raltegravir	***CYP2D6***	rs3892097	22	42524947	A	0.152	1.000	20.59 (1.51 — 280.40)	0.023
		CYP2D6	rs1065852	22	42526694	T	0.174	1.000	16.40 (1.21 — 222.90)	0.036
		SLC22A2	rs316019	6	160670282	T	0.109	1.000	17.83 (1.07 — 297.10)	0.045

[a]Physical position (bp); [b]Minor allele; [c]Minor Allele Frequency; [d]p-value for Hardy-Weinberg equilibrium test; [e]Odds ratio for CNS adverse event occurrence adjusted for covariates [sex, age, BMI, integrase inhibitor (in All group), and integrase inhibitor duration]

Table 1: SNPs significantly associated with CNS adverse event occurrence (continued)

CNS adverse event	Drug	Gene	SNP	Chromosome	BP[a]	A1[b]	MAF[c]	HWE[d]	OR (95% CI)[e]	*p*-value
Headache	**ALL**	***GSTP1***	rs1695	11	67352689	G	0.347	1.000	3.53 (1.46 — 8.55)	0.005
		SLCO1B1	rs2306283	12	21329738	G	0.477	0.499	0.42 (0.19 — 0.92)	0.031
		ABCB1	rs3213619	7	87230193	C	0.028	1.000	18.37 (1.86 — 181.90)	0.013
Insomnia	**ALL**	***VKORC1***	rs9934438	16	31104878	A	0.318	0.723	2.70 (1.14 — 6.38)	0.024
		VKORC1	rs9923231	16	31107689	T	0.318	0.723	2.70 (1.14 — 6.38)	0.024

[a]Physical position (bp); [b]Minor allele; [c]Minor Allele Frequency; [d]p-value for Hardy-Weinberg equilibrium test; [e]Odds ratio for CNS adverse event occurrence adjusted for covariates [sex, age, BMI, integrase inhibitor (in All group), and integrase inhibitor duration]

Anxiety Occurrence. Anxiety was reported in 29.5% of the comprehensive grouping; while dolutegravir, elvitegravir and raltegravir groups presented with 33.3%, 17.4%, and 34.8%, respectively. No significant interaction was found between regimen and anxiety occurrence (p=0.368). In the comprehensive group, three SNPs, one protective (rs1143672) and two increasing occurrences (rs9934438 and rs9923231), showed association with increased anxiety occurrence (Table 1). Additionally, rs99234438 was in LD (r^2=1.00) with rs9923231. When regimens were tested as individual groups, there were no significantly associated SNPs.

Fatigue Occurrence. The occurrence of fatigue was frequent in each of the groups (comprehensive; 33.0%; dolutegravir; 33.3%; elvitegravir, 30.4%; and raltegravir; 34.8%). As such, no association was found between fatigue and regimen (p=1.000). Three SNPs, rs1128503 (decreased occurrence), rs2070673 (increased occurrence) , and rs1902023 (increased occurrence), were associated with fatigue (Table 1) in the comprehensive group. Concerning the dolutegravir and elvitegravir patients, fatigue occurrence was not associated with any SNPs following multiple logistic regression. Raltegravir regimen grouping revealed three SNP associations (rs3892097, rs1065852, and rs316019) with increased fatigue occurrence.

Headache Occurrence. Headaches occurred most frequently in the dolutegravir group (33.3%) followed by the raltegravir group (17.4%) then the elvitegravir group (8.7%) with overall occurrence being 22.7% of all patients. No association (p=0.066) was determined between regimen and occurrence. While no logistic associations were found among the individual regimens, three SNP-headache occurrence associations (Table 1) were discovered in the comprehensive group. These ranged to from decreasing occurrence (rs3213619) to slight elevation (rs1695) to larger increased frequency of occurrence (rs3213619).

Insomnia Occurrence. Insomnia was reported in 19.3% of patients overall which was matched well with the 19.0% seen in dolutegravir. Meanwhile, elvitegravir and raltegravir demonstrated differing rates with 8.7% and 30.4%, respectively. No association (p=0.190) was determined to exist through Fisher's exact testing. As shown in Table 1, one gene contained SNPs (rs9934438 and rs9923231) which, when analyzed across regimens in the comprehensive group, were significantly associated with increased insomnia occurrence. As noted earlier the SNPs, rs9934438 and rs9923231, were in LD (r^2=1.00). Individual drug groups showed no associations.

Gastrointestinal adverse events

Diarrhea Occurrence. With an overall occurrence in 29.5% of patients, dolutegravir, elvitegravir, and raltegravir grouping yielded 30.9%, 26.1%, and 30.4%, respectively. As the presence of diarrhea was rather evenly distributed among groups, no association (p=0.955) between this adverse event and regimen was found. One SNP (rs4680) exhibited a protective association in the comprehensive group (Table 2); meanwhile, no other diarrhea-SNP associations were found.

Table 2: SNPs significantly associated with GI adverse event occurrence

GI Adverse Event	Drug	Gene	SNP	Chromosome	BP[a]	A1[b]	MAF[c]	HWE[d]	OR (95% CI)[e]	*p*-value
Diarrhea	**ALL**	***COMT***	rs4680	22	19951271	G	0.455	0.534	0.42 (0.19 — 0.91)	0.027
Nausea	**ALL**	***GSTP1***	rs1695	11	67352689	G	0.347	1.000	2.82 (1.09 — 7.29)	0.033
		NAT2	rs1208	8	18258316	G	0.398	0.447	2.64 (1.07 — 6.50)	0.034

[a]Physical position (bp); [b]Minor allele; [c]Minor Allele Frequency; [d]p-value for Hardy-Weinberg equilibrium test; [e]Odds ratio for GI adverse event occurrence adjusted for covariates [sex, age, BMI, integrase inhibitor (in All group), and integrase inhibitor duration]

Nausea Occurrence. Nearly one in five of all patients (18.2%) experienced nausea. Dolutegravir grouping exhibited slightly higher occurrence at 23.8%; while elvitegravir and raltegravir both showed an occurrence of 13.0%. No significance (p=0.459) was found between regimen and nausea occurrence. In the cumulative regimen group, two SNPs, rs1695 and rs1208 (Table 3), were associated with increased nausea occurrence; however, individual drug grouping eliminated associations.

Discussion

The purpose of this study was to examine the effects of individual characteristics on adverse event occurrence in the use of INSTI regimen. Successful completion of this project sought to identify methods for predicting and possibly preventing negative outcomes.

Central Nervous System Adverse Events

Abnormal Dream Occurrence. When dolutegravir (50 mg) was dosed once daily to healthy patients over five days, 8% (1/12) of patients experienced abnormal dreams (16). This report is similar to the finding in our cohort at 9.5%. The occurrence of abnormal dreams in elvitegravir found in our study (4.3%) was lower than those previously reported by Wohl *et al.*. That study reported 15% and 16% occurrence in a sample size of 348 patients following 96 and 144 weeks, respectively, when elvitegravir in combination with cobicistat, emtricitabine, and tenofovir disoproxil fumarate was dosed (8). More patients may have had a higher frequency of alternate genotypes than those observed in our study. In the STARTMRK and BENCHMRK trials under all causalities, 7.5% and 0.9% experienced abnormal dreams when taking raltegravir (17). This was much lower than our 30.4%.

The SNP, rs1143672, appears to have not been previously associated with abnormal dreams. The *ABCB1* SNPs which were associated with abnormal dream occurrence in the raltegravir group may be related to an alteration of raltegravir concentrations in cerebrospinal fluid (18). A higher, although nonsignificant (p=0.4419), trough concentration of raltegravir has been reported in patients with genotypes differing from the homozygous G alleles (255±161 ng/mL vs 441±525 ng/mL) (19). In that study, rs1128503 did not influence raltegravir trough concentration nearly as much (480±348 ng/mL vs 404±505 ng/mL; p=0.8019). The protective effects seen in our study should be studied further to determine the interaction.

Anxiety Occurrence. In a 96 weeks study of dolutegravir plus a nucleoside reverse transcriptase inhibitor backbone, 5% of patients presented with anxiety (20); while in a study using dolutegravir/abacavir/lamivudine, anxiety has been reported in 2% of patients (21). The difference seen in our study (33.3%) could be the duration of the specific regimens or the influence of concurrent medications drugs. The percentage of patients presenting with anxiety occurrence in our study (17.4%) was higher than those reported previously with elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate dosing 6% (94) and 10% (23). This could be the inclusion of other medications which were not considered in this study. In the SAILING study concerning raltegravir, 2% experienced anxiety; while 6% was discovered in the SPRING-2 ART cohort (24). The occurrence (34.8%) was much higher in our raltegravir grouping.

Our results indicate that, overall, anxiety occurred across the tested INSTIs. The protective effect of rs1143672, in the SLC15A2 gene, with both abnormal dreams and anxiety may indicate changes in drug transport are contributing to these adverse events. *VKORC1* SNPs occur frequently across variables; however, the mechanism of interaction, if valid, is unknown.

Fatigue Occurrence. The percentage of patients experiencing fatigue while taking dolutegravir in our study was 33.3%; while fatigue occurred in 6% and 4% of dolutegravir patients in two previous studies by Molina *et al.* (20) and Cahn *et al.* (9), respectively. In previous clinical studies concerning elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate, fatigue was reported in 13% and 15% of patients at 96 weeks and 144 weeks, respectively (8). Even in the elvitegravir group, our findings of fatigue were twice (30.4%) those in the previous study. Fatigue was reported in 7% of raltegravir patients in one study (9) and 3.9% of the patients from the STARTMRK study (17); while our values were more than five times higher at 34.8%. One explanation of the high values in our study is that we relied on patient reported occurrence without expansive clinical examination. Thus, the fatigue may have alternate causes. Regardless of mechanism, we found no association between regimen and fatigue occurrence in this population.

The SNPs associated with fatigue in the comprehensive group showed relatively low variation as opposed to those in the raltegravir group. The results of this study seem to indicate that a few SNPs can strongly predict fatigue occurrence with raltegravir; however, the extent of the 95% confidence intervals are quite high. Upon confirmation in a larger population, the variation will likely be reduced as more individuals with differing genotypes may be present.

Headache Occurrence. Seventeen percent of patients reported headaches when dosed with dolutegravir (20) compared with 9% in another study (9); while 3% experienced headaches when given dolutegravir/abacavir/lamivudine (21). All values were much lower than that observed in our study (33.3%). Our 8.7% occurrence of headaches within the elvitegravir group was much lower than the 16% (at 96 weeks) and 18% (at 144 weeks) occurrence when given the elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate regimen (8). Headache

occurrence was reported in 9% of raltegravir patients in one study (9); while the STARTMRK study yielded 9.3% (20). Both lower than our 17.4%. Although different, no significant association between regimen and outcome was not found which matches our results of no association within individual groups. The strong positive association seen with rs3213619, which likely occurs due to alteration in drug transport, had high variability; however, upon confirmation, this SNP may be clinically important for those wishing to avoid headaches.

Insomnia Occurrence. Molina and colleagues reported insomnia in 8% of patients receiving dolutegravir (20); meanwhile, Walmsley *et al*. showed 4% occurrence when using dolutegravir/abacavir/lamivudine (21). This is less than half of the percentage found in our study (19.0%). Our insomnia occurrence (8.7%) was somewhat less than the 11% and 12% reported for elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate at 96 and 144 weeks (8). Also, the four percent of patients experienced moderate-to-severe insomnia in a previous study concerning raltegravir (25) was much lower than our 30.4%. This may have been because we included any occurrence of insomnia rather than moderate to severe. The high variability across regimens would explain the absence of significant association between regimen and insomnia. Once again *VKORC1* shows association, but the interaction is unknown.

Gastrointestinal Adverse Events

Diarrhea Occurrence. Nearly one third of our patients (30.9%) reported diarrhea in the dolutegravir group. Previously 18% of dolutegravir receiving patients reported diarrhea in the study by Molina *et al.* (20) and 20% in the study by Cahn and colleagues (9); meanwhile 5% was reported when dolutegravir/abacavir/lamivudine was given (21). We found nearly identical occurrence (26.1%) of diarrhea as those reported for elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate (25% and 26%) at 96 and 144

weeks (8). Diarrhea was reported in 18% of raltegravir patients (9); while the STARTMRK study reported 5.0% (20). The values are much smaller than our 30.4%. The distribution of diarrhea in our patients was relatively uniform; thus, explaining the lack of association between regimen and diarrhea. As catechol-o-methyltransferase, which had a significant SNP in the comprehensive analysis, is involved in neurotransmitter metabolism the mechanism of interaction with diarrhea not evident; however, the effect is not large (26).

Nausea Occurrence. Nausea was reported in 17% of patients receiving dolutegravir alone (20); 8% in another study (9); and 2% in a dolutegravir/abacavir/lamivudine study (21). While in our study 23.8% of patients reported nausea, this was similar to the first study, but much larger than the latter two. Previously, elvitegravir/cobicistat/emtricitabine/tenofovir disoproxil fumarate showed 22% (96 weeks) and 23% (144 weeks) occurrence of nausea (8). These values are actually a bit larger than our 13%. Nausea has been previously reported in 8% of raltegravir patients (9) and 8.5% in the STARTMRK results (20). Once again, our value is a bit higher at 13%. As the values reported in our study are relatively close across regimens, no association was found in Fisher's exact testing. The associated SNPs in the comprehensive group both increase the likelihood of nausea, but the mechanism unclear.

In addition to the limitations previously reported (15), such as small sample size, this study depended on patient-reported adverse event occurrence which may be limited by patient recall. These events were also left to the determination of the patient rather than clinical workup. Also, we may have missed important SNPs as this was not a genome wide association study.

These results indicate that pharmacogenetics may play an important role in predicting the adverse effect profile of integrase inhibitor-based regimens. As the identification of patient outcome determinants contributes to better utilization of medication, especially in first line

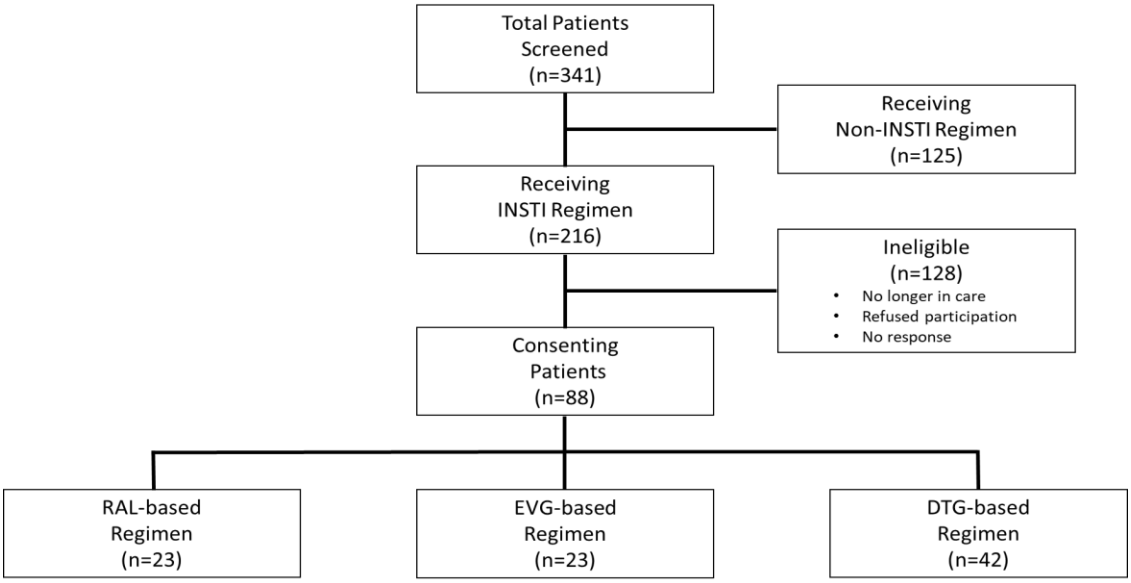
therapies such as INSTIs, these exploratory findings provide support for further examination of precision medicine in HIV pharmacotherapy. In conclusion, the associations found in this study strengthen the need for further assessment within the HIV positive population of factors contributing to unfavorable patient outcomes.

Methods

Subject Recruitment

This study, as previously reported (15), was approved by the East Tennessee State University (ETSU)/VA Medical Campus Institutional Review Board (IRB). Patients were recruited as delineated in Figure 1. Briefly, non-pregnant adults (≥18 years) on an INSTI regimen for HIV-1 were eligible. Following the receipt of informed consent, side effect occurrence, including central nervous system-related (abnormal dream, anxiety, fatigue, headache, and insomnia) and gastrointestinal-related (diarrhea and nausea), was treated as a binary outcome (occurrence vs nonoccurrence) regardless of frequency was assessed with a questionnaire.

Figure 1: Subject Recruitment Schematic



Dolutegravir grouping included patients receiving 50 mg of dolutegravir in combination with abacavir (600 mg)/lamivudine (300 mg) in a single tablet or dosed with either tenofovir disoproxil fumarate (300 mg)/emtricitabine (200 mg) or tenofovir alafenamide (25 mg)/emtricitabine (200 mg). The elvitegravir grouped patients were administered either elvitegravir (150 mg)/cobicistat (150 mg)/tenofovir disoproxil fumarate (300 mg)/emtricitabine (200 mg) or elvitegravir (150 mg)/cobicistat (150 mg)/tenofovir alafenamide (10 mg)/emtricitabine (200 mg) once daily. Raltegravir (400 mg) taken every 12 hours along with tenofovir disoproxil fumarate (300 mg)/emtricitabine (200 mg) or tenofovir alafenamide (25 mg)/emtricitabine (200 mg) every 24 hours. Some patients were also receiving protease inhibitors in addition to the INSTI and background regimens.

Pharmacogenetic Analysis

An iPLEX® ADME PGx Panel v1.0 (Sequenom, San Diego, CA) was utilized to interrogate patient SNPs. HAPLOVIEW software was used to determine Hardy-Weinberg equilibrium (HWE), minor allele frequency (MAF), and pairwise linkage disequilibrium (LD) statistics (r^2) (27). The additive genetic association model was employed in this study with the most frequent allele being considered as a reference.

Statistical Analysis

De-identified patient information was stored in the ETSU version of REDCap (Research Electronic Data Capture), a secure web-based application (28). Fisher's exact tests were used to evaluate independence of binary adverse event outcomes among regimens and genotypes using SPSS v25 (29). Clinical outcome associations with genotype were analyzed using multiple logistic regression with inclusion of covariates [age, BMI, sex, regimen duration, and specific regimen (in the comprehensive group)] via PLINK v1.07 (30). A p-value of greater than 0.05 was set as the statistical cutoff. Correction for multiple testing across Fisher's exact tests and logistic regression was not conducted due to the exploratory nature of this study (31).

Study Highlights

o What is the current knowledge on the topic?

HIV antiretrovirals, which successfully suppress viral load, require life-long dosing. As such, the occurrence of adverse events and the avoidance thereof have risen in importance. The first three available integrase strand transfer inhibitors (INSTI) have each displayed, although relatively rare, discontinuation-worthy adverse events.

o What question did this study address?

This study sought to analyze genetic influence, through single nucleotide polymorphism (SNP) association, on the occurrence of such adverse events in INSTI dosing.

o What does this study add to our knowledge?

Abnormal dreams may be related to raltegravir. Additionally, several SNP associations were discovered with various central nervous system gastrointestinal adverse events. These results may point to additional considerations in regimen selection.

o How might this change clinical pharmacology or translational science?

These associations, if confirmed in larger studies, may help practitioners to tailor patient regimens with greater accuracy.

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CHAPTER 4

CONCLUSIONS

The purpose of this study was to test our hypothesis that particular drug outcomes would be influenced by the pharmacogenetics of an individual. In order to complete this project, we evaluated INSTI drug exposure in HIV-1 patients, documented genetic polymorphisms, collected clinical outcomes, and performed association analyses.

Drug Exposure

As outlined in Chapter 2, subjects in our study underwent C_{trough} sample collection to fulfill specific aim one. Although this method of drug exposure determination was undoubtedly less complicated than comprehensive pharmacokinetic sampling, subjects, especially those on strict dosing schedules, were typically reluctant to alter their routine. Thus, many potential participants were disqualified. As C_{trough} sample concentrations are characteristically low, analysis also proved to be a difficulty. Also, high variability was seen; however, this was expected in this drug class (Rizk et al., 2012). Additionally, differences in food intake (amount, time relative to dosing, and composition) may have also contributed to the variability (Lampiris, 2012; Olin et al., 2012).

Owing to the lack of intra- and inter-day data as well as the few samples that yielded concentrations within respective standard curves (dolutegravir; n=23; elvitegravir, n=15; and raltegravir, n=6), these data were only tested against genotype. While associations were found only with dolutegravir, this may be a function of the others having much less variation within genotype in the already small sample size. However, as stated earlier, the associations (Table 1.2) have not been reported prior to this study which may point to a new route of investigation. As with the other results of this study future confirmation will give a more definitive answer.

Genetic Analysis

The second specific aim was conducted as delineated in Chapter 2. Although alternative, less invasive methods of DNA collection are available, the sampling used in this study was concurrent with the whole blood collection for C_{trough} determination. The completion of this second aim was successful; however, in future studies the use of genome wide association studies (GWASs) may be more beneficial as one-to-one SNP to outcome relationships are rare. As opposed to individual SNP studies, several SNP studies (such as the present project), and whole exome studies (which analyze variation within segments of the genome which code for protein), a GWAS allows for more a comprehensive look into variations which may contribute to several outcomes. Expression analysis would also be very helpful as the amount of protein, in most cases, has a proportionate influence on activity (Elens et al., 2013; Okubo et al., 2013; Wang et al., 2011). Additionally, SNPs may not give the complete picture. A SNP alone, for example, may show full functionality regarding genotype; however, a regulating sequence may be inoperative.

Clinical Outcomes

The third aim of this project was carried out via two routes: electronic medical record review and questionnaire administration. Firstly, we decided to gather the results of patient metabolic panels as these were conducted routinely. The hepatic function outcomes (ALP, ALT, and AST) along with renal outcomes (BUN and eGFR) were gathered successfully (as outlined in Chapter 2). A majority of these values were found to reside within the normal ranges apart from ALT. These results likely indicate that INSTI do not generally have a large influence on hepatic and renal parameters which is helpful as other concurrent drugs may. The elevation in ALT may have been elicited through concurrent disease states or conditions. Overall, toleration

was high in terms of hepatic and renal outcomes. Secondly, a questionnaire was administered, as explained in Chapter 3. Included in the questionnaire, apart from typically demographic information, was the question of which adverse events occurred over the previous two weeks. In these outcomes, only abnormal dream occurrence was significantly related to regimen in which case raltegravir showed a higher occurrence.

In addition to the hepatic and renal information for the collection time and baseline values, consideration of more information regarding co-administered drugs may increase the accuracy of prediction. For example, subject outcomes may be dependent based on the duration of various background regimens. This suggestion would be more practical in a multiple personnel setting which would allow for evaluation of various aspects of data collection. Another improvement would be to have adverse events evaluated for relatedness to drug administration by a physician rather than relying on subject reported events. Subjects may, for example, suffer from migraine headaches or have an underlying psychological disorder which manifests in anxiety or insomnia. Thus, the occurrence of an adverse event may have been reported, but due to a preexisting condition or other factor rather than the drug of interest.

Association Analyses

Finally, association analyses were conducted, in completion of specific aim 4, between genotype information and the variables collected from pharmacokinetic and clinical outcomes observation. First, in terms of hepatic outcomes we saw numerous associations between genotype and ALP, ALT, and AST (Tables 1.3, 1.4, and 1.5). However, the significant values were found primarily in the comprehensive regimen grouping. This may have been a result of smaller insignificant associations coalescing into significance. Regardless of reasoning, this study was intended to open new avenues of investigation rather than concretely describe

relationships. As some of the polymorphisms presented with relatively large effects on respective levels, these may need to be examined further. Secondly, there were many SNP associations found with BUN levels (Table 1.6) with primarily minor effects and the three SNPs in the comprehensive group seemed to improve renal function as measured by eGFR (Table 1.7). Elvitegravir grouping found SNP associations with lower eGFR. As noted earlier the presence of cobicistat and tenofovir may have contributed to these outcomes (D. E. Murrell et al., 2015).

Thirdly, several SNP associations were found across CNS (Table 2.1) and GI (Table 2.2) event occurrence. Abnormal dreams associations may have been associated with penetration of raltegravir into cerebrospinal fluid (Tsuchiya et al., 2014). Our results also indicate that, overall, anxiety occurred across the tested INSTIs. Drug transportation may help to explain the abnormal dreams and anxiety as changes were seen when alleles changed in some SNPs. Fatigue also had associations, but the variations were large when considering raltegravir grouping. Headache prediction was also possible; however, the high variation lessens the strength of the result. Insomnia, diarrhea, and nausea occurrence had associated SNPs; however, as with the other associations the mechanisms are unclear.

As noted in Chapters 2 and 3, this study has limitations. The first being the low sample size, in terms of regimen group size and the occurrence of different alleles, which may have influenced study outcomes. A case-control study design may be more helpful in the future. Another limitation is that SNPs that occur more in different people groups may have been missed in this relatively homogenous population. A third limitation is the adverse event recording method. Our method did not take into account alternative explanations and were not evaluated for relatedness by a physician.

In conclusion, we determined some patient outcomes were associated with several SNPs. The fact that many have been previously unreported may be related to perceived clinical significance; however, our results add to the understanding of possibly related factors. Additionally, we found that raltegravir may need to be monitored in terms of abnormal dreams. As INSTIs are frontline regimens, information which may inform regimen choices is important for the prescribing physician as well as the patient.

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APPENDIX

SUPPLEMENTARY TABLES

Table S1: Abnormal dream occurrence in associated SNPs by allele

Regimen	SNP	Allele	Abnormal dream occurrence	
			No	Yes
ALL	rs1143672 (A>G)	AA	19	6
		GA	36	6
		GG	21	0
Raltegravir	rs1128503 (C>T)	CC	4	4
		CT	10	3
		TT	2	0

Table S2: Anxiety occurrence in associated SNPs by allele

Regimen	SNP	Allele	Anxiety occurrence	
			No	Yes
ALL	rs1143672 (G>A)	GG	16	5
		GA	34	8
		AA	12	13
	rs9934438 (G>A)	GG	33	9
		GA	24	12
		AA	5	5
	rs9923231 (C>T)	CC	33	9
		CT	24	12
		TT	5	5

Table S3: Fatigue occurrence in associated SNPs by allele

Regimen	SNP	Allele	Fatigue occurrence	
			No	Yes
ALL	rs1128503 (C>T)	CC	19	16
		CT	30	13
		TT	10	0
	rs2070673 (T>A)	TT	41	14
		TA	17	12
		AA	1	3
	rs1902023 (G>T)	GG	20	6
		GT	27	10
		TT	12	13
Raltegravir	rs3892097 (G>A)	GG	13	3
		GA	2	5
		AA	0	0
	rs1065852 (C>T)	CC	13	3
		CT	2	4
		TT	0	1
	rs316019 (G>T)	GG	14	4
		GT	1	4
		TT	0	0

Table S4: Headache occurrence in associated SNPs by allele

Regimen	SNP	Allele	Headache occurrence	
			No	Yes
ALL	rs1695 (A>G)	AA	32	5
		AG	32	9
		GG	4	6
	rs2306283 (G>A)	GG	18	4
		GA	36	4
		AA	14	12
	rs3213619 (T>C)	TT	66	17
		TC	2	3
		CC	0	0

Table S5: Insomnia occurrence in associated SNPs by allele

Regimen	SNP	Allele	Insomnia occurrence	
			No	Yes
ALL	rs9934438 (G>A)	GG	38	4
		GA	26	10
		AA	7	3
	rs9923231 (C>T)	CC	38	4
		CT	26	10
		TT	7	3

Table S6: Diarrhea occurrence in associated SNPs by allele

Regimen	SNP	Allele	Diarrhea occurrence	
			No	Yes
ALL	rs4680 (A>G)	AA	15	13
		AG	29	11
		GG	18	2

Table S7: Nausea occurrence in associated SNPs by allele

Regimen	SNP	Allele	Nausea occurrence	
			No	Yes
ALL	rs1695 (A>G)	AA	33	4
		AG	33	8
		GG	6	4
	rs1208 (A>G)	AA	31	3
		AG	30	8
		GG	11	5

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	Derek E. Murrell, John B. Bossaer, Ronald L. Carico, Sam Harirforoosh, and David B. Cluck. 2016. Isavuconazonium sulfate: a triazole prodrug for invasive fungal infections. International Journal of Pharmacy Practice. 2017 Feb;25(1):18-30. International Journal of Pharmacy Practice Top 20 most downloaded recent papers 2016-2017
	Derek E. Murrell and Sam Harirforoosh. 2016. Improving the renal profile of a human immunodeficiency virus drug combination through prodrug alteration. Journal of Integrative Nephrology & Andrology 2016; 3(1):1-2.
	Derek E. Murrell, Jonathan Moorman, and Sam Harirforoosh. 2015. Stribild: a review of component characteristics and combination drug efficacy. European Review of Medical and Pharmacological Sciences. 2015;19(5):904-14.
In preparation:	Derek E. Murrell, Jonathan P. Moorman, David B. Cluck, Stacy D. Brown, Ke-Sheng Wang, Michelle M. Duffourc, and Sam Harirforoosh. Exploratory genetic association of pharmacokinetics and selected clinical outcomes of HIV integrase inhibitors. (In preparation)
	Derek E. Murrell, David B. Cluck, Jonathan P. Moorman, Ke-Sheng Wang, Michelle M. Duffourc, and Sam Harirforoosh. Association of pharmacogenetics and HIV integrase inhibitor adverse events: an exploratory study. (In preparation)

Selected Presentations:

Derek E. Murrell, Ke-Sheng Wang, David B. Cluck, Jonathan P. Moorman, and Sam Harirforoosh. Genetic Polymorphisms in ABCB1, SLC15A2, and VKORC1 Genes are Associated with Several Adverse Events in HIV Integrase Stand Transfer Inhibitor Usage (poster presentation). 2017 American Association of Pharmaceutical Scientists Annual Meeting and Exposition. San Diego, CA. November 2017

Derek E. Murrell, David Cluck, Jonathan P. Moorman, Sam C. Karpen, and Sam Harirforoosh. Comparison of Side Effect Profiles Among Integrase Strand Transfer Inhibitor Regimens (poster presentation). 2017 Annual Meeting of the American College of Clinical Pharmacology. San Diego, CA. September 2017

Derek E. Murrell, David Cluck, Jonathan P. Moorman, Sam C. Karpen, and Sam Harirforoosh. Evaluation of Renal and Hepatic Outcomes in HIV+ Individuals following Tenofovir Disoproxil Fumarate/Emtricitabine or Tenofovir Alafenamide/Emtricitabine plus Dolutegravir Regimens (poster presentation). 2017 Canadian Society for Pharmaceutical Sciences Annual Symposium. Montreal, QC. May 2017

Derek E. Murrell, David Cluck, Jonathan P. Moorman, Sam C. Karpen, and Sam Harirforoosh. Comparison of Renal Effects following Elvitegravir, Cobicistat, Emtricitabine, and Tenofovir Disoproxil Fumarate or Tenofovir Alafenamide Regimens in HIV-infected Patients (poster presentation). 2017 Canadian Society for Pharmaceutical Sciences Annual Symposium. Montreal, QC. May 2017

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