CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

214429Orig1s000

MULTI-DISCIPLINE REVIEW

Summary Review
Office Director
Cross Discipline Team Leader Review
Clinical Review
Non-Clinical Review
Statistical Review
Clinical Pharmacology Review

NDA Summary Review

NDA#	214429 Class 1 Resubmission, Response to Complete Response
Applicant	Drugs for Neglected Diseases *initiative*
Date of Submission	May 18, 2021
PDUFA Goal Date	July 18, 2021
Division/Office	Division of Anti-Infectives (DAI)/Office of Infectious Diseases (OID)
Established/Proper Name	Fexinidazole
Pharmacologic Class	Nitroimidazole antimicrobial
Dosage Form/Strength	Oral Tablet, 600 mg
Proposed Indication	Treatment of both first-stage (hemolymphatic) and second-stage (meningoencephalitic) human African trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* in patients 6 years of age and older and weighing at least 20 kg
Regulatory Action	Approval

Background

Fexinidazole is a member of the 5-nitroimidazole group of compounds proposed for the treatment of both stage 1 (hemolymphatic) and stage 2 (meningoencephalitic) human African Trypanosomiasis caused by *Trypanosoma brucei gambiense* (g-HAT) in patients 6 years of age and older and weighing at least 20 kg. Due to the decreased efficacy observed in patients with severe second stage HAT (identified by having a cerebrospinal fluid white blood cell count (CSF-WBC) >100 cells/µL) due to *T. brucei gambiense* disease, fexinidazole tablets should only be used in these patients if there are no other available treatment options.

The proposed oral dosing is weight based and the treatment course for g-HAT is 10 days for both stage 1 and stage 2 disease. For patients weighing ≥ 35 kg, the first 4 days utilize a daily loading dose (1,800 mg) followed by a daily maintenance dose (1,200 mg) for the remaining 6 days. For patients weighing ≥ 20 kg to < 35 kg, the loading and maintenance doses are 1,200 mg and 600 mg, respectively. The drug product will be supplied as 600 mg tablets in 14 or 24 count blister packs.

An initial application for the proposed indication was submitted by Sanofi-Aventis U.S. LLC (Sanofi) on March 12, 2020. The basis for potential approval was three completed trials conducted in early and late stage g-HAT. As noted in the review of the initial submission, the Applicant had provided adequate evidence of efficacy and safety for the proposed indication, however, no final agreement on labeling language could be reached by the PDUFA goal date; thus the Complete Response was issued on November 12, 2020.

With this current submission, the Applicant has responded to remaining labelling issues and has submitted a safety update that highlights new safety information received between the reporting

period of April 1, 2020 (the cutoff of the 120-day safety update for the initial application) and January 31, 2021 (the new cutoff date). This memorandum will discuss pertinent new safety findings presented in the safety update as well as discuss the status of current labelling negotiations. Of note, in May 2021 a transfer of ownership of the NDA occurred from Sanofi to Drugs for Neglected Diseases initiative (DNDi).

Current Submission

The three pivotal trials (DNDiFEX004, DNDiFEX005, and DNDiFEX006) have already been completed and have previously been reviewed and described (see Table 62 of the NDA 214429 Multi-Disciplinary Review signed into DARRTS on 11/12/2020 for additional details). No new safety data from these trials has been submitted with the current submission.

Currently, there are three ongoing clinical trials of fexinidazole, with a total of 248 subjects exposed to fexinidazole. Some of the deaths, SAEs, and treatment discontinuations were reported in the original NDA submission (as part of the initial submission or 120 Day Safety Update). Thus, with this submission, the Applicant's focus was on data that were newly available during the 10-month reporting period described above. There are no final clinical study reports (CSR) available for these studies, so only new safety data available as of the above cutoff date were included in this submission. The newly reported safety findings are presented by trial:

• **Study DNDi-FEX-09-HAT (009):** an uncontrolled trial in any stage g-HAT patients ≥ 6 years old, conducted in the Democratic Republic of Congo (DRC) and Guinea. Study 009 has completed enrollment and is ongoing.

New safety information from Study 009:

- A death was reported in a newborn of a 21-year-old mother treated with fexinidazole. The mother became pregnant > 2 months after her last exposure to fexinidazole. A normal male neonate was born 367 days after the last fexinidazole dose but died 4 days later due to a presumed neonatal infection. A causal relationship is unlikely given the lengthy period between these events and the mother's exposure to fexinidazole.

- An SAE of moderate "Transverse presentation" of the fetus was reported in a pregnant 26-year-old g-HAT patient 367 days after the last dose of fexinidazole; this patient was not exposed to fexinidazole during pregnancy and gave birth to a normal male newborn by C-section. A causal relationship is unlikely given the lengthy period between these events and the patient's exposure to fexinidazole.

- Follow up information was provided on 4 previously reported children exposed to fexinidazole during pregnancy and 2 other previously reported cases of maternal fexinidazole exposure before pregnancy. In all 6 cases, the babies were found to be developing normally.

- Follow up information was provided on seven previously reported babies who were breastfed during maternal fexinidazole exposure. All seven children were found to be developing normally.

• **Study DNDi-FEX-07-HAT (007):** an uncontrolled trial in patients ≥ 6 years old and ≥ 20 kg with any stage of human African trypanosomiasis caused *Trypanosoma brucei rhodesiense* (r-HAT), conducted in Uganda and Malawi. Enrollment in this study is ongoing.

New safety information from study 007:

- A 55-year-old male developed severe community acquired pneumonia 232 days after his last dose of fexinidazole. The patient was admitted to the hospital and received IV/oral antimicrobial therapy with ceftriaxone and metronidazole and was discharged nine days later. The patient slowly recovered over the next 2-3 months. A causal relationship is unlikely given the lengthy period between these events and the patient's exposure to fexinidazole.

• **Study DNDi-FEX-12-CH (012):** a multiple treatment regimen cohort trial (with comparison to historical placebo) in patients with chronic indeterminate Chagas Disease (CD), conducted in Spain. Study 012 has been completed and unblinded but final data analysis is still ongoing. No new safety information was reported.

(b) (4)

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Lastly, the Applicant reviewed published literature relevant to class safety findings for the above reporting period, and no new safety findings were noted.

Safety conclusions

From the Applicant's reporting of newly available safety information derived from ongoing trials, postmarketing surveillance, and a review of published literature, no changes in the previous fexinidazole safety assessment are warranted.

Labelling discussions

Labeling changes addressed and resolved each of the Agency's concerns as outlined in the benefit-risk assessment in the original NDA 214429 Multi-Disciplinary Review, as follows:

1) The potential for fexinidazole induced hepatotoxicity. Elevations in liver test values were observed in a Phase 2 trial of fexinidazole for Chagas disease (CD) (DNDiCHFEXI001). Although significant elevations in serum transaminases were not observed in the g-HAT trials, the potential exists for hepatotoxicity and elevations of liver tests. Agreement was reached with the Applicant regarding a proposed warning in the product labeling to communicate this risk (Section 5.5)

2) Fexinidazole-induced neutropenia. In the DNDiFEX004 trial for g-HAT, 15/264 (5.7%) patients treated with fexinidazole experienced neutropenia (< 1000 WBC/μL) after treatment, whereas 4/130 (3.1%) NECT-treated subjects experienced neutropenia. Agreement was reached with the Applicant regarding a proposed warning in the product labeling to communicate this risk (Section 5.4)

3) The potential for fexinidazole-induced neuropsychiatric adverse reactions. Adult patients treated with fexinidazole tablets reported a higher percentage of Central Nervous System (CNS) and psychiatric-related adverse reactions than those treated with NECT in the DNDiFEX004 clinical trial. Agreement was reached with the Applicant regarding a proposed warning in the product labeling to communicate this risk (Section 5.3); in addition, an agreement was reached to describe the risk of psychotic reactions with the concomitant use of disulfiram (Section 5.7).

Additional labeling changes agreed by both the Agency and the Applicant included:

- Additional demographic information (such as age and BMI) from subjects in studies 004, 005, and 006 has been included in section 6.1 Clinical Trials Experience.
- Revision of Table 2 in section 6.1 Clinical Trials Experience to list system organ classes (SOCs) in alphabetical order and AEs within each SOC by decreasing incidence in the fexinidazole arm. Also, in this table, the Agency recommended that the Applicant delete (b) (4)
- Removal of a reference to a (b) (4)
- Deletion of a statement in section 8.1 (b) (4)

Post Marketing Requirements (PMR):

A total of three PMRs were issued.

There are currently no available human data to inform the safety of fexinidazole during pregnancy. The Applicant agreed to submit the results of an ongoing descriptive study collecting prospective and retrospective data in women exposed to fexinidazole during pregnancy to evaluate the long-term safety of fexinidazole in women exposed during pregnancy and the effects upon their children:

- **PMR 3952-1:** Submit the results of your current worldwide descriptive study that is collecting prospective and retrospective data in women exposed to Fexinidazole during pregnancy to assess risk of pregnancy and maternal complications, adverse effects on the developing fetus and neonate, and adverse effects on the infant.

Fexinidazole is an inhibitor of hepatic CYP3A4 based on findings from in vitro studies and mechanistic model-based analysis as noted in the original NDA review (Section 6). Therefore, the Clinical Pharmacology team recommended, and the Applicant agreed to conduct a study to assess the potential drug-drug interaction between fexinidazole and cytochrome P450 3A4 enzyme drug substrates:

- **PMR 3952-2:** Conduct a clinical drug-drug interaction (DDI) trial to evaluate the effect of repeat doses of Fexinidazole Tablets on the pharmacokinetics of midazolam, which is a cytochrome P450 (CYP) 3A4 sensitive drug substrate, in healthy subjects.

The PK of fexinidazole and its pharmacologically active M1 and M2 metabolites is currently uncharacterized in subjects with hepatic impairment. The Applicant agreed to conduct a study to determine fexinidazole/metabolite exposure in subjects with hepatic impairment:

- **PMR 3952-3:** Conduct a clinical pharmacokinetic (PK) trial to evaluate the PK of fexinidazole and the M1 and M2 metabolites in subjects with hepatic impairment who fall in the Child-Pugh (CP) categories of mild (CP-A) and moderate (CP-B) impairment.

Conclusions and Recommendation:

The updated safety information provided by the Applicant has been reviewed, and the safety and efficacy assessments in the original NDA review are unchanged. Trial DNDiFEX004 adequately supported noninferiority of fexinidazole to NECT in late second stage g-HAT. The clinical success rates in the mITT population at 18 months were 239/262 (91.2%) in the fexinidazole group and 124/127 (97.6%) in the NECT group. Fexinidazole's clinical success rate was inferior to NECT (difference -6.4% [95% CI: -11.6, -0.1%]); however, the trial achieved the primary endpoint of noninferiority since the lower 95% confidence limit of -11.6% was within the prespecified NI margin of -13%.

Patients with severe disease, as defined by a baseline CSF-WBC count > 100 cells/μL had lower success rates in the fexinidazole group (86.9%) as compared to the NECT group (98.7%). In the subgroup of patients with a baseline CSF-WBC count ≤ 100 cells/μL, the success rates were numerically higher in the fexinidazole treatment arm (98.0%) than the NECT treatment arm (95.9%). A limitation of use statement was agreed upon to advise against the use of fexinidazole for the treatment of severe stage 2 g-HAT, unless there were no other treatment options, as well as a warning to provide additional context regarding the risk. Supportive information for early stage g-HAT as well as data from pediatric patients from 6 to less than 15 years of age were provided by two prospective, single-arm trials.

Safety was assessed in the clinical trials for g-HAT as well as trials for other indications. The use of fexinidazole for the treatment of g-HAT was associated with neuropsychiatric adverse reactions, prolonged QT interval, and tolerability issues (nausea and vomiting). Hepatotoxicity and neutropenia were reported with fexinidazole exposures greater than recommended for the treatment of g-HAT. Overall, the safety risks found to be acceptable given the high morbidity and mortality associated with g-HAT. With the agreed upon changes, the product labeling adequately conveys risks associated with fexinidazole treatment. An approval action is recommended and the signatory concurs.

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Clinical Reviewer, DAI: Shrimant Mishra, MD, MPH
Cross-Discipline Team Leader, DAI: Edward Weinstein, MD, PhD
Deputy Division Director, DAI: Dmitri Iarikov, MD, PhD
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NDA Multidisciplinary Review and Evaluation

Application Type	NDA
Application Number(s)	214429
Priority or Standard	Priority
Submit Date(s)	March 12, 2020
Received Date(s)	March 12, 2020
PDUFA Goal Date	November 12, 2020
Division/Office	Division of Anti-Infectives/Office of Infectious Diseases
Review Completion Date	November 12, 2020
Established/Proper Name	Fexinidazole
(Proposed) Trade Name	Fexinidazole tablets
Pharmacologic Class	Nitroimidazole Antimicrobial
Code name	HOE239
Applicant	sanofi-aventis U.S. LLC
Dosage form	Oral Tablet
Applicant proposed Dosing Regimen	(b) (4)
Applicant Proposed Indication(s)/Population(s)	Treatment of both first-stage (hemolymphatic) and second-stage (meningoencephalitic) human African trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* in (b) (4)
Recommendation on Regulatory Action	Complete Response
Recommended Indication(s)/Population(s) (if applicable)	Treatment of both the first-stage (hemolymphatic) and second-stage (meningoencephalitic) human African trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense* in patients 6 years of age and older and weighing at least 20 kg.
Recommended Dosing Regimen	Patients weighing greater than or equal to 35 kg: 1,800 mg for 4 days followed by 1,200 mg for 6 days Patients weighing greater than or equal to 20 kg to less than 35 kg: 1,200 mg for 4 days followed by 600 mg for 6 days

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OB = Office of Biostatistics
DAI = Division of Anti-Infectives
OPQ = Office of Pharmaceutical Quality
DPMH = Division of Pediatric and Maternal Health
OPDP = Office of Prescription Drug Promotion
OSI = Office of Scientific Investigations
OSE = Office of Surveillance and Epidemiology
DEPI = Division of Epidemiology
DMEPA = Division of Medication Error Prevention and Analysis
DRM = Division of Risk Management
DPV = Division of Pharmacovigilance
QT-IRT = Interdisciplinary Review Team for Cardiac Safety Studies
OSIS = Office of Study Integrity and Surveillance

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Glossary

AE	adverse event
ALT	alanine aminotransferase reaction
AST	Aspartate aminotransferase
AUC	area under the curve
BA	bioavailability
BE	bioequivalence
BLA	biologics license application
BMI	body mass index
CAR	Central African Republic
CATT	Card Agglutination Test for Trypanosomiasis
CD	Chagas Disease
CDC	centers for disease control and prevention
CI	confidence interval
CL	clearance
CNS	Central Nervous System
COA	clinical outcome assessment
CSF	cerebrospinal fluid
CTC	capillary tube centrifugation
CTCAE	Common Terminology Criteria for Adverse Event
CYP	cytochrome P
DDI	drug-drug interaction
DDIRC	Drug-Drug Interaction Risk Calculator
DFMO	difluoromethylornithine
DNDi	Drugs for Neglected Diseases initiative
DRC	Democratic Republic of the Congo
ECG	electrocardiogram
EMA	European Medical Agency
EOH	end of hospitalization
EOT	end-of-therapy
FDA	Food and Drug Administration
GD	gestation day
HAT	human African Trypanosomiasis
hERG	human electro retinogram
IND	investigational new drug
ITT	intent-to-treat
IU/L	international units per liter
LC-MS/MS	Liquid Chromatography with tandem mass spectrometry
LD	lactation day
mAECT	mini-anion exchange centrifugation technique
mAECT-BC	mini-anion exchange centrifugation technique – buffy coat

mHCT	microhematocrit capillary centrifugation test
mITT	modified intent-to-treat
NDA	new drug application
NECT	nifurtimox eflornithine combination therapy
NI	noninferiority
NOAEL	no-observed-adverse-effect level
NTR	nitroreductase
OCP	Office of Clinical Pharmacology
PI	prescribing information
PK	pharmacokinetics
PL	product labeling
PMR	postmarketing requirement
PPK	Population Pharmacokinetics
PREA	Pediatric Research Equity Act
QD	once daily
R	predicted in vivo ratio
REMS	risk evaluation and mitigation strategy
RI	renal impairment
SAE	serious adverse event
SGE	special government employee
SOC	system organ class
TEAE	treatment-emergent adverse event
ULN	upper limit of normal
WBC	white blood cell
WHO	World Health Organization

1. Executive Summary

1.1. Product Introduction

Fexinidazole is a member of the 5-nitroimidazole group of compounds proposed for the treatment of both stage 1 (hemolymphatic) and stage 2 (meningoencephalitic) human African Trypanosomiasis caused by *Trypanosoma brucei gambiense* (g-HAT) in patients 6 years of age and older and weighing at least 20 kg. The proposed oral dosing is weight based and the treatment course for g-HAT is 10 days for both stage 1 and stage 2 disease. For patients weighing ≥ 35 kg , the first 4 days utilize a daily loading dose (1,800 mg) followed by a daily maintenance dose (1,200 mg) for the remaining 6 days. For patients weighing ≥ 20 kg to < 35 kg, the loading and maintenance doses are 1,200 mg and 600 mg, respectively. The drug product is proposed to be supplied as 600 mg tablets in 14 or 24 count blister packs.

1.2. Conclusions on the Substantial Evidence of Effectiveness

The Applicant has provided substantial evidence of effectiveness to support approval of fexinidazole for the treatment of first and second stage g-HAT in patients aged 6 years and older who weigh 20 kg or more. A single adequate and well-controlled noninferiority trial demonstrated that fexinidazole was noninferior to nifurtimox-eflornithine combination therapy (NECT) for the treatment of late second-stage g-HAT. Two single-arm trials provided supportive clinical data: a trial in patients ≥15 years of age with stage 1 or early stage 2 g-HAT and a trial in pediatric patients ≥15 years of age with either stage of the disease. Additional supportive information was provided by in vitro microbiology studies, and animal models of infection demonstrating the activity of fexinidazole.

Trial DNDiFEX004 (NCT01685827) was a randomized, open-label, active-controlled trial that compared fexinidazole to NECT in patients with late second stage g-HAT. Efficacy was assessed as clinical success (cure or probable cure) at 18 months in the modified intent-to-treat (mITT) population, which included all patients who received at least a single dose of study medication and excluded patients who were lost to follow-up due to geopolitical unrest. The difference in the success rates (fexinidazole - NECT), was -6.4% (95% confidence interval (CI): -11.6%, -0.1%). While the success rate in the fexinidazole treatment arm was significantly lower than in the NECT arm, fexinidazole met the statistical criteria for noninferiority (NI) to NECT since the lower bound of this confidence interval was greater than the pre-specified NI margin of -13%. Cure rates at 24 months were similar to the results at 18 months.

Supportive information for the single adequate and well-controlled trial, was provided by two prospective, single-arm trials in g-HAT that used the same fexinidazole dosing regimen in adults as the comparative trial. DNDiFEX005 (NCT02169557) studied patients ≥ 15 years of age with stage 1 and early stage 2 g-HAT and DNDiFEX006 (NCT02184689) enrolled pediatric patients aged 6 to 15 years old and weighing at least 20 kg with any stage of the disease. Treatment success proportions at 12 months in all patients were 98.7% (227/230, 95% CI [96.2%, 99.7%])

in DNDiFEX005 and 97.6% (122/125, 95% CI [93.1%, 99.5%]) in DNDiFEX006. The results were similar between stage 1 and stage 2 subgroups of g-HAT, and the results observed at 12 months remained consistent at 18 months.

Due to lack of agreement with the Applicant on product labeling, the NDA will receive a complete response during this review cycle.

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

In NDA 214429, the Applicant is seeking approval of fexinidazole tablets for the treatment of first and second stage g-HAT in patients aged 6 years or older who weigh 20 kg or more. Fexinidazole is an orally-available 5-nitroimidazole that is bioactivated by *T. brucei* to generate toxic reactive amines. The proposed dosing is weight-based with a 10-day treatment course for both stage 1 and stage 2 g-HAT. The first 4 days utilize a daily loading dose (1,800 mg for patients ≥ 35 kg) followed by a daily maintenance dose (1,200 mg for patients ≥ 35 kg) for the remaining 6 days. For patients weighing ≥20 kg to <35 kg, the loading and maintenance doses are 1,200 mg and 600 mg, respectively.

The primary data to support the efficacy of fexinidazole for the treatment of g-HAT were from a single adequate and well-controlled randomized NI trial in patients ≥ 15 years of age with late stage 2 g-HAT (DNDiFEX004). The trial inclusion criteria required patients to have evidence of parasites in blood, lymph and/or cerebrospinal fluid (CSF) at enrollment. If testing for parasites in CSF was negative, a CSF WBC count >20 cells/µL was required to confirm late second-stage HAT. Patients (n=394) were randomized in a 2:1 ratio to a 10-day treatment regimen of either fexinidazole tablets (n=264) or NECT (n=130). The trial evaluated clinical outcomes of patients at 3, 6, 12, 18, and 24 months after the end of treatment, with clinical success at 18 months designated as the primary endpoint. Clinical success was based upon a cure, or a probable cure as judged by prespecified criteria that incorporated patient vital status, absence of trypanosomes in any body fluid, resolution of signs and symptoms of trypanosomiasis, and evaluation of CSF WBC count. The primary analysis population was the mITT population defined as all subjects who received at least one dose of study drug except for five subjects who fled an area of armed conflict and for whom no post-treatment data were available. The pre-specifed NI margin of 13% was considered acceptable to support the indication based on a conservative estimate of the treatment effect of 17.1% for clinical outcome at 18 months.

The clinical success rates were 239/262 (91.2%) in the fexinidazole group and 124/127 (97.6%) in the NECT group; the difference in response rates between the two treatment groups (fexinidazole minus NECT) was -6.4% (95% CI: -11.6, -0.1%) at 18 months in the mITT population. The trial achieved the primary endpoint of noninferiority since the lower 95% confidence limit of -11.6% was within the prespecified NI margin of -13%. While a treatment effect could be demonstrated for fexinidazole, it was significantly less effective than NECT as the upper boundary of the 95% CI was less than 0. A subgroup analysis revealed that patients with severe disease, as defined by a baseline CSF-WBC count > 100 cells/µL had lower success rates in the fexinidazole group (86.9%) as compared to the NECT group (98.7%). In the subgroup of patients with a baseline CSF-WBC count ≤ 100 cells/µL, the success

rates were numerically higher in the fexinidazole treatment arm (98.0%) than the NECT treatment arm (95.9%). Secondary analyses produced similar results as the primary analysis, and examined clinical success rates at 24 months, as well as subgroup analyses by age, sex, and other disease characteristics at baseline.

There were two additional clinical studies that provided supportive data. DNDiFEX005 was a prospective, multicenter, open-label, single-arm study in patients ≥15 years of age with stage-1 (CSF-WBC ≤5 cells/µL) or early stage-2 g-HAT (CSF-WBC 6 to 20 cells/µL). This study was considered a "plug-in" study because patients were recruited at the same sites by the same investigators as the DNDiFEX004 trial with the same dosing and similar criteria for clinical success. The result for the primary endpoint, the overall clinical success rate at 12 months, was 98.7%, 95% CI [96.2%, 99.7%]. The response rates were similar for stage 1 (186/189 [98.4%]) and early stage 2 disease (41/41 [100%]). A secondary endpoint of clinical outcome at 18 months resulted in an overall success rate of 97.8%. DNDiFEX006 was a prospective, multicenter, open-label, single-arm "plug-in" study in pediatric patients from 6 to less than 15 years of age weighing over 20 kg with either stage 1 or 2 g-HAT. As in the previous study, the primary endpoint was the proportion of clinical success at 12 months following the end of treatment. The overall proportion of patients who achieved clinical success at 12 months was 122/125 (97.6%). Results from subgroup analyses were generally similar to the overall study result when analyzed by disease stage, disease severity, patient age and sex. The overall clinical success rate at 18 months was 98.4%. g-HAT is generally considered to be a fatal disease, but a conservative estimate for a spontaneous cure rate based upon historical trials with less effective treatment regimens, including both stage 1 and stage 2 g-HAT patients, was 90.4% at 12 months. Both trials were able to exclude this historical control rate.

Trial DNDiFEX004 was the primary source for safety data since it was the only comparative trial. The interpretation of the treatment emergent adverse events (TEAEs) was complicated by background disease symptoms, concomitant medications, and a 24-month period of patient observation following 10 days of study drug administration. Overall, there was a numerical imbalance in mortality that did not favor the fexinidazole arm (9/264 [3.4%])) as compared to the NECT arm (2/130 [1.5%]) at month 24 (difference 1.9%, 95% CI [-2.4%, 5.2%]). Although most deaths occurred after treatment was completed, two deaths did occur during treatment in the fexinidazole arm, while no deaths occurred during treatment in the NECT arm. No deaths were considered to be related to the study drug by the investigator. In the subpopulation of patients with less severe g-HAT (CSF-WBC count ≤100 cells/µL at baseline), the mortality rate at 24 months was numerically lower in the fexinidazole arm (2/103 [1.9%]) than the NECT treatment arm (2/50 [4.0%]). A total of 73 serious adverse events (SAEs) were reported in the trial with a similar incidence in both treatment groups: SAEs were reported in 31/264 patients (11.7%) in the fexinidazole arm and in 13/130 patients (10.0%) who received NECT. Most SAEs occurred after the treatment period, and most were considered unrelated to treatment. In the fexinidazole treatment arm, two discontinuations occurred due to patient deaths; there were no permanent treatment

discontinuations in the NECT arm. Overall, TEAEs were reported in 93% of study subjects and were generally similar between fexinidazole and NECT treatment arms in terms of incidence, severity and relatedness to study drug. Adverse events (AEs) occurring in at least 5% of fexinidazole subjects and at a 3% or greater incidence than the NECT arm were headache, tremor, dizziness, nausea, dyspepsia, abdominal pain upper, salivary hypersecretion, hypocalcemia, hypoalbuminemia, asthenia, chest pain, feeling hot, insomnia, and neck pain. An increased incidence of neuropsychiatric AEs were noted in the fexinidazole treatment arm, ranging from insomnia and mood disorders to psychotic events.

In addition, the following safety issues were identified and reviewed in this NDA:

- The potential for fexinidazole induced hepatotoxicity (see section 8.2.4 of this review). A warning for hepatotoxicity was proposed by the Applicant based upon elevations in liver laboratory test values observed in a Phase 2 trial of fexinidazole for Chagas disease (CD) (DNDiCHFEXI001). The exposure to fexinidazole was higher in many patients in the CD trial than g-HAT, with the highest dose group receiving 1800 mg/day for 8 weeks. Of note, in a Phase 1 study, one healthy subject who received 3600 mg of fexinidazole x 14 days (twice the loading dose) experienced transaminase elevations 30 times above the upper limit of normal (ULN) after completing treatment. No subjects in the g-HAT clinical trials had a serum transaminase value greater than 5x ULN. The proportion of subjects with AST values between 1x and 3x ULN was greater at baseline (50/619 [8.1%]) than at the end of treatment for g-HAT (20/619 [3.2%]). Although significant elevations in serum transaminases were not observed in the g-HAT trials, the potential exists for elevations of liver laboratory tests. An agreement could not be reached with the Applicant regarding a proposed warning in the product labeling to communicate this risk.

- Fexinidazole-induced neutropenia (see section 8.2.4 of this review). In the DNDiFEX004 trial for g-HAT, 15/264 (5.7%) patients treated with fexinidazole experienced neutropenia (< 1000 WBC/µL) after treatment, whereas 4/130 (3.1%) NECT-treated subjects experienced neutropenia. One fexinidazole-treated subject had a nadir < 500 WBC/µL. All cases occurred in patients with a baseline absolute neutrophil count < 5000 WBC/µL at baseline, and there were no infection-related complications reported due to neutropenia. The mean changes in absolute neutrophil count from baseline to end of treatment within each treatment arm were not significant, and did not meaningfully differ between the treatment arms. The safety data from the CD trial provided some information on the potential for neutropenia with greater fexinidazole exposures. In the CD trial, SAE of neutropenia was reported in 8/40 (20%) subjects, and was assessed as related to the study drug. Based on the available clinical trial data for the treatment of g-HAT, there is potential for neutropenia, and an

agreement could not be reached with the Applicant regarding the communication of this information in the Warnings and Precautions section of the product label.

- Fexinidazole undergoes significant hepatic metabolism (see section 6.2.1 of this review), but hepatically impaired patients were not studied in the clinical trials or pharmacokinetic studies. The Applicant proposed (b) (4) in the prescribing information. Due to the uncertainty of dosing hepatically impaired patients and the potential for hepatotoxicity at higher exposure levels, the proposed contraindication was revised to "patients with hepatic impairment" and additional information was added to section 8.7 (Hepatic Impairment). The Applicant agreed to Postmarketing requirements to examine fexinidazole pharmacokinetics in mild to moderately hepatically impaired subjects as well as drug-drug interactions with midazolam, a cytochrome P450 (CYP) 3A4 drug substrate (see section 13 of this review).

- The potential for fexinidazole-induced neuropsychiatric adverse reactions was reviewed (see section 8.2.4 of this review). Adult patients treated with fexinidazole tablets reported a higher percentage of Central Nervous System (CNS) and psychiatric-related adverse reactions than those treated with NECT in the DNDiFEX004 clinical trial. There was an increased incidence in insomnia, headache, and tremor in the fexinidazole arm as compared to the NECT arm. Psychiatric-related AEs occurring in the fexinidazole arm included depression, agitation, personality change, and suicidal ideation. Of note, the nifurtimox prescribing information contains a warning for worsening of neurological and psychiatric conditions. While few of the adverse events were serious, most events were considered by the investigator to be study drug-related. The Applicant agreed to the inclusion of a warning in the prescribing information to help inform healthcare providers of the risk of neuropsychiatric adverse reactions; however, an agreement had not been reached with the Applicant regarding warning language describing the risk of psychotic reactions with the concomitant use of disulfiram.

There were uncertainties and limitations to the study results with regard to efficacy and safety. Efficacy was primarily assessed in one comparative NI trial in late stage-2 g-HAT in adults that utilized an open-label design. NECT is not approved by the FDA; however, it is referenced by the World Health Organization (WHO) as a combination treatment for HAT and adequate information existed on its efficacy in order to justify a noninferiority margin. Therefore, NECT was an acceptable comparator for a NI assessment. The supporting data to extend the indication to include stage 1 disease and pediatric patients relied on two historically-controlled single arm trials; while randomized, comparative data are preferred, a comparison to less effective historical regimens was considered a conservative approach since untreated g-HAT is a fatal disease. Furthermore, a drug with

efficacy in patients with neurological disease (stage 2) would be expected to be effective for the treatment of patients with less advanced hemolymphatic disease (stage 1). For pediatric patients, extrapolation of efficacy from an adult population is reasonable since the characterstics of the disease and response to therapy would be expected to be similar. Fexinidazole is an orally administered monotherapy for both stages of g-HAT, as compared to the NECT regimen, which contains an injectable (eflornithine), and is only available from the CDC for second stage disease. Fexinidazole may provide a treatment option for patients who cannot tolerate NECT, or for patients who lack vascular access. With regard to safety, the study populations consisted of patients in Sub-Saharan Africa which may not reflect some characteristics of the US population. For example, international travelers from the US may differ from the study population in terms of age (only 7 patients in the study were ≥65 years), physical characteristics such as body mass index (BMI) (median BMI was 18.9), as well as general health and nutrition. Patients with hepatic impairment were excluded from the clinical studies, and fexinidazole undergoes extensive hepatic metabolism. Dosing for hepatically impaired patients was not established, and the potential for drug-drug interactions was not fully examined.

Although noninferiority of fexinidazole compared to NECT was demonstrated in an adequate and well-controlled trial, the trial also indicated that fexinidazole's efficacy was inferior to NECT. The decrement in efficacy and increased mortality could be addressed in product labeling; however, an agreement could not be reached with the Applicant regarding a limitation of use statement and warning language to describe the identified risks of heptatotoxicity, neutropenia, and psychotic reactions with concomitant use of disulfiram.

Due to lack of agreement with the Applicant on product labeling the NDA will receive a complete response during this review cycle.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	Human African Trypanosomiasis caused by *T.b. gambiense* (g-HAT) is considered to be fatal without treatment, though data are limited both in terms of the uniformity of fatal outcomes and duration required for such outcomes. Significant morbidity, including severe neuropsychiatric deterioration is typical. The disease is restricted to	Given the significant morbidity and mortality associated with this disease, effective therapies are needed.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	endemic areas of sub-Saharan Africa; however, it may sometimes be seen in returning travelers and immigrants to the United States. There are two stages of the disease. In the first stage, infection is primarily limited to the bloodstream and lymphatics and does not involve the CNS (hemolymphatic stage; Stage 1). During this stage, infected individuals may be fairly asymptomatic or may have mild to moderate symptoms including intermittent fevers, headaches, muscle and joint aches, pruritus, lymph node swelling, and weight loss. After a period of 1-2 years, the infection spreads to the CNS (meningo-encephalitic stage; Stage 2), at which time an infected individual starts showing neurological manifestations of disease such as personality changes, progressive confusion, daytime sleepiness, and coma.	
Current Treatment Options	The only approved therapy is eflornithine for stage 2 g-HAT. Pentamidine is used off label for stage 1 g-HAT, and NECT is an unapproved therapy for stage 2 disease.	There are limited therapies available to treat g-HAT, and there is no single therapy for both stages of disease. In addition, the current treatment options are associated with significant toxicities. A safe and effective treatment option that is orally available and indicated for both g-HAT stages would address an area of clinical need.
Benefit	Trial DNDiFEX004 was a randomized, open-label, active-controlled trial that compared fexinidazole to NECT in patients with late second stage g-HAT. Efficacy was assessed as clinical success (cure or probable cure) at 18 months. The difference in the success rates	Fexinidazole appears to effectively treat Stage 1 disease of g-HAT as well as less severe (CSF WBC ≤ 100 cells/µL) Stage 2 disease and provides an oral treatment

Dimension	Evidence and Uncertainties	Conclusions and Reasons
	(fexinidazole - NECT), was -6.4% (95% confidence interval (CI): -11.6, -0.1%). While the success rate in the fexinidazole treatment arm was significantly lower than in the NECT arm, fexinidazole met the statistical criteria for noninferiority (NI) to NECT. A subgroup analysis revealed that patients with severe stage 2 disease, as defined by a baseline CSF-WBC count > 100 cells/µL had lower success rates in the fexinidazole group (86.9%) as compared to the NECT group (98.7%). Supportive evidence from two prospective, single-arm trials in g-HAT were also provided. DNDiFEX005 studied patients ≥ 15 years of age of age with stage 1 and early stage 2 g-HAT and DNDiFEX006 enrolled pediatric patients aged 6 to 15 years old and weighing at least 20 kg. Treatment success proportions at 12 months in all patients were 98.7% (227/230, 95% CI [96.2%, 99.7%]) in DNDiFEX005 and 97.6% (122/125, 95% CI [93.1%, 99.5%]) in DNDiFEX006.	option for such patients. It appears to be less effective than NECT for severe stage 2 disease (CSF WBC >100 cells/µL) and should only be used to treat these patients if there are no other available treatment options.
Risk and Risk Management	As stated above, patients with severe stage 2 disease, as defined by a baseline CSF-WBC count > 100 cells/µL had lower success rates in the fexinidazole group as compared to the NECT group. The use of fexinidazole for the treatment of g-HAT was associated neuropsychiatric adverse reactions, prolonged QT interval, and tolerability issues (nausea and vomiting). Hepatotoxicity and neutropenia were reported with fexinidazole exposures greater than recommended for the treatment of g-HAT.	An agreement was not reached with the Applicant regarding the communication of serious risks in the prescribing information. A limitation of use statement was proposed by the Agency to advise against the use of fexinidazole for the treatment of severe stage 2 g-HAT, unless there were no other treatment options as well as a warning to provide additional context regarding the risk. Other adverse reactions recommended to be included in the

Dimension	Evidence and Uncertainties	Conclusions and Reasons
		warnings section of product labeling include QT prolongation, neuropsychiatric adverse events, neutropenia, and the potential for hepatotoxicity. Additional risk evaluation and mitigation strategies (REMS) are not recommended.

1.4. Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

□	The patient experience data that were submitted as part of the application include:			Section of review where discussed, if applicable
	□	Clinical outcome assessment (COA) data, such as		
		□	Patient-reported outcome (PRO)	
		□	Observer reported outcome (ObsRO)	
		□	Clinician reported outcome (ClinRO)	
		□	Performance outcome (PerfO)	
	□	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
	□	Patient-focused drug development or other stakeholder meeting summary reports		
	□	Observational survey studies designed to capture patient experience data		
	□	Natural history studies		
	□	Patient preference studies (e.g., submitted studies or scientific publications)		
	□	Other: (Please specify):		
□	Patient experience data that were not submitted in the application, but were considered in this review:			
	□	Input informed from participation in meetings with patient stakeholders		
	□	Patient-focused drug development or other stakeholder meeting summary reports		
	□	Observational survey studies designed to capture patient experience data		

	□	Other: (Please specify):	
X	Patient experience data were not submitted as part of this application.		

2. Therapeutic Context

2.1. Analysis of Condition

Human African Trypanosomiasis (HAT) is a disease caused by the protozoan hemoflagellates of the genus *Trypanosoma*. Two subspecies that are morphologically indistinguishable cause distinct disease patterns. *Trypanosoma brucei gambiense* (*T.b. gambiense)* and *Trypanosoma brucei rhodesiense* (*T.b. rhodesiense*) cause West African Sleeping Sickness, or g-HAT, and East African Sleeping Sickness, or r-HAT, respectively. While these two disease entities have distinct epidemiological and clinical characteristics, the focus of this application being *T.b. gambiense,* this section will focus only on g-HAT. *T.b. gambiense* is transmitted by the tsetse fly and makes up the vast majority (95 to 98%) of sleeping sickness cases in Africa (Centers for Disease Control and Prevention). An infected fly transmits the parasite to humans when taking a blood meal. Once inside a human, the infective trypomastigote undergoes further development and multiplies by binary fission, eventually entering the bloodstream, lymphatics and eventually, the cerebrospinal fluid and the central nervous system (CNS). The cycle is repeated when an uninfected tsetse fly takes a blood meal from an infected human. Humans are the main reservoir of the protozoan. While West African Sleeping Sickness is most heavily concentrated in central African countries such as Democratic Republic of the Congo and Central African Republic (CAR), it is also found in West African nations such as the Ivory Coast and Guinea, as well as a few East African Countries such as Uganda and Sudan. Tsetse flies inhabit thickets/woodlands or the vegetation near streams. In general, g-HAT is considered a rural disease affecting people near water sources with occupations such as fishing, mining, hunting, and farming.

HAT cases have been steadily decreasing and in 2016 numbered 2164. Recent estimates state that roughly 750,000 individuals are at high to very high risk of disease. However, underreporting is suspected due to a variety of factors, including fear of treatment and impediments to surveillance such as armed conflict. Most cases occur in adults, although pediatric cases are not uncommon and may represent a quarter of cases. Very few cases are imported into the United States (<100 in the decade 2000 to 2010); most of which are *T.b. rhodesiense*.

There are two major stages of infection with *T.b. gambiense*. In the first stage, infection is primarily limited to the bloodstream and lymphatics and does not involve the CNS (hemolymphatic stage; Stage 1). During this stage, infected individuals may be fairly asymptomatic or may have mild to moderate symptoms including intermittent fevers, headaches, muscle and joint aches, pruritus, lymph node swelling, and weight loss. Patients often do not seek care and active suspicion of disease is typically required. After a variable time period that can range from one to two years, the infection spreads to the CNS (meningo-encephalitic stage; Stage 2), at which time an infected individual starts showing neurological manifestations of disease such as personality changes, progressive confusion, daytime

sleepiness, and coma. Other signs such as partial paralysis, loss of balance/ataxia may also occur. If left untreated, death occurs at variable times (generally around 3 years). Stage 2 disease can be further subdivided into early and late Stage 2 depending on the presence of trypanosomes in the CSF as well the number of WBCs in the CSF.

The natural history of the disease is variable and understanding of the history is limited by rapid initiation of treatment as soon as a diagnosis is made. The mean duration of Stage 2 disease is estimated at around 8 months. Although the natural history of the disease generally ends in death, it is not fully understood and there are reported cases of subjects with spontaneously resolved disease, particularly in West Africa.

The gold standard for diagnosis involves finding the parasite in body fluids or tissue by microscopy, often through an aspirate of the cervical node. Other diagnostic methods are primarily used for screening purposes. Once a diagnosis of West African Sleeping Sickness is made, a lumbar puncture is indicated to evaluate for CNS involvement/stage of disease (World Health Organization (WHO) criteria include threshold levels of CSF WBC and proteins for diagnosis; parasites may also be seen in this fluid) (World Health Organization 2019).

2.2. Analysis of Current Treatment Options

The treatment is dependent on the stage of infection. In Stage 1 of the disease, intramuscular pentamidine is the preferred agent, although other treatment alternatives such as suramin and melarsoprol are available. In the USA, the CDC recommends a 7-day regimen of intramuscular pentamidine. Pentamidine does not cross the blood brain barrier and thus is not effective for Stage 2 disease. It is generally well-tolerated but can have adverse effects including hypoglycemia, diarrhea, nausea, and vomiting. Suramin is not often used because it can cause severe reactions in patients co-infected with *Onchocerca volvulus*. In the second stage, the WHO-recommended treatment is a 10-day combination regimen of oral nifurtimox and intravenous eflornithine (NECT), which generally requires hospitalization. In the United States, the NECT regimen is not approved for treatment though it can be obtained through the CDC. Eflornithine alone is approved in the US for treatment though it is not currently marketed and also can be obtained from the CDC (daily intravenous (IV) infusions of eflornithine for 14 days). Both NECT and eflornithine alone are associated with adverse reactions including nausea, vomiting, neuropsychiatric events, fever, bone marrow suppression, seizures, and diarrhea. Melarasoprol is a rarely used, highly toxic agent used in relapsed/refractory disease; treatment has been associated with encephalopathy and deathThe treatment options currently available for g-HAT are summarized in section 1.3 above and are briefly resummarized here.

Table 1. Summary of Treatment Armamentarium Relevant to Proposed Indication

Product(s) Name	Relevant Indication	Year of Approval	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues	Other Comments
FDA approved treatments [combine by pharmacologic class, if relevant]						
Ornidyl (Eflornithine Hydrochloride)	Stage 2 g-HAT (meningoencephalitic stage)	1990	400 mg/kg/day divided into 4 infusions every 6 hours x 14 days	Cure Rate: 90-95%[2]	The main adverse reactions are fever, pruritus, hypertension, nausea, vomiting, diarrhea, abdominal pain, headaches, myelosuppression (anemia, leukopenia, thrombocytopenia) and, more rarely, seizures that are generally isolated and respond to treatment.	Never marketed or distributed in the United States; can be obtained through CDC request Burdensome treatment regimen requiring IV access.

Product(s) Name	Relevant Indication	Year of Approval	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues	Other Comments
Other Treatments – [Combine by Pharmacologic class, if relevant]						
NECT (nifurtimox eflornithine combination therapy)	Stage 2 g-HAT	Not approved in the US; introduced in 2009 worldwide	nifurtimox 5 mg/kg (15 mg/kg/day) in po tid dosing x 10 days; eflornithine 200 mg/kg IV infusion bid (400 mg/kg/ day) x 7 days	Cure Rates 95-98%[2]	The most common adverse reactions are abdominal pain, nausea, vomiting, and headache. There is a risk of seizures, and occasional psychotic reactions and hallucinations. Diarrhea and vomiting are frequent (> 50% of treated patients) but do not warrant cessation of treatment. Other described adverse effects include tremor (5-10%), headache, bone marrow suppression (anemia, leukopenia), vertigo, and ear troubles.	Can be obtained through WHO/CDC in the US. Burdensome treatment regimen requiring IV access

Product(s) Name	Relevant Indication	Year of Approval	Dosing/ Administration	Efficacy Information	Important Safety and Tolerability Issues	Other Comments
Pentamidine	Stage 1 g-HAT	Not approved in the US for this indication (approved for treatment of *Pneumocystis Carinii* pneumonia)	Pentamidine 4 mg/kg IV x 7 days.	Cure Rates 93-98%[2]	Possible immediate reactions include hypotension in about 10% of patients, with dizziness and sometimes collapse and shock. After intravenous injection, hypotension can be as frequent as in 75% of cases. Occasional adverse reactions are nausea or vomiting and pain at the injection site. Sterile abscesses or necrosis may occur at the site of intramuscular injection. Reported systemic reactions include azotemia due to nephrotoxicity, leukopenia, raised liver function enzymes, hypoglycemia, and hyperglycemia. Persistent diabetes is a rare but feared event. Severe adverse events (SAEs)such as anaphylaxis and acute pancreatitis are extremely rare.	Available off label in the United States

Source: World Health Organization 2019
Abbreviations: CDC, Centers for Disease Control and Prevention g-HAT, human African trypanosomiasis due to *T. b. gambiense;* IV, intravenous; po, by mouth; tid, three times daily; WHO, World Health Organization

3. Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Fexinidazole has not been marketed for human use in any indication in the United States. Fexinidazole is a new chemical entity. Fexinidazole was granted orphan designation on April 4, 2016 for Human African Trypanosomiasis (HAT) or sleeping sickness. HAT is included in the list of diseases eligible for a tropical disease PRV.

The Applicant, sanofi-aventis U.S. LLC, submitted the new drug application (NDA) 214429 for Fexinidazole (b) (4) Tablets, 600 mg on March 12, 2020. The submission included a request for a tropical disease priority review voucher.

3.2. Summary of Presubmission/Submission Regulatory Activity

The fexinidazole development project is a partnership between the Drugs for Neglected Diseases initiative (DNDi) and sanofi-aventis U.S. LLC. The goal of the development project is to develop fexinidazole for the treatment of HAT in remote areas of Sub-Saharan African countries without rapid access to hospital care. The initial clinical development plan was discussed with the European Medicines Agency (EMA), WHO, and the FDA.

Sanofi-aventis Groupe submitted Pre-IND 110365 for HOE239 (fexinidazole) oral formulation to the Division of Special Pathogens and Transplant Products on October 26, 2010. The indication requested was for the treatment of late-stage HAT due to *T.b. gambiense*. The Sponsor's proposed clinical development plan consisted of one Phase 2/3 pivotal, noninferiority trial comparing fexinidazole with the reference treatment, NECT, in patients 15 years of age and older with late stage HAT (FEX004). The Division strongly recommended a developmental approach which included either an additional Phase 2 or an additional confirmatory Phase 3 study. In addition, the FDA provided clinical pharmacology, nonclinical, statistical, and microbiology guidance.

On July 8, 2016, a Type C meeting was held to discuss the adequacy of the revised clinical development program for registration of fexinidazole for the treatment of HAT due to *T.b. gambiense* in adults and children 6 years of age and older and weighing more than 20 kg in the United States. The revised clinical development program consisted of a multicenter, open-label, randomized, noninferiority (NI), study (Phase 2/3) comparing fexinidazole and NECT in adult patients with late stage HAT (DNDiFEX004) and two open-label, single-arm cohort studies in adult patients with early/intermediate HAT (DNDiFEX005), and in all HAT stages for children aged 6 to less than 15 years of age (DNDiFEX006). The Division concluded that in general, the revised clinical program appeared adequate to support an NDA submission.

The Office of Pharmaceutical Quality provided written response to questions regarding starting materials on April 18, 2019.

A Chemistry, Manufacturing and Controls Pre-NDA meeting was held on October 3, 2019. OPQ agreed that the Sponsor's overall drug substance control strategy for impurities, the proposed quality attributes in the specification for the proposed drug substance, the drug product formulation and packaging to support comparability of the Phase 3 clinical supplies, the proposed commercial product, the tests and controls included in the proposed drug product specification, and the primary stability studies appeared to be acceptable, but noted that all information would be reviewed in detail during the NDA review. OPQ also noted that additional biopharmaceutic information would be required.

A Clinical Pre-NDA meeting was held on September 20, 2019 to reach agreement on the plans for an NDA including the study pooling strategy and the format of the clinical datasets. The indication was expanded to include treatment of first-stage (hemolymphatic) and second-stage (meningo-encephalitic) HAT due to *Trypanosoma brucei gambiense* in adults and children 6 years of age and older and weighing 20 kg or more. As noted earlier, the clinical program for fexinidazole consisted of three studies: pivotal study DNDiFEX004 entitled, "Efficacy and Safety of Fexinidazole Compared to nifurtimox-eflornithine combination therapy (NECT) in Patients with Late Stage-2 HAT due to *T.b. gambiense*: a Pivotal, Noninferiority, Multicenter, Randomized, Open-Label Study," and two additional uncontrolled cohort studies, DNDiFEX005, "Efficacy and Safety of Fexinidazole in Patients with Stage-1 or Early Stage 2 HAT due to *T.b. gambiense*: A Prospective, Multicenter, Open-Label Cohort Study, Plug-in to the Pivotal Study," and DNDiFEX006, "Efficacy and Safety of Fexinidazole in Children at Least 6 Years Old and Weighing over 20 kg with HAT due to *T.b. gambiense*: A Prospective, Multicenter, Open Study, Plug-in to the Pivotal Study." The Division agreed that the proposed studies appeared adequate to support an NDA submission. No agreements for late submissions were made.

The Division did not agree with the Sponsor's plan (b) (4) and recommended that only study DNDiFEX004 be used for comparative safety analysis as it is the only trial with a comparator arm. The Division requested additional information regarding the Sponsor's analyses of the in vivo drug-drug interaction (DDI) studies conducted with caffeine and omeprazole.

The Division also provided guidance regarding clinical datasets, parasitological methods, site inspections, the tropical disease priority review voucher program, statistics, nonclinical pharmacology/toxicology, and clinical pharmacology.

On April 17, 2020, the Division of Medication Errors and Prevention (DMEPA) communicated to the Sponsor that their proposed proprietary name, Fexinidazole (b) (4) is redundant because of the product's established name and does not serve as a unique identifier for the drug product. On April 23, 2020, the Sponsor submitted a request to withdraw the proposed proprietary name, Fexinidazole (b) (4), and to use the established name, Fexinidazole, in its place.

4. Significant Issues From Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

Inspections were conducted remotely by OSI for three Democratic Republic of Congo sites. Their summary findings are presented below. The results of the inspections are generally acceptable and should not impact efficacy and safety review findings. Sites 01, 02, and 04 were located in the Democratic Republic of the Congo (DRC). Due to travel restrictions to the DRC, remote regulatory assessments (in lieu of full GCP site inspections) were conducted at the Applicant (sanofi-aventis) headquarters located at: 255 Corporate Drive, Bridgewater, New Jersey, 08807. Certified hardcopies of study documents were scanned into the electronic master file (eTMF). Video conferencing via WebEx, document sharing via an online platform (Box.com), and read-only access to the online trialmaster file were used in these remote investigations. Drs. Helene Mahenzi Mbembo, Medard Ilunga Wa Kyhi, and Steven Lumeya Vuvu underwent remote regulatory assessments of the clinical investigator's practices and procedures to determine compliance with applicable regulations in support of this NDA. Based on the results of these investigations, the study DNDiFEX004 appears to have been conducted adequately, and the data generated by these clinical investigators generally appear reliable in support of the proposed indication. However, it should be noted that Dr. Vuvu's site enrolled six possibly ineligible subjects (all randomized to the fexinidazole group). Only two of the six events (i.e., the two subjects who lacked confirmatory lumbar puncture) were reported as protocol deviations, and all six subjects were included in the final analysis.

OSI recommended that the six subjects (all of whom were randomized to the fexinidazole group) who lacked confirmatory LP (Subject #' (b) (6) and (b) (6) and with body mass index (BMI) exclusionary criteria (Subjects #'s (b) (6) and (b) (6) should be excluded from the per protocol analysis set. OSI also recommended a sensitivity analysis to assess the robustness of the results, taking the protocol deviations for these six subjects into account.

4.2. Product Quality

The NDA, as amended, has provided sufficient CMC information to assure the identity, strength, purity, and quality of the proposed drug product, fexinidazole tablets. Fexinidazole tablet, 600 mg, is an immediate release tablet. The drug product formulation contains conventional excipients of compendial grade. The fexinidazole tablets are packaged in blister packs made of aluminum foil with peelable aluminum foil lidding. The stability information submitted in the NDA supports the 48-month expiration dating for the drug product to be stored in the proposed blister packs below 30°C. Information provided in the NDA for fexinidazole drug substance and the drug product was found to be adequate. The manufacturing and testing facilities for this NDA are deemed acceptable and an overall "Approve" recommendation was entered into Panorama by the Office of Pharmaceutical Manufacturing Assessment (OPMA) on July 31, 2020.

The NDA is recommended for approval by the Office of Pharmaceutical Quality (OPQ). For details refer to the OPQ Integrated Quality Assessment dated August 12, 2020.

The following CMC postmarketing commitment (PMC) has been agreed to with the Applicant:

Develop a procedure for the [redacted] (b) (4) and establish it as a first GMP step in the manufacturing process for fexinidazole drug substance.

4.3. Clinical Microbiology

The clinical microbiology information, based on the nonclinical studies as well as parasitological assessments in the clinical studies, is summarized below (for details see Section 16.4).

4.3.1. Nonclinical Studies

The activity of fexinidazole and its two metabolites, fexinidazole sulfoxide and fexinidazole sulfone, was assessed using three subspecies of *T. brucei,* namely *T. brucei brucei*, *T. brucei gambiense* and *T. brucei rhodesiense*. Two of the subspecies, namely, *T. brucei gambiense* and *T. brucei rhodesiense*, infect humans; *T. brucei brucei*, closely resembles the human-infective subspecies of *T. brucei* and infects animals such as cattle and wildebeest but does not appear to be pathogenic to humans.

Mechanism of Action

The mechanism by which fexinidazole, a nitro-containing drug, and its metabolites (fexinidazole sulfoxide and fexinidazole sulfone) exhibit activity against *T. brucei* has not been investigated. Studies with *T. brucei brucei* and other protozoans suggest that the nitroreductase (NTR) enzyme plays an important role in the bioactivation of fexinidazole resulting in the generation of reactive amines and damage to DNA and proteins (for details see Section 16.4.1.1).

In Vitro Activity

The in vitro activity of fexinidazole and its metabolites was measured in axenic cultures of the trypanosomes of different strains/isolates of *T. brucei brucei, T. brucei gambiense,* and *T. brucei rhodesiense*. The methods for measurement of in vitro sensitivity of *T. brucei* are not standardized and limited to testing in research laboratories. The results show that based on the inhibition of growth, the 50% inhibitory concentration (IC_{50}) values for fexinidazole against the strains of *T. brucei brucei, T. brucei gambiense,* and *T. brucei rhodesiense*, were ≤ 2.86, 0.93, and 0.94 µg/mL, respectively. Based on a small number of strains tested, the activity of fexinidazole, fexinidazole sulfoxide, and fexinidazole sulfone appear to be similar.

The activity of fexinidazole or its metabolites against the trypanosomes is cidal and appears to be concentration- and time-dependent. The maximum killing of *T. brucei rhodesiense* in vitro required up to 48 hours of exposure to the drug.

For details see Section 16.4.1.2.

In Vivo Activity (Animal Studies)

The activity of fexinidazole was measured in acute and chronic murine infection models of HAT. Although the route of infection in mice is intraperitoneal, several aspects of the disease in *T. brucei*-infected animal models mimic human disease. Briefly, there is a progressive increase in parasitemia after infection with the bloodstream trypanosomes. However, the time to peak parasitemia varies depending on the *T. brucei* subspecies, the strain, and the inoculum concentration of trypanosomes used for infection. CNS involvement occurs when the infection is allowed to develop beyond 15 to 20 days resulting in the development of meningoencephalitis and death of the mice. Generally, the acute infection models mimic the early Stage 1 HAT (hemolymphatic stage) whereas the chronic infection models mimic the late Stage 2 HAT (meningo-encephalitic stage, when parasites have disseminated to the brain). CNS infection in untreated mice can result in hind leg paralysis and death. Relapse can occur after discontinuation of treatment, when the trypanosomes escape the action of the drug(s), including drugs that do not enter the brain.

Studies show that fexinidazole was effective in curing mice infected with any of the three subspecies tested. Cure was based on evidence of parasites in blood and/or brain of the surviving mice. In the acute infection model, no relapse was reported in any study under the experimental conditions tested. However, in the chronic infection model, relapse was reported in some of the studies, which is probably due to the presence of the residual parasites in the brain.

One study (Kaiser et al. 2011) reported reduced activity of the metabolites, compared to fexinidazole, in mice with acute infection due to *T. brucei rhodesiens*e. All the fexinidazole-treated mice survived, and no relapse was observed until Day 60 under the experimental conditions tested. However, survival was lower in mice treated with fexinidazole sulfoxide (50%) and fexinidazole sulfone (25%), and relapse was observed; the mice treated with fexinidazole sulfoxide and fexinidazole sulfone relapsed at>38.5 days and >31.5 days, respectively.

For details see Section 16.4.1.3.

Drug Resistance and Cross-Resistance

In vitro studies suggest a potential for the development of resistance by *T. brucei* parasites to fexinidazole. The mechanism of resistance to fexinidazole appears to be similar to other nitro-containing drugs such as nifurtimox, that includes reduced expression of *Type I* NTR.

Studies suggest cross-resistance between fexinidazole and nifurtimox. Fexinidazole-resistant clones of *T. brucei brucei*, generated in vitro, were resistant to nifurtimox; similarly, nifurtimox-resistant clones were resistant to fexinidazole, both in vitro and in vivo. Although the clinical relevance of these findings is not known, the potential for the development of resistance to fexinidazole in patients previously treated with NECT exists.

For details see Sections 16.4.1.5 and 16.4.1.6.

4.3.2. Parasitological Assessment in Clinical Studies

The parasitological testing in the 3 clinical trials, conducted in areas known to be endemic for *T. brucei gambiense*, include detection of

- anti-*T. brucei gambiense* antibodies by the Card Agglutination Test for Trypanosomiasis (CATT), as well as
- motile parasites in blood, lymph node aspirate, and/or CSF.

Initial screening of the participants was performed by card agglutination test for trypanosomiasis (CATT) followed by confirmation of diagnosis based on microscopic evidence of parasites by the concentration tests in fresh blood, lymph node aspirate, and/or CSF. All parasitological assessments were performed at the investigational sites or based on certified report from the mobile team (for details see Section 16.4.2.1).

Based on epidemiological findings, *T. brucei gambiense* is known to be prevalent in DRC and CRC.

The parasitological tests used by the Applicant are adequate for diagnosis and follow-up. All enrolled patients had evidence of parasites in blood, lymph node aspirate, and/or CSF. Early and late Stage 2 patients without evidence of parasites in the CSF had 6 to 20 and >20 WBC/µL of CSF, respectively, suggestive of inflammatory response to infection. At the end of 10 days of treatment, only one patient in the fexinidazole treatment group in Study DNDiFEX004 had detectable parasites in the blood. At Month 3 follow-up, there was no evidence of parasites in any of the body fluid tested (blood, lymph node aspirate, and/or CSF), in any patient in all three studies. By Month 18, confirmed relapse, based on evidence of parasites in any body fluid, was reported in 11 of the 23 late Stage 2 patients who failed fexinidazole treatment in Study DNDiFEX004; of these 11 patients, 9 had evidence of parasites in the CSF. None of the patients that failed treatment in Studies DNDiFEX005 or DNDiFEX006 were confirmed relapses.

For details see Sections 16.4.2 and 8.

A clinical trial (Study DNDi-FEX-07-HAT) in patients with *T. brucei rhodesiense* is ongoing in Malawi and Uganda.

4.3.3. Conclusions

Fexinidazole and its metabolites (fexinidazole sulfoxide and fexinidazole sulfone) are active against *T. brucei gambiense* and *T. brucei rhodesiense* in vitro as well as in acute and chronic murine animal models of infection. The clinical studies support the effectiveness of fexinidazole against *T. brucei gambiense* in patients with Stage 1, early Stage 2, or late stage 2 HAT. A clinical trial (Study DNDi-FEX-07-HAT) in patients with *T. brucei rhodesiense* is ongoing.

Studies with *T. brucei* and other protozoans such as *Leishmania donovani* and *Trypanosoma cruzi* suggest that the mechanism of action and resistance to fexinidazole is similar to other nitro-containing drugs such as nifurtimox. Cross-resistance between fexinidazole and nifurtimox appears to be due to down regulation of Type I NTR, important for bioactivation of nitro-containing drugs. Although the clinical relevance of these findings is not known, the potential

for the development of resistance to fexinidazole in patients previously treated with NECT cannot be ruled out.

4.4. Devices and Companion Diagnostic Issues

Not applicable to this NDA; no companion diagnostics or devices are being evaluated in conjunction with this NDA.

5. Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

The nonclinical safety profile of fexinidazole was evaluated in primary, secondary, and safety pharmacology studies in rats, dogs, and human tissues. Repeat-dose toxicology studies were conducted in rats and dogs for up to 28 days. Fertility and pre- and postnatal developmental studies were conducted in rats; embryo-fetal developmental studies in rats and rabbits; in vitro genetic toxicology studies in *Salmonella* strains and cultured human peripheral blood lymphocytes, and in vivo studies in mouse bone marrow. In vivo and in vitro pharmacokinetics studies were conducted to evaluate the absorption, distribution, metabolism, and excretion of fexinidazole in rats, dogs, mice, hamsters, and monkeys.

Pharmacokinetics

Fexinidazole was well-distributed with higher steady-state volume of distributions than total body water in the mouse (42 L/kg), rat (4.7 L/kg), and dog (3.2 L/kg). Fexinidazole had moderate oral bioavailability (30 % in rats) and the T_{max} of the parent drug was 0.5 to 2 hours after oral administration across the species tested. The drug was highly bound to plasma protein (88 to 95 %) in all species examined and had wide tissue distribution in rodents. The highest level of fexinidazole-related radioactivity was measured 2 hours after dosing in the intestinal wall, stomach, liver, adrenal glands, and kidney. Concentrations in some tissues were higher than in blood. Fexinidazole penetrated the blood brain barrier with a brain T_{max} of between 2 and 8 hours after dosing in rats. The level of fexinidazole in brain was approximately 0.6 times the levels in plasma. Fexinidazole is metabolized to two main metabolites, fexinidazole sulfoxide (M1) and fexinidazole sulfone (M2). Nonclinical studies were considered adequate to assess the effects of these metabolites since levels in rats and dogs were higher than levels observed in humans.

Fexinidazole was almost completely metabolized before elimination since no parent drug was recovered in urine and only minimal amounts were recovered in feces. Most of the radioactive dose was eliminated via fecal and urinary excretion (59% and 30% of the oral dose, respectively, over 96 hours). The C_{max} and AUC values for drug-related material in milk in response to a single dose of fexinidazole administered to pregnant rats was about 80% of that measured in plasma. Fexinidazole appears to transfer to pups through milk since the drug was detected in the plasma of neonates on lactation day 4, after oral administration of fexinidazole to lactating rats.

Safety Pharmacology

Fexinidazole did not result in any biologically significant general behavioral effects in the Irwin test or effects on respiratory parameters following single oral doses of up to 300 mg/kg, although minimal reduction in mobility and slight to moderate palpebral closure were observed at 1000 mg/kg. Although there was no clear evidence of dose-related Qt prolongation in dogs after single doses of fexinidazole up to 1000 mg/kg, the **h**uman ***E**ther-à-go-go*-**R**elated **G**ene

(hERG) assay showed that fexinidazole sulfone, the fexinidazole metabolite, reduced the residual current by 33 % at 9.3 µg/mL. Plasma levels of fexinidazole sulfone in patients receiving the recommended dose of fexinidazole (C_{max} 19.6 µg/mL) were about two times the levels shown to affect hERG potassium channels. Fexinidazole sulfone levels at clinical exposures could therefore contribute to the Qt prolongation described in patients in Section 5.2 of the Fexinidazole Prescribing information.

Repeat-Dose Toxicology Studies

The liver and thymus were identified as the target organs of toxicity in repeat-dose toxicity studies in rats and dogs at doses of 50, 200, and 800 mg/kg/day for 28 days.

In rats, all doses were associated with increased minimal to slight centrilobular hepatocellular hypertrophy (not entirely reversible at 800 mg/kg after 2 weeks drug-free). The 800 mg/kg dose was associated with reduced body weights (in males only) and reversibly increased liver weights. The no-observed-adverse-effect level (NOAEL) was estimated to be 200 mg/kg for males (0.7 times the clinical exposure, which is approximately 7 µg*hr/mL) and 800 mg/kg for females (similar to the clinical exposure).

In dogs, alkaline phosphatase and cholesterol increased and thymus weights decreased at all doses. In addition, the 800 mg/kg dose was associated with increased severity of thymus involution at the end of dosing. Increased severity of thymus involution and decreased thymus weights persisted till the end of the reversibility period at 800 mg/kg. Reduced cellularity of bone marrow was seen in one mid- and one high-dose male. Ovary weights were increased at all doses, but this was ascribed to the immaturity of the ovary in all control animals. No NOAEL could be determined due to reduced thymus weights at all doses.

Genetic Toxicology and Carcinogenicity

Fexinidazole was mutagenic (positive in strains TA98, TA100, and TA1535 in the presence and absence of S9 and TA1537, and TA102 in the presence of S9) but was negative in the in vitro micronucleus assays in cultured human peripheral blood lymphocytes up to 180 µg/mL (+/-S9), and the in vivo micronucleus assay in the bone marrow of treated mice (2 oral doses up to 2000 mg/kg). The Applicant argues that the positive finding in the Ames assay does not indicate a risk to humans because bacterial nitroreductase enzymes contribute to the mutagenicity of fexinidazole and equivalent enzymes in mammalian cells cannot nitroreduce compounds with low redox potential and generate mutagenic by-products. As evidence, the Applicant points to the fact that higher levels of fexinidazole are required to induce revertant colonies in nitroreductase-deficient Salmonella strains TA98NR and TA100NR. This reviewer notes that even the nitroreductase-deficient strains the Applicant tested in the Ames assay were positive; therefore, the positive mutagenicity results will be described in the prescribing information. No carcinogenicity studies have been conducted with fexinidazole, but it is structurally similar to another widely used genotoxic antibiotic, metronidazole, which is used for similar duration and has been shown to have carcinogenic potential.

Reproductive Toxicology

Fexinidazole was not associated with effects on fertility. Daily oral doses of fexinidazole to rats for 14 days (females) or 28 days (males) prior to cohabitation and during cohabitation had no effects on male or female reproductive performance, on the estrous cycle or sperm, or on embryo/fetal viability. The NOEL for fertility and early embryonic development in rats was 600 mg/kg/day, the highest dose tested. The exposure at the NOAEL was estimated at 9 µg*hr/mL, similar to the clinical exposure.

Developmental toxicity studies were conducted in rats and rabbits, at doses resulting in levels of fexinidazole, M1 and M2 metabolite up to about 2 times the clinical exposure based on area under the curve (AUC) comparisons. Delayed ossification-reduced fetal weights and reduced placental weights were observed at 800 mg/kg in the presence of maternal toxicity (reduced maternal body weights). There was no effect on prenatal development in the rat up to the daily dose of 200 mg/kg, similar to the clinical dose based on AUC comparisons.

In rabbits, administration of oral fexinidazole at 20 and 40 mg/kg to pregnant females during organogenesis resulted in abortions in animals that experienced prolonged, severe reductions in food consumption and moderate reductions in body weight. The abortions were therefore ascribed to the maternal toxicity. Excessive maternal toxicity is not uncommon when rabbits are used in embryo-fetal toxicity testing of antibiotics. The rabbit does not therefore serve as a good model for embryo-fetal toxicity testing of antibiotics. Although M1 and M2 metabolites were detected at all doses, no detectable fexinidazole was observed at 10 mg/kg, and concentrations close to the lower limit of quantification of the bioanalytical method were measured at 40 mg/kg. Based on these findings, the NOAEL for maternal toxicity and embryofetal development in the rabbit was 10 mg/kg/day, equivalent to a dose of less than 0.01 times the maintenance dose based on C_{max} comparisons).

Fexinidazole administration to pregnant rats at 600 mg/kg, from Gestation Day (GD) 6 to Lactation Day (LD) 21, resulted in reduced body weights in F0 dams and slight delays in the mean age for balanopreputial cleavage in F1 male rats and for vaginal opening in F1 female rats. The NOAEL for maternal toxicity and pre- and postnatal development was 200 mg/kg, similar to the clinical dose based on AUC comparisons.

The sponsor has provided enough nonclinical safety information on fexinidazole to support approval for marketing in the U.S.

5.2. Referenced NDAs, BLAs, DMFs

None

5.3. Pharmacology

Primary Pharmacology

Fexinidazole is orally bioavailable and converted to two main active metabolites: fexinidazole sulfoxide (M1) and fexinidazole sulfone (M2). The two metabolites have equivalent in vitro activities against *T. b. rhodesiense* and *T. b. gambiense* compared to fexinidazole.

The trypanocidal mechanism of action of fexinidazole remains unclear. Bioactivation appears to be mediated by reduction of fexinidazole by NADH-dependent, type I nitroreductase (NTR) enzymes in trypanosomatids. The reduction by type I NTR leads to an unsaturated open-chain nitrile product but the fexinidazole reduction product is responsible for its cidal activity is unknown. Please see Section 4.3 (Clinical Microbiology) for further information.

Secondary Pharmacology

Fexinidazole and its metabolites were evaluated in various in vitro receptor binding, ion channel, and enzyme assays, as well as binding sites on proteins involved in uptake processes and CNS regulatory processes. Fexinidazole had little effect on receptor binding and enzyme activity at 10 µM except at the muscarinic receptors where it inhibited binding to the muscarinic M_1 and M_2 receptors by 52% and 65%, respectively.

Table 2. Binding of Fexinidazole and Its Metabolites (Fexinidazole Sulfone and Fexinidazole Sulfoxide) at Muscarinic Receptors 10 µM, Study # 14929

	% Inhibition of Control Specific Binding		
Receptor	Fexinidazole	Fexinidazole Sulfone (M1) Metabolite	Fexinidazole Sulfoxide (M2)
Muscarinic receptor 1	52	13	22
Muscarinic receptor 2	65	17	29

Source: Study # 14929

Safety Pharmacology

Fexinidazole, Fexinidazole Sulfoxide, Fexinidazole Sulfone: Effect on hERG Tail Current Recorded From Stably Transfected HEK 293 Cells. Study # 0507-2007

Table 3. Effect on hERG Tail Current Recorded From Stably Transfected HEK 293 Cells, Fexinidazole, Fexinidazole Sulfoxide, and Fexinidazole Sulfone, Study # 0507-2007

Treatment	Concentration (µM)	Concentration (µg/mL)	Residual Current
Fexinidazole	1	0.28	100
	5	1.4	94
	30	8.4	89
Fexinidazole sulfoxide	1	0.3	87
	5	1.5	101
	30	8.9	98
Fexinidazole sulfone	1	0.3	96
	5	1.6	91
	30	9.3	67

Source: Study # 0507-2007

Fexinidazole sulfone reduced the residual current by 33 % at 9.3 µg/mL. In healthy adult subjects, the C_{max} for fexinidazole sulfone was 19.6 µg/mL following administration of fexinidazole tablets at the recommended dose of 1800 mg once daily for 4 days under fed conditions. Based on the hERG data, clinical doses could lead to fexinidazole sulfone levels which could theoretically impact potassium channel function.

Fexinidazole: Effects on Cardiovascular Parameters After Oral Administration to the Beagle Dog. Study #0506-2007

To evaluate the potential cardiovascular effects of fexinidazole, male beagle dogs (n=4) were administered vehicle or drug at escalating doses of 100, 300, and 1000 mg/kg with a 4-day interval between each test item dose. No mortality, morbidity, or effects on body weight/food consumption were noted. Assessments of cardiovascular function were based on hemodynamic and electrocardiographic (ECG) parameters where telemetry data were continuously recorded for at least 60 minutes prior to dosing and through at least 24 hours after treatment. Calculated parameters included systolic blood pressure, diastolic blood pressure, average blood pressure, heart rate, ECG intervals (RR, PR, QRS and QT, ms), body temperature.

Although no plasma drug levels were evaluated in this study, plasma levels determined as part of another 3-day toxicity study showed systemic exposure (both in terms of C_{max} and AUC) reached a plateau at 500 mg/kg/day. No biologically significant fexinidazole-related effects were noted on qualitative or quantitative ECG or hemodynamic parameters. A transient increase in mean Qt interval at the low dose was not seen at the mid and high doses. This was at least partly driven by one animal which showed wide variations in Qt beginning in the predose period. Body temperature was moderately increased in a dose-dependent manner, between 2 and 5 hours after treatment at doses of 300 and 1000 mg/kg (+0.6 and +1.0°C, respectively). The NOAEL was 100 mg/kg. No drug-related cardiovascular effects were observed.

Fexinidazole: Effects on General Behavior (Irwin's test) and Body Temperature in the Male Rat after Oral Administration. Study # 0508-2007

To evaluate the neurological effects of fexinidazole, twenty-four male Rat/Crl:CD(SD)BR rats were randomized to four groups (6 rats/group) and administered a single dose by oral gavage of vehicle (5% Tween 80 in 0.5% methyl cellulose 400 cP (Methocel) or fexinidazole at 100, 300, or 1000 mg/kg. Effect on Irwin's test was assessed before treatment and 2 and 24 hours after vehicle or fexinidazole.

Evaluations included home cage observations (including piloerection, convulsions), open field observations (including mobility, startle response), manipulation observations (including grip strength, corneal reflex), close observations (including pupil size, salivation), and final observations (including pain response, aggressiveness). No fexinidazole-related effects were observed for clinical signs or body temperature at 100 or 300 mg/kg. At 1000 mg/kg, two animals showed a minimal reduction in mobility and three animals presented with slight to moderate palpebral closure. One animal showed these signs at 24 hours after treatment. Unlike the dog study, body temperature was unaffected.

Based on these results, the NOAEL was 300 mg/kg. Body temperature was not affected at any dose.

Fexinidazole: Effects on Respiratory Parameters in the Unrestrained Conscious Male Rat After Oral Administration. Study # 0509-2007

To evaluate the respiratory effects of fexinidazole, male Crl:CD(SD)BR rats were randomized to four groups (eight rats/group) and administered a single dose by oral gavage of vehicle or fexinidazole at 100, 300 or 1000 mg/kg. The effect of treatment on respiratory function was assessed by placing the animals in individual plethysmographic chambers and recording respiratory parameters for 4 hours after treatment.

Respiratory parameters were acquired continuously at the sampling frequency of 500 Hz by Notocord HEM 3.4 software. Basal values were calculated as the mean of values extracted every 5 minutes from 30 to 10 minutes before treatment. After treatment, values were extracted every 30 minutes up to 4 hours. No mortality, morbidity, or signs of toxicity were observed. No biologically significant fexinidazole-related changes in respiratory parameters were observed. Based on these results, the NOEL for respiratory function in rats is 1000 mg/kg.

5.4. ADME/PK

Type of Study	Major Findings
Absorption Fexinidazole: Evaluation of the pharmacokinetics and of the bioavailability after single IV and oral administration to male Sprague Dawley rats. Study 0327-2007	**Table 4. Fexinidazole Plasma Pharmacokinetics in Rats**
Distribution	

	Analyte					
	Fexinidazole		M1		M2	
Plasma Pharmacokinetics	**5 mg/kg IV**	**10 mg/kg Oral**	**5 mg/kg IV**	**10 mg/kg Oral**	**5 mg/kg IV**	**10 mg/kg Oral**
$C_{0.033}$ (µg/mL)	3.7	-	-	-	-	-
C_{max} (µg/mL)	-	0.2	3.1	3.5	2.1	2.7
T_{max} (h)	0.03	2	1	2	5	6
T_{last} (h)	6	8	6	8	6	8
$AUC_{0\text{-}t(last)}$ (µg.h/mL)	1.2	0.6	9.4	16	9.6	14
$T_{1/2,z}$ (h)	1.7	1.7	-	-	-	-
$AUC_{0\text{-}\infty}$ (µg.h/mL)	1.2	0.7	-	-	-	-
CL (L/h/kg)	4.6	-	-	-	-	-
Vss (L/kg)	4.7	-	-	-	-	-
F% (AUC0-∞)	-	30	-	-	-	-

Source: Study 0327-2007
Abbreviations: $AUC_{0\text{-}t(last)}$, area under the plasma/serum concentration versus time curve from time zero to the time corresponding to the last quantifiable concentration; $AUC_{0\text{-}\infty}$, total drug exposure across time; $C_{0.033}$, CL, clearance; C_{max}, maximum serum concentration; F, bioavailability; IV, intravenous; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; T_{last}, time of last measurable concentration; T_{max}, time at which the C_{max} is observed; V_{ss}, volume of distribution at steady state

Type of Study	Major Findings
Fexinidazole: Determination of Tissue Distribution by Whole Body Autoradiography following Single Oral Administration of [^{14}C]-Fexinidazole to Rats. Study # 0510-2007	**Table 5. Mean Tissue Concentration (µg Eq/G) After 800 mg/Kg Oral Dose to Male Rats**

	Mean Tissue Concentration (µg Eq/g)			
Tissue/organ	**2h**	**8h**	**24h**	**48h**
Adrenal glands	540	465	83	24
Blood	303	273	51	14
Bone	38	24	17	NI
Bone marrow	289	211	75	15
Brain	160	149	22	6
Brown fat	276	252	67	24
Eyes	76	111	73	30
Harderian glands	310	294	53	21
Heart	342	297	62	18
Intestine wall	5281	505	104	NI
Kidney	458	420	103	29
Liver	555	439	95	22
Lungs	304	250	51	13
Lymph node	281	234	46	NI
Muscle	331	233	50	17
Pancreas	396	306	56	12
Pituitary	315	243	41	NI
Prostate	404	268	51	NI
Salivary glands	309	246	49	11
Seminal vesicles	410	292	53	NI
Skin	269	228	58	17
Spinal cord	133	147	23	7
Spleen	323	225	56	15
Stomach	620	307	79	NI
Testis	172	149	31	9
Thymus	263	21	42	11
Thyroid	317	264	NI	NI
Urinary bladder	373	284	315	NI

Source: Study # 0510-2007
Abbreviations: NI, not identifiable

Type of Study	Major Findings
Study to investigate the pharmacokinetics and blood brain penetrability of Fexinidazole following intravenous and oral administration in the mouse. Study # DND/01	

Table 6. Mean Plasma: Brain Ratios of Fexinidazole, M1 and M2

Route of Administration	Sample Time (Min)	Mean Plasma: Brain Ratios of Fexinidazole, M1 and M2			
		Plasma	Brain Fexinidazole	Brain M1	Brain M2
1 mg/kg Intravenous	5	1.0	0.6	0.045	0
	15	1.0	0.8	NST	NST
	30	1.0	1.0	0.064	0
25 mg/kg PO	15	1.0	0.8	NST	NST
	30	1.0	1.2	0.078	0.05
	60	1.0	1.2	0.12	0.06

Source: Study # DND/01
Abbreviations: M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; NST, no sample taken; PO, by mouth

Table 7. Mean Brain Plasma Concentrations of Fexinidazole, M1 and M2 (µg/G)

Route of Administration	Sample Time (min)	Mean Brain Plasma Concentrations of Fexinidazole, M1 and M2 (µg/g)		
		Brain Fexinidazole	Brain M1	Brain M2
1 mg/kg Intravenous	5	0.09	0.04	BLQ
	15	0.02	NST	NST
	30	0.01	0.058	BLQ
25 mg/kg PO	15	0.38	NST	NST
	30	0.27	1.1	0.16
	60	0.25	1.6	0.39

Source: Study # DND/01
Abbreviations: M1, Fexinidazole sulfoxide; M2, Fexinidazole sulfone; NST, no sample taken

Table 8. Plasma Protein Binding by Fexinidazole and Its Major Metabolites M1 and M2 at 10µM

Test Compound	% Bound					
	Mouse	Rat	Hamster	Rabbit	Dog	Human
Fexinidazole	88	91	94	93	91	94
M1	35	42	47	49	27	38
M2	47	63	60	65	35	39

Source: Study # DND/01
Abbreviations: M1: Fexinidazole sulfoxide M2: Fexinidazole sulfone

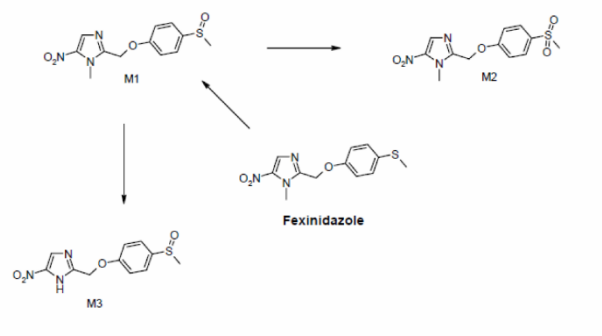
Metabolism	

Type of Study

Fexinidazole: Evaluation of the In Vitro Cross Species Intrinsic Clearance and Metabolism with Mouse, Rat, Dog, Monkey and Human Hepatocytes. Study # 0141-2007

Major Findings

Figure 1. Possible Metabolic Pathway



Source: Study # 0141-2007
Abbreviations: M1, Fexinidazole sulfoxide; M2, Fexinidazole sulfone; M3, N-des-methyl fexinidazole sulfoxide

Table 9. Metabolites Forming at T=0 and After 30 and 120 Minutes of Incubation With Rat Dog and Human Hepatocytes (% of Total Drug-Related Material)

	Time (mins)								
	Rat			Dog			Human		
Compound %	**0**	**30**	**120**	**0**	**30**	**120**	**0**	**30**	**120**
Parent	68	-	-	50	-	-	83	2	<1
M1	32	90	68	50	94	84	17	96	95
M2	-	10	32	-	4	15	-	2	4
M3	-	-	-	-	1	1	-	<1	-

Source: Study # 0141-2007
-: indicates reduction in parameters compared to control.
Abbreviations: M1, Fexinidazole sulfoxide; M2, Fexinidazole sulfone; M3, N-des-methyl fexinidazole sulfoxide

Excretion

Fexinidazole: Determination of Excretion Balance following Single Oral Administration of [^{14}C]-Fexinidazole to Rats. Study # 0162-2008

Table 10. Fexinidazole-Related Radioactivity (as a % of the Excreted Dose) Excreted in Rats Given a Single Oral 800 mg/Kg Dose of [^{14}C]-Fexinidazole

Fexinidazole-Related Radioactivity (% Excreted Dose)	**Excretion Route**		
	Urine	**Feces**	**Cage Washing**
0-8h	5.2	NA	-
8-24h	16	29	1.9
24-48h	7.4	24	0.7
40-72h	1.2	5	0.1
72-96h	0.3	0.5	0.1
0-96	30	59	2.8

Type of Study: TK data from general toxicology studies

Major Findings

Table 11. 28-Day Oral Toxicity Study in the Rat, Study # 0504-2007

$T_{1/2}$	2-4 hours*
Accumulation	Females: 2x (28 days dosing up to 200 mg/kg). Males: 4x to 6x (28 days dosing up to 200 mg/kg). No accumulation at 800 mg/kg
Dose proportionality:	Approximately dose proportional

Source: Study # 0504-2007
Abbreviation: $t_{1/2}$, half-life

Table 12. 3-Day Repeated Oral PK Study in Rat, Study 0513-2007

Fexinidazole Dose (mg/kg)	AUC (µ*h/mL) 500	1000
Fexinidazole	9.2	9.4
M1	271	287
M2	503	567

Source: Study 0513-2007
Abbreviations: AUC, area under the time concentration curve; M1, Fexinidazole sulfoxide; M2, Fexinidazole sulfone

Table 13. Fexinidazole: Evaluation of Pharmacokinetics Following repeat doses in dogs

$T_{1/2}$	2 hours***
Accumulation	No accumulation
Dose proportionality:	Approximately dose proportional

Source: Study # 0505-2007
Abbreviation: $t_{1/2}$, half-life

Type of Study: TK data from reproductive toxicology studies

* Fexinidazole: DRF oral embryofetal and Pre- and Postnatal Development Toxicity study in the rat. Study # 0340-2008

** Fexinidazole: Evaluation of Pharmacokinetics Following Oral Administration in the Rabbit. Study # 0049-2010

Major Findings

Rat*
Mean AUC: 2.6, 7.5, 17.3 µg*h/mL at 50, 200 and 800 mg/kg/day.

Rabbit**
AUC: Fexinidazole not quantifiable at 10 or 40 mg/kg

Table 14. Fexinidazole Plasma Pharmacokinetics in Rabbits

	Analyte					
	Fexinidazole		M1		M2	
Fexinidazole Dose/ Route	10 mg/kg	40 mg/kg	10 mg/kg	40 mg/kg	10 mg/kg	40 mg/kg
Plasma pharmacokinetics						
C_{max} (µg/mL)	ND	0.007	1.4	2.6	2.5	4.1
T_{max} (h)	ND	1.5	1.0	1.0	1.7	3.3
$AUC_{0\text{-}t(last)}$ (µg.h/mL)	ND	ND	2.8	8.5	16	48

Source: Study # 0049-2010
Abbreviation: $AUC_{0\text{-}t\ (last)}$, area under the plasma/serum concentration versus time curve from time zero to the time corresponding to the last quantifiable concentration; C_{max}, maximum serum concentration; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; T_{max}, time at which the C_{max} is observed;

5.5. Toxicology

5.5.1. General Toxicology

Study title/ number: 28-Day Oral Toxicity Study in the Rat (Study # 0504-2007)

Key Study Findings:

- High dose animals showed reduced body weights and reversibly increased liver weights.
- Increased minimal to slight centrilobular hepatocellular hypertrophy at all doses. (not entirely reversible at 800 mg/kg after 2 weeks drug-free)
- The NOAEL dose was 200 mg/kg (AUC4.6 µg*h /mL about 0.7 times the clinical exposure) in the males due to reduced body weights at 800 mg/kg and 800 mg/kg in the females (AUC7.9, similar to the clinical exposure)

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 15. Methods of 28-Day Oral Toxicity Study in the Rat, Study # 0504-2007

Parameters	Details
Dose and frequency of dosing:	0, 50, 200, 800 mg/kg
Route of administration:	Oral gavage
Formulation/vehicle:	5% Tween 80/0.5% Methyl cellulose 400 cP (Methocel) suspension, water for injection
Species/strain:	Rat
Number/sex/group:	10-15/sex/group
Age:	6 weeks
Satellite groups/unique design:	3/sex/group (toxicokinetic group)
Deviation from study protocol affecting interpretation of results:	No

Observations and Results: Changes From Control

Parameters	Major Findings
Mortality	No drug-related mortality
Clinical signs	No drug-related clinical signs
Body weights	**Table 16. Body Weight Compared to Control Rats on Day 28** Dose / Male / Female LD / -3 % / +1 % MD / -7 % / +1 % HD / -10 % / -2 % Abbreviations: HD, high dose; LD, low dose; MD, mid dose
Ophthalmoscopy	No drug-related changes in ophthalmoscopy findings.

<table>
<tr><th>Parameters</th><th>Major Findings</th></tr>
<tr><td>Hematology</td><td>Day 29
HD: Absolute neutrophils -47 % (males)
Day 43
HD: Absolute neutrophils +11 % (males)</td></tr>
<tr><td>Clinical chemistry</td><td>No drug-related effects</td></tr>
<tr><td>Urinalysis</td><td>No drug-related effects</td></tr>
<tr><td>Gross pathology</td><td>No drug-related effects</td></tr>
<tr><td>Organ weights</td><td>Table 17. Absolute Liver Weights (% Increase, Males and Females Combined)
<table><tr><th>Day</th><th>LD</th><th>MD</th><th>HD</th></tr><tr><td>28</td><td>+8</td><td>+8</td><td>+21</td></tr><tr><td>43</td><td>-</td><td>-</td><td>+2</td></tr></table>Note: Absolute liver weights were not evaluated in recovery animals.
-: indicates reduction in parameters compared to control.
Abbreviations: HD, high dose; LD, low dose; MD, mid dose</td></tr>
<tr><td>Histopathology
Adequate battery: Yes</td><td>Day 28: Increased minimal to slight centrilobular hepatocellular hypertrophy compared to control, all doses
Day 43: Increased minimal centrilobular hypertrophy at HD (only drug-treated group evaluated)</td></tr>
<tr><td>Pharmacokinetics</td><td>Table 18. Fexinidazole Pharmacokinetics ($AUC_{(0\text{-}Last)}$, µg*H /mL)
<table><tr><th>Dose</th><th colspan="2">Day 1</th><th colspan="2">Day 28</th></tr><tr><th>(mg/kg)</th><th>Male</th><th>Female</th><th>Male</th><th>Female</th></tr><tr><td>50</td><td>0.2</td><td>0.8</td><td>0.9</td><td>1.6</td></tr><tr><td>200</td><td>1.1</td><td>3.2</td><td>4.4</td><td>4.8</td></tr><tr><td>800</td><td>10.0</td><td>15.7</td><td>11.9</td><td>7.9</td></tr></table>Abbreviation: $AUC_{(0\text{-}last)}$, area under the plasma/serum concentration versus time curve from time zero to the time corresponding to the last quantifiable concentration
Table 19. Fexinidazole Pharmacokinetics C_{max}, (µg/mL)
<table><tr><th>Dose</th><th colspan="2">Day 1</th><th colspan="2">Day 28</th></tr><tr><th>(mg/kg)</th><th>Male</th><th>Female</th><th>Male</th><th>Female</th></tr><tr><td>50</td><td>0.04</td><td>0.2</td><td>0.1</td><td>0.4</td></tr><tr><td>200</td><td>0.2</td><td>0.6</td><td>0.3</td><td>0.9</td></tr><tr><td>800</td><td>1.5</td><td>1.5</td><td>0.5</td><td>0.7</td></tr></table>Abbreviation: C_{max}, maximum serum concentration</td></tr>
</table>

General Toxicology: Additional Studies

Study title/ number: Fexinidazole: 28-Day Oral Toxicity Study in the Dog (Study # 0505-2007)

Key Study Findings

- Bodyweight reduced in high dose females
- Decreased thymus weights at all doses, with increased severity of thymus involution at the end of the reversibility period at 800 mg/kg

No NOAEL could be determined for this study.

Conducting laboratory and location:	(b) (4)
GLP compliance: Yes	Yes

Table 20. Methods of 28-Day Oral Toxicity Study in the Dog, Study # 0505-2007

Parameter	Details
Dose and frequency of dosing:	0, 50, 200, 800 mg/kg
Route of administration:	Oral gavage
Formulation/vehicle:	5% Tween 80/0.5% Methyl cellulose 400 cP (Methocel) suspension, water for injection
Species/strain:	Dog/beagle
Number/sex/group:	3/sex/group
Age:	1 year old
Satellite groups/unique design:	2/sex/group (2-week recovery group)
Deviation from study protocol affecting interpretation of results:	No

Observations and Results: Changes From Control [include major findings only]

Parameters	Major Findings
Mortality	No drug-related mortality
Clinical signs	No drug-related clinical signs
Body weights	**Table 21. Mean Bodyweight in HD Dogs Compared to Control Days 28 and 40 (Recovery)** Day: Male / Female Day 28: -0 % / -8 % Day 40: -0 % / -15 % Abbreviations: HD, high dose
Ophthalmoscopy	No drug-related changes in ophthalmoscopy findings.
Hematology	**Table 22. Lymphocytes Count Change Compared to Controls** Control: LD / MD / HD Day 28: -14% / -23% / -31% Day 42: - / - / -22% Note: males and females combined - Lymphocytes count was not evaluated in low- and mid-dose recovery animals. Abbreviations: HD, high dose; LD, low dose; MD, mid dose **Table 23. Neutrophil Count Change Compared to Controls** Control: LD / MD / HD Day 28: -8% / -8% / -20% Day 42: - / - / -18% Note: males and females combined - Neutrophil count was not evaluated in low- and mid-dose recovery animals Abbreviations: HD, high dose; LD, low dose; MD, mid dose

<table>
<tr><th>Parameters</th><th>Major Findings</th></tr>
<tr><td>Clinical chemistry</td><td>
Table 24. Alkaline Phosphatase Levels (IU/L, Males and Females Combined)
<table>
<tr><th>Day</th><th>Control</th><th>LD</th><th>MD</th><th>HD</th></tr>
<tr><td>Day 28</td><td>67</td><td>+13 %</td><td>+19 %</td><td>+29</td></tr>
<tr><td>Day 43</td><td>70</td><td>-</td><td>-</td><td>+0%</td></tr>
</table>
- Alkaline Phosphatase levels were not evaluated in low- and mid-dose recovery animals
Abbreviations: HD, high dose; LD, low dose; MD, mid dose

Table 25. Cholesterol Levels (mg/Dl, Males and Females Combined)
<table>
<tr><th>Day</th><th>Control</th><th>LD</th><th>MD</th><th>HD</th></tr>
<tr><td>Day 28</td><td>114</td><td>+18%</td><td>+25%</td><td>+47%</td></tr>
<tr><td>Day 43</td><td>120</td><td>-</td><td>-</td><td>+15 %</td></tr>
</table>
- Cholesterol levels were not evaluated in low- and mid-dose recovery animals
Abbreviations: HD, high dose; LD, low dose; MD, mid dose
</td></tr>
<tr><td>Urinalysis</td><td>No drug-related effects</td></tr>
<tr><td>Gross pathology</td><td>No drug-related effects</td></tr>
<tr><td>Organ weights</td><td>
Table 26. Absolute Ovary Weights (% Change Compared to Controls)
<table>
<tr><th>Day</th><th>LD</th><th>MD</th><th>HD</th></tr>
<tr><td>Day 28</td><td>+140 %</td><td>+115 %</td><td>+115 %</td></tr>
<tr><td>Day 43</td><td>-</td><td>-</td><td>-20 %</td></tr>
</table>
Abbreviations: HD, high dose; LD, low dose; MD, mid dose

Table 27. Absolute Thymus Weights Compared to Controls
<table>
<tr><th>Day</th><th>Control</th><th>LD</th><th>MD</th><th>HD</th></tr>
<tr><td>Day 28</td><td>3.3</td><td>-6%</td><td>-44 %</td><td>-42 %</td></tr>
<tr><td>Day 43</td><td>2.6</td><td>-</td><td>-</td><td>-40 %</td></tr>
</table>
Abbreviations: HD, high dose; LD, low dose; MD, mid dose
</td></tr>
<tr><td>Histopathology
Adequate battery: Yes</td><td>
Day 28
MD: Minimally reduced cellularity of the bone marrow. (1 male)
HD: Moderately reduced cellularity of the bone marrow, severe involution of thymus, atrophy of the adipose tissue, reduced body weight compared to predose (one male)

Day 43
HD: Increased severity of involution in the thymus of both sexes compared with controls. Reduced body weight.

All control animals had immature ovaries with no corpora lutea. All drug-treated animals had mature ovaries, and all had corpora lutea except one low-dose animal.
</td></tr>
</table>

Parameters	Major Findings
Pharmacokinetics	**Table 28. Fexinidazole Pharmacokinetics ($AUC_{(0\text{-}Last)}$, µg*H /mL)**

Dose (mg/kg)	Day 1 Male	Day 1 Female	Day 28 Male	Day 28 Female
50	0.14	0.24	0.12	0.09
200	0.42	0.45	0.40	0.38
800	0.78	0.90	0.74	0.96

Abbreviation: $AUC_{(0\text{-}last)}$, area under the plasma/serum concentration versus time curve from time zero to the time corresponding to the last quantifiable concentration

Table 29. Fexinidazole Pharmacokinetics C_{max}, (µg/mL)

Dose (mg/kg)	Day 1 Male	Day 1 Female	Day 28 Male	Day 28 Female
50	0.03	0.04	0.02	0.04
200	0.05	0.08	0.06	0.07
800	0.1	0.18	0.09	0.10

Abbreviation: C_{max}, maximum serum concentration

5.5.2. Genetic Toxicology

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Study title/ number: Reverse Mutation in Nine Histidine-Requiring Strains of Salmonella typhimurium: Bacterial Reverse Mutation Test (Ames test) (Study # 2647/021)

Key Study Findings:

- Positive in strains TA98, TA100, and TA1535 in the presence and absence of S9 and TA1537 and TA102 in the presence of S9
- Positive in nitroreductase deficient variants (TA98NR, TA100NR, TA1535NR and YG7127) with reduced mutagenic response compared to the parent strains.

GLP compliance: Yes, with the following exceptions, which did not adversely affect the validity of the study: (1) the existence of a concentration response was checked by nonstatistical analysis up to limiting levels (2) the compound was considered positive if a data set showed a significant response in Dunnett's which was concentration related.

Test system: *Salmonella typhimurium* strains TA98, TA98NR, TA100, TA100NR, TA1535, TA1535NR, YG7127, TA1537, and TA102 up to 1000 µg/plate; (*+/- S9)*

Study is valid: Yes

Table 30. Effect of Fexinidazole on the Number of His^+ Revertant Colonies Without S9 Mix. (Strains TA98, TA98NR, TA100, TA100NR, TA1535, TA1537, TA102)

Fexinidazole µg/plate	Number of His^+ Revertant Colonies (Mean ±SEM or SD) Salmonella typhimurium Strain TA98	TA98NR	TA100	TA100NR	TA1535	TA1537	TA102
0	21±7	29 ±2	104 ±3	90 ±7	14 ±5	7 ±1	305 ±28
1	32 ±16	18 ±2	124 ±5	84 ±3	19 ±6	8 ±2	229 ±7
4	21 ±6	24 ±6	160 ±6***	88 ±14	14 ±3	7 ±2 (M)	275 ±7

Fexinidazole µg/plate	Number of His+ Revertant Colonies (Mean ±SEM or SD) Salmonella typhimurium Strain TA98	TA98NR	TA100	TA100NR	TA1535	TA1537	TA102
20	23 ±6	18 ±3	320 ±16***	97 ±8	39 ±5***	8 ±4 (M)	288 ±9
100	32 ±4	24 ±4	1301 ±43***	121 ±8***	98 ±13***	9 ±6(M)	297 ±15
350	123 ±13***	32 ±16	1879 ±181***	148 ±8****	72 ±11***	0 ±0 (M)	331 ±21
500	162 ±21***	36 ±5	1998 ±109*** (V)	190 ±9***	9 ±1 (V)	0 ±0 (M)	256 ±41
1000	13 ±9 (P, M) 21 ±2 (P, M)		227 ±25*** (P, M, V)	231 ±24*** (P)	3 ±3(P, M, V)	(T, P)	57 ±15 (P)
Positive control[a]	NQO: 388 ±9 2NF: 1813 ±91 NFT: 123 ±15	NQO: 333 ±11 2NF: 656 ±131 NFT: 23 ±3	NaN_3: 899 ±83 MTZ: 1244 ±30 NFT: 698 ±19	NaN_3: 798 ±79 MTZ: 139 ±13 NFT: 239 ±10	NAN_3: 680 ±37 NFT: 21 ±2	AAC: 510 ±139 NFT: 13 ±3	MMC: 742 ±45 NFT: 459 ±36

Source: Study # 2647/021:
Toxic effects and precipitate: Toxicity observed in strain TA1537; precipitation observed in strains TA1537 and TA102
Comments: NTR^+ strains: TA98, TA100, TA1535, TA1537; TA102; NTR- strains: TA98NR, TA100NR
[a] AAC: 50 µg/plate; MMC: 0.2 µg/plate; NFT: 5 µg/plate
* p ≤0.05
** p ≤0.01
*** p ≤0.005
Abbreviations: 2NF, 2-nitrofluorene; AAC, 9-aminoacridine; M, manually counted; MMC, mitomycin C, MTZ, NaN_3, sodium azide; NFT, nitrofurantoin; NQO, nitroquinoline-oxide; P, precipitation occurred; S9, metabolic activation; SD, standard deviation; SEM, standard error of the mean; T, toxicity (no revertant colonies); V, very thin background bacterial lawn

Table 31. Effect of Fexinidazole on the Number of His^+ Revertant Colonies Without S9 Mix. (TA1535, TA1535NR, YG7127)

Metabolizing System	Test Article	Concentration	Number of His+ Revertant Colonies (Mean ±SEM or SD) TA 1535	TA 1535 NR	YG 7127
Without S9	Fexinidazole	0	15 ± 4	12 ± 4	11 ± 2
		1	18 ± 2	12 ± 2	12 ± 4
		4	18 ± 3	15 ± 3	8 ± 3
		20	35 ± 4**	12 ± 6	16 ± 2
		100	91 ± 3**	13 ± 3	17 ± 8
		350	28 ± 3**	11 ± 3	24 ± 7**
		500	(T, P)	17 ± 5	34 ± 7**
	Positive control[a]		NaN_3 626 ± 4 NFZ 75 ± 11 NFT 14 ± 4	NaN_3 583 ± 29 NFZ 33 ± 2 NFT 13 ± 4	NaN_3 625 ± 27 NFZ 49 ± 10 NFT 15 ± 6

Source: Study # 2647/021
Toxic effects and precipitate: toxicity observed in strain TA1535; precipitation observed in strains TA1535, TA1535NR, and YG7127
[a] AAN: 5 µg/plate; NaN3: 2 µg/plate; NFT: 5 µg/plate; NFZ: 10 µg/plate
* p ≤0.05
** p ≤0.01
*** p ≤0.005
Abbreviations: NaN_3, sodium azide; NFT, nitrofurantoin; NFZ, nitrofurazone; P, precipitation occurred; S9, metabolic activation; SD, standard deviation, SEM, standard error of the mean; T, toxicity (no revertant colonies)

Table 32. Effect of Fexinidazole on the Number of His+ Revertant Colonies With S9 Mix. (Strains TA98, TA98NR, TA100, TA100NR, TA1535, TA1537, TA102)

Fexinidazole µg/plate	Number of His+ Revertant Colonies (Mean ±SEM or SD) Salmonella typhimurium Strain TA98	TA98NR	TA100	TA100NR	TA1535	TA1537	TA102
0	34 ±6 20	18 ±3	116 ±5	111 ±3	15 ±4	13 ±3	240 ±23
1	47 ±8	16 ±5	210 ±22***	114 ±4	25 ±5*	16 ±4	187 ±21
4	45 ±4	19 ±5	436 ±22***	123 ±6	49 ±5***	20 ±5	225 ±2
20	89 ±7***	12 ±3	1341 ±79***	161 ±8***	150 ±7***	21 ±4*	298 ±22
100	401 ±10***	9 ±2	1816 ±95***	275 ±15***	74 ±15***	57 ±10***	543 ±27***
350	47 ±34	27 ±2	275 ±49***	457 ±13***	(T)	(T)	707 ±60***
500	63 ±13 (V)	34 ±11***	(T)	546 ±13***	(T)	(T)	1008 ±78*** (V)
1000	38 ±6 (P, M, V)	36 ±8*** (P, S, M)	(T, P)	717 ±44*** (P)	(T, P)	(T, P)	1284 ±195*** (P, V)
Positive control[a]	B[a]P: 575 ±12	B[a]P: 253 ±5	AAN: 1564 ±122	AAN: 1822 ±68	AAN: 301 ±1	AAN: 113 ±16	AAN: 720 ±173

Source: Study # 2647/021
Toxic effects and precipitate: Toxicity observed in strain TA100 and TA1535; precipitation observed in strains TA98, TA98NR, TA100, TA100NR, and TA1535
* p ≤0.05
** p ≤0.01
*** p ≤0.005
[a] B[a]P: 10 µg/plate; AAN: 5 µg/plate (20 µg/plate for TA102)
Abbreviations: AAN, 2-aminoanthracene; M: manually counted; P, precipitation occurred; S, slight thinning of background bacterial lawn; S9, metabolic activation; SD, standard deviation; SEM, standard error of the mean; T, toxicity (no revertant colonies); V, very thin background bacterial lawn

Table 33. Effect of Fexinidazole on the Number of His+ Revertant Colonies With S9 Mix. (TA1535, TA1535NR, YG7127)

Metabolizing System	Test Article	Concentration	Number of His+ Revertant Colonies (Mean ±SEM or SD) TA 1535	TA 1535 NR	YG 7127
With S9	Fexinidazole	0	17 ± 4	17 ± 6	9 ± 2
		0.2	18 ± 4	13 ± 4	16 ± 5
		1	30 ± 4	15 ± 4	15 ± 2
		4	52 ± 12	19 ± 4	18 ± 6
		20	139 ± 27	16 ± 6	32 ± 7
		100	60 ± 3	22 ± 6	90 ± 14
		350	(T)	51 ± 8	111 ± 48
	Positive control[a]		AAN 338 ± 6	AAN 397 ± 6	AAN 280 ± 16

Source: Study # 2647/021
Toxic effects and precipitate: toxicity observed in strain TA1535; precipitation observed in strains TA1535, TA1535NR, and YG7127
* p ≤0.05
** p ≤0.01
*** p ≤0.005
[a] AAN: 5 µg/plate; NaN3: 2 µg/plate; NFT: 5 µg/plate; NFZ: 10 µg/plate
Abbreviations: AAN: 2-aminoanthracene; S9, metabolic activation; SD, standard deviation; SEM, standard error of the mean; T, toxicity (no revertant colonies)

Study title/ number: Reverse Mutation in Four Histidine-Requiring Strains of *Salmonella typhimurium*: Bacterial Reverse Mutation Test (Ames test) (Study # 8201845).

Key Study Findings:

- Dose-related increases in revertant numbers seen in TA 102 and YG 7168 (+/- S9)
- Negative with TA 1537 and YG 7167 (+/- S9)
- Nitroreductase-deficient variants such as YG7127 showed reduced mutagenic response compared to the parent strains.

GLP compliance: Yes.

Test system: *Salmonella typhimurium* NTR$^+$ strains: (TA1537 and TA102) and NTR- strains (YG7167, YG7168) up to 1000 µg/plate; *+/- S9]*

Study is valid: Yes

Table 34. Effect of Fexinidazole on the Number of His$^+$ Revertant Colonies Without S9 Mix. (Mean ±SEM or SD)

	Number of His$^+$ Revertant Colonies (Mean ±SEM or SD) Salmonella typhimurium Strain			
Fexinidazole µg/plate	**TA1537**	**YG7167**	**TA102**	**YG7168**
0	22.0 ±5.1	5.6 ±5.1	278.0 ±11.4	178.2 ±10.4
1.58	17.0 ±5.0	12.7 ±5.5*	278.0 ±14.2	178.7 ±17.2
5	45 ±4	19 ±5	436 ±22***	123 ±6
15.8	22.3 ±7.1	11.3 ±1.2*	324.7 ±108.6	240.7 ±18.0**
50	25.0 ±4.6	9.7 ±4.0	313.3 ±9.8	195.3 ±8.0
100	23.3 ±6.4	10.0 ±2.6	-	-
158	20.0 ±4.6	7.3 ±1.5	338.7 ±17.6	250.0 ±13.5**
500	11.7 ±4.0 (M)	9.7 ±1.2	436.7 ±27.7**	293.0 ±12.5**
1000	-	-	332.0 ±62.3	254.7 ±29.5** (P)
Positive control[a]	AAC: 118.0 ±33.9 NFT: 33.3 ±5.9	AAC: 98.7 ±40.6 NFT: 7.7 ±4.0	MMC: 508.0 ±20.0 NFT: 581.7 ±24.2 AAI: 460.3 ±21.0	MMC: 767.0 ±15.6 NFT: 467.3 ±13.3 AAI: 262.7 ±4.0

Source: Study # 8201845
Toxic effects and precipitate: Precipitation observed in strain YG7168 at 1000 µg/plate
Comments: NTR$^+$ strains: TA1537, TA102; NTR- strains: YG7167, YG7168
* p≤0.05, ** p≤0.01
[a] AAC: 50 µg/plate for TA1537, YG7167; NFT: 5 µg/plate for TA1537, YG7167, TA102, YG7168; MMC: 0.2 µg/plate for TA102, YG7168; AAI: 280 µg/plate for TA102, YG7168
Abbreviations: AAC, 9-aminoacridine; AAI, aristolochic acid; M, manually counted; MMC: mitomycin C; NFT: nitrofurantoin; P: precipitation occurred; S9: metabolic activation; SD: standard deviation; SEM: standard error of the mean

Table 35. Effect of Fexinidazole on the Number of His$^+$ Revertant Colonies With S9 Mix. (Mean ±SEM or SD)

	Number of His$^+$ Revertant Colonies (Mean ±SEM or SD) Salmonella typhimurium Strain			
Fexinidazole µg/plate	**TA1537**	**YG7167**	**TA102**	**YG7168**
0	24.4 ±5.3	14.6 ±2.9	253.4 ±19.5	223.6 ±22.6
1.58	21.0 ±4.6	13.0 ±1.7	281.0 ±5.3	247.7 ±18.2
5	22.0 ±3.6	9.3 ±7.5	301.3 ±14.3	253.3 ±26.1
15.8	28.3 ±3.2	6.0 ±1.0	351.0 ±11.4**	295.0 ±11.1

Fexinidazole µg/plate	Number of His+ Revertant Colonies (Mean ±SEM or SD) Salmonella typhimurium Strain TA1537	YG7167	TA102	YG7168
50	30.7 ±5.1	7.7 ±2.1	450.7 ±10.5**	294.7 ±49.2
100	38.3 ±3.1*	15.3 ±7.2	-	-
158	20.7 ±7.6	13.3 ±3.8	824.3 ±132.1**	456.7 ±27.7**
500	(T)	5.7 ±2.9 (S)	1348.3 ±23.8**	665.3 ±94.9**
1000	-	-	1444.7 ±129.5** (P, S)	932.7 ±194.8** (P, S)
Positive control[a]	AAC: 118.0 ±33.9 NFT: 33.3 ±5.9	AAC: 98.7 ±40.6 NFT: 7.7 ±4.0	MMC: 508.0 ±20.0 NFT: 581.7 ±24.2 AAI: 460.3 ±21.0	MMC: 767.0 ±15.6 NFT: 467.3 ±13.3 AAI: 262.7 ±4.0

Source: Study # 8201845
Toxic effects and precipitate: toxicity observed in strain TA1537 at 500 µg/plate; precipitation observed in strains TA102 and YG7168 at 1000 µg/plate
Comments: NTR+ strains: TA1537, TA102; NTR- strains: YG7167, YG7168
* p≤0.05, ** p≤0.01
[a] AAC: 50 µg/plate for TA1537, YG7167; NFT: 5 µg/plate for TA1537, YG7167, TA102, YG7168; MMC: 0.2 µg/plate for TA102, YG7168; AAI: 280 µg/plate for TA102, YG7168
Abbreviations: AAC, 9-aminoacridine; AAI, aristolochic acid; MMC, mitomycin C; NFT, nitrofurantoin; P, precipitation occurred; S, slight thinning of background bacterial lawn; S9, metabolic activation; SD, standard deviation; SEM, standard error of the mean; T, toxicity (no revertant colonies)

In Vitro Assays in Mammalian Cells

Study title/ number: Induction of Micronuclei in Cultured Human Peripheral Blood Lymphocytes (Study # 2647/022)

Key Study Findings: Negative

GLP compliance: Yes

Test system: Human peripheral blood lymphocytes; up to 180 µg/mL; +/-S9]

Study is valid: Yes

In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Study title/ number : Induction of Micronuclei in the Bone Marrow of Treated Mice (Study # 2647/23)

Key Study Findings: Negative

GLP compliance: Yes

Test system: Mouse, bone marrow micronuclei; 2 oral doses up to 2000 mg/kg]

Study is valid: Yes

5.5.3. Carcinogenicity

Carcinogenicity studies are not indicated for fexinidazole and were not conducted. However, structurally related compounds such as metronidazole have exhibited carcinogenic potential in lifetime animal studies. In the absence of fexinidazole carcinogenicity studies, a decision was made not to put a statement in the Physician's prescribing information under section 5:

Warnings and precautions. Instead, the carcinogenicity section of label was edited to state: No carcinogenicity study was performed with fexinidazole. Carcinogenicity has been observed in mice and rats treated chronically with nitroimidazole-class drugs which are structurally similar to fexinidazole. It is unclear if the positive tumor findings in lifetime rodent studies indicate a risk to patients taking a 10-day treatment of fexinidazole for HAT. (b) (4)

5.5.4. Reproductive and Developmental Toxicology

Study title/ number: Fexinidazole: Oral Fertility Study in the Male and Female Rat (Study # 2014-0197)

Key Study Findings

- There were no adverse effects on male fertility, female fertility, or early embryonic development at any dose.
- The NOAEL was 600 mg/kg/day where the exposure was estimated at 9 µg*hr/mL, 1.3 times the clinical exposure based on AUC comparisons.

Conducting laboratory and location	(b) (4)
GLP compliance:	Yes

Table 36. Methods of Fexinidazole: Oral Fertility Study in the Male and Female Rat. Study # 2014-0197

Parameter	Details
Dose and frequency of dosing:	0 (vehicle), 50, 200 and 600 mg/kg. Daily.
Route of administration:	Oral gavage
Formulation/vehicle:	5% Tween 80 in 0.5% Methyl cellulose 400 cP (Methocel) suspension
Species/strain:	Rat, Sprague Dawley
Number/sex/group:	24
Study design:	Male: Dosed 28 days prior to start of cohabitation, during the mating period and up to the sacrifice of the females. Females: Dosed for 14 days prior to mating, during the mating period and up to GD7.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameters	Major Findings
Mortality	No deaths.
Clinical signs	No drug-related clinical signs
Body weights	No biologically significant differences in body weight.
Necropsy findings	No biologically significant differences from control

Embryo-Fetal Development

Study title/ number: Fexinidazole: Oral Embryofetal Developmental Toxicity Study in the Rat (Study #0243-2009)

Key Study Findings

- Delayed ossification and reduced fetal and placental weights were observed at 800 mg/kg in the presence of maternal toxicity (reduced maternal body weights).
- There were no drug-related malformations at any dose. Visceral examinations were not performed at the mid- and low-dose groups because no visceral findings were noted in the high dose group.
- The NOAEL for fetal development was 200 mg/kg, similar to the clinical dose based on AUC comparisons. The NOAEL for maternal toxicity was 200 mg/kg.

Conducting laboratory: (b) (4)

GLP compliance: Yes

Table 37. Methods of Fexinidazole: Oral Embryofetal Developmental Toxicity Study in the Rat. Study #0243-2009

Parameter	Details
Dose and frequency of dosing:	0, 50, 200, and 800 mg/kg/day
Route of administration:	Oral gavage
Formulation/vehicle:	5% Tween 80 in 0.5% Methyl cellulose 400 cP
Species/strain:	Rat, Sprague Dawley
Number/sex/group:	24
Satellite groups:	Toxicokinetics (3 females/group)
Study design:	Fexinidazole was given orally, once daily, to pregnant rats from GD 6 to GD 17 or from GD 6 to postpartum Day 7.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameters	Major Findings
Mortality	No deaths.
Clinical signs	Yellow (fexinidazole-colored) feces at 800 mg/kg/day from GD 7-8 up to GD 18-20.
Maternal body weights	HD: -10 to -19%, GD 10 to GD20

Parameters	Major Findings
Necropsy findings	**Table 38. Cesarean Section Data**

Weight	Control	LD	MD	HD
Placental weight	0.5	+1	-2%	-15%
Fetal body weight (g)	3.7	-1%	-1%	-16 %

Abbreviations: LD, low dose; MD, mid dose; HD, high dose

Parameters	Major Findings
Necropsy findings	

Table 39. Offspring Malformations

	# of Fetuses (# of Litters Affected)			
Fexinidazole Dose Group	**Control**	**LD**	**MD**	**HD**
External malformations				
Examined	309 (24)	337 (24)	298 (23)	327 (23
Generalized edema	0 (0)	0	0 (0)	1 (1)
Omphalocele	0 (0)	0	0 (0)	1 (1)
Malpositioned right forelimb	0 (0)	0	0 (0)	1 (1)
Total	0 (0)	0	0 (0)	1 (1)
Visceral malformations				
Examined	157 (24)	-	-	164 (23)
Diaphragmatic hernia	0 (0)	-	-	1 (1)
Small lungs	0 (0)	-	-	1 (1)
Microphthalmia	0 (0)	-	-	1 (1)
Total	0 (0)	-	-	2 (2)
Visceral variations				
Subcutaneous edema	1 (1)	-	-	0 (0)
Subcutaneous hemorrhage	1 (1)	-	-	0 (0)
Sub palpebral hemorrhage	0 (0)	-	-	1 (1)
Ectopic kidneys	2 (2)	-	-	2 (2)
Dilated renal pelvis	4 (4)	-	-	3 (3)
Dilated ureters	1 (1)	-	-	2 (2)
Total	8 (7)	-	-	6 (6)
Skeletal malformations				
Examined	152 (24)	-	157 (23)	163 (23)
Marked and diffuse retardation of ossification	1 (1)	-	1 (1)	1 (1)
Total	1 (1)	-	1 (1)	1 (1)
Skeletal malformations	1 (1)	-	1 (1)	1 (1)
Skeletal variations				
Asymmetrical ossification of sternebrae	0 (0)	-	0 (0)	2 (2)
Unossified 1st thoracic centrum	1 (1)	-	1 (1)	5 (3)
Reduced ossification caudal centra	2 (2)	-	1 (1)	9 (7)
Total skeletal variations	58 (21)	-	67 (20)	61 (23)

Reviewer note: Mid- and low-dose fetuses were not examined for visceral abnormalities as no relevant differences between high-dose and control fetuses were revealed.
Abbreviations: LD, low dose; MD, mid dose; HD, high dose

Parameters	Major Findings
Pharmacokinetics	**Table 40. GD 17 $AUC_{(0\text{-}24h)}$ for Fexinidazole, M1 and M2**

GD 17 $AUC_{(0\text{-}24h)}$	Fexinidazole Dose (µ*h/mL) 50	200	800
Fexinidazole	2.1	NE*	11.5
M1	73	NE*	389
M2	94	293	795

*Not evaluated
Abbreviations: GD, gestational day; AUC**(0-24h)**, area under the plasma concentration-time curve from time zero to 24 hours; M1, Fexinidazole sulfoxide; M2 Fexinidazole

Study title/ number: Fexinidazole: Oral Embryofetal Developmental Toxicity Study in the Rabbit (Study # 0244-2009)

Key Study Findings

- Abortions were observed at 20 and 40 mg/kg in the presence of maternal toxicity (reduced bodyweight/bodyweight gain and extremely reduced food consumption)
- Based on these findings, the NOAEL for maternal toxicity and embryofetal development in the rabbit was 10 mg/kg/day, equivalent to a dose of less than 0.01 times the clinical dose based on pharmacokinetics comparisons.
- Excessive maternal toxicity is not uncommon when rabbits are used in embryo-fetal toxicity testing of antibiotics. The rabbit does not therefore serve as a good model for embryo-fetal toxicity testing of antibiotics.

Conducting laboratory: (b) (4)

GLP compliance: Yes

Table 41. Methods of Fexinidazole: Oral Embryofetal Developmental Toxicity Study in the Rabbit. Study # 0244-2009

Parameter	Details
Dose and frequency of dosing:	0, 10, 20, and 40 mg/kg/day
Route of administration:	Oral gavage
Formulation/vehicle:	5% Tween 80 in 0.5% Methyl cellulose 400 cP
Species/strain:	Rabbit, New Zealand White
Number/sex/group:	20
Study design:	Fexinidazole was given orally, once daily, to pregnant rabbits from GD 6 to GD 20.
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Parameters	Major Findings
Mortality	No treatment-related deaths.
Clinical signs	MD: 1 abortion

Parameters	Major Findings
	HD: 2 abortions
Food consumption	Food consumption in animals which aborted MD: mean -82 % GD6 to GD 21 HD: mean -96%, GD14 to GD24
Body weights	Animals which aborted showed reduced body weight compared to controls MD: -25% HD: -16%
Necropsy findings	
Cesarean section data	Post Implantation Loss HD:+61 % compared to control
Offspring	**Table 42. External Anomalies in Offspring**

Fetal Gross External Anomalies	Fexinidazole Dose (mg/kg) 0 (Vehicle)	10	20	40
Gastroschisis				
Litter incidence	1	0	0	0
Fetal incidence	1	0	0	0
Absent tail				
Litter incidence	1	0	0	0
Fetal incidence	1	0	0	0
Imperforate anus				
Litter incidence	1	0	0	0
Fetal incidence	1	0	0	0
Malrotated limb(s)				
Litter incidence	1	1	0	1
Fetal incidence	1	1	0	1
Short limb(s)				
Litter incidence	0	0	0	1
Fetal incidence	0	0	0	1
Fusion/agenesis of paw(s)				
Litter incidence	0	0	0	1
Fetal incidence	0	0	0	1
Fetal visceral anomalies				
Absent kidney				
Litter incidence	1	0	0	0
Fetal incidence	1	0	0	0
Malpositioned kidney				
Litter incidence	1	0	0	0
Fetal incidence	1	0	0	0
Fetal skeletal anomalies				
Marked disorganization of Sacro caudal vertebrae (fusion/agenesis)				
Litter incidence	1	NE	NE	0
Fetal incidence	1	NE	NE	0
Marked disorganization of right forelimb and girdle (small/misshapen/ unossified bones)				

Parameters	Major Findings				
	Litter incidence	0	NE	NE	1
	Fetal incidence	0	NE	NE	1
	Agenesis of paw(s)				
	Litter incidence	0	NE	NE	1
	Fetal incidence	0	NE	NE	1
	Reviewer's note: Skeletal abnormalities were not evaluated in the low and mid doses since there were no significant findings at the high dose affecting more than 1 animal or 1 litter.				
	Abbreviations: LD: low dose; MD: mid dose; HD: high dose; NE: not evaluated.				

Prenatal and Postnatal Development

Study title/ number: Fexinidazole: Oral Pre- and Postnatal Developmental Toxicity Study in the Rat (Study # 0245-2009)

Key Study Findings

- The mean age for balanopreputial cleavage in selected F1 male rats and for vaginal opening in selected F1 female rats was slightly delayed at 600 mg/kg.
- The NOAEL for maternal toxicity and pre- and postnatal development was 200 mg/kg, similar to the clinical dose based on AUC comparisons.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 43. Methods of Fexinidazole: Oral Pre- and Postnatal Developmental Toxicity Study in the Rat. Study # 0245-2009

Parameter	Details
Dose and frequency of dosing:	0, 50, 200, 600 mg/kg/day
Route of administration:	Oral gavage
Formulation/vehicle:	5% Tween 80 in 0.5% Methyl cellulose 400 cP (Methocel) suspension
Species/strain:	Rat, Sprague Dawley
Number/sex/group:	24
Study design:	Fexinidazole was administered to pregnant females, from GD 6 to LD 21. Pups were culled to 8 pups per litter on LD 4 and nursed for 21 days. Pups were observed for survival, growth, and development and functional tests were performed. After weaning, one male and one female F1 pups per dam were observed for survival, growth, and development. Rats were tested for their reproductive ability at sexual maturity. All F1 females were subjected to cesarean section at midpregnancy and the uterine content was examined and recorded.

Parameter	Details
Deviation from study protocol affecting interpretation of results:	No

Observations and Results

Generation	Major Findings
F0 Dams	**Table 44. Body Weight of F0 Dams** **Body Weight Gain (g)** / **Control** / **LD** / **MD** / **HD** GD 6-10: 19 / 20 / 15 / -19 GD 6-14: 40 / 42 / 36 / 7 Abbreviations: HD, high dose; LD, low dose, MD, mid dose **Table 45. Food Consumption of F0 Dams** **Food Consumption (g/rat/day)** / **Control** / **LD** / **MD** / **HD** GD 6-10: 22 / 22 / 29 / 8 GD 10-14: 23 / 23 / 22 / 16 Abbreviations: HD, high dose; LD, low dose, MD, mid dose
F1 Generation	HD: Body weight -10 %, PND 28-63 Plasma concentrations (ng/mL) of Fexinidazole, sulfoxide and sulfone metabolites in pups on LD 4 after oral administrations of 50, 200 and 600 mg/kg/day fexinidazole to lactating rats. **Table 46. Plasma Concentrations in F1 Generation** **LD4 Plasma Concentrations (ng/mL)** / **Fexinidazole Dose 50** / **200** / **600** Fexinidazole: 1.3 / 2.2 / 9.4 Fexinidazole sulfoxide: 42 / 116 / 297 Fexinidazole sulfone: 650 / 1997 / 4621 **Table 47. Mean Age of F1 Generation** **Mean Age (Days)** / **Control** / **LD** / **MD** / **HD** Balanopreputial separation: 39.7 / 40.8 / 41.1 / 41.8 Vaginal opening: 31.8 / 31.8 / 32.1 / 32.5 Abbreviations: HD, high dose; LD, low dose, MD, mid dose
F2 Generation	No drug-related effects

6. Clinical Pharmacology

6.1. Executive Summary

The clinical pharmacology review of this NDA for Fexinidazole tablets primarily focused on the following assessments:

- A bioequivalence (BE) study that assessed and compared the bioavailability (BA) of fexinidazole and the systemic exposure to its active metabolites from a 600 mg tablet formulation used in Phase 3 clinical trial and the final to-be-marketed 600 mg tablet formulation
- The Applicant-proposed drug product labeling language for the following clinical pharmacology aspects:
  - Dosage and administration instructions for patients who experience vomiting during treatment
  - Dosage adjustments for patients with renal impairment
  - Drug-drug interactions and management

6.1.1. Recommendations

The Office of Clinical Pharmacology (OCP) has reviewed the information provided by the Applicant in NDA 214429 for Fexinidazole tablets and recommends approval of this NDA.

Table 48. Office of Clinical Pharmacology Review Issues and Recommendations

Review Issue	Recommendations and Comments
Pivotal or supportive evidence of effectiveness	Pivotal evidence of effectiveness is derived from a Phase 3 Study DNDiFEX004 that evaluated the efficacy and safety of Fexinidazole tablets in adults with late stage-2 human African Trypanosomiasis due to *T. b. gambiense* (g-HAT). Study DNDiFEX004 used nifurtimox-eflornithine combination therapy (NECT) as a reference arm. Additional supportive evidence of effectiveness is derived from an open-label study in adults with early stage-2 or stage-1 g-HAT (Study DNDiFEX005) and from an open-label study in children aged ≥6 years and weighing ≥20 kg with disease at any stage (Study DNDiFEX006).
General dosing instructions	The proposed dosing regimen is based on a patient's body weight as given below: **Table 49. Dosing Regimen** **Body Weight Group** / **Dose in mg/Day (Number of Fexinidazole 600 mg Tablets)** / **Treatment Duration (Days)** ≥35 kg: Loading dose: 1800 (3) — 4 days; Maintenance dose: 1200 (2) — 6 days ≥20 and <35 kg[a]: Loading dose: 1200 (2) — 4 days; Maintenance dose: 600 (1) — 6 days [a] Six years of age or older Fexinidazole tablets are to be taken once daily for 10 days with food at about the same time each day.

Review Issue	Recommendations and Comments
	This proposed fexinidazole dosing is same as the dosing regimen that was evaluated in the abovementioned three efficacy and safety studies. From a clinical pharmacology perspective, the Applicant's proposed fexinidazole dosing regimen is acceptable for the treatment of g-HAT in patients ≥ 6 years of age and weighing ≥ 20 kg. During the clinical trials, vomiting occurred in ~29-68% of patients who received fexinidazole treatment. The Applicant proposes: • If a first event of vomiting occurs after receiving (b) (4) of Fexinidazole tablets, (b) (4) the next dose the following day using the recommended treatment schedule. (b) (4) The Applicant's proposal related to dosing instructions for patients who experience vomiting is acceptable from a clinical pharmacology perspective. See Section 6.3.2.2 for additional details.
Dosing in patient subgroups (intrinsic and extrinsic factors)	Renal impairment (RI): (See Section 6.3.2.3 for complete details) • No dosage adjustments for patients with mild or moderate RI • Avoid the use of Fexinidazole tablets in patients with severe RI Hepatic impairment (HI): (See Section 6.3.2.3 for complete details) • The Applicant did not conduct a HI PK study and excluded g-HAT patients with HI, including laboratory liver test abnormalities, from the efficacy / safety trials. • The Applicant proposed (b) (4) • This is deemed acceptable to the Clinical Pharmacology review team. Alcohol use: (See Section 6.3.2.4 for complete details) • Fexinidazole is a nitroimidazole class compound. Based on its chemical class, consumption of alcoholic beverages should be avoided during treatment with Fexinidazole tablets. QT Prolongation: (See IRT-QT review for complete details) Fexinidazole treatment should be avoided in patients who: • Have QTcF interval greater than 470 msec and/or a history of Torsades de pointes, congenital long QT syndrome, cardiac arrhythmias, uncompensated heart failure, or family history of sudden death • Are receiving other drugs known to prolong the QT interval, block cardiac potassium channels, and/or induce bradycardia Recommendations for managing drug-drug interactions: Interactions with fexinidazole as the perpetrator (See Section 6.3.2.4 for complete details): • Due to CYP3A4 inhibition by fexinidazole, concomitant use of drugs metabolized by CYP3A4 should be avoided. • Due to inhibition by fexinidazole, concomitant use of drugs metabolized by CYP1A2 and/or CYP2C19 as well as drugs that are substrates of

Review Issue	Recommendations and Comments
	OCT2, OAT1, OAT3, MATE1, and/or MATE2-K transporters should be used with caution. If fexinidazole is coadministered with these drugs, patients should be monitored for adverse reactions associated with these drugs. • Due to CYP2B6 induction by fexinidazole, concomitant use of drugs metabolized by CYP2B6 should be avoided. If coadministration cannot be avoided, patients should be monitored for lack of efficacy of these drugs. Interactions with fexinidazole as the victim (See Section 6.3.2.4 for complete details) • Concomitant use of drugs that induce CYP450 should be avoided. • Concomitant use of drugs that inhibit CYP450 should be avoided. If coadministration cannot be avoided, patients should be monitored for lack of efficacy of fexinidazole treatment.
Labeling	The content and format of the proposed labeling language is not aligned with the current OCP labeling guidance. The Review team has recommended revisions to the Applicant's proposed product labeling for Fexinidazole tablets.
Bridge between the to-be-marketed and clinical trial formulations	The to-be-marketed 600 mg Fexinidazole tablet formulation is different from the 600 mg fexinidazole tablet formulation that was used in the Phase 3 clinical trial. The Applicant has conducted a BE study that assessed and compared the BA of fexinidazole and the systemic exposure to its active metabolites from both formulations. Findings from the study showed that the to-be-marketed 600 mg tablet formulation is BE to the Phase 3 clinical trial tablet formulation. See Section 6.3.2.1 for additional details.

6.1.2. Postmarketing Requirements and Commitments

The Clinical Pharmacology review team recommends following two clinical studies as postmarketing requirements (PMRs):

- A clinical DDI study of Fexinidazole tablets and oral midazolam
  - Rationale: The findings from in vitro and mechanistic model-based predictions show a significant potential for fexinidazole to inhibit metabolism of CYP3A4 drug substrates. Thus, a clinical DDI study between Fexinidazole tablets and the sensitive CYP3A4 substrate, oral midazolam, will help to better quantify the magnitude and potential risk of an interaction between fexinidazole and CYP3A4 substrates.
- A clinical pharmacokinetics (PK) study in subjects with hepatic impairment
  - Rationale: The PK of fexinidazole and its pharmacologically active M1 and M2 metabolites in subjects with hepatic impairment is unknown. Fexinidazole is extensively metabolized by multiple CYP450 enzymes in the liver and hepatic metabolism and/or excretion likely accounts for a substantial portion of the elimination of the parent drug and/or M1 and M2 metabolites. M2 has relatively higher systemic exposure compared to fexinidazole or M1, and therefore, is likely playing a key role in the drug's desired pharmacological activity. The biotransformation pathway of M1 to M2 has not been completely characterized. In

addition, M2 plasma concentrations have also been associated with the increased risk of QTc interval prolongation. Therefore, a PK study in subjects with hepatic impairment is warranted to determine if alterations in PK exposure to fexinidazole, M1, and/or M2 are observed and whether dosage adjustment of Fexinidazole tablets is needed in such subjects.

6.2. Summary of Clinical Pharmacology Assessment

6.2.1. Pharmacology and Clinical Pharmacokinetics

Fexinidazole tablets are an unscored tablet formulation containing 600 mg fexinidazole. Once absorbed, fexinidazole gets converted to two active metabolites: fexinidazole sulfoxide (M1) and fexinidazole sulfone (M2). Both metabolites are postulated to have similar in vitro activity against Trypanosoma parasites as reported for fexinidazole.

To characterize the PK of fexinidazole, M1, and M2, the Applicant has conducted multiple clinical studies in healthy Sub-Saharan African adults. Individual reviews of all clinical PK studies can be found in Section 16.3.3. The following is the summary of findings related to the key clinical pharmacology aspects.

Absorption

Following administration of 1800 mg once daily with food for 4 days, median T_{max} on Day 4 for fexinidazole and M1 was 4 hours whereas for M2 was 6 hours. In a single dose study, following administration of 1200 mg fexinidazole with high-fat meal, the relative exposures (AUC) of fexinidazole, M1, and M2 were approximately 4- to 5-fold higher compared to the AUCs under fasting conditions. AUC of active metabolites M1 and M2 were 11- and 34-fold higher, respectively, compared to the parent drug fexinidazole.

Distribution

Plasma protein binding estimates for fexinidazole, M1, and M2 are 98%, 41%, and 57%, respectively. The estimated means (range) of CSF to plasma ratios for M1 and M2 were 0.53 (0.1 to 2.2) and 0.36 (0.1 to 0.8), respectively.

Metabolism

Fexinidazole is extensively metabolized by hepatic CYP450 enzymes. See Table 51 for additional details.

Excretion

Urinary excretion pathway does not play a significant role in the elimination of fexinidazole, M1, or M2 from the body.

6.2.2. General Dosing and Therapeutic Individualization

General Dosing

The Applicant-proposed dosing regimen of Fexinidazole tablets is based on the patient's body weight with a minimum age and weight cutoff of 6 years and 20 kg, as summarized in Table 50.

Table 50. Applicant-Proposed Fexinidazole Dosing Regimen (to-Be-Given With Food Once Daily at About the Same Time of the Day for 10 Days)

Body Weight Group	Dose in mg/ Day (Number of Tablets)	Treatment Duration (Days)
≥35 kg	Loading dose: 1800 (3)	4
	Maintenance dose: 1200 (2)	6
≥20 and <35 kg[a]	Loading dose: 1200 (2)	4
	Maintenance dose: 600 (1)	6

[a] Six years or older

Dosage and Administration Instructions

The Applicant proposes to include the following for the Dosage and Administration section of Fexinidazole tablets labeling, :

1. [redacted] (b) (4)
2. If a first event of vomiting occurs after receiving Fexinidazole tablets, do not redose. [redacted] (b) (4) the next dose the following day using the recommended treatment schedule. [redacted] (b) (4) .
3. [redacted] (b) (4)

During the clinical trials that evaluated the efficacy and safety of the Applicant-proposed fexinidazole dosing regimen, vomiting occurred in ~29 to 68% of patients. Therefore, the Applicant's first and third proposals as listed above are acceptable.

Regarding the second proposal, the Applicant has not submitted [redacted] (b) (4) . Therefore, the inclusion of statement, i.e., [redacted] (b) (4) is not acceptable. Regarding the instruction of not redosing patients if a first event of vomiting occurs after receiving Fexinidazole tablets, this language is acceptable from a clinical pharmacology perspective because of the following:

- The Reviewer's analysis suggests that the overall rates of "early vomiting", i.e., within 1 to 2 hours of dosing, that may warrant redosing were low compared to the overall vomiting rates observed in clinical trials.
- There is a potential of safety risks if a patient gets redosed erroneously.

See Section 6.3.2.2 for additional details.

Effect of Fexinidazole on CYP450 Substrates:

The Applicant has conducted a clinical DDI study that evaluated the effect of repeated co-administration of fexinidazole 1800 mg once daily (QD) for 4 days followed by 1200 mg for 1 day under fed conditions with single dose administration of 100 mg of caffeine (probe substrate of CYP1A2) and 20 mg of omeprazole (probe substrate of CYP2C19) in healthy male and female subjects of African sub-Saharan origin. The study results showed that mean caffeine AUC was 2.1-fold higher with no significant increase in C_{max}. Mean C_{max} and AUC of omeprazole were approximately 2-fold higher (See Table 59). Therefore, if coadministration with CYP1A2 and 2C19 substrates cannot be avoided, patients should be monitored for adverse reactions associated with these drugs.

Findings from a static mechanistic model-based analysis predict that fexinidazole may significantly increase AUC of sensitive CYP3A4 substrates. Therefore, concomitant use of drugs metabolized by CYP3A4 should be avoided. Note the Applicant did not conduct a dedicated clinical DDI study with a CYP3A4 substrate, such as midazolam.

An in vitro study showed that that fexinidazole may induce CYP2B6 in vivo and decrease the systemic PK exposure of CYP2B6 substrates. Therefore, concomitant use of fexinidazole with drugs metabolized via CYP2B6 should be avoided. If coadministration cannot be avoided, patients should be monitored for the lack of efficacy of these drugs.

Effect of Fexinidazole on Drug Transporters

In in vitro studies, fexinidazole was found to inhibit OCT2, OAT1, OAT3, MATE1, and MATE2-K. M1 was found to inhibit OAT3, MATE1, and MATE2-K. M2 was found to inhibit OCT2, OAT1, OAT3, MATE1, and MATE2-K. Coadministration of drugs that are substrates of these aforementioned transporters with fexinidazole may increase the plasma concentrations of these drugs. Therefore, concomitant use of drugs that are substrates of these transporters should be avoided. If coadministration cannot be avoided, patients should be monitored for adverse reactions associated with these drugs.

Effect of Other Drugs on Fexinidazole PK

Multiple CYP450 enzymes, including CYP3A4 and flavin monooxygenase, are involved in the metabolism of fexinidazole to its pharmacologically active M1 and M2 metabolites. The complete biotransformation pathway of M1 to M2 has not been characterized by the Applicant. Concomitant use of CYP450 inducers may increase the systemic exposure to M1 and M2. It is noteworthy that the observed increase in QTcF following fexinidazole treatment was found to be associated with M2 plasma concentrations (See IRT-QT review for complete details). Therefore, the Clinical Pharmacology review team recommends that concomitant use of drugs that induce CYP450 be avoided with Fexinidazole tablets because of potential safety concerns with increased metabolite PK exposure.

The Applicant has not conducted any clinical drug interaction studies with CYP450 inhibitors. Therefore, there is lack of information on the effect of drugs that are inhibitors of CYP450 on fexinidazole PK. The review team recommends that concomitant use of Fexinidazole tablets

should be avoided with drugs that are inhibitors of CYP450. If coadministration cannot be avoided, patients should be monitored for lack of efficacy due to potential for decreased plasma concentrations of the pharmacologically active M1 and M2 metabolites.

Outstanding Issues

None.

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. General Pharmacology and Pharmacokinetic Characteristics

The available clinical pharmacology and PK information for Fexinidazole tablets is summarized in Table 51. The Applicant has not conducted a human mass balance study using radiolabeled fexinidazole. In addition, the effect of renal or hepatic impairment on fexinidazole PK has not been evaluated. The Applicant has conducted exploratory assessments to characterize fexinidazole's disposition and excretion in humans and the findings from these assessments are summarized in Table 51.

Table 51. Clinical Pharmacology and Pharmacokinetic Summary

Parameter	Summary
Pharmacodynamics Cardiac electrophysiology	Concentration-dependent QTcF prolongation was observed with oral administration of fexinidazole tablets. Based on the exposure-response relationship, the mean (upper 90% confidence interval) increase in QTcF is predicted to be 19.0 msec (23.3 msec) at the approved recommended dosing regimen in adults. The observed increase in QTcF appears to be associated with the M2 metabolite of fexinidazole. See IRT-QT review for complete details.
Pharmacokinetics	**Table 52. Pharmacokinetic Parameters Estimates for Fexinidazole, M1, and M2 Following Administration of Fexinidazole Tablets**

Pharmacokinetic Parameters	Day	Fexinidazole	M1	M2
Mean (±SD) C_{max}, mcg/mL	Day 1	1.6 (±0.4)	8.1 (±2.2)	7.5 (±3.3)
	Day 4	0.8 (±0.3)	8.0 (±2.3)	19.6 (±5.4)
	Day 10	0.5 (±0.2)	5.9 (±2.1)	12.5 (±3.5)
Mean (±SD) AUC_{0-24} hours, mcg*h/mL	Day 1	14.3 (±2.6)	102.3 (±28.5)	110.1 (±41.1)[a]
	Day 4	11.6 (±2.2)	127.9 (±49.2)	391.5 (±126.7)[a]
	Day 10	7.0 (±2.5)	84.2 (±36.3)	252.4 (±73.6)

Note: 1800 mg once daily for 4 days, then 1200 mg once daily for 6 days to healthy adult subjects under fed conditions (N = 12)
[a] AUC_{0-t}
Abbreviations: C_{max}, maximum serum concentration; AUC_{0-24}, area under the plasma concentration-time curve from time zero to 24 hours; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; SD, standard deviation

Absorption

Table 53. Absorption Parameter Estimates for Fexinidazole, M1, and M2 Following Administration of Fexinidazole Tablets

Absorption Parameter	Day	Fexinidazole	M1
Median T_{max} (range) on day 4, hours	4 (0-9)	4 (0-6)	6 (0-24)

Note: 1800 mg once daily for 4 days, then 1200 mg once daily for 6 days to healthy adult subjects under fed conditions (N = 12)
Abbreviations: M1, Fexinidazole sulfoxide; T_{max}, time at which the C_{max} is observed

Effect of food[b,c]

Compared to the AUC in fasted state, the AUC of fexinidazole, M1, and M2 were approximately 4- to 5-fold higher following administration with high-fat meal.

Compared to the AUC in fasted state, the AUC of fexinidazole, M1, and M2 were approximately 2- to 3- fold higher following administration with meals with different levels of fat content, i.e., Plumpy nuts® meal with ~60% calories from fat and Rice plus Beans meal with ~1% calories from fat.

Distribution

Table 54. Distribution Parameter Estimates for Fexinidazole, M1, and M2 Following Administration of Fexinidazole Tablets

Distribution Parameters	Day	Fexinidazole	M1
Apparent volume of distribution on day 4, L	3222 (±1199)	NA	NA
Plasma protein binding	98%	41%	57%
CSF concentrations at 24 hours after the last fexinidazole dose on day 10, mcg/mL	NA	0.91 to 1.53	6.17 to 7.08
Range of mean CSF to plasma ratios	NA	0.50 to 0.52	0.31 to 0.5

Abbreviations: CSF, cerebro spinal fluid; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; NA, not applicable

Elimination

Table 55. Elimination Parameter Estimates for Fexinidazole, M1, and M2 Following Administration of Fexinidazole Tablets

Elimination Parameter	Day	Fexinidazole	M1
Mean (±SD) Day 10 Half-life, hours	15 (±6)	16 (±6)	23 (±4)
Mean (±SD) Apparent Clearance on Day 4, L/hour	161 (±37)	NA	NA

Abbreviations: M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; SD, standard deviation

Metabolism

Fexinidazole	Fexinidazole is metabolized to M1 by several CYP450 enzymes, including CYP3A4, and flavin monooxygenase.
Active metabolites	Complete pathway of M1 to M2 metabolism has not been characterized; however, several CYP450 enzymes including, CYP3A4, and flavin monooxygenase, are suggested to be involved. M2 is not further metabolized. The AUC_{0-24} of M1 and M2 are 11- and 34-fold higher, respectively, than that of fexinidazole.

Excretion Urine	Less than 3.2% of a given dose of Fexinidazole tablets, primarily as M1 and M2 metabolites.
Drug interaction (study type)	
CYP450 inhibition	Fexinidazole inhibits CYP1A2 (in vivo), CYP2B6 (in vitro), CYP2C19 (in vitro and in vivo), CYP2D6 (in vitro), and CYP3A4/5 (in vitro); M1 has the inhibits CYP2C19 (in vitro). Findings from a static mechanistic model-based analysis predicted that in vivo, fexinidazole may have significant drug-drug interactions with CYP3A4 substrates and may not have drug interactions with CYP2D6 substrates.
CYP450 induction	Fexinidazole and M1 have the potential to induce CYP2B6 in vitro.
Transporters inhibition	Fexinidazole inhibits OATP1B1, OATP1B3, OCT2, OAT1, OAT3, MATE1, and MATE2-K (all in vitro) transporters. M1 inhibits OAT3, MATE1, and MATE2-K (all in vitro). M2 inhibits OCT2, OAT1, OAT3, MATE1, and MATE2-K (all in vitro).

Source: Compiled by the Reviewer.
[a] AUC_{0-t}
[b] The effect of food was evaluated following administration of a single 1200 mg dose with a meal containing approximately 963 Kcal with 62% of total calories from fat, 17% from protein, and 21% from carbohydrate. (n=12)
[c] The effect of food was evaluated following administration of a single 1200 mg dose with Plumpy nuts® meal (~500 Kcal including ~60% from fat) and with Rice plus Beans meal (~393 Kcal including ~1% from fat). (n=11)
NA: Not available or not applicable
Abbreviations: C_{max}= maximum plasma concentration; T_{max}= time to maximum concentration; CSF= cerebrospinal fluid; CYP= cytochrome P450 enzymes; AUC_{0-24} hours= area under the plasma concentration-time curve from time zero to 24 hours, AUC_{0-t}= area under the plasma concentration-time curve from time zero to the last timepoint with measurable analyte concentrations.

6.3.2. Clinical Pharmacology Questions

6.3.2.1. Does the Clinical Pharmacology Program Provide Supportive Evidence of Effectiveness?

The pivotal evidence of effectiveness for this NDA submission is derived from a Phase 3 study (DNDiFEX004) that evaluated the efficacy and safety of fexinidazole in adults with late stage-2 g-HAT. Additional supportive evidence of effectiveness is derived from an open-label study in adults with early stage-2 or stage-1 g-HAT (Study DNDiFEX005) and from an open-label study in children aged ≥6 years and weighing ≥20 kg with disease at any stage (Study DNDiFEX006). The clinical pharmacology program provides supportive evidence of effectiveness by demonstrating the bioequivalence of the proposed to-be-marketed tablet formulation to the Phase 3 clinical trial tablet formulation. The Applicant has conducted a BE study (Study DNDiHATFEX008) that assessed and compared the BA of fexinidazole and the systemic exposure to its active metabolites from both formulations. The findings from the study are discussed below. PK exposure-response analyses were not conducted by the Applicant or the Clinical Pharmacology review team for the efficacy or safety endpoints, due to the lack of varying dosing regimens studied in clinical trials and numerically high rates of successful treatment outcomes with the Applicant-proposed dosing regimen (see Section 8.1.4 for more details).

Study DNDiHATFEX008

This was a single-dose, replicate cross-over study that enrolled 30 sub-Saharan African male subjects who received a single 1200 mg fexinidazole dose as two 600 mg tablets in a cross-over

manner (Total four treatments over two periods) under fed conditions. BE assessment findings from Study DNDiHATFEX008 are summarized in Table 56. See Section 16.3.3 for additional details on the study design and findings.

Table 56. Summary of the Bioavailability Assessment, Study DNDiHATFEX008

Parameters	% Point Estimates (90% CI, n=30) 2 x 600 mg To-Be-Marketed Tablets /2 x 600 mg Clinical Trial Tablets
Fexinidazole	
$AUC_{0\text{-}t.last}$	88 (82-95)
C_{max}	90 (83-98)
M1	
$AUC_{0\text{-}t.last}$	92 (87-98)
C_{max}	93 (88-98)
M2	
$AUC_{0\text{-}t.last}$	90 (85-96)
C_{max}	91 (85-96)

Source: Adapted from study report
Abbreviations: $AUC_{0\text{-}t.last}$, area under the concentration versus time curve up to last concentration timepoint; CI, confidence interval; C_{max}, maximum plasma concentration; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; n, number of subjects in subgroup

The findings from Study DNDiHATFEX008 indicated that the BA of fexinidazole and systemic exposure to its active metabolites from the to-be-marketed formulation is ~10% lower than that of the Phase 3 clinical trial tablet formulation. Nonetheless, the point estimates and associated 90% CIs were contained within (b) (4) % which showed that the to-be-marketed 600 mg tablet formulation is bioequivalent to the Phase 3 clinical trial tablet formulation.

6.3.2.2. Is the Proposed Dosing Regimen Appropriate for the General Patient Population for Which the Indication Is Being Sought?

Applicant-Proposed Dosing Regimen

Yes, the proposed dosing regimen is appropriate for the general patient population, i.e., g-HAT patients ≥ 6 years and weighing ≥ 20 kg.

The Applicant-proposed dosing regimen was evaluated in Study DNDiFEX004, which is a pivotal clinical study for this NDA. Study DNDiFEX004 evaluated the efficacy and safety of fexinidazole in adults with late stage-2 g-HAT. Study DNDiFEX004 also evaluated an FDA nonapproved comparator treatment arm: nifurtimox-eflornithine combination therapy. See Section 8.1 for comparison of efficacy and safety findings from both the treatment arms of Study DNDiFEX004.

The Applicant-proposed dosing regimen was evaluated in Study DNDiFEX005, which evaluated the efficacy of fexinidazole treatment in adults with early stage-2 or stage-1 g-HAT and in Study DNDiFEX006 that evaluated the efficacy of fexinidazole treatment in children aged ≥6 years and weighing ≥20 kg with disease at any stage. See Section 8.1 for efficacy and safety findings for the proposed fexinidazole dosing regimen.

The Applicant's Proposal for Dosing in Patients Who Experience Vomiting

During the abovementioned clinical trials, vomiting occurred in ~29 to 68% of patients after receiving fexinidazole treatment. The Applicant proposes to include the following information in the Fexinidazole tablet product labeling:

- If a first event of vomiting occurs after receiving Fexinidazole tablets, do not redose. (b) (4) next dose the following day using the recommended treatment schedule. (b) (4)

The Applicant has not submitted (b) (4) therefore, a recommendation to remove this statement was sent to the Applicant.

For reviewing the Applicant's proposal of not redosing a patient in the event of vomiting, the Reviewer performed additional analysis utilizing the data from three clinical studies that provide evidence of effectiveness, i.e., Studies DNDiFEX004, DNDiFEX005, and DNDiFEX006. First, the response/success rates to treatment were compared between patients who experienced vomiting and who did not. The findings from this analysis suggested that vomiting did not have a notable impact on the response rates in any of the three studies (Table 57).

Table 57. Vomiting and Response/Success Rates in Studies DNDiFEX004, DNDiFEX005, and DNDiFEX006

			Response/Success Rate % (n)	
Study	**Timepoint**	**Vomiting Rate % (n/N)**	**Vomiting**	**No Vomiting**
DNDiFEX004	Month 24	29% (75/262)	89% (67)	90% (168)
DNDiFEX005	Month 18	42% (97/230)	99% (96)	97% (129)
DNDiFEX006	Month 18	68% (84/123)	99% (83)	97% (38)

Source: Adapted from study report
Abbreviations: N, number of subjects; n, number of subjects in subgroup

Further review of the study protocols of these studies indicated that during the conduct of these studies, patients were redosed, which was in contrast to the Applicant's product labeling proposal to not redose. The redosing instructions from study protocols are summarized below:

Studies DNDiFEX004 and DNDiFEX005

A full dose is to be re-administered if vomiting happened within 30 minutes of treatment. Patients vomiting between 30 and 60 minutes are to be re-administered with two tablets during loading dose period (first four days) or one tablet during maintenance dose period (last six days). Note that these re-administered doses are partial and not the full loading or maintenance doses, respectively.

Study DNDiFEX006:

A full dose is to-be re-administered if vomiting happened within two hours after treatment administration.

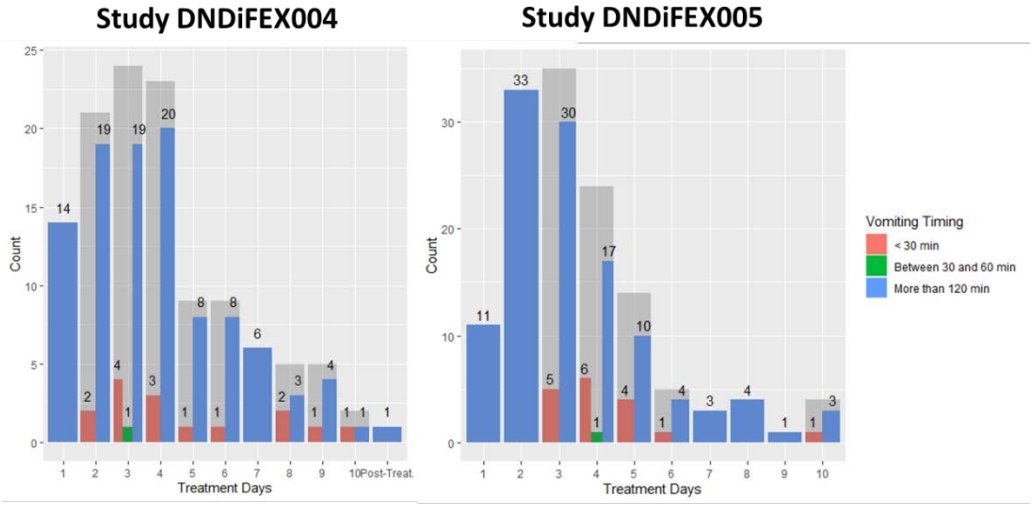
To understand the number of patients who had experienced early vomiting, i.e., vomiting within 60 minutes (Studies DNDiFEX004 and DNDiFEX005) or 120 minutes (Study DNDiFEX006) of dosing, and were redosed, the frequency and timing of the vomiting occurrences in all three studies were evaluated by the Reviewer. The findings from the Reviewer's analysis suggested that most incidences of vomiting occurred within the loading dose period of 4 days including the occurrences that were categorized as early vomiting (Figure 2). In Study DNDiFEX004, 16 incidences of early vomiting were from 14 (~5%) of the total patients who received fexinidazole treatment including two patients with two incidences of early vomiting. In Study DNDiFEX005, 18 incidences of early vomiting were from 15 (7%) of the total patients who received fexinidazole treatment including three patients with early vomiting incidences twice. In Study DNDiFEX006, 37 incidences of early vomiting were from 27 (22%) of the total patients who received fexinidazole treatment including six patients and two patients with early vomiting incidences twice and thrice, respectively.

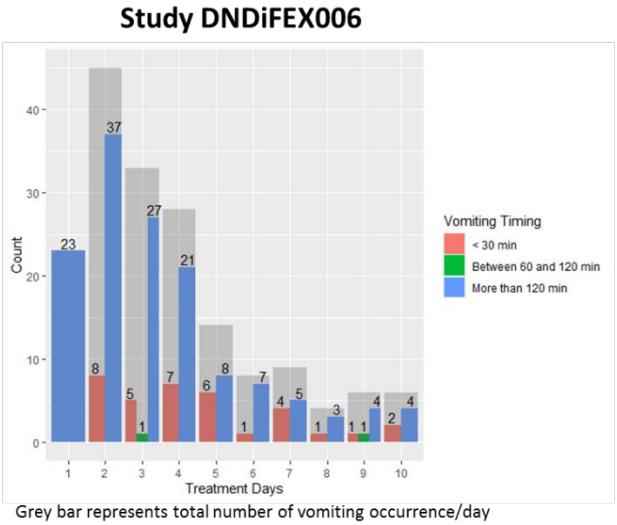
Since patients who experienced early vomiting were redosed, information on the response/success rates for patients who experienced early vomiting and had not been redosed was not available. Therefore, from an efficacy perspective, it is not possible to discern the impact of the redosing on response/success rates in these patient groups.

From a safety perspective, most of the early vomiting occurred during the loading dose period (Figure 2). Findings from Study DNDiFEX003 showed that higher systemic PK exposures to fexinidazole and its active metabolites may increase the risk of poor tolerability to fexinidazole treatment. Study DNDiFEX003 had evaluated a higher fexinidazole loading dosage regimen (2400 mg QD for 6 days), i.e., 600 mg (1 tablet) higher/day, than the Applicant is proposing for adult patients with g-HAT and lead to discontinuation of the study. Therefore, in the Reviewer's opinion, the safety data from Study DNDiFEX003 suggest a potential for safety risks with fexinidazole if a patient experiencing early vomiting gets redosed erroneously, leading to a higher than recommended total dose and/or higher plasma concentrations of parent fexinidazole and metabolites.

It is noteworthy that vomiting was not reported on Day 1 of treatment in any of the three studies and most occurrences of vomiting were not early vomiting. This suggests that the mechanism of action for vomiting may not be the result of tolerability issues at the gastrointestinal level.

Figure 2. Timing and Frequency of Vomiting Incidences Observed in Studies DNDiFEX004, DNDiFEX005, and DNDiFEX006





Grey bar represents total number of vomiting occurrence/day
Source: Reviewer's analysis

If a second event of vomiting occurs after administration of Fexinidazole tablets, the Applicant proposes to include the following labeling language in the Dosage and Administration section:

- (b) (4)

The observed rate of patients experiencing early vomiting more than once was low (~2%; 13 of 619 patients) in the three clinical efficacy and safety studies.

Overall, the Applicant's proposal to not redose patients if a first or second event of vomiting occurs after receiving fexinidazole treatment is acceptable from a Clinical Pharmacology perspective for the following reasons:

- Potential for safety risks if a patient gets redosed erroneously, leading to a higher than recommended total dose and/or higher plasma concentrations of parent fexinidazole and metabolites
- Relatively lower rates of early vomiting observed in patients receiving the proposed fexinidazole dosing regimen

6.3.2.3. Is an Alternative Dosing Regimen or Management Strategy Required for Subpopulations Based on Intrinsic Patient Factors?

Renal Impairment

The Pharmacometrics (PM) Reviewer used data generated from a population PK analysis to assess the impact of renal impairment on the AUC of fexinidazole, M1, and M2. As shown in Table 58, renal function does not appear to affect fexinidazole, M1, or M2 AUC estimates after stratification by age and weight. Any differences in AUC appear to be within the range of variability, and there are no consistent trends towards an increase or decrease in AUC with increasing renal impairment. Therefore, no dosage adjustment is needed for patients with mild or moderate renal impairment (eGFR 30 to 89 mL/min/1.73m^2). There was insufficient information to robustly assess the AUC in patients with severe renal impairment (eGFR < 30 mL/min/1.73m^2). The Review team recommends avoiding the use of Fexinidazole tablets in patients with severe renal impairment. See Section 16.3.4 for more details.

Table 58. Mean (CV%) Fexinidazole and Metabolites AUC in Patients Receiving Fexinidazole Stratified by Age, Weight, and Renal Impairment.

				AUC (mcg*h/mL)		
Age (yr)	Weight (kg)	Renal Function	n	Fexinidazole	M1	M2
<18	<35	Normal	68	3.03 (58%)	111 (28%)	371 (37%)
		Mild	24	2.33 (62%)	105 (27%)	335 (36%)
		Moderate	1	1.49	85.9	348
		Severe	2	3.42 (63%)	117 (5%)	379 (37%)
	≥35	Normal	21	4.12 (56%)	145 (16%)	494 (28%)
		Mild	7	4.51 (68%)	150 (44%)	464 (48%)
		Moderate	1	3.24	143	448
≥18	<35	Normal	2	3 (36%)	170 (43%)	496 (17%)
	≥35	Normal	121	3.79 (44%)	154 (27%)	485 (36%)
		Mild	58	3.67 (48%)	148 (27%)	428 (32%)
		Moderate	12	3.6 (37%)	141 (30%)	454 (36%)

Source: Reviewer's analysis.
Abbreviations: AUC, area under the time concentration curve; CV, coefficient of variation; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; n, number of subjects in subgroup; yr, year

Hepatic Impairment

Fexinidazole is extensively metabolized by the liver; however, the Applicant has not conducted a clinical study to assess the effect of hepatic impairment on the PK of fexinidazole, M1, or M2.

The Applicant proposes [redacted] (b) (4) The Applicant's proposal is acceptable.

6.3.2.4. Are There Clinically Relevant Food-Drug or Drug-Drug Interactions, and What Is the Appropriate Management Strategy?

Yes, there are clinically relevant food-drug and drug-drug interactions with Fexinidazole tablets.

Food Effect

The food-effect PK study (Study DNDiFEX001 Part II) demonstrated that when a single 1200 mg Fexinidazole tablet dose was administered with a high calorie and high fat meal, C_{max} and AUC estimates for fexinidazole were 466% and 428% of the estimates following dosing under fasting conditions. Fexinidazole tablets are proposed to be administered with food. See Section 16.3.3 for additional details on the design and findings of the food effect PK study. Of note, Fexinidazole tablets were given with food in clinical efficacy studies that support this NDA. Thus, the Clinical Pharmacology review team agrees with the Applicant's proposed labeling to administer Fexinidazole tablets with food.

Drug-Drug Interactions

The Applicant has conducted in vitro studies, a static mechanistic model-based analysis, and a clinical DDI cocktail study to characterize the DDI potential with Fexinidazole tablets. Findings from these studies and the review of Applicant-proposed management strategies are discussed below.

Effect of Fexinidazole on Other Drugs

The findings from an in vitro study (Study ICH0106, See Section 16.3.2) showed that fexinidazole inhibits CYP1A2, -2B6, -2C8, -2C9, -2C19, -2D6, and -3A4/5 with IC_{50} values of 22.3, 26.5, 147, 118, 0.863, 98.5, and 35.4 mcM, respectively. M1 was found to be inhibit CYP1A2, -2C19, and -2D6 with IC_{50} values of 142, 10.8, and 278 mcM.

CYP1A2 and CYP2C19 Inhibition

To further characterize the DDI potential with CYP1A2 and CYP2C19 substrates, the Applicant has conducted a single dose DDI study with cocktail of caffeine and omeprazole used as probe substrates for CYP1A2 and CYP2C19, respectively. The PK of caffeine and omeprazole was evaluated and compared after administering a single dose of 100 mg caffeine and 20 mg omeprazole without (reference arm) and with (DDI arm) fexinidazole. In the DDI arm fexinidazole treatment was 1800 mg fexinidazole QD for 4 days plus 1200 mg fexinidazole on Day 5 and cocktail probes were administered on Day 4. The observed caffeine C_{max} and AUC estimates in the DDI arm were ~119% and ~200%, respectively, compared to the reference arm (Table 59). For omeprazole, both C_{max} and AUC estimates were ~200% in the DDI arm compared to the reference arm (Table 59).

Table 59. Exposure Parameter Ratios for Caffeine and Omeprazole Following a Single Dose of 100 mg Caffeine and 20 mg Omeprazole With (DDI Arm) and Without (Reference Arm) Fexinidazole Treatment

Parameters	% Point Estimates DDI Arm (With Fexinidazole)/ Reference Arm (Without Fexinidazole)
Caffeine	
AUC	205 (166-252)
C_{max}	119 (114-124)
Omeprazole	
AUC	205 (142-295)
C_{max}	195 (131-291)

Source: Adapted from study report
Abbreviations: AUC, area under the time concentration curve; C_{max}, maximum plasma concentration; DDI, drug-drug interactions

Based on these findings, the Applicant proposes to include the following DDI-related language in the Section 7 of the Fexinidazole tablets product labeling (PI):

(b) (4)

The Review team agrees with the Applicant's proposal in principle and recommends to include the following revision to Section 7 of the Fexinidazole tablets PI:

Drugs Metabolized by CYP1A2 or CYP2C19	
Examples (not fully inclusive):	CYP1A2: Duloxetine, Tacrine, Tizanidine, Theophylline CYP2C19: Lansoprazole, Mephenytoin, Diazepam
Clinical Impact	Increased risk for adverse reactions associated with increased concentrations of the drug due to inhibition of either CYP1A2 or CYP2C19 by fexinidazole.
Prevention or Management	Monitor for adverse reactions associated with these drugs when used concomitantly with fexinidazole tablets.

CYP2D6 and CYP3A4 Inhibition

To further characterize the DDI potential with CYP2D6 and CYP3A4 substrates, the Applicant used a static mechanistic model-based approach using Drug-Drug Interaction Risk Calculator (DDIRC), Pharmapendium®. See Section 16.3.2 for the review of the mechanistic model used in the DDIRC (from the Drug-Drug Interaction Risk Calculator User Guide) and its comparison to the model recommended in 2020 FDA guidance on in vitro drug interaction studies. The Applicant-submitted predictions show that in vivo, fexinidazole treatment would not significantly increase the PK exposure of CYP2D6 probe or sensitive substrate drugs such as metoprolol (mean predicted interaction ratio of 1.1-fold), desipramine (1.1-fold),

nebivolol (1.1-fold), propafenone (1.1-fold), risperidone (1.1-fold), and venlafaxine (1.3-fold). However, the predictions from DDIRC showed that fexinidazole treatment would increase the exposure of sensitive or probe CYP3A4 drugs, having a notable intestinal first pass metabolism such as lovastatin (mean predicted interaction ratio of 8-fold), simvastatin (4-fold), midazolam (2-fold), and nisoldipine (6-fold), and saquinavir (2.5-fold).

The Reviewer did not have access to abovementioned DDIRC. Therefore, the Applicant's exact analysis could not be repeated, nor the prediction findings submitted in the file could be verified. To predict the potential DDI between fexinidazole and CYP2D6 and CYP3A4 substrates, the Reviewer used an OCP/FDA internal Microsoft Excel-based tool that assesses the DDI prediction using static mechanistic models. The internal DDI prediction tool utilizes the models as described in the above-mentioned FDA guidance document. See Section 16.3.2 for additional details on reviewer's analysis. The results from the internal DDI tool predicted an increase in the AUC of midazolam and dextromethorphan by 2.37-fold and 1.1-fold, respectively. These findings were comparable to the predictions from DDIRC, which predicted an AUC increase of 2.2-fold for midazolam and 1.12-fold for dextromethorphan.

Based on the findings from the model-based approach, the Applicant proposes to include the following DDI-related language in Section 7 of the Fexinidazole tablets PI:

(b) (4)

The Review team partially agrees with the Applicant's proposal and recommends including the following revision to Section 7 of the Fexinidazole tablets PI:

Drugs Metabolized by CYP3A4	
Examples (not fully inclusive):	Lovastatin, Simvastatin, Nisoldipine, Saquinavir, Midazolam
Clinical Impact	Increased risk for adverse reactions associated with increased concentrations of the drug due to inhibition of CYP3A4 by fexinidazole.
Prevention or Management	Avoid concomitant use with Fexinidazole tablets.

CYP450 Induction

The findings from an in vitro study (Study XT093051, See Section 16.3.2) showed that fexinidazole and M1 induce CYP1A2 mRNA expression. Fexinidazole also inhibits CYP1A2 and the findings from the in vivo DDI cocktail study that included caffeine showed that the net effect of fexinidazole treatment on CYP1A2 would be inhibition (Table 59). Fexinidazole and M1 have also been shown to induce CYP2B6 in vitro. Based on the observed induction potential towards CYP2B6, the Applicant proposes to include the following DDI related language in Section 7 of the Fexinidazole tablets PI:

(b) (4)

The Review team agrees with the Applicant's proposal in principle and recommends including the following revision to Section 7 of the Fexinidazole tablets PI:

Drugs Metabolized by CYP2B6	
Examples (not fully inclusive):	Bupropion, Efavirenz
Clinical Impact	Increased risk for the lack of efficacy associated with decreased plasma concentrations of the drug due to induction of CYP2B6 by fexinidazole
Prevention or Management	Avoid concomitant use with Fexinidazole tablets. If coadministration cannot be avoided, monitor for lack of efficacy of these drugs.

Drug Transporters Inhibition

The findings from an in vitro study (Study TRI0039, See Section 16.3.2) showed that fexinidazole inhibits OATP1B1, OATP1B3, OCT2, OAT1, OAT3, MATE1, and MATE2-K with IC_{50} values of 69.8 (72 with preincubation), 121 (59 with preincubation), 68.7, 38.9, 267, 33.5, 2.08, and 4.38 mcM, respectively. M1 inhibits OAT3, MATE1, and MATE2-K with IC_{50} values of 113, 53.9, and 44.9 mcM, respectively. M2 inhibits OCT2, OAT1, OAT3, MATE1, and MATE2-K with IC_{50} values of 282, 202, 71.8, 44.3, 18.1, and 39.3 mcM, respectively.

OATP1B1 and OATP1B3

The Applicant further characterized the potential for inhibition of the hepatic transporters OATP1B1 and OATP1B3 by fexinidazole using the methods described in the FDA in vitro DDI study guidance document. In the Applicant's analysis, the predicted interaction ratio R is <1.1 for both of these transporters (see Table 60 below). (b) (4)

Table 60. Predicted Risk of In Vivo Interaction (Inhibition) of Fexinidazole on Hepatic Transporters: OAPT1B1 or OATP1B3

Transporter	IC_{50} (µM)	$I_{in,max,u}$	Predicted in vivo R	Risk of in vivo interaction
OATP1B1	69.8	4.5	1.07	No (R<1.1)
OATP1B3	59.1	4.5	1.08	No (R<1.1)

Abbreviations: IC_{50}, half-maximal inhibitory concentration; $I_{in,max}$, estimated maximum plasma inhibitor concentration at the inlet to the liver; **OATP**, organic anion transporting polypeptide; **R**, predicted ratio of the victim drug's AUC in the presence and absence of the investigational drug as the inhibitor

$$R^{a} = 1 + (f_{u,p}{}^{b} \times I_{in,max}{}^{c}) / IC_{50}{}^{c} \geq 1.1$$

a R is the predicted ratio of the victim drug's AUC in the presence and absence of the investigational drug as the inhibitor
b $f_{u,p}$ is the unbound fraction in plasma
c $I_{in,max}$ is the estimated maximum plasma inhibitor concentration at the inlet to the liver. It is calculated as: $I_{in,max} = (I_{max} + (F_a F_g \times k_a \times dose)) / Q_h / R_B$
d IC_{50} is the half-maximal inhibitory concentration

For fexinidazole:

I_{max} = 0.18 µM; $F_a F_g$ = 1; Dose = 1800 mg; k_a = 0.6 h^{-1}; Q_h = 88 L/h, R_B = 0.61; $f_{u,p}$ = 0.063; $I_{in,max,u}$ = 4.5 µM.

Source: From an Applicant submitted Clinical Pharmacology Summary Document

The Reviewer performed an independent analysis using an internal FDA/OCP Microsoft excel-based tool that utilizes the models as described in the above-mentioned FDA guidance document. The findings from the Reviewer's analysis had similar predicted in vivo ratio (R), i.e., 1.08 for OATP1B1 and 1.09 for OATP1B3. The FDA guidance document notes that the investigational drug has the potential to inhibit OATP1B1/3 in vivo if the R value is ≥ 1.1. (b) (4) is not acceptable. The Review team recommends replacing the Applicant-proposed statement from Section 12 of the Fexinidazole tablets PI with the following under Drug Interaction Studies-> In Vitro Studies -> Transporter Systems:

- In vitro, fexinidazole has the potential to inhibit drugs that are substrates of OATP1B1 and OATP1B3.

OCT2, OAT1, OAT3, MATE1, and MATE2-K

The Applicant further characterized a potential for inhibition of the renal transporters OCTs, OATs, and MATEs by fexinidazole, M1, and M2, using the methods described in the FDA in vitro DDI study guidance document. In the Applicant's analysis, at least one analyte was found to have a risk to inhibit each of the renal transporters (Table 61).

Table 61. Predicted Risk of In Vivo Interaction (Inhibition) of Fexinidazole on Renal Transporters

Transporter	Compound	IC_{50} (µM)	$I_{max,u}$	$I_{max,u}/IC_{50}$	In vivo risk of interaction
OCT1	M2	284	38.8	0.14 (≥0.1)	Yes
OCT2	M1	251	17.0	0.07	
	M2	203	38.8	0.19 (≥0.1)	Yes
OAT1	M1	>251	17.0	0.08	
	M2	72.1	38.8	0.54 (≥0.1)	Yes
OAT3	M1	113	17.0	0.15 (≥0.1)	Yes
	M2	44.3	38.8	0.86 (≥0.1)	Yes
MATE1	Fexinidazole	2.08	0.18	0.09 (≥0.02)	Yes
	M1	54.2	17.0	0.31 (≥0.02)	Yes
	M2	18.1	38.8	2.1 (≥0.02)	Yes
MATE2-K	Fexinidazole	4.43	0.18	0.04 (≥0.02)	Yes
	M1	44.9	17.0	0.39 (≥0.02)	Yes
	M2	39.3	38.8	1.0 (≥0.02)	Yes

Abbreviations: IC_{50}, half-maximal inhibitory concentration; **MATE**, multi-antimicrobial extrusion protein; **OAT**, organic anion transporter; **OCT**, organic cation transporter

There is a risk of in vivo interaction if: Imax,u/IC50 is ≥0.1 for OCT1 (TBC), OCT2, OAT1, OAT3 or Imax,u/IC50 is ≥0.02 for MATEs

Source: From an Applicant submitted Clinical Pharmacology Summary Document

Based on these findings (Table 61), the Applicant proposes to include the following DDI-related language for Section 7 of the Fexinidazole tablets PI:

(b) (4)

The Review team agrees with the Applicant's proposal in principle and recommends including the following revision to Section 7 of the Fexinidazole tablets PI:

Drugs Substrates of OCT2, OAT1, OAT3, MATE1, and MATE2-K Transporters	
Examples(not fully inclusive):	Metformin, Dofetilide, Adefovir, Cefaclor, Furosemide
Clinical Impact	Increased risk for adverse reactions associated with increased concentrations of the drug due to inhibition of these transporters by fexinidazole
Prevention or Management	Avoid concomitant use with Fexinidazole tablets. If coadministration cannot be avoided, monitor for adverse reactions associated with these drugs.

Effect of Other Drugs on Fexinidazole PK

CYP3A4 Inhibitors or Inducers

Fexinidazole is extensively metabolized and multiple CYP450 are involved in its metabolism. The Applicant has not conducted in vivo DDI studies with CYP3A4 inhibitors or CYP3A4 inducers. Due to the lack of information in this regard, the Applicant proposes to include the following DDI-related language in Section 7 of the Fexinidazole tablets PI:

(b) (4)

The Applicant's proposal is not acceptable. M2 has relatively higher exposure (in plasma and CSF) as compared to fexinidazole and M1 and is likely playing a key role in the drug's desired pharmacological activity. It is also noteworthy that M2 plasma concentrations have been associated with the increased risk of QTc interval prolongation. In vitro studies suggest that fexinidazole is metabolized to M1 by several CYP450 enzymes, including CYP3A4 and flavin monooxygenase. However, the complete biotransformation pathway of M1 to M2 has not been characterized. Therefore, the overall interaction potential between a CYP3A4 inhibitor and fexinidazole remains unknown, including information regarding whether and to what extent CYP3A4 inhibitors or inducers affect the M2 plasma concentrations. Therefore, the review team recommends including the following revision to Section 7 of the Fexinidazole tablets PI:

Drugs that induce CYP450	
Examples (not fully inclusive):	Rifampin, Phenytoin, St. John's Wort, Carbamazepine
Clinical Impact	Increased risk for adverse reactions associated with increased systemic exposure to the M1 and M2 metabolites of fexinidazole. M2 plasma concentrations have been associated with the increased risk of QT interval prolongation.
Prevention or Management	Avoid concomitant use with Fexinidazole tablets.
Drugs that are inhibitors of CYP450	
Examples (not fully inclusive):	Clarithromycin, Itraconazole, Voriconazole, Erythromycin, Fluconazole,
Clinical Impact	Multiple CYP450 enzymes are involved in the metabolism of fexinidazole to its pharmacologically active M1 and M2 metabolites. Although no clinical drug interaction studies were performed with CYP450 inhibitors, the formation of the M1 and M2 metabolites may be decreased.
Prevention or Management	Avoid concomitant use with Fexinidazole tablets. If coadministration cannot be avoided, monitor for lack of efficacy of fexinidazole due to potential for decreased plasma concentrations of its active metabolites.

Effect of Alcohol Use with Fexinidazole

Fexinidazole is a nitroimidazole class compound. Nitroimidazoles are associated with causing a disulfiram-like reaction (Antabuse effect) when taken with alcohol. These reactions are characterized by flushing, rash, peripheral edema, nausea, and headache. Based on this interaction risk associated with this chemical class, the Applicant proposes (b) (4) .

- *Reviewer Comment: Based on internal discussion with the Clinical review team, consumption of alcoholic beverages during treatment with Fexinidazole tablets* (b) (4) *, precautionary language will state to avoid consumption of alcohol during treatment and for at least 48 hours after completing therapy with Fexinidazole tablets.*

7. Sources of Clinical Data and Review Strategy

7.1. Table of Clinical Studies

Table 62. Listing of Key Clinical Trials Relevant to This NDA/BLA

Trial Identity	NCT No.	Trial Design	Regimen/ Schedule/ Route	Study Endpoints	Treatment Duration/ Follow-Up	No. of Patients Enrolled	Study Population	No. of Centers and Countries
Controlled studies to support efficacy and safety								
DNDiFEX004	01685827	Phase 2/3, open-label, randomized, active-controlled study	Fexinidazole: 1800 mg (3 x 600-mg tablets), once daily for 4 days (Days 1 through 4), followed by 1200 mg (2 x 600-mg tablets), once daily for 6 days (Days 5 through 10) Oral NECT: Nifurtimox 120-mg tablets administered orally at a dose of 15 mg/kg/day, in 3 divided doses for 10 days (Days 1 through 10) DFMO injectable solution administered as a 2-hour IV infusion at a dose of 400 mg/kg/day, in 2 divided doses, for 7 days (Days 1 through 7)	Treatment outcome (success/failure assessed at 18 months after the end of therapy (EOT)	10 days/ EOT (Day 11) EOH (Day 13-18), Week 9, Months 3, 6, 12, 18, and 24	Fexinidazole: 264 NECT: 130	Patients (≥15 years of age) with late stage-2 g-HAT Late stage 2 disease defined as: Presence of parasites in blood, lymph node, or CSF. If no parasites in CSF, then CSF WBC must be > 20 cells/µL.	10 centers/2 countries (DRC and CAR)

Trial Identity	NCT No.	Trial Design	Regimen/ Schedule/ Route	Study Endpoints	Treatment Duration/ Follow-Up	No. of Patients Enrolled	Study Population	No. of Centers and Countries
DNDiFEX005	02169557	Open-label, single-arm study	Fexinidazole: Same as in DNDiFEX004	Treatment outcome (success/failure assessed at 12 months after the end of therapy	10 days/ EOT (Day 11 or 12), EOH (Day 11-18), Week 9, Months 6, 12, 18	Fexinidazole: Total: 230 Stage-1: 189 Early stage-2: 41	Patients (≥15 years of age) with stage-1 and early stage-2 g-HAT Stage-1 Disease: Parasites in blood or lymph node and CSF WBC ≤5 cells/µL. Early stage- 2 Disease: Parasites in Blood or lymph node and CSF WBC 6-20 cells/µL	8 centers /1 country (DRC)
DNDiFEX006	02184689	Open-label, single-arm study	Fexinidazole: Body weight ≥20 kg and <35 kg 1200 mg (2 x 600-mg tablets) once daily for 4 days (Days 1 through 4) 600 mg (1 x 600-mg tablet) once daily for 6 days (Days 5 through to 10) Body weight ≥35 kg (same as in adults in DNDiFEX004)	Treatment outcome (success/failure assessed at 12 months after the end of therapy	10 days/ EOT (Day 11, 12), EOH (Day 13-18), Week 9, Months 6, 12, 18	Fexinidazole: Total: 125 Stage-1: 69 Early stage-2: 19 Late stage-2: 37	Children (aged between 6 and 15 years) with stage-1 and stage-2 g-HAT (disease definition as above)	8 centers /1 country (DRC)

Trial Identity	NCT No.	Trial Design	Regimen/ Schedule/ Route	Study Endpoints	Treatment Duration/ Follow-Up	No. of Patients Enrolled	Study Population	No. of Centers and Countries
Related study to support safety								
DNDiCHFEXI001	NCT02498782	Double-blind, randomized, placebo-controlled	HD (2 Weeks): 1800 mg/day for 2W, then placebo for 6W (total dose 25.2 g) HD (4 Weeks): 1800 mg/day for 4W then placebo for 4W (total dose 50.4 g) HD (8 Weeks): 1800 mg/day for 8W (total dose 100.8 g) LD (2 Weeks): 1200 mg/day for 2W, then placebo for 6W (total dose 16.8 g) LD (4 Weeks): 1200 mg/day for 4W then placebo for 4W (total dose 33.6 g) LD (8 Weeks): 1200 mg/day for 8W (total dose 67.2 g)	Parasitological cure rate by serial negative qualitative PCR at the end of treatment (EOT) and sustained parasitological clearance until 6 months of follow-up	8 weeks / 54 weeks	47 (40 fexinidazole; 7 placebo)	Patients 18 to 50 years old with Chagas Disease	2 centers / 1 country (Bolivia)

Source: Reviewer's summary
Abbreviations: CAR, Central African Republic; CSF, cerebral spinal fluid; DFMO: alpha-difluoromethylornithine nifurtimox and Ornidyl®; DRC, Democratic Republic of the Congo; EOT, end of therapy; EOH, end of hospitalization; g-HAT: human African Trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense*; HD, high dose; IV, intravenous; LD, low dose; NECT: Nifurtimox-Eflornithine combination therapy; WBC, white blood cell

7.2. Review Strategy

In this NDA, evidence of clinical efficacy is based primarily on a single, randomized, open-label clinical efficacy trial DNDiFEX004 in patients 15 to 71 years of age with late stage-2 g-HAT, which evaluated 264 patients randomized to receive fexinidazole compared with 130 patients randomized to NECT.

The overall objective of the fexinidazole clinical program was to give the same treatment to all g-HAT patients, without the need of CSF-based staging of the disease. Therefore, one single-arm trial DNDiFEX005 was conducted in patients ≥15 years of age with stage-1 or early stage-2 g-HAT disease and another trial DNDiFEX006 in children aged 6 to 14 years with any stage of the disease. These two single-arm trials provided supportive evidence for efficacy, and will only be reviewed briefly in this document since they were uncontrolled trials.

For each trial, there was a follow-up study in order to collect additional data after the primary efficacy endpoint. The results of these follow-up studies will be included in relevant sections in each main trial and no separate sections will be included for follow-up studies.

8. Statistical and Clinical and Evaluation

8.1. Review of Relevant Individual Trials Used to Support Efficacy

8.1.1. DNDiFEX004

Trial Design

This was an open-label, active-controlled, randomized, multicenter trial to assess the efficacy and safety of fexinidazole compared with NECT in patients aged ≥ 15 years with late stage-2 g-HAT.

Randomization was centralized (performed by one center). Eligible patients were randomized by site to receive fexinidazole (test drug) or NECT (active comparator) in a 2:1 ratio. The treatment regimens were as follows:

- Fexinidazole: fexinidazole 1800 mg (3 x 600-mg tablets) administered orally, once daily for 4 days (Days 1 through 4; LD), followed by 1200 mg (2 x 600-mg tablets) administered orally, once daily for 6 days (Days 5 through 10; maintenance dose), with food
- NECT: nifurtimox tablets administered orally, three times daily at a dose of 15 mg/kg/day for 10 days (Days 1 through 10) plus Ornidyl® (DFMO; eflornithine infusion) administered twice daily as a 2-hour intravenous (IV) infusion at a dose of 400 mg/kg/day for 7 days (Days 1 through 7), with food.

The total duration of treatment was 10 days (D1 to D10). An end-of-therapy (EOT) assessment was performed on Day 11 (EOT visit). Patients were hospitalized until the end of hospitalization

(EOH) visit on Day 13 to Day 18. Patients with satisfactory clinical status were permitted to leave the hospital from Day 13 onwards (up to Day 18). Initially, follow-up visits took place at 3, 6, 12, 18 months (primary time point), and 24 months after the EOT visit. An additional earlier visit at Week 9 was added as a safety precaution in Protocol Amendment 3 dated February 9, 2015, during the conduct of the trial.

The primary efficacy analysis occurred once all subjects had reached 18 months. Follow-up continued until all subjects were followed up to Month 24. The last patient completed the study on April 26, 2017. This additional follow-up of all subjects out to 24 months was called the follow-up study to the primary study.

This was an open-label study because the route of administration and the dosing regimens were different for the two treatment groups. However, the Applicant, data management personnel, and study statistician were blind to treatment assignment to ensure data integrity.

There were prescreening and screening procedures. The corresponding inclusion and exclusion criteria were as follows:

Inclusion Criteria

- 15 years of age or older
- Male or female
- Able to ingest at least one complete meal per day (or at least one sachet of Plumpy'Nut®)
- Karnofsky score >50
- Late stage-2 HAT due to *T. b. gambiense* with confirmed evidence of parasite in the blood and/or lymph and/or CSF, certified by the report from the mobile team, with details on examinations performed and CSF WBC count, or performed at the investigational site; in case of no parasites in the CSF, CSF WBC count needed to be > 20 cells/µL.
- Having a permanent address and able to comply with the schedule of follow-up visits
- Signed informed consent form

Exclusion Criteria

- Severe malnutrition, defined as BMI <16 kg/m^2
- Unable to take medication by the oral route
- Pregnancy or breastfeeding
- Clinically significant medical condition that could, in the opinion of the Investigator, have jeopardized the patient's safety or participation in the study, including, but not limited to significant liver or cardiovascular disease, suspected or proven active infection, central nervous system trauma or seizure disorder, coma, or consciousness disturbances
- Severely deteriorated general status, including as a result of cardiovascular shock, respiratory distress, or end-stage disease

- Any condition that affected the patient's ability to communicate with the Investigator as required to complete the study
- Any contraindication to imidazole drugs, i.e., known hypersensitivity to imidazoles, or to NECT, i.e., known hypersensitivity to DFMO
- Prior treatment for HAT
- Prior enrolment in the study
- Foreseeable difficulty complying with follow-up, including migrant worker, refugee status, itinerant trader
- History of alcohol or drug addiction
- Clinically significant laboratory test abnormality, including, for example:
- Aspartate aminotransferase (AST) and/or alanine aminotransferase (ALT) >2 times the upper limit of normal (ULN)
- Total bilirubin >1.5 times ULN
- Severe leukopenia at <2000/mm3
- Potassium <3.5 mmol/L
- Pregnancy confirmed by a positive urine pregnancy test within 24 hours prior to the start of treatment
- Unstable abnormalities on ECG
- QTcF ≥450 ms, on automatic reading on 2 successive ECGs in resting position, performed to 20 minutes apart
- Patients not tested for malaria and/or not having received appropriate treatment for malaria
- Patients not having received appropriate treatment for soil-transmitted helminthiasis

The following criteria were considered to be temporary exclusion criteria:

- Traumatic lumbar puncture, i.e., red blood cells visible in CSF; repeat lumbar puncture could be performed 48 hours later
- Recovery period after treatment for malaria and/or treatment for helminthiasis, i.e., approximately 3 days
- Abnormalities on laboratory tests or ECG that could be controlled within a few days after the initial assessment. If the value returned to normal or was considered not clinically significant, the patient could be included in the study.

All subjects were tested for malaria prior to starting g-HAT treatment. If positive, they were treated with artemether and lumefantrine and then underwent at least a 3-day recovery period post treatment prior to starting study medication. Similarly, all subjects were treated for soil-based helminthiasis with either albendazole or mebendazole with a similar recovery period.

Study Endpoints

Primary Efficacy Endpoint

The primary efficacy endpoint was treatment outcome (success/failure) assessed at 18 months after EOT, based on the WHO criteria with the following adaptation: a death was considered a

failure in this study, regardless of the cause. In case of missing lumbar puncture at 18 months, a more detailed decision tree was used, involving data such as the outcome at 24 months. The algorithm of classification that was used to categorize patients as “success” or “failure” for the primary efficacy endpoint at 18 months is presented in Figure 3.

The outcome at 18 months was considered a failure in the following cases:

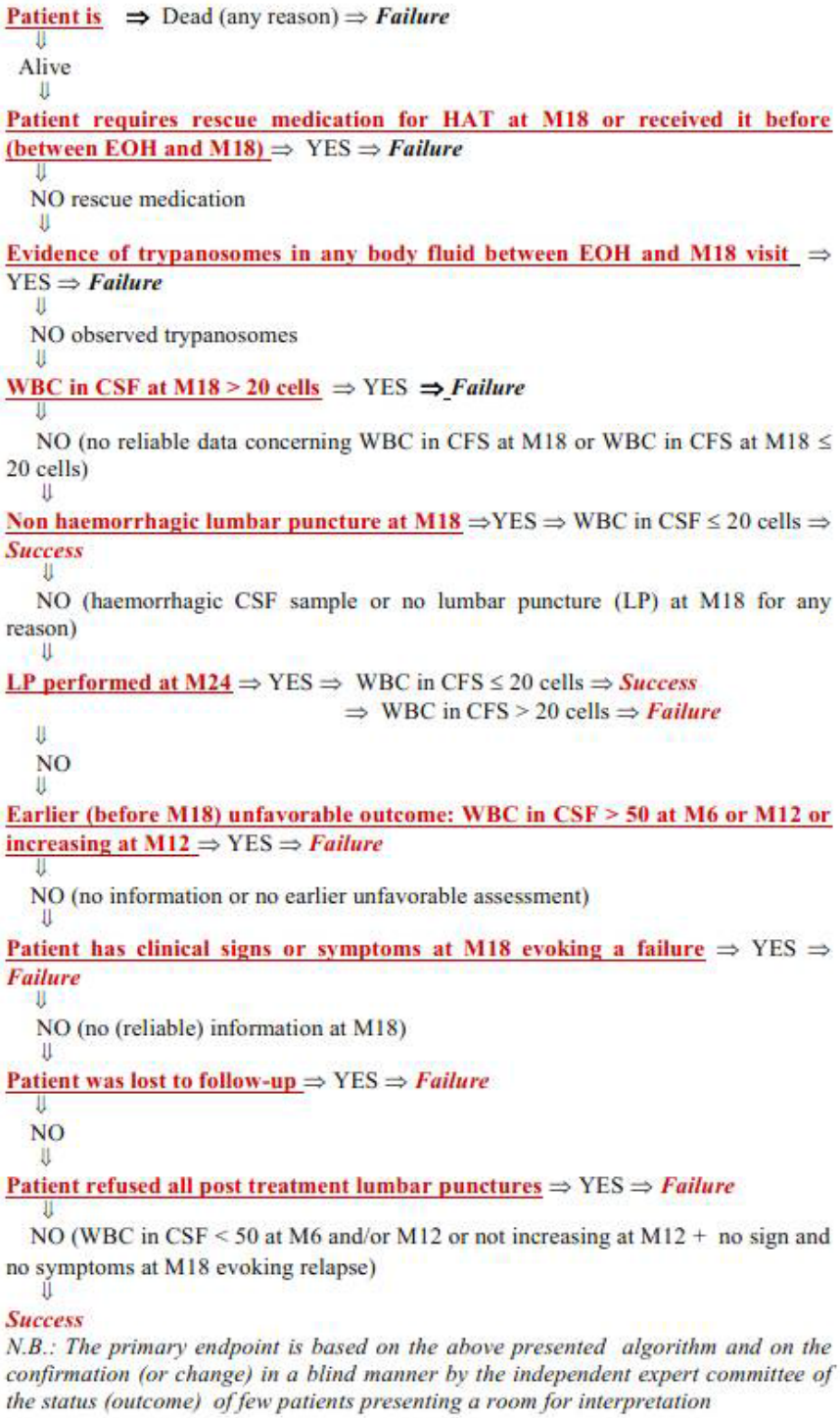
- Death (for any reason)
- Relapse: evidence of trypanosomes in any body fluid
- Probable relapse: no evidence of trypanosomes in any body fluid but CSF WBC >20 cells/µL; no evidence of parasites in the blood or lymph and refusal to undergo lumbar puncture (or CSF sample was hemorrhagic), but required rescue treatment, in the opinion of the Investigator, because of a marked deterioration in his/her clinical status that was unlikely to be related to a disease other than HAT; patient refused lumbar puncture at 18 months and the outcome had been assessed as unfavorable at an earlier time point; or withdrawal from the study due to probable relapse at any time point earlier than 18 months
- Patient lost to follow-up (except patients in an area of armed conflict)

The outcome at 18 months was considered a success in the case of:

- Cure: patient alive, with no evidence of trypanosomes in any body fluid, and CSF WBC ≤20 cells/µL
- Probable cure - for patients who refused a lumbar puncture (or who had a hemorrhagic CSF sample) at 18 months: no parasites in the blood or lymph, and a satisfactory clinical condition without clinical signs or symptoms (or clinical status unlikely to be due to HAT), a WBC count in CSF <50 cells/µL at 6 and/or 12 months, and not increasing at 12 months, as long as there was no indication of a relapse up to 24 months and if no definitive failure (presence of trypanosomes) had been observed before in any body fluid.

Subjects with questionable outcome due to the difficulty in interpreting signs and symptoms in the absence of lumbar puncture at 18 months using this algorithm were adjudicated in a blind manner by three independent experts.

Figure 3. Algorithm of Classification to Categorize Patients as Success or Failure for the Primary Efficacy Endpoint (18 Months)



Source: Figure 4 of Study Report
Abbreviations: CSF, cerebrospinal fluid; EOH, end of hospitalization; HAT, Human African Trypanosomiasis; M, month; WBC, white blood cell

Secondary Efficacy Endpoints

Secondary efficacy endpoints included outcomes (success/failure) of treatment as assessed at Day 11 (24 hours after EOT), 3, 6, 12 months (early efficacy time point), and 24 months (late efficacy time point), after EOT.

For the early efficacy endpoints (≤12 months), the definition of success and failures were different at each time point (with increasingly stricter criteria for success). For the endpoint at 24 months, the definitions of success and failure were the same as for the primary efficacy endpoint if a lumbar puncture was performed or if a definitive failure occurred before.

Analysis Populations

The intent-to-treat (ITT) population comprised all randomized patients who received at least one dose of study treatment. This population was used to perform a sensitivity analysis on the primary efficacy endpoint. It was also used to perform all safety analyses.

The modified intent-to-treat (mITT) population comprised all ITT patients, except those who fled an area of armed conflict and for whom no post-treatment data were available. In order to maintain the planned sample size due to the exclusion of 5 subjects, 4 additional subjects were randomized.

Statistical Analysis Plan

Sample Size Calculation

The initially planned sample size was 510 with a 2:1 randomization ratio (fexinidazole:NECT), with an assumed success rate of 94% for NECT and 89% for fexinidazole, a one-sided Type I error rate of 0.025, a noninferiority margin of -13%, and a statistical power of 87.8%. In 2013, after a few months of enrollment, the Applicant determined that the planned sample size would be difficult to reach, due to slower enrollment than expected. An Expert Committee meeting was held on June 24, 2013 to discuss this issue. The Expert Committee recommended a sample size reduction to 390, using a lower statistical power of 80%, other things being unchanged.

Given this change in sample size in an ongoing open-label trial, we explored the information that was known at the time of the decision. At that time, 96 (i.e., 65 plus 31) patients were randomized and all had a success at EOT, and 25 (i.e., 17 plus 8) subjects reached 6 months of which all had successful outcome at 6 months. No subjects reached 12 months or the primary time point of 18 months at the time of the decision to change the sample size. Therefore, the effect of change in sample size on the integrity of the trial was not a concern.

Primary Efficacy Analysis

The primary efficacy analysis (outcome at 18 months) was performed on the mITT population. Statistical analysis of the primary efficacy endpoint, to assess the difference in response rates between the two treatment groups (fexinidazole minus NECT), was performed by calculating the 2-sided Pocock adjusted CI for the difference. Because of the planned interim analysis

(described below), the calculated critical Z-value for the primary endpoint of the interim analysis and final analysis was 2.178; therefore, the two-sided significance level (alpha) was equal to 0.0294 for both analyses. We conducted an exact test when there were fewer than 5 failures in a treatment group.

The noninferiority margin for the difference in success proportions between the two treatment groups was -13%. If the lower bound of the adjusted CI of the treatment difference (fexinidazole minus NECT) was ≥−13%, the noninferiority of fexinidazole was concluded. We considered the choice of this margin was appropriate as detailed in the noninferiority margin justification below.

Methods for handling missing data are described below.

In Protocol Amendment #4 dated November 17, 2016, the primary analysis population was ITT and the mITT population was not defined yet. In December 2016, the primary analysis changed from an ITT to mITT population for exclusion of subjects due to war conflict. Database lock was performed on January 5, 2017 and unblinding of the study took place one day later.

Early, Primary and Late Endpoints: Time Course of the Failure/Success Proportion

A Kaplan-Meier curve presented the cumulative failure rate over time for the 2 treatment groups. The fexinidazole cumulative failure rate curve was compared to that of NECT (from the current study) using a log-rank test.

Secondary Analysis of the Primary Efficacy Endpoint: Analysis of Site Effect

A secondary analysis of the primary efficacy endpoint was a stratified analysis by site. The Breslow Day test was conducted to test the interaction between treatment and sites.

Additional analyses were to be conducted related to Site 06 (Batangafo, CAR) to assess whether the war conditions impacted the results.

Methods for handling missing data for efficacy endpoint at 18 months

The protocol and statistical analysis plan defined a primary method for handling missing data as well as sensitivity analyses for the efficacy endpoint at 18 months. Imputation methods considered both missing lumbar punctures and patients being lost to follow-up.

The primary imputation method was defined in the protocol as:

- Probable success (considered as a success) if the patient refused the lumbar puncture at 18 months, showed no signs or symptoms of HAT at 18 months, did not report any sign of relapse later, and had a favorable evolution at the last available assessment. Otherwise, the outcome was considered as a failure. The outcome for any patient lost to follow-up at 18 months or before was also imputed as a failure. The algorithm of classification provided the status at 18 months for all patients.

After database lock, changes were made to the primary imputation method to consider experts' adjudication. In the study report, the primary analysis used the primary imputation

method with the adjudication by 3 independent experts. This impacted the results of five patients. This change of the primary analysis method after database lock could be problematic. We will assess the impact of this change by also considering the primary imputation in the ITT population as initially planned.

Three additional methods were defined as follows:

- Second method: Imputing a failure in the absence of a lumbar puncture at 18 months, in addition to imputing subjects who are lost to follow-up as failures.
- Third method: No imputation of missing data. This method was used for the analysis of time course of success/failure (including Kaplan-Meier approach). In the Kaplan-Meier approach, the observation was censored at the first missing outcome provided further assessments were all missing.
- Fourth method: A subject with missing outcome data, either lost to follow-up (but with a CSF WBC count at 6 and/or 12 months) or with missing lumbar puncture at 18 months, had their outcome imputed based on the predicted probability of relapse. The predicted probability of relapses was based on logistic models using CSF WBC count at baseline, 6 and/or 12 months, age and sex, based on the Médecins Sans Frontières (MSF) data of approximately 2000 patients. If the predicted probability of relapse was missing, the 24-month outcome was used, if available, or if the 24-month outcome was missing, the patient was considered a failure.

Interim Analysis

An interim analysis was added in February 9, 2015 (Protocol Amendment 3) at the recommendation of an Expert Committee. If the interim analysis of efficacy of fexinidazole was not inferior to NECT with 195 patients at 12-month follow-up, an early submission for registration would be made, assuming an overwhelming efficacy, i.e., 95% at 12 months in both treatment arms. The same noninferiority margin of -13% and justification of the Type I error were used as in the primary efficacy analysis of the outcome at 18 months. Regardless of the results of the interim analysis, the study would continue to obtain a precise idea of the relapse rate and the rate of intercurrent events in the long term. When the interim analysis report was finalized on May 1, 2015, all subjects were already randomized.

The interim database lock took place on April 21, 2015 with a database cut-off date of January 8, 2015. The interim analysis of efficacy, conducted by an independent statistician not otherwise involved in the study and belonging to another company, showed that noninferiority of fexinidazole to NECT on the primary endpoint (difference in success proportion at 12 months) was not met. The difference in the ITT population was -9.41% (111/129 [86.05%] versus 63/65 [94.45%] with a 97.06% CI [-18.09%, -0.73%]). The study continued as originally planned.

Futility analyses were planned for when specified lower treatment success rates were met. However, futility analyses were not performed because the conditions were not met.

Protocol Amendments

There were 4 amendments. Only Amendment 1, the initial protocol, was introduced before enrollment of patients and the subsequent amendments were introduced before database lock and unblinding of the Sponsor and trialists. In Amendment 3 on February 9, 2015, the sample size was reduced to 390 patients and an interim analysis of efficacy and safety was added to be performed when 195 patients reached 12 months of follow-up.

Compliance with Good Clinical Practices

This study was performed in compliance with ICH (E6) Good Clinical Practices.

Financial Disclosure

One principal investigator received arrangement in order to participate in international congresses and trainings. The Applicant and DNDi did not believe any bias was introduced by these agreements.

Patient Disposition

The study was initiated (first patient enrolled) on October 7, 2012, the 18-month visit (last patient completed) was completed on November 29, 2016, and the 24-month visit was completed on April 26, 2017. A total of 410 patients from 10 sites in two countries were prescreened and all eligible 395 patients, except for one patient who committed suicide before randomization, were included in the study and randomized. All randomized patients received at least one dose and were included in the ITT population. Five patients (2 assigned to fexinidazole and 3 assigned to NECT) were excluded from the mITT population, due to fleeing an area of armed conflict and having no post-treatment data. These subjects were from the only study site located in CAR and were randomized between April and December of 2013. All other 7 ITT subjects from this site were randomized between March and October of the same year. At the time of the primary efficacy analysis at 18 months, 81.5% of subjects had already completed the 24-month visit. In the follow-up study, 91.6% completed the 24-month visit. The majority (97%) of the subjects available at month 18 had lumbar puncture for a CSF laboratory test.

Table 63. Patient Disposition, DNDiFEXI004

Disposition	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
Randomized	264	130	394
ITT/Treated	264	130	394
mITT	262	127	389
Treatment completer	260 (96.5)	127 (97.7)	387 (98.2)
Completing month-18 visit	246 (93.2)	125 (96.2)	371 (94.2)
Premature discontinuation before 18 months	18	5	23
Consent withdrawal	1	0	1
Death	6	2	8
Treatment failure	8	0	8
Lost to follow-up, including fleeing an area of armed conflict and had no data post-treatment	3	3	6

Disposition	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
Completion of month-24 visit at data lock for the 18-month primary analysis	206 (78.0)	112 (86.2)	318 (80.7)
Ongoing at the end of the data lock for the 18-month primary analysis	32 (12.1)	13 (10.0)	45 (11.4)
Premature discontinuation at data lock for the 18-month primary analysis	26 (9.8)	5 (3.8)	31 (7.9)
Follow-up study			
Completion of month-24 visit	237 (89.8)	124 (95.4)	361 (91.6)
Premature discontinuation	27 (10.2)	6 (4.6)	33 (8.4)
Consent withdrawal	1 (0.4)	0	1 (0.3)
Death	9 (3.4)	2 (1.5)	11 (2.8)
Treatment failure (rescue treatment)	12 (4.5)	0	12 (3.0)
Lost to follow-up	5 (1.9)	4 (3.1)	9 (2.3)
Completed visit			
Month 12	246 (93.2)	122 (93.8)	368 (93.4)
Month 18	241 (91.3)	121 (93.1)	362 (91.9)
Month 24	237 (89.8)	124 (95.4)	361 (91.6)
Lumbar puncture			
Month 18			
Yes	233 (88.3)	119 (91.5)	352 (89.3)
No	8 (3.0)	2 (1.5)	10 (2.5)
Month 24			
Yes	231 (87.5)	119 (91.5)	350 (88.8)
No	5 (1.9)	3 (2.3)	8 (2.0)
Missing	1 (0.4)	2 (1.5)	3 (0.8)

Source: Adapted from Figure 6 in Study Report.
Numbers are in n (% of randomized patients)
Abbreviations: ITT, intent-to-treat; mITT, modified intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy

Protocol Violations/Deviations

None of the patients in the fexinidazole group had any major protocol deviations. In the NECT group, one patient (Patient (b) (6)) had a major protocol deviation because of a severely deteriorated general status, which was a pre-exclusion criterion. The patient subsequently died. This patient was included in all the analyses presented in this section.

Demographic Characteristics

Demographic characteristics are included in the table below. The majority of patients were male. The mean age was 34.7 years with a range of 15 to 71. Only 7 patients were ≥65 years. Race information was not collected. The majority of subjects (97%) were from DRC. Height, weight, and body mass index were balanced between the two treatment groups.

Table 64. Demographic Characteristics of the Primary Efficacy Analysis, ITT Population, DNDiFEXI004

Characteristic	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
Sex, n(%)			
Male	161 (61.0)	80 (61.5)	241 (61.2)
Female	103 (39.0)	50 (38.5)	153 (38.8)

Characteristic	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
Age (years)			
Mean (SD)	34.5 (12.6)	35.3 (13.2)	34.8 (12.8)
Median	33.0	33.5	33.0
Range	15, 71	15, 68	15, 71
Age group, n(%)			
< 65 years	259 (98.1)	128 (98.5)	387(98.2)
≥ 65 years	5 (1.9)	2 (1.6)	7 (1.8)
Height (cm)			
Mean (SD)	161.71 (9.31)	161.82 (11.2)	161.74 (9.96)
Median	162	161	162
Range	131, 189.5	131, 196	131, 196
Weight (kg)			
Mean (SD)	50.52 (8.18)	50.74 (9.63)	50.59 (8.68)
Median	50	48.25	50
Range	30, 74	33, 82	30, 82
Body mass index (kg/m^2)			
Mean (SD)	19.22 (2.35)	19.24 (2.42)	19.22 (2.37)
Median	18.9	18.75	18.9
Range	16, 29.4	16, 28.3	16, 29.4
Country, n(%)			
Central African Republic (CAR)	8 (3.0)	4 (3.1)	12 (3.0)
Democratic Republic of the Congo (DRC)	256 (97.0)	126 (96.9)	382 (97.0)

Source: Table 10 in Study Report
Abbreviations: ITT, intent-to-treat; N, number of subjects; n, number of subjects in subgroup; NECT, nifurtimox eflornithine combination therapy; SD, standard deviation

Other Baseline Characteristics

Medical history at screening is shown in the table below. The most commonly reported terms (observed in at least 10% of patients) were hypoalbuminemia, hyperkalemia, hypocalcemia, hyponatremia, and headache. The differences in these terms between the two treatment groups were within 6.5% and were not statistically significant.

Table 65. Medical History at Screening: Preferred Term Reported in at Least 2% of Patients in Either Group, ITT Population, DNDiFEX004

Preferred Term	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
Any medical history	240 (90.9)	112 (86.2)	352 (89.3)
Metabolism and nutrition disorders	184 (69.7)	84 (64.6)	268 (68.0)
Hypoalbuminemia	124 (47.0)	54 (41.5)	178 (45.2)
Hyperkalemia	80 (30.3)	46 (35.4)	126 (32.0)
Hypocalcemia	28 (10.6)	12 (9.2)	40 (10.2)
Hyponatremia	24 (9.1)	16 (12.3)	40 (10.2)
Hypercreatininaemia	5 (1.9)	4 (3.1)	9 (2.3)
Hyperproteinemia	4 (1.5)	5 (3.8)	9 (2.3)
Nervous system disorders	67 (25.4)	34 (26.2)	101 (25.6)
Headache	46 (17.4)	26 (20.0)	72 (18.3)
Tremor	10 (3.8)	3 (2.3)	13 (3.3)
Palmomental reflex	7 (2.7)	2 (1.5)	9 (2.3)

Preferred Term	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
Blood and lymphatic system disorders	61 (23.1)	22 (16.9)	83 (21.1)
Anemia	32 (12.1)	6 (4.6)	38 (9.6)
Leukocytosis	11 (4.2)	8 (6.2)	19 (4.8)
Thrombocytopenia	9 (3.4)	5 (3.8)	14 (3.6)
Splenomegaly	7 (2.7)	1 (0.8)	8 (2.0)
Investigations	45 (17.0)	27 (20.8)	72 (18.3)
Blood alkaline phosphatase decreased	9 (3.4)	7 (5.4)	16 (4.1)
Blood urea decreased	9 (3.4)	5 (3.8)	14 (3.6)
Blood albumin decreased	6 (2.3)	2 (1.5)	8 (2.0)
Aspartate aminotransferase increased	6 (2.3)	1 (0.8)	7 (1.8)
Blood creatinine decreased	2 (0.8)	3 (2.3)	5 (1.3)
Musculoskeletal and connective tissue disorders	33 (12.5)	19 (14.6)	52 (13.2)
Back pain	18 (6.8)	13 (10.0)	31 (7.9)
Neck pain	17 (6.4)	4 (3.1)	21 (5.3)
Gastrointestinal disorders	29 (11.0)	20 (15.4)	49 (12.4)
Gastritis	6 (2.3)	5 (3.8)	11 (2.8)
Abdominal pain	5 (1.9)	4 (3.1)	9 (2.3)
Dyspepsia	2 (0.8)	4 (3.1)	6 (1.5)
General disorders and administration site conditions	27 (10.2)	4 (3.1)	31 (7.9)
Pyrexia	12 (4.5)	1 (0.8)	13 (3.3)
Asthenia	8 (3.0)	1 (0.8)	9 (2.3)
Gastritis	6 (2.3)	5 (3.8)	11 (2.8)
Abdominal pain	5 (1.9)	4 (3.1)	9 (2.3)
Dyspepsia	2 (0.8)	4 (3.1)	6 (1.5)
Infections and infestations	29 (11.0)	16 (12.3)	45 (11.4)
Filariasis	5 (1.9)	3 (2.3)	8 (2.0)
Renal and urinary disorders	14 (5.3)	12 (9.2)	26 (6.6)
Leukocyturia	7 (2.7)	8 (6.2)	15 (3.8)
Hematuria	5 (1.9)	3 (2.3)	8 (2.0)
Proteinuria	4 (1.5)	3 (2.3)	7 (1.8)
Injury, poisoning and procedural complications	17 (6.4)	5 (3.8)	22 (5.6)
Procedural pain	11 (4.2)	5 (3.8)	16 (4.1)
Skin and subcutaneous tissue disorders	12 (4.5)	6 (4.6)	18 (4.6)
Pruritus	9 (3.4)	1 (0.8)	10 (2.5)
Pityriasis	2 (0.8)	4 (3.1)	6 (1.5)
Vascular disorders	8 (3.0)	9 (6.9)	17 (4.3)
Hypertension	4 (1.5)	6 (4.6)	10 (2.5)
Hypotension	3 (1.1)	3 (2.3)	6 (1.5)
Psychiatric disorders	11 (4.2)	4 (3.1)	15 (3.8)
Abnormal behavior	4 (1.5)	3 (2.3)	7 (1.8)
Social circumstances	9 (3.4)	5 (3.8)	14 (3.6)
Menopause	9 (3.4)	5 (3.8)	14 (3.6)
Surgical and medical procedures	11 (4.2)	3 (2.3)	14 (3.6)
Appendicectomy	6 (2.3)	2 (1.5)	8 (2.0)
Respiratory, thoracic and mediastinal disorders	9 (3.4)	3 (2.3)	12 (3.0)
Cough	6 (2.3)	2 (1.5)	8 (2.0)
Reproductive system and breast disorders	3 (1.1)	3 (2.3)	6 (1.5)

Source: Table 11 in Study Report
Numbers are in n(%)
Abbreviations: ITT, intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy

History of clinical signs and symptoms of HAT at inclusion in the ITT population are displayed in the table below. More than 50% of patients reported headache, pruritus, sleepiness, weight loss, and asthenia, which did not differ between the two treatment groups (less in 3% in prevalence). The signs and symptoms less frequently observed in the fexinidazole group than in the NECT group were amenorrhea, insomnia, shaking, and other signs/symptoms. The signs and symptoms more frequently observed in the fexinidazole group than in the NECT group were language disorder and pruritus.

Table 66. History of Clinical Signs and Symptoms of HAT at Inclusion, ITT Population, DNDiFEX004

Signs/Symptoms	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
Headache			
Yes	188 (71.2)	93 (71.5)	281 (71.3)
No	76 (28.8)	37 (28.5)	113 (28.7)
Sleepiness			
Yes	146 (55.3)	72 (55.4)	218 (55.3)
No	118 (44.7)	58 (44.6)	176 (44.7)
Impotence (men only)	161	80	241
Yes	52 (32.3)	27 (33.8)	79 (32.8)
No	107 (66.5)	53 (66.3)	160 (66.4)
Not done	2 (1.2)	0	2 (0.8)
Amenorrhea (women only)	103	50	153
Yes	38 (36.9)	22 (44.0)	60 (39.2)
No	65 (63.1)	28 (56.0)	93 (60.8)
Convulsion			
Yes	6 (2.3)	4 (3.1)	10 (2.5)
No	258 (97.7)	126 (96.9)	384 (97.5)
Fever			
Yes	86 (32.6)	44 (33.8)	130 (33.0)
No	178 (67.4)	86 (66.2)	264 (67.0)
Insomnia			
Yes	92 (34.8)	60 (46.2)	152 (38.6)
No	172 (65.2)	70 (53.8)	242 (61.4)
Behavioral disorder			
Yes	38 (14.4)	22 (16.9)	60 (15.2)
No	226 (85.6)	108 (83.1)	334 (84.8)
Language disorder			
Yes	33 (12.5)	12 (9.2)	45 (11.4)
No	230 (87.1)	118 (90.8)	348 (88.3)
Not done	1 (0.4)	0	1 (0.3)
Pruritus			
Yes	155 (58.7)	71 (54.6)	226 (57.4)
No	109 (41.3)	59 (45.4)	168 (42.6)
Shaking			
Yes	87 (33.0)	49 (37.7)	136 (34.5)
No	177 (67.0)	81 (62.3)	258 (65.5)
Walk disorder			
Yes	51 (20)	26 (20)	77 (19.5)
No	213 (80)	104 (80)	317 (80.5)
Anorexia			
Yes	51 (19.3)	24 (18.5)	75 (19.0)
No	213 (80.7)	106 (81.5)	319 (81.0)

Signs/Symptoms	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
Nausea			
Yes	24 (9.1)	12 (9.2)	36 (9.1)
No	240 (90.9)	118 (90.8)	358 (90.9)
Diarrhea			
Yes	7 (1.5)	2 (1.5)	9 (2.3)
No	257 (98.5)	128 (98.5)	385 (97.7)
Weight loss			
Yes	146 (55.3)	71 (54.6)	217 (55.1)
No	118 (44.7)	59 (45.4)	177 (44.9)
Asthenia			
Yes	145 (54.9)	71 (54.6)	216 (54.8)
No	119 (45.1)	59 (45.4)	178 (45.2)
Other			
Yes	77 (29.2)	43 (33.1)	120 (30.5)
Not reported	187 (70.8)	87 (66.9)	274 (69.5)

Source: Table 12 in Study Report.
Note: Numbers are in n(%).
Abbreviations: HAT, Human African Trypanosomiasis; ITT, intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy

Laboratory findings are summarized in the table below. All patients, except for four patients in the fexinidazole group, were positive in CATT, a serological test. These 4 patients met the criteria for late stage-2 g-HAT by either presenting with trypanosomes in CSF or having CSF WBC count >20 cells/µL. Trypanosomes were detected in the lymph node in 36% of patients and in the blood in 13.5% to 25.2% of patients depending on the methods used (capillary tube centrifugation (CTC), mini-anion exchange centrifugation technique (mAECT), or mini-anion exchange centrifugation technique – buffy coat (mAECT-BT)). 12 subjects (3.0%) had CSF WBC ≤ 20 cells/µL at baseline, a positive card agglutination test for trypanosomiasis (CATT) result and presence of trypanosomes in CSF (slightly higher proportion occurring in the NECT arm). Importantly, similar percentages of patients in both arms (66.2 to 69.3%) had trypanosomes detected in the CSF. In terms of severity of disease, the mean and median CSF WBC at baseline were similar in both arms and > 100 WBC cells/µL.

Table 67. Laboratory Findings Regarding the Diagnosis and Staging of HAT, ITT Population, DNDiFEX004

Laboratory Findings	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
CATT, n(%)			
Positive	260 (98.5)	130 (100.0)	389 (99.0)
Negative	3 (1.1)	0 (0.0)	3 (0.8)
Not done	1 (0.4)	0 (0.0)	1 (0.3)
CATT dilution, n(%)			
1/4	22 (8.4)	10 (7.7)	32 (8.1)
1/8	6 (2.3)	8 (6.2)	14 (3.6)
1/16	90 (34.2)	44 (33.8)	134 (34.1)
Not done	145 (55.1)	68 (52.3)	213 (54.2)
Missing	1 (0.4)	0	1 (0.3)

Laboratory Findings	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
Lymph node sampling, n(%)			
Positive	100 (37.9)	41 (31.5)	141 (35.8)
Negative	28 (10.6)	18 (13.8)	46 (11.7)
Not done	136 (51.7)	71 (54.6)	207 (52.7)
CTC, n(%)			
Positive	68 (25.9)	31 (23.8)	99 (25.1)
Negative	93 (35.4)	56 (43.1)	149 (37.8)
Not done	103 (39.0)	43 (33.1)	146 (37.1)
mAECT, n(%)			
Positive	55 (20.8)	25 (19.2)	80 (20.3)
Negative	17 (6.4)	10 (7.7)	27 (6.9)
Not done	192 (72.7)	95 (73.1)	287 (72.8)
mAECT-BC, n(%)			
Positive	29 (11.0)	24 (18.5)	53 (13.5)
Negative	12 (4.6)	10 (7.7)	22 (5.6)
Not done	223 (84.5)	96 (73.8)	319 (81.0)
Trypanosomes/CSF, n(%)			
Positive	175 (66.3)	90 (69.2)	265 (67.3)
Negative	88 (33.3)	40 (30.8)	128 (32.5)
Not done	1 (0.4)	0 (0.0)	1 (0.3)
WBC/mm^3/CSF			
Mean (SD)	378.04 (670.56)	317.07 (427.62)	357.92 (601.46)
Median	164.5	144.0	157.0
Range	4, 7545	6, 2890	4, 7545
≤20, n(%)	4 (1.5)	8 (6.2)	12 (3.0)

Source: Adapted from Table 13 in Study Report
Abbreviations: CATT, card agglutination test for trypanosomiasis; CSF, cerebrospinal fluid; CTC, capillary tube centrifugation; HAT, human African Trypanosomiasis; ITT, intent-to-treat; mAECT, mini-anion exchange centrifugation technique; mAECT-BC = mini-anion exchange centrifugation technique – buffy coat; N, number of subjects; n, number of subjects in subgroup; SD, standard deviation; WBC = white blood cell; CTC =capillary tube centrifugation

Vital signs, Karnofsky Index score, and general health status were balanced between the two treatment groups and the majority of subjects had a baseline Karnofsky Index score ≥70%.

Use of prior medications among more than 2% of subjects is shown in the table below. Every subject took a prior medication. As directed under the protocol, almost all subjects took a benzimidazole derivative. Similarly, paracetamol, artemether, lumefantrine were other commonly reported medications in more than 22% of subjects. In general, use of prior medications was comparable between the two treatment groups.

Table 68. Prior Medications in at Least 2% of Patients, ITT Population, DNDiFEX004

Medications	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
Any previous medication	264 (100.0)	130 (100.0)	394 (100.0)
Benzimidazole derivatives	262 (99.2)	130 (100.0)	392 (99.5)
Albendazole	137 (51.9)	68 (52.3)	205 (52.0)
Mebendazole	125 (47.3)	62 (47.7)	187 (47.5)
Anilides	94 (35.6)	48 (36.9)	142 (36.0)
Paracetamol	94 (35.6)	48 (36.9)	142 (36.0)
Artemisinin and derivatives, combinations	58 (22.0)	32 (24.6)	90 (22.8)
Artemether, lumefantrine	58 (22.0)	32 (24.6)	90 (22.8)

Medications	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
Phenothiazines with aliphatic side-chain	7 (2.7)	3 (2.3)	10 (2.5)
Chlorpromazine	7 (2.7)	3 (2.3)	10 (2.5)
Aluminium compounds	4 (1.5)	5 (3.8)	9 (2.3)
Aluminium hydroxide	4 (1.5)	5 (3.8)	9 (2.3)

Source: Table 15.1.17.a in Study Report.
Numbers are in n(%)
Abbreviations: ITT, intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy

Treatment Compliance, Concomitant Medications, and Rescue Medication Use

In the ITT population, only two patients did not fully complete treatment with fexinidazole due to death, and all patients with NECT fully completed treatment. The most frequently used concomitant medications included paracetamol, diazepam, chlorpromazine, and metoclopramide, which were taken by a slightly larger percentage of subjects in the fexinidazole than in the NECT group.

Table 69. Concomitant Medications in at Least 2% of Patients, ITT Population, DNDiFEX004

Medications	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
Any concomitant medication	150 (56.8)	67 (51.5)	217 (55.1)
Anilides	98 (37.1)	42 (32.3)	140 (35.5)
Paracetamol	98 (37.1)	42 (32.3)	140 (35.5)
Benzodiazepine derivatives	37 (14.0)	11 (8.5)	48 (12.2)
Diazepam	36 (13.6)	11 (8.5)	47 (11.9)
Midazolam	1 (0.4)	0	1 (0.3)
Phenothiazines with aliphatic side-chain	23 (8.7)	7 (5.4)	30 (7.6)
Chlorpromazine	23 (8.7)	7 (5.4)	30 (7.6)
Propulsives	18 (6.8)	4 (3.1)	22 (5.6)
Metoclopramide	17 (6.4)	4 (3.1)	21 (5.3)
Metoclopramide hydrochloride	1 (0.4)	0	1 (0.3)
Aluminium compounds	13 (4.9)	4 (3.1)	17 (4.3)
Aluminium hydroxide	12 (4.5)	4 (3.1)	16 (4.1)
Aluminium oxide	1 (0.4)	0	1 (0.3)
Aluminium phosphate	0	1 (0.8)	1 (0.3)
Penicillins with extended spectrum	12 (4.5)	5 (3.8)	17 (4.3)
Amoxicillin	10 (3.8)	5 (3.8)	15 (3.8)
Ampicillin	4 (1.5)	0	4 (1.0)
Acetic acid derivatives and related substances	8 (3.0)	5 (3.8)	13 (3.3)
Diclofenac	7 (2.7)	3 (2.3)	10 (2.5)
Paracetamol, diclofenac	2 (0.8)	1 (0.8)	3 (0.8)
Indometacin	0	1 (0.8)	1 (0.3)
Solutions for parenteral nutrition	10 (3.8)	2 (1.5)	12 (3.0)
Glucose	10 (3.8)	2 (1.5)	12 (3.0)
Ascorbic acid (vitamin), plain	8 (3.0)	2 (1.5)	10 (2.5)
Ascorbic acid	8 (3.0)	2 (1.5)	10 (2.5)
Propionic acid derivatives	8 (3.0)	2 (1.5)	10 (2.5)
Ibuprofen	8 (3.0)	2 (1.5)	10 (2.5)
Salt solutions	7 (2.7)	3 (2.3)	10 (2.5)
Sodium chloride	7 (2.7)	3 (2.3)	10 (2.5)

Medications	Fexinidazole (N=264)	NECT (N=130)	Total (N=394)
Solutions affecting the electrolyte	5 (1.9)	5 (3.8)	10 (2.5)
Sodium lactate, potassium chloride, calcium chloride	5 (1.9)	5 (3.8)	10 (2.5)
Glucose, sodium chloride	1 (0.4)	1 (0.4)	1 (0.8)
Third-generation cephalosporins	9 (3.4)	1 (0.8)	10 (2.5)
Ceftriaxone	9 (3.4)	1 (0.8)	10 (2.5)
Cefotaxime	1 (0.4)	0	1 (0.3)
Pyrazolones	4 (1.5)	5 (3.8)	9 (2.3)
Metamizole sodium	4 (1.5)	3 (2.3)	7 (1.8)
Caffeine, propyphenazone	0	1 (0.8)	1 (0.3)
Noramidopyrine	0	1 (0.8)	1 (0.3)

Source: Table 15.3.10.a in Study Report
Numbers are in n(%)
Abbreviations: ITT, intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy

Rescue treatment was NECT for patients treated with fexinidazole and prespecified extended NECT for patients treated with NECT. For the 12 patients in the fexinidazole group with treatment failure, rescue treatment was initiated at the 6-month visit (n=1), between 6 and 12 months (n=2), at the 12-month visit (n=2), between 12 and 18 months (n=3), at the 18-month visit (n=3), or after 18 months (n=1). None in the NECT group received rescue treatment due to treatment failure.

Efficacy Results – Primary Endpoint

The Applicant's primary efficacy analysis was conducted in the mITT population. The Applicant also conducted a sensitivity analysis excluding all subjects from Site 06. The results are shown in the table below. In this analysis, two subjects (in the fexinidazole group) were considered as failures, due to loss to follow-up and consent withdrawal prior to 18 months. The lower bounds of the CIs were greater than -13%, demonstrating that fexinidazole was noninferior to NECT. However, despite fexinidazole being statistically noninferior to NECT using a -13% noninferiority margin, it was also found to be significantly less effective than NECT (i.e., the upper bound of the CI excluded 0).

Table 70. Primary Efficacy Endpoint Analysis: Success Proportion at 18 Months Using the Primary Imputation Method and Experts' Adjudication, mITT Population, DNDiFEX004

Analysis	Fexinidazole (N=264)	NECT (N=127)	Difference [97.06% CI]
MITT analysis (Applicant's primary)	239/262 (91.2%)	124/127 (97.6%)	-6.4% [-11.2%, -1.6%]*
MITT analysis excluding all of site 06	238/256 (92.0%)	123/126 (97.6%)	-4.7% [-9.2%, -0.1%]

Source: Tables 20 and Table 31.
*The exact CI was [-11.6%, -0.1%] for the primary analysis.
Abbreviations: CI, confidence interval; mITT, modified intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy

The ITT population was used by the Applicant as a secondary analysis for the primary efficacy endpoint, which included five patients from Site 06 who fled an area of armed conflict and had no post-treatment data analysis (two patients in the fexinidazole group and three patients in the NECT group). These five patients were considered as failures in this analysis. The lower

limits of the CIs for the difference in success proportion between treatments met the noninferiority margin of -13%. The reasons for failures in the fexinidazole were treatment failure, loss to follow-up, consent withdrawal, and death.

Table 71. Primary Efficacy Endpoint Analysis- Success Proportion at 18 Months, ITT Population, DNDiFEX004

Outcome	Fexinidazole (N=264)	NECT (N=130)	Difference [97.06% CI]
Success at 18 months*	239 (90.5%)	124 (95.4%)	-4.9% [-10.5%, 0.8%]
Failure at 18 months	25	6	
Rescue 6 to <12 months	3	0	
Rescue at 12 months	4	0	
Rescue >12 to <18 months	1	0	
Rescue at 18 months	4	0	
WBC in CSF >20 cells/µL	2	0	
Trypanosomes	1	0	
Loss to follow-up	3	3	
Consent withdrawal	1	0	
No post-trt lumbar puncture	0	1	
Death	6	2	

Source: Reviewer's analysis based on Table 24 in Study Report
*ITT analysis: Tables 22 and 23 in Study Report.
Abbreviations: CI, confidence interval; CSF, cerebral spinal fluid; ITT, intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy; trt, treatment; WBC, white blood cell

Based on the update of the study with complete 24-month follow-up data, the results at 18 months were the same as those in the primary efficacy analysis. The results at 24 months are reported in the table below for both the ITT and mITT populations. Results at 24 months were similar to those at 18 months.

Regarding the differences between the 18-month to 24-month results in the fexinidazole group, 7 successes became failures at 24 months (3 deaths, 2 WBC in CSF >20 cells/µL [probable relapses], 2 lost to follow-up) and 3 failures became successes (two subjects with WBC in CSF >20 to <20 cells/µL, and one subject with no trypanosomes). So, at 24 months there were 4 more failures in the fexinidazole group. There were no changes in the NECT group. Table 72 reports the results for both 12 and 24 months.

Table 72. Success Proportions at 12 and 24 Months, DNDiFEX004

	Fexinidazole		NECT		Difference	
Treatment	mITT (N=262)	ITT (N=264)	mITT (N=127)	ITT (N=130)	mITT	ITT
12-month						[95% CI]
Success	239 (91.2%)	239 (90.5%)	125 (98.4%)	125 (96.2%)	-7.2% [-11.6%, -1.%]	-5.6% [-10.5%, 0.%]
24-month						[97.06% CI]
Success	235 (89.7%)	235 (89.0%)	124 (97.6%)	124 (95.4%)	-7.9% [-13.3, -1.1%]	-6.4% [-12.2%, 0.%

Source: Reviewer's analysis and Table 36 in Study Report and Table 9 in Follow-Up Study Report
Note: If a cell was less than 5, an exact method was used to calculate a CI
Abbreviations: CI, confidence interval; ITT, intent-to-treat; mITT, modified intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy

Data Quality and Integrity

The review team did not audit the case report forms or clinical data. Analysis of treatment effects by study site did not reveal any heterogeneity. No significant financial arrangements or interests with study site investigators were identified.

Efficacy Results – Secondary and Other Relevant Endpoints

Mortality Analysis

There were 6 and 2 deaths in the fexinidazole and NECT groups by Month 18, respectively. There were 3 more deaths in the fexinidazole group between months 18 and 24. The timing and reasons of deaths are shown in Table 73. At Month 24, the difference was 1.9%, with a 95% CI [-2.4%, 5.2%], although not statistically significant. For more details of deaths, please see the safety review section.

Table 73. Timing of and Reasons for Deaths, ITT Population, DNDiFEX004

Time	Fexinidazole (N=264)	NECT (N=130)
Before EOH	2 (poisoning; pneumonia aspiration)	0
EOH to 3 months	3 (poisoning; pneumonia & influenza; unknown)	1 (hypoglycemia)
6 to 12 months	1 (starvation & hypoglycemia)	1 (cardio-respiratory arrest)
18 to 24 months	3 (amelobastoma; alcohol poisoning; Inguinal hernia strangulated)	0
Total	9 (3.4%)	2 (1.5%)
Difference [exact 95% CI]		1.9% [-2.4%, 5.2%]

Source: Reviewer's analysis
All of these subjects, except for the two early deaths, were considered treatment successes prior to deaths
Abbreviations: CI, confidence interval; EOH, end of hospitalization; ITT, intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy

Analysis of Site Effect, With or Without Site 06

As mentioned above, this study included 10 sites. Table 74 contains the results by site. Site 06 was the only site in CAR, and some subjects from this site were excluded from the mITT population due to fleeing an area of armed conflict. Analyses were conducted with and without this site. With Site 06, in the ITT population, Breslow-Day test for homogeneity of the odds ratios across sites provided a p-value of 0.21, indicating there was not strong enough evidence to reject the homogeneity. A Cochran-Mantel-Haenszel common risk difference was -4.9% with a 97.06% CI of [-9.7%, -0.2%], p-value=0.0487. Without Site 06, Breslow-Day test for homogeneity test was not significant either and the common risk difference results were similar, with a common risk difference of -4.7% with a 97.06% CI of [-9.3%, -0.1%], p-value=0.0573. With or without this site, based on the CIs, fexinidazole was significantly worse than NECT, although the noninferiority of fexinidazole to NECT was confirmed. The Applicant conducted the analyses using the mITT population and obtained similar results.

Table 74. Success Proportion at 18 Months by Study Site, ITT Population, DNDiFEX004

Site	Fexinidazole (N=264)	NECT (N=130)
01	32/34 (94.1%)	17/17 (100%)
02	38/44 (86.4%)	21/22 (95.5%)
03	53/60 (88.3%)	29/30 (96.7%)
04	38/38 (100%)	18/18 (100%)
05	20/21 (92.5%)	11/11 (100)
06*	1/8 (12.5%)	1/4 (25%)
07	15/15 (100%)	5/6 (83.3%)
08	14/14 (100%)	7/7 (100%)
09	22/24 (91.7%)	12/12 (100%)
10	6/6 (100%)	3/3 (100%)

Source: Reviewer's analysis and Table 30 in Study Report
* 2 fexinidazole subjects and 3 NECT subjects were considered as failures due to lack of follow-up information. These five subjects are excluded from the mITT population due to fleeing an area of conflict.
Abbreviations: ITT, intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy

Impact of Missing Values

There are two types of missing data with this trial, missing lumbar puncture and losses to follow-up. In the primary efficacy analysis in the ITT population, 241 (91.2%) and 121 (93.1%) subjects in the two groups had an 18-month visit. There were 10 (8 plus 2) subjects who had the 18-month visit, with no lumbar puncture at 18 months. These were imputed as successes, based on data from Month 24 and/or based on adjudication by the experts.

The Applicant conducted sensitivity analyses using different imputation methods in the mITT population. The reviewer conducted similar analyses in the ITT population as shown in the following Table 75, with similar results. The results of these analyses were consistent with the primary analysis.

Table 75. Secondary Endpoints and Sensitivity Analysis at 18 Months

Method	Fexinidazole (N=264)	NECT (N=130)	Difference [97.06% CI]
Primary imputation (mITT)	237/262 (90.5%)	124/127 (97.6%)	-7.2% [-12.4%, -0.6%]
Primary imputation (ITT)	237/264 (89.8%)	124/130 (95.4%)	-5.6% [-11.4%, 1.5%]
Second method (ITT)	226/264 (85.6%)	119/130 (91.5%)	-5.9% [-12.9%, 2.3%]
Fourth method (ITT)	236/264 (89.4%)	124/130 (95.4%)	-6.0% [-11.8%, 0.9%]
Fourth method with outcome at 24 months (ITT)	238/264 (90.2%)	125/130 (96.2%)	-6% [-11.6%, -0.9%]

Source: Adapted from Table 41 in Study Report. The ITT sensitivity analyses were performed by the reviewer.
Note: If a cell was less than 5, an exact method was used to calculate a CI
Abbreviations: CI, confidence interval; ITT, intent-to-treat; mITT, modified intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy

A third method was conducted using Kaplan-Meier estimation in the mITT population where there was no imputation of missing data. Failure-free rates at 18 months were estimated to be 93.1% for fexinidazole and 98.4% for NECT. A log-rank test for the time to failure in days in the mITT population indicated that there was a statistically significant difference between the two treatment groups (p=0.008).

Additional Analyses Conducted on the Individual Trial

Subgroup Analyses by Age, Sex, and Disease Characteristics

Subgroup analysis results are shown in Table 76. Among those younger than 45 years of age, the proportion of success in the fexinidazole group was numerically lower than in the NECT group, while in the older age group they were similar between the two treatment groups. The treatment effects were similar between males and females. As stated earlier, information on race was not collected.

Outcome was also assessed by baseline characteristics related to disease severity, WBC in CSF, fever, shaking, headache, and Karnofsky score. With WBC in CSF ≤100 cells/µL at baseline, a higher proportion of success was observed in the fexinidazole group than in the NECT group; while in the group with higher WBC counts, the opposite result was observed. Treatment effects did not appear to be affected by fever at baseline. For patients with shaking, headache, or a lower Karnofsky score (poorer performance status), the differences in success proportions between the two treatment groups were greater (and favored NECT) compared to patients without shaking, headache, or higher Karnofsky scores. Subjects with more severe disease at baseline appeared to be doing worse on fexinidazole compared to NECT, with the variable WBC in CSF showing the clearest relationship.

Note that the effect seen with age appeared to be related to disease severity and not age. There was a higher percentage of subjects in those < 45 years of age who had WBC in CSF > 100 cells/µL (68%) compared to in those ≥ 45 years of age (40%). When looking at success proportions at 18 months by both age and WBC in CSF, the lower proportion of success with fexinidazole compared to NECT was seen in subjects with WBC in CSF > 100 cells/µL regardless of age category. In subjects with WBC in CSF ≤ 100 cells/µL, fexinidazole's effect was similar to or numerically higher than that of NECT, also, regardless of age.

Table 76. Subgroup Analysis of the Success Proportions at 18 Months, ITT Population, DNDiFEX004

Characteristics	Fexinidazole (N=264)	NECT (N=130)
Age (years)		
<45	184/205 (89.8%)	92/96 (95.8%)
≥45	55/59 (93.2%)	32/34 (94.1%)
Sex		
Male	145/161 (90.1%)	76/80 (95.0%)
Female	94/103 (91.3%)	48/50 (98.0%)
WBC in CSF (cells/µL)		
≤100	100/103 (97.1%)	47/50 (94.0%)
>100	139/161 (86.3%)	77/80 (96.3%)
Age and WBC in CSF		
Age <45, WBC ≤100	62/65 (95.4%)	31/32 (96.9%)
Age <45, WBC >100	122/140 (87.1%)	61/64 (95.3%)
Age ≥45, WBC ≤100	38/38 (100%)	16/18 (88.9%)
Age ≥45, WBC >100	17/21 (81.0%)	16/16 (100%)
Fever		
Yes	77/86 (89.5%)	42/44 (95.5%)
No	162/178 (91.0%)	82/86 (95.4%)

Characteristics	Fexinidazole (N=264)	NECT (N=130)
Shaking		
Yes	75/87 (86.2%)	47/49 (95.9%)
No	164/177 (92.7%)	77/81 (95.1%)
Headache		
Yes	167/188 (89.8%)	88/93 (94.6%)
No	72/76 (94.7%)	36/37 (97.3%)
Karnofsky		
60-80%	159/178 (88%)	92/95 (96%)
90-100%	80/84 (95%)	32/32 (100%)

Source: Reviewer's analysis
Abbreviations: CSF, cerebral spinal fluid; ITT, intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy; WBC, white blood cell

As noted in Table 76 above, along with the reduced efficacy in the overall population, there was an increase in deaths in the fexinidazole arm compared to NECT. Table 77 shows the results of deaths by baseline WBC in CSF. As can be seen from the table, though the number of deaths was smaller, the relationship was similar with what was seen with treatment success, with a smaller difference (fexinidazole – NECT) seen in subjects with lower WBC in CSF at baseline and a larger difference in subjects with higher WBC in CSF at baseline.

Table 77. Subgroup Analysis of Deaths by 24 Months by Baseline WBC in CSF, ITT Population, DNDiFEX004

WBC in CSF (cells/µL)	Fexinidazole (N=264)	NECT (N=130)	Difference Exact 95% CI
≤100	2/103 (1.9%)	2/50 (4.0%)	-2.1% [-12.0%, 4.1%]
>100	7/161 (4.3%)	0/80 (0%)	4.3% [-1.0%, 8.8%]

Source: Reviewer's analysis
Abbreviations: CI, confidence interval; CSF, cerebral spinal fluid; ITT, intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy; WBC, white blood cell

These results were similar in the mITT population.

Table 78. Subgroup Analysis of the Death by 24 Months and Success Proportions at 18 Months, mITT Population, DNDiFEX004

Outcome	WBC in CSF (cells/µL)	Fexinidazole (N=262)	NECT (N=127)	Difference Exact 95% CI
Success	≤100	100/102 (98.0%)	47/49 (95.9%)	2.1% [-4.1%, 12.3%]
	>100	139/160 (86.9%)	77/78 (98.7%)	-11.8% [-18.3%, -2.1%]
Death	≤100	2/102 (2.0%)	2/49 (4.1%)	-2.1% [-12.3%, 4.1%]
	>100	7/160 (4.4%)	0/78 (0%)	4.4% [-0.9%, 8.9%]

Source: Reviewer's analysis
Abbreviations: CI, confidence interval; CSF, cerebral spinal fluid; mITT, modified intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy; WBC, white blood cell

Justification of the Noninferiority Margin

In order to interpret the noninferiority trial, a conservative estimate of the effect of NECT compared to no treatment is needed. This information, obtained from outside the current

noninferiority trial, is used to justify the noninferiority margin. Our noninferiority margin justification relies on one trial of NECT compared to a less effective treatment regimen (Priotto et al. 2006), as placebo-controlled trials were not available. Other trials of NECT used an effective control, eflornithine, which are not helpful in determining the noninferiority margin for the current noninferiority trial.

In the trial by Priotto et al. 2006, the study design, study population and cure were defined similarly as in the current trial DNDiFEX004, except for the timing of the primary efficacy endpoint (18 months for DNDiFEX004 and 24 months for Priotto et al. 2006) and the imputation method for the primary efficacy endpoint. However, in the current NDA, month-24 results were also assessed and there was a second imputation method which considers the absence of lumbar puncture at 18 months as a failure, which was similar to the study by Priotto et al. 2006.

The trial by Priotto et al. 2006, was a randomized, open-label clinical trial in Uganda that assessed three treatments for late-stage g-HAT in patients 5 to 62 years old. A total of 54 patients with confirmed second-stage *T. b. gambiense* infection with trypanosomes detected in CSF with any CSF leukocyte count, or trypanosomes detected in blood or lymph node fluid with more than five leukocytes per microliter in CSF were randomized to either melarsoprol and nifurtimox (M plus N), melarsoprol and eflornithine (M plus E) or NECT (same dose and duration as in DNDiFEX004). Enrollment stopped with fewer than 20 patients per arm. The planned sample size was to be 145 per arm, but enrollment was suspended due to unacceptable toxicity in one of the 3 arms (high fatality in the M plus N arm). The primary outcome was the cure rate. The following were regarded as therapeutic failures: (1) deaths in temporal relation to treatment (within 30 days of treatment start) and (2) relapses of HAT or death compatible with HAT within the 24 months of follow-up. All deaths due to disease without clearly established alternative causality were regarded as compatible with HAT. The results indicated in Table 79 were obtained.

Table 79. Cure Rates at 24 Months From Priotto et al. 2006

Cure Rates	M plus N N=18	M plus E N=19	NECT N=17
24-month success	8/18 (44.4%)	13/19 (68.4%)	16/17 (94.1%)
95% CI vs. NECT	(18.7%, 74.1%)	(-2.4%, 51.5%)	

Source: Table 12 in the article. CIs are from reviewer's analysis
Abbreviations: M plus E, melarsoprol and eflornithine; M plus N, melarsoprol and nifurtimox; N, number of subjects; NECT, nifurtimox eflornithine combination therapy; CI, confidence interval

The data comparing NECT to M plus N could support a conservative estimate of the treatment effect (referred to as M1) of 18.7% at 24 months. We note the possible bias due to the trial stopping early due to increased mortality on the M plus N arm. However, we also note the large treatment effect and the large variability due to the small sample size. We assume that the failure of M plus N is due to lack of treatment effect as opposed to toxicity of the treatment (six deaths, 2 relapses, 1 changed treatment, 1 partial follow-up). Assuming M plus N is more effective than no treatment, 18.7% would be a conservative estimate of the treatment effect of NECT.

To determine if this margin is suitable for 18 months, the primary endpoint in DNDiFEX004, we compared 18-month results with 24-month results from previous trials. As no clinical trials of

NECT, other than the current NI trial, DNDiFEX004, had an outcome at 18 months, we considered other treatment as well.

First, we will investigate the treatment effect change over time for NECT, eflornithine, and melarsoprol, the three commonly used drugs for Stage 2. The table below shows a summary of historical efficacy data of Stage 2 treatment from the Applicant.

Table 80. Historical Efficacy Data of Stage-2 Treatments

Therapy Name	Patients Treated[c]	Failure-Free Rates[a] Based on Kaplan-Meier Estimates [95% CI] Based on Log-Log Transformation 6 Months	12 Months	18 Months	24 Months
NECT[b]	756	96.8% [95.3%, 98.0%]	95.5% [93.6%, 96.9%]	94.7% [92.9%, 96.2%]	NA
Eflornithine	1337	93.70% [92.0%, 95.4%]	90.37% [88.3%, 92.4%]	89.17% [87.0%, 91.3%]	86.76% [84.4%, 89.2%]
Melarsoprol	7505	81.42% [80.2%, 82.6%]	75.54% [74.2%, 76.9%]	74.41% [73.0%, 75.8%]	72.37% [71.0%, 73.8%]

Source: Adopted from Table 27 from Summary of Clinical Efficacy and updated based on the submission of September 14, 2020.
[a] The "failure-free rate" is not exactly equivalent to the "success rate" as defined by the algorithm (and adjudication committee in DNDiEX004). The failure-free rate considers patients with confirmed failure. A patient lost to follow-up is not a confirmed failure (he/she would be censored in the Kaplan-Meier analysis) and is not considered as a failure by the algorithm that focuses on the confirmed success. A patient lost to follow- up is not a confirmed success either. The most recent estimates are provided in this table.
[b] Success rates are reported for the 2 NECT studies (Valverde et al. 2013, Priotto et al. 2009) that did not provide 24-month outcome. Eflornithine and melarsoprol's data were from (Hümbelin 2006)
[c] Except in the pivotal NECT study (N = 143 aged ≥15 years), studies enrolled pediatric and adult patients
Abbreviations: CI, confidence interval; NECT, nifurtimox eflornithine combination therapy

The differences between eflornithine and melarsoprol at 12, 18, and 24 months from the above table were relatively stable at 15%. Therefore, we think the noninferiority margin at 24 months would be applicable to 18 months. We believe it is reasonable to use the M1 of 18.7% at 24 months for M1 at 18 months.

An alternative method for calculating M1 is to compare NECT with melarsoprol using the data in the above table. A conservatively low estimate for failure free rate at 18 months for NECT is 92.9% (the lower limit of the 95% CI), while a conservatively high estimate for melarsoprol is 75.8% (the upper limit of the 95% CI). The difference between these two estimates is 17.1%. This is an alternative estimate for M1 at 18 months.

In conclusion, based on a small controlled trial, NECT was superior to M plus N leading to a conservative treatment effect of 18.7% at 24 months. Based on data comparing 18- and 24-month results, we believe that this estimate is relevant for an 18-month treatment effect as well. An additional method for calculating a treatment effect led to an estimate of 17.1%. Therefore, M1 is conservatively estimated as 17.1%, the smaller of the two estimates. The Applicant proposed a 13% margin for the current NI trial is acceptable for the primary endpoint at Month 18, as it is smaller than 17.1%. This allows us to conclude that fexinidazole is effective and it is within a more clinically justified margin which allows for a smaller amount of loss of efficacy to the control.

8.1.2. DNDiFEX005

This trial was a prospective, multicenter, open-label, single-arm study in patients with stage-1 or early stage-2 HAT due to *T. b. gambiense*. The trial was conducted between April 30, 2014 and November 30, 2016 in 8 centers in DRC. This study and DNDiFEX006 (discussed below) were considered as "plug-in" studies because patients were recruited at the same sites by the same investigators as in the pivotal study DNDiFEX004, thereby facilitating the pooling of the data and allowing within-site comparisons by the Applicant. Because there were no new safety concerns and satisfactory CSF concentrations of fexinidazole were seen based on the preliminary blinded data obtained in the pivotal study, this study of fexinidazole in less seriously affected patients was conducted.

The fexinidazole regimen was the same as used in the pivotal study. Study visits included EOT (Day 11), EOH (Day 11 to 18), Week 9, Months 6, 12, and 18.

Patients were stratified by WBC in CSF into two strata:

- Stage-1 HAT: CSF WBC ≤5 cells/µL
- Early stage-2 HAT: CSF WBC 6 to 20 cells/µL

This was an uncontrolled study so there was no blinding.

The primary endpoint was the treatment outcome (success or failure) assessed at 12 months after the EOT, based on the adapted WHO criteria. If the patient was not a definitive failure at 12 months and if a lumbar puncture was performed at 18 months but not 12 months, then the outcome at 18 months was carried backward.

In the present study, the limit of unacceptable success proportion for fexinidazole treatment at 12 months (i.e., the lower bound of the 95% CI) was set at 80%. The Applicant calculated this limit by subtracting the margin of 13% used in DNDiFEX004 to the success proportion expected with the reference treatments at 12 months (93%), which was estimated by combining the historical success proportions of NECT in stage-2 patients [94% (577/614) at 18 months] and pentamidine in stage-1 patients [91% (207/2524) at 18 months]. See discussion in Section 8.1.4 regarding historical control estimates.

The secondary efficacy endpoints were the outcome (either success or failure) of treatment as assessed at 24 hours, and 6 and 18 months after the EOT.

The intent-to-treat (ITT) population comprised all patients who received at least one dose of study treatment. This population was used to perform the primary efficacy analysis and was the main population for efficacy and safety analyses.

Results

The ITT population included 230 subjects. Four subjects prematurely discontinued the study due to death (3 before 12 months and 1 between 12 and 18 months). The first database lock occurred once all subjects reached 12 months. Subjects were then continued to be followed for

18 months in a follow-up study. In the follow-up study, 98.3% of patients completed the 18-month visit. No additional withdrawals were observed.

Table 81. Patient Disposition, DNDiFEX005

Patient Disposition	All	Stage-1	Early Stage-2
Preselected	238	195	43
ITT	230 (100%)	189 (100%)	41 (100%)
Completed 10-day treatment	230	189	41
Reached 12-month primary time point	227 (98.7%)	186 (98.4%)	41 (100%)
Prematurely withdrawal	3	3	0
Death	3	3	0
Reached 18-month by end of first data lock for primary efficacy analysis	157 (68.3%)	132 (69.8%)	25 (61.0%)
Prematurely withdrawal	1	0	1
Death	1	0	1
Ongoing at the time of first data lock for primary efficacy analysis	69 (30.0%)	54 (28.6%)	15 (36.6%)
Follow-up study			
Reached 18-month additional time point	226 (98.3%)	186 (98.4%)	40 (97.6%)
Additional prematurely withdrawal (death) from follow-up study	0	0	0

Source: Adapted from Figure 5 in Study Report and Figure 3 in follow-Up Study Report
Abbreviation: ITT, intent-to-treat

No major protocol deviations were reported, and all patients received the correct dose. Equal numbers of males and females were included in the trial. Age ranged from 15 to 73 years. Weight ranged from 29 to 79 kg. WBC in CSF ranged from 1 to 5 in stage-1 HAT patients and 6 to 19 in early stage-2 HAT patients.

Table 82. Demographic Factors and WBC in CSF at Baseline, ITT Population, DNDiFEX005

Demographics	Stage-1 (N=189)	Early stage-2 (N=41)	Total (N=230)
Sex, n(%)			
Female	96 (50.8%)	19 (46.3%)	115 (50.0%)
Male	93 (49.2%)	22 (53.7%)	115 (50.0%)
Age (years)			
Mean (SD)	34.16 (15.72)	35.15 (14.37)	34.33 (15.46)
Median	34	35	34
Range	15, 73	15, 64	15, 73
Weight (kg)			
Mean (SD)	49.59 (8.39)	53.26 (8.27)	50.24 (8.47)
Median	49	54	50
Range	29, 79	36, 71	29, 79
WBC in CSF (cells/µL)			
Mean (SD)	2.68 (1.23)	9.88 (4.00)	3.97 (3.41)
Median	2	8	3
Range	1, 5	6, 19	1, 19

Source: Tables 9 and 13 in Study Report
Abbreviations: CSF, cerebral spinal fluid; ITT, intent-to-treat; N, number of subjects; n, number of subjects in subgroup; SD, standard deviation; WBC, white blood cell

More than 20% of patients had headache (67.0%), weight loss (36.2%), amenorrhea (23.7% of women), fever (25.2%), asthenia (23.1%) and pruritus (22.2%).

The overall success proportion at 12 months was 98.7%, 95% CI [96.2%, 99.7%], which was significantly higher than the expected 91%. As the lower bound of the 95% CI for the overall success proportion was greater than 80%, the primary study endpoint as defined by the Applicant was met.

Table 83. Success Proportion at 12 Months According to HAT Stage Using the Primary Imputation Method, ITT Population, DNDiFEX005:

Success Proportion	Stage-1 (N=189)	Early Stage-2 (N=41)	Total (N=230)
Success	186 (98.4%)	41 (100%)	227 (98.7%)
95% CI	[95.4%, 99.7%]	[91.4%, 100.0%]	[96.2%, 99.7%]

Source: Table 23 in Study Report
Abbreviations: CI, confidence interval; HAT, human African Trypanosomiasis; ITT, intent-to-treat; N, number of subjects

The 3 patients considered as failures were in stage-1 HAT and died before the 12-month time point, which were considered by the Applicant as not related to the treatment and not related to the disease (meningeal disorder and encephalitis on Day 54, shock on Day 202, and Crohn's disease and peritonitis on Day 345).

The treatment outcome at 18 months as a secondary efficacy endpoint is shown in the table below.

Table 84. Success Proportion at 18 Months According to HAT Stage Using the Primary Imputation Method, ITT Population, DNDiFEX005:

Success Proportion	Stage-1 (N=189)	Early Stage-2 (N=41)	Total (N=230)
Success	185 (97.9%)	40 (97.6%)	225 (97.8%)
95% CI	[94.7%, 99.4%]	[87.1%, 99.9%]	[95.0%, 99.3%]

Source: Table 8 in Study Report
Abbreviations: CI, confidence interval; HAT, human African Trypanosomiasis; ITT, intent-to-treat; N, number of subjects

There were 2 additional failures at 18 months than at 12 months:

- One patient (early stage-2 HAT): The change of status was due to death of a patient between 12 and 18 months from anemia, pulmonary sepsis and nephropathy.
- One patient (stage-1 HAT): The change of status was caused by a rise in the CSF WBC above 20 cells/µL at 18 months, probably due to meningitis.

Though no patients were lost to follow-up, there were 5 patients who did not have a lumbar puncture at 12 months or were not already a clinical failure. In the primary analysis the results of their 18-month lumbar puncture was used. Sensitivity analyses considering alternative methods for handling this missing information were considered and all analyses were consistent with the primary analysis given the small number of subjects with missing information.

Given the high success rates, analyses by gender and age were consistent with the overall efficacy results.

8.1.3. DNDiFEX006

This was a prospective, multicenter, single-arm, open-label, Phase 2/3, plug-in trial in children at least 6 years old (and < 15 years) and weighing over 20 kg with HAT due to *T.b. gambiense* of any stage. This was conducted between May 3, 2014 and November 22, 2016 in 8 centers in DRC.

Children were stratified by CSF WBC into three strata:

- Stage-1 HAT: evidence of trypanosomes in the blood or lymph, no trypanosomes in the CSF, and CSF WBC ≤5 cells/µL
- Early stage-2 HAT: evidence of trypanosomes in the blood or lymph, no trypanosomes in the CSF, and CSF WBC 6 to 20 cells/µL
- Late stage-2 HAT: evidence of trypanosomes in the blood or lymph, and either CSF WBC >20 cells/µL or trypanosomes in the CSF (adapted from WHO for clinical studies on HAT).

Doses were based on body weight:

- Body weight ≥20 kg and <35 kg
  - 1200 mg (2 x 600-mg tablets) once daily for 4 days (Days 1 through 4; loading dose)
  - 600 mg (1 x 600-mg tablet) once daily for 6 days (Days 5 through to 10; maintenance dose)
- Body weight ≥35 kg (same as in adults)
  - 1800 mg (3 x 600-mg tablets) once daily for 4 days (Days 1 through 4; loading dose)
  - 1200 mg (2 x 600-mg tablets) once daily for 6 days (Days 5 through to 10; maintenance dose).

Fexinidazole was administered with food and the total duration of treatment was 10 days. Visits included EOT (Day 11, 12), EOH (Day 13 to 18), Week 9, Months 6, 12, and 18.

The primary efficacy endpoint was treatment outcome (success/failure) as assessed at 12 months after the EOT, based on the adapted WHO criteria. If the patient was not a definitive failure at 12 months and if a lumbar puncture was performed at 18 months but not 12 months, then the outcome at 18 months was carried backward.

The intent-to-treat (ITT) population comprised all patients who received at least one dose of study treatment. This population was used to perform the primary efficacy analysis and was the main population for efficacy and safety analyses.

As in the previous study, an 80% success proportion at 12 months was used as the cut-off point for efficacy. It was expected that the success proportion would be statistically significantly higher than 80%. See discussion in section 8.1.4 regarding historical control estimates.

Results

A total of 130 patients were preselected (screened), and 125 were included in the study: 69 with stage-1, 19 with early stage-2 and 37 with late stage-2 HAT. One patient died between 12 and 18 months.

Table 85. Patient Disposition, DNDiFEX006

Patient Disposition	All	Stage-1	Early Stage-2	Late Stage-2
Preselected	130	73	19	38
ITT	125	69	19	37
Completed 10-day treatment	125	69	19	37
Reached 12-month primary time point	124	68	19	37
Prematurely withdrawal	1	1	0	0
Death	1	1	0	0
Reached 18-month additional time point	124	68	19	37

Source: Adapted from Figure 5 in Study Report
Abbreviation: ITT, intent-to-treat

Two patients had major deviations, one with late stage-2 HAT having a history of HAT treatment in the previous 2 years and one with stage-1 HAT having a treatment deviation (the wrong number of tablets where administered: 18 instead of 14).

About 54% of patients were male. The mean age was 11 years, and the range was 6 to 14 years.

Table 86. Demographic Factors and CSF WBC at Baseline, ITT Population, DNDiFEX006

Demographics	Stage-1 (N=69)	Early Stage-2 (N=19)	Late Stage-2 (N=37)	Total (N=125)
Sex, n(%)				
Female	36 (52.2)	7 (36.8)	15 (40.5)	58 (46.4)
Male	33 (47.8)	12 (63.2)	22 (59.5)	67 (53.6)
Age (years)				
Mean (SD)	11.0 (2.3)	10.8 (2.2)	10.5 (2.3)	10.8 (2.3)
Median	11	10	10	11
Range	6, 14	7, 14	6, 14	6, 14
Weight (kg)				
Mean (SD)	29.2 (7.7)	27.9 (7.9)	26.8 (6.8)	28.3 (7.5)
Median	27.0	25.0	25.0	27.0
Range	20, 54	20, 46	20, 47	20, 54
CSF WBC (cells/µL)				
Mean (SD)	2.9 (1.2)	10.8 (4.6)	223.8 (188.7)	69.48 (143.0)
Median	3	9	193	5
Range	1, 5	6, 20	5, 857	1, 857

Source: Tables 9 and 13 in Study Report
Abbreviations: CSF, cerebral spinal fluid; ITT, intent-to-treat; N, number of subjects; n, number of subjects in subgroup; SD, standard deviation; WBC, white blood cell

More than 20% of patients had headache (55.2%), fever (45.2%), weight loss (38.4%), drowsiness (33.6%), asthenia (31.2%), other (21.6%), and pruritus (21.6%).

The success proportion at 12 months were 97.6%. The lower limit of the 95% CI was higher than 80%, the unacceptable success proportion.

Table 87. Success Proportion at 12 Months According to HAT Stage Using the Primary Imputation Method, ITT Population, DNDiFEX006

Success Proportion	Stage-1 (N=69)	Early Stage-2 (N=19)	Late Stage-2 (N=37)	Total (N=125)
Success	68 (98.6%)	18 (94.7%)	36 (97.3%)	122 (97.6%)
95% CI	[92.2%, 99.9%]	[74.0%, 99.9%]	[85.8%, 99.9%]	[93.1%, 99.5%]

Source: Tables 19 & 25 in Study Report.
Abbreviations: CI, confidence interval; HAT, human African Trypanosomiasis; ITT, intent-to-treat; N, number of subjects

Three treatment failures were death due to dyspnea and injury (stage-1, caused by a traumatic aggression, unrelated to treatment considered by the Applicant) on Day 182, and CSF WBC counts 36 and 26 cells/µL at 12 months (early and late stage-2), respectively.

Results at 18 months is shown in the table below. One patient with early stage-2 HAT was changed from failure to success due to a CSF WBC change to ≤20 cells/µL, so the success proportions at 18 months were increased to 98.4% for total patients and to 100% for patients with early stage-2 HAT.

Table 88. Success Proportion at 18 Months According to HAT Stage Using the Primary Imputation Method, ITT Population, DNDiFEX006

Success Proportion	Stage-1 (N=69)	Early Stage-2 (N=19)	Late Stage-2 (N=37)	Total (N=125)
Success	68 (98.6%)	19 (100%)	36 (97.3%)	123 (98.4%)
95% CI	[92.2%, 99.9%]	[82.4%, 100%]	[85.8%, 99.9%]	[94.3%, 99.8%]

Source: Table 7 in Follow-Up Study Report
Abbreviations: CI, confidence interval; HAT, human African Trypanosomiasis; ITT, intent-to-treat; N, number of subjects

Though no patients were lost to follow-up, 2 patients did not have a lumbar puncture or a definitive failure and had the results of their 18-month lumbar puncture used to impute their 12-month results (considered as successes in the primary analysis). Sensitivity analysis of the outcomes at 12 months using different imputation methods were conducted and the results were consistent with the overall results, given the small amount of missing data.

Results by CSF WBC at baseline are displayed in the table below. The success proportions were slightly lower in patients with a higher CSF WBC count. However, due to the small sample size and uncontrolled data, it is not possible to make a reliable conclusion.

Efficacy results by age and sex at 12 months were consistent with the overall efficacy results (one failure in each stage in patients with ages of 12, 14 and 12 years, respectively) , as the success proportions were so high.

Table 89. Success Proportion at 12 Months by Baseline CSF Using the Primary Imputation Method, ITT Population, DNDiFEX006

	Success, n/N(%) [95% CI]	
CSF WBC (cells/µL)	Late Stage-2	All Stages
≤100	10/10 (100%)	96/98 (98.0%) [92.8%, 99.8%]
>100	26/27 (96.3%)	26/27 (96.3%) [81.0%, 99.9%]

Source: Reviwer's analysis
Abbreviations: CI, confidence interval; CSF, cerebral spinal fluid; ITT, intent-to-treat; N, number of subjects; n, number of subjects in subgroup; WBC, white blood cell

8.1.4. Assessment of Efficacy Across Trials

Assessment of efficacy of fexinidazole for the treatment of HAT was based on three trials: one open-label, active-controlled trial with NECT and two single-arm, open-label trials. The first trial was conducted in 394 patients aged 15 years or older with late stage-2 g-HAT. Single-arm trial DNDiFEX005 was conducted in stage-1 and early stage-2 g-HAT patients aged 15 years or older (n=230). Single-arm trial DNDiFEX006 was conducted in stage-1 and early and late stage-2 g-HAT children at least 6 years old and < 15 years and weighing over 20 kg (n=125). These three trials included all stages of g-HAT and patients aged between 6 and 71 years old.

Primary Endpoints

In the three trials, the primary efficacy endpoint was treatment outcome (success/failure) at either 12 or 18 months, though all three trials assessed both time points. For the pivotal trial, it was at 18 months, and for the two single-arm trials it was at 12 months.

The table below reports the results of 12, 18-month, and 24-month endpoints. As the pivotal trial included only patients with late stage-2 g-HAT, the success proportions at 18 months were lower for this trial than in the two-single arm trials with patients in early stages, which included both patients with stage-1 and early stage-2 g-HAT and one of which only included 30% of patients with late stage-2 g-HAT. At 12 months, the two single-arm trials showed similar and higher success proportions.

Table 90. Summary of Treatment Outcome at Different Time Points, ITT Population

Time Points	DNDiFEX004 (Late Stage-2) Fexinidazole (N=264)	NECT (N=130)	DNDiFEX005 (Stage-1 and Early Stage-2) Fexinidazole (N=230)	DNDiFEX006 (Children - All Stages) Fexinidazole (N=125)
12 months				
Success (%)	239 (90.5%)	125 (96.2%)	**227 (98.7%)**	**122 (97.6%)**
95% CI			**[96.2%, 99.7%]**	**[93.1%, 99.5%]**
Difference [95% CI]		-5.6% [-10.5%, -0.8%]		
18 months				
Success (%)	**239 (90.5%)**	**124 (95.4%)**	225 (97.8%)	123 (98.4%)
[95% CI]			[95.0%, 99.3%]	[94.3%, 99.8%]
Difference [97.06% CI]		**-4.9% [-10.5%, 0.8%]**		
24 months				
Success (%)	235 (89.0%)	124 (95.4%)		
Difference [97.06% CI]		-6.4% [-12.2%, 0.6%]		

Source: Reviewer's summary of individual study results
Bold font was for the primary efficacy analysis in the trial
Abbreviations: CI, confidence interval; ITT, intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy

Secondary and Other Endpoints

The success proportions at 12 and 24 months for DNDiFEX004 and at 18 months for the two single-arm trials are displayed in the above table. As the follow-up interval increased, treatment effects were relatively stable within each trial.

In trial DNDiFEX004, there were nine deaths (3.41%) in the fexinidazole group and 2 deaths (1.54%) in the NECT group. In DNDiFEX005, there were 3 deaths in stage-1 g-HAT patients and one death in early stage-2 g-HAT patients (overall 1.74%). In DNDiFEX006, there was one death in a stage-1 g-HAT patient (overall 0.8%).

Subpopulations

Primary analyses in the three trials by age and gender are summarized in the table below. As the trials were conducted in Africa, no race information was available and no analysis by race was performed. Very few patients were > 65 years old, so a cutoff of 45 years was used instead. As discussed regarding DNDiFEX004, lower efficacy was seen with fexinidazole in subjects < 45 years old. This appeared to be due to younger subjects having a higher severity of disease and fexinidazole showing reduced efficacy in more severe disease. No clear differences in treatment effects were identified between males and females.

Table 91. Primary Efficacy Analysis by Age and Sex in Three Trials, ITT Population

	DNDiFEX004 18 Months		DNDiFEX005 12 Months	DNDiFEX006 12 Months
Demographics	**Fexinidazole (N=264)**	**NECT (N=130)**	**Fexinidazole (N=230)**	**Fexinidazole (N=125)**
Age (years)				
6 to <15				122/125 (97.6%)
15 to <45	184/205 (89.8%)	92/96 (95.8%)	160/162 (99.8%)	
45+	55/59 (93.2%)	32/34 (94.1%)	67/68 (98.5%)	
Sex				
Male	145/161 (90.1%)	76/80 (95.0%)	112/115 (97.4%)	66/67 (98.5%)
Female	94/103 (91.3%)	48/50 (98.0%)	115/115 (100%)	56/58 (96.6%)

Source: Reviewer's analysis
Abbreviations: ITT, intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy

The comparative trial, DNDiFEX004, showed that fexinidazole had improved efficacy in late stage-2 g-HAT subjects with less severe disease at baseline. The table below shows the results by CSF WBC for DNDiFEX004 and DNDiFEX006 (late stage-2 only). Note that in DNDiFEX006 the only death occurred in stage-1 g-HAT subjects.

Table 92. Success and Death Proportions by Baseline CSF WBC in Patients With Late Stage-2 G-HAT, ITT Population

	DNDIFEX004		DNDiFEX006
Baseline CSF WBC (cells/µL)	**Fexinidazole (N=264)**	**NECT (N=130)**	**Fexinidazole (N=37)**
Success			
≤100	100/103 (97.1%)	47/50 (94.0%)	10/10 (100%)
>100	139/161 (86.3%)	77/80 (96.3%)	26/27 (96.3%)

Baseline CSF WBC (cells/µL)	DNDIFEX004 Fexinidazole (N=264)	NECT (N=130)	DNDiFEX006 Fexinidazole (N=37)
Death			
≤100	2/103 (1.9%)	2/50 (4.0%)	
>100	7/161 (4.3%)	0/80 (0%)	

Source: Reviewer's analysis
Abbreviations: CSF, cerebral spinal fluid; g-HAT, human African trypanosomiasis due to *T. b. gambiense*; ITT, intent-to-treat; N, number of subjects; NECT, nifurtimox eflornithine combination therapy; WBC, white blood cell

Estimates of Spontaneous Cure Rates at 12 Months for Stage 1 and 2 g-HAT

In order to interpret the uncontrolled trials, we need to have some estimates of the spontaneous success rates. Note that since uncontrolled trials do not contain a concurrent control, this type of design has an inability to control bias and interpretation is limited to situations where the effect of the treatment and untreated control is large and the usual course of disease is highly predictable. In this NDA, these uncontrolled trials are considered supportive of the more interpretable noninferiority trial.

As the two uncontrolled trials, DNDiFEX005 and DNDiFEX006, used a 12-month efficacy in the primary analysis, we focused on the cure rates at 12 months.

For stage-1 g-HAT, we identified two randomized clinical trials (Burri et al. 2016 and Pohlig et al. 2016), which compared pafuramidine with standard pentamidine. Burri et al. 2016 included subjects 15 years and older and Pohlig et al. 2016 included subjects 12 years and older. As lower cure rates were observed in the pafuramidine group, we used this less effective regimen for the estimates of spontaneous cure rates. The patient populations and the primary efficacy endpoints were similar to the two uncontrolled trials, DNDiFEX005 and DNDiFEX006, except that DNDiFEX006 included subjects as young as 6 years and that both studies also included subjects with stage-2 disease. In the first trial, the parasitological cure rate at 12 months was 83% (30/36) in the pafuramidine group, ignoring 5 patients lost to follow-up. In the second trial, the cure rate at 12 months was 86.8% (118/136) in the pafuramidine group in the ITT population. Using the data from these two trials, a meta-analysis yielded an upper limit of 90.43% for the estimated spontaneous cure rate at Month 12 in stage-1 g-HAT.

For stage-2 g-HAT, there was a small study by Na-Bangchang et al. 2004 in subjects 18 years and older. Eflornithine 100 mg and 125 mg/kg was administered every 6 hours for 14 days, orally (not IV). The 12-month cure was 9/12 (75%) and 10/13 (77%). Using these two arms in the clinical trial in a meta-analysis, the upper limit of the combined cure rate was 88.8%, which could be used as a conservative estimate for the spontaneous cure rate at Month 12 at Stage 2. Additionally, Table 80 reports an upper bound of 82.6% for melarsoprol.

Therefore, as a conservative estimate, for a trial including both stage-1 and stage-2 g-HAT patients, a cure rate of 90.43% (the larger one of the two estimates of 82.6% and 90.43%) could be used as the spontaneous cure rate at Month 12. Note that since these are treated rates and not spontaneous cure rates, they are very conservative. It should be noted that despite these high estimated rates of spontaneous cure, clinically g-HAT disease is thought of as being uniformly fatal within 2 to 3 years. Thus, significant questions about the spontaneous cure rate

of g-HAT remain. Regardless, even if the high estimated spontaneous cure rates are used as a comparator, both trials were able to exclude this larger historical control rate of 90.43%.

8.1.5. Integrated Assessment of Effectiveness

Discussions of effectiveness of g-HAT treatment are complex and rely on multiple parameters including ability to eradicate the parasite, toxicity associated with drug administration, and the burden of the treatment regimen itself, particularly in resource-poor settings where g-HAT is endemic (and applicable to a smaller degree to treatment in the United States). In the clinical development program for fexinidazole, efficacy was primarily assessed in one comparative noninferiority trial in late stage-2 g-HAT in adults. Though efficacy was demonstrated in this comparative trial by showing that fexinidazole was noninferior to NECT using a justified noninferiority margin of 13%, the trial also demonstrated that fexinidazole's efficacy was inferior to NECT. In addition, the number of deaths was higher in the fexinidazole arm compared to NECT. Importantly, this decrease in efficacy and increase in deaths appeared related to more severe disease at baseline; failures in the fexinidazole arm outpaced those in the NECT arm regardless of type of failure (deaths, detection of trypanosomes in the CSF, need for rescue medication, etc.). Thus, it cannot be reasonably concluded that fexinidazole has equivalent success as NECT in treating more severe late stage 2 g-HAT. Still, given the burden of treatment with NECT (requirement for lumbar puncture for staging, intravenous access, and complicated treatment regimen), it may be preferable in some instances (very resource poor setting, inability to be monitored in an inpatient setting, etc.) to use fexinidazole in such cases. Also, fexinidazole provides an alternative treatment in patients with NECT intolerance. In subjects who had WBC in CSF at baseline of ≤ 100 cells/µL, the efficacy of fexinidazole was similar to NECT as was the number of deaths.

Two uncontrolled trials were considered supportive of these overall results.

Thus, given the general objective of the Applicant to develop a treatment that adequately treats all stages of g-HAT disease and eliminates the need for staging, the fexinidazole clinical development program only partly met its objective. Still, it appears to provide an effective treatment for early stage disease and less severe stage-2 disease and provides an alternative, potentially less burdensome (albeit less effective) treatment option for subjects with more severe stage-2 disease at baseline.

8.2. Review of Safety

As noted in the table below, the sponsor's safety evaluation draws upon several studies that span multiple phases (1 to 3), indications (g-HAT, CD, and visceral leishmaniasis), locations, study population sizes, and distinct populations within the g-HAT indication (stage 1 and 2 g-HAT disease in adults and pediatric subjects). The sponsor has provided individual and pooled analyses for its three main g-HAT studies (studies 004, 005, and 006), and for the purposes of this review, attention was focused on those studies as well; these studies dealt with treating the proposed indication at the proposed fexinidazole dosing regimen. Other studies, such as PK studies and studies in other indications, were reviewed but are not explicitly described unless

findings were pertinent to adverse events of special interest, adverse events referred to in labelling, unique findings, etc. Among the main g-HAT studies themselves, this reviewer has given primary emphasis to study 004 and has not drawn from pooled analyses. Because study 004 is the only controlled study in the proposed indication and because this study focused on primarily older (≥ 15 years old) patients with late stage 2 g-HAT, pooling with studies 005 and 006 was not considered appropriate given their uncontrolled design and distinct study populations (stage 1 and early/late stage 2 g-HAT disease in adult and pediatric populations). Thus, study 004 safety findings are described in detail while safety findings in studies 005 and 006 are discussed at a summary level and in relation to the main safety findings in study 004. The sponsor table below provides an overview of the various studies that make up the safety database.

Note: as the ITT and safety populations for studies 004, 005, and 006 are identical, the demographics of the safety population is not discussed here as it has already been discussed in the earlier efficacy section 8.1.1

Table 93. Table of Studies Included in Fexinidazole Safety Database

Study name and status	Design	Purpose	Treatment / study duration	Cohorts planned	Patient number included	Fexinidazole formulation	Study drug and dose
Clinical studies in the g-HAT indication (included in the pooled safety analysis completed to the primary endpoint)							
DNDiFEX004 complete	Phase II/III, pivotal, multicenter, non-inferiority, randomized, open-label (blinded for the sponsor, data management personnel, study statistician and statistician consultant), unbalanced parallel groups (2:1 ratio), conducted in CAR and DRC	Efficacy and safety of fexinidazole compared to NECT therapy in late stage-2 g-HAT patients ≥15 years old	10 days / 25 months	2 cohorts (randomized 2:1 to receive fexinidazole)	394 (264 fexinidazole; 130 NECT)	Oral tablet (600 mg)	Fexinidazole: 1800 mg/day (3 tablets) D1-4 (loading dose) and 1200 mg/day (2 tablets) D5-10 (maintenance dose) Reference treatment NECT: oral nifurtimox (15 mg/kg/day) for D1-10 + infusion of DFMO (400 mg/kg/day) for D1-7 Both in fed conditions
DNDiFEX005 complete	Phase II/III, multicenter, open-label, cohort study, conducted in DRC	Efficacy of treatment at 1-year follow-up in stage-1 or early stage-2 g-HAT patients ≥15 years old	10 days / 19 months	Single group of patients	230	Oral tablet (600 mg)	Fexinidazole: 1800 mg/day (3 tablets) D1-4 (loading dose) and 1200 mg/day (2 tablets) D5-10 (maintenance dose) in fed conditions
DNDiFEX006 complete	Phase II/III, multicenter, open-label, cohort study in patients between 6 and <15 years old, conducted in DRC	Efficacy and safety of a 10-day treatment for g-HAT at stage-1, early or late stage-2 in children ≥6 years old, weighing ≥20 kg and able to swallow tablets	10 days / 19 months	Single group of patients – treatment administered by body weight	125	Oral tablet (600 mg)	Fexinidazole (fed conditions) Body weight ≥20 kg and <35 kg (low dose): 1200 mg/day (2 tablets) D1-4 + 600 mg/day (1 tablet) D5-10 Body weight ≥35 kg (same dose as adults): 1800 mg/day (3 tablets) D1-4 + 1200 mg/day (2 tablets) D5-10

Study name and status	Design	Purpose	Treatment / study duration	Cohorts planned	Patient number included	Fexinidazole formulation	Study drug and dose
Clinical studies in other patient populations							
DNDiFEXIVL001 complete (early termination due to a high frequency of relapses)	Phase II, open-label, proof-of-concept in patients 15 to 60 years old, conducted in Sudan	Efficacy and safety in patients with primary VL in Sudan	10 days / 18 months	1 cohort	14	Oral tablet (600 mg)	Fexinidazole: 1800 mg/day (3 tablets) D1-4 + 1200 mg/day (2 tablets) D5-10 in fed conditions
DNDiCHFEXI001 complete (early termination of recruitment for safety reasons)	Phase II, double-blind, randomized, placebo-controlled in patients 18 to 50 years old, conducted in Bolivia	Proof-of-concept, safety and efficacy of 6 regimens of fexinidazole in patients with CD	8 weeks [a] / 54 weeks	7 cohorts: 1 placebo arm and 6 fexinidazole arms	47 (40 fexinidazole; 7 placebo)	Oral tablet (600 mg)	Fexinidazole (fed conditions): HD (2W): 1800 mg/day for 2W, then placebo for 6W (total dose 25.2 g) HD (4W): 1800 mg/day for 4W then placebo for 4W (total dose 50.4 g) HD (8W): 1800 mg/day for 8W (total dose 100.8 g) LD (2W): 1200 mg/day for 2W, then placebo for 6W (total dose 16.8 g) LD (4W): 1200 mg/day for 4W then placebo for 4W (total dose 33.6 g) LD (8W): 1200 mg/day for 8W (total dose 67.2 g)

Study name and status	Design	Purpose	Treatment / study duration	Cohorts planned	Patient number included	Fexinidazole formulation	Study drug and dose
Clinical pharmacology studies							
DNDiFEX001 Part I complete	Phase I, randomized, double-blind, placebo-controlled in healthy subjects of sub-Saharan African origin, 18 to 45 years old, conducted in France	Single ascending dose safety tolerability and PK data	One single dose / 9 days	9 cohorts of 8 subjects Per cohort: 6 subjects active treatment; 2 subjects placebo	72 (54 fexinidazole; 18 placebo)	Oral suspension	Fexinidazole (fasting conditions, single dose): 100 mg, 200 mg, 400 mg, 800 mg, 1200 mg, 1800 mg, 2400 mg, 3000 mg, 3600 mg
DNDiFEX001 Part II complete		Bioavailability of tablets versus oral suspension and assessment of food effect	3 single doses / 39 days	1 cohort (crossover, washout)	13	Oral suspension versus tablet	Fexinidazole (3-way crossover; single doses, 14-day washout period): **A**:1200 mg fasting conditions, oral suspension **B**:1200 mg fasting conditions, oral tablets **C**:1200 mg fed conditions, oral tablets
DNDiFEX001 Part III complete		Multiple ascending doses	14 days / 30 days	3 cohorts of 8 subjects Per cohort: 6 with active treatment; 2 with placebo	27 (21 fexinidazole; 6 placebo)	Oral tablet (600 mg)	Fexinidazole (fasting conditions, multiple ascending doses from Day 1-14): 1200 mg/day, 2400 mg/day, 3600 mg/day
DNDiFEX002 complete	Phase I, randomized, open-label, in healthy subjects of sub-Saharan African origin,18 to 45 years old, conducted in France	Impact of food on relative bioavailability	3 single doses / 37 days	1 cohort (crossover, washout)	12	Oral tablet (600 mg)	Fexinidazole (3-way crossover; 1200 mg in 3 conditions, 14-day washout period): **A**:fasting **B**:fed (meal type 1: Plumpy'nut®) **C**:fed (meal type 2: rice and beans without fat)

Study name and status	Design	Purpose	Treatment / study duration	Cohorts planned	Patient number included	Fexinidazole formulation	Study drug and dose
DNDiFEX003 complete	Phase I, double-blind, placebo-controlled, randomized in healthy subjects of sub-Saharan African origin, 18 to 45 years old, conducted in France	PK of oral fexinidazole	10 days / 26±2 days	2 cohorts of 18 subjects Per cohort: 12 subjects fexinidazole; 6 subjects placebo	30 (22 fexinidazole; 8 placebo)	Oral tablet (600 mg)	Fexinidazole (fed conditions, multiple ascending dose): *Dose group 1*: 1800 mg/day D1-4 + 1200 mg/day D5-10 *Dose group 2*: 2400 mg/day D1-4 + 1200 mg/day D5-10
DNDiHATFEX008 complete	Phase I, open-label, randomized, replicate design in healthy subjects of sub-Saharan African origin, 18 to 45 years old, conducted in France	Bioequivalence of reference versus proposed marketed formulation of fexinidazole	2 sequences, 2 treatments, 4 periods: 4 single doses (2 test and 2 reference) / 7 weeks	2 cohorts/ sequences: TRTR or RTRT	30	Oral tablet (600 mg)	Fexinidazole (fed conditions, single dose, 14-day washout period): *Reference (Aptuit, Italy)*: 1200 mg *Test (AAI Pharma, USA)*: 1200 mg
INT15307 complete	Phase I, open-label, non-randomized, crossover study in healthy subjects of sub-Saharan African origin, 18 to 55 years old, conducted in France	Drug-drug interaction study: effect of repeated oral doses of fexinidazole on the pharmacokinetic of a single dose of a CYP probe cocktail (caffeine and omeprazole)	2 treatments, 2 periods: Period 1 of 2 days and Period 2 of 6 days / 2 months	1 cohort	12	Oral tablet (600 mg, AAI Pharma, USA)	Period 1: CYP probe cocktail alone, fed conditions, single oral dose: omeprazole 20 mg + anhydrous caffeine base 100 mg on Day 1 Period 2: Fexinidazole oral dose in fed conditions, 1800 mg/day from Day 1 to Day 4 and 1200 mg on Day 5, with single dose of CYP probe cocktail on Day 4 A 2-day washout period

Study name and status	Design	Purpose	Treatment / study duration	Cohorts planned	Patient number included	Fexinidazole formulation	Study drug and dose
Ongoing studies at the safety data cut-off date (07 September 2019; no CSRs available)							
DNDi-FEX-09-HAT ongoing	Phase IIIb, open-label, multicenter, cohort study in any stage g-HAT patients ≥6 years old [b], conducted in DRC and Guinea	Efficacy and safety of a 10-day treatment in any stage g-HAT patients ≥6 years old [b]	10 days / 36 months	Single group of patients – treatment administered by body weight	174 planned; 174 patients treated at the cut-off date	Oral tablet (600 mg)	Fexinidazole (fed conditions): Body weight ≥20 kg and <35 kg (low dose): 1200 mg/day (2 tablets) D1-4 + 600 mg/day (1 tablet) D5-10 Body weight ≥35 kg (same dose as adults): 1800 mg/day (3 tablets) D1-4 + 1200 mg/day (2 tablets) D5-10
DNDiCHFEX12 ongoing	Phase II, randomized, double-blind, multicenter study in CD patients, conducted in Spain	Safety and efficacy of fexinidazole in patients with chronic indeterminate CD	10 days / around 14 months	3 cohorts with active treatment; historical comparison with placebo	45 planned; 45 enrolled; 44 patients treated at the cut-off date	Oral tablet (600 mg)	Fexinidazole (fed conditions): Group A: 600 mg/day for 10 days (total dose of 6.0 g) Group B: 1200 mg/day for 3 days + placebo for 7 days (total dose of 3.6 g) Group C: 600 mg/day for 3 days + 1200 mg/day for 4 days + placebo for 3 days (total dose of 6.6 g)

Source: Summary of Clinical Safety, Table 1
Abbreviations: CAR, Central African Republic; CD, Chagas Disease; D, day; DFMO, alpha-difluoromethylornithine nifurtimox and Ornidyl®; DRC, Democratic Republic of the Congo; g-HAT, Human African trypanosomiasis (HAT) due to *Trypanosoma brucei gambiense;* HD, high dose; LD, low dose; NECT, nifurtimox-eflornithine combination therapy; PK, pharmacokinetics; VL- Visceral Leishmaniasis, W, week

Please note that Study 009 (ongoing g-HAT study) was not examined in detail and is only referred to as pertinent to the overall safety findings.

8.2.1. Review of the Safety Database

Overall Exposure

g-HAT

The Applicant table below highlights the safety exposure from the three g-HAT trials (DNDiFEX 004, 005, and 006).

Table 94. Safety Exposure, Studies 004, 005, and 006

Description	Statistics	DNDiFEX004 NECT Late stage 2 (N=130)	DNDiFEX004 Fexi Late stage 2 (N=264)	DNDiFEX005 Fexi Stage 1 and early 2 (N=230)	DNDiFEX006 Fexi Children all stages (N=125)	All Fexinidazole (N=619)
Randomized or Included	n (%) - Yes	130 (100.0)	264 (100.0)	230 (100.0)	125 (100.0)	619 (100.0)
Premature treatment discontinuation	n (%) - No	130 (100.0)	262 (99.2)	230 (100)	125 (100.0)	617 (99.7)
	n (%) - Yes	0 (0.0)	2 (0.8)	0 (0.0)	0 (0.0)	2 (0.3)
Premature study discontinuation	N	130	264	230	125	619
	n (%) - No	124 (95.4)	237 (89.8)	226 (98.3)	124 (99.2)	587 (94.5)
	n (%) - Yes	6 (4.6)	27 (10.2)	4 (1.7)	1 (0.8)	32 (5.2)
Reason of withdrawal n (% of withdrawals)	N	6	27	4	1	32
	Lost to follow up	4 (66.7)	5 (18.5)	0 (0.0)	0 (0.0)	5 (15.6)
	Death	2 (33.3)	9 (33.3)	4 (100.0)	1 (100.0)	14 (43.8)
	Consent withdrawal	0 (0.0)	1 (3.7)	0 (0.0)	0 (0.0)	1 (3.1)
	Treatment failure [a]	0 (0.0)	12 (44.4) [b]	0 (0.0)	0 (0.0)	12 (37.5)

Abbreviations: Fexi=fexinidazole; g-HAT=human African trypanosomiasis due to *T.b. gambiense*; ITT=intent-to-treat; NECT=nifurtimox-eflornithine combination therapy.

a Treatment failure is defined as patients with proven or probable g-HAT relapse at any time during follow-up who received rescue treatment.

b A 13th case of fexinidazole treatment failure was observed 3 days after the additional 24-month visit (Patient 401_042).

Note: Text highlighted in grey corresponds to updated data with follow-up report. No changes for the other studies.

Source: 5.3.5.3 Pooled safety analysis, Table 14.1.1.b. and 5.3.5.1 Study DNDiFEX004 FU 1, 3 to 15 Study report body, Section 5.1.1

Source: Table 6, Summary of Clinical Safety

As can be noted in the table above, the ITT population and safety population of the three studies are identical (i.e., all randomized subjects in these studies received a dose of study medication). Taken together, there were 619 subjects who received fexinidazole in the three studies; 264 (43%) of these subjects were from the controlled study 004. Please note that only two subjects discontinued treatment. Both of these subjects were in the fexinidazole arm and discontinued study medication due to death of the patient (death described in further detail in section 8.2.3). Study discontinuations occurred mostly in study 004 and were primarily due to death, treatment failure, and loss to follow-up. 361 (58%) of the 619 fexinidazole subjects had Stage 2 disease, the majority of which was late stage 2 disease. 516 (83%) of the 619 fexinidazole subjects took the higher fexinidazole dose (1800 mg loading dose on Days 1 to 4 followed by 1200 mg maintenance dose on Days 5 to 10).

In study 004, fexinidazole treatment duration was a mean of 10 days with a mean cumulative dose of 14.4 gm of fexinidazole. For NECT, the cumulative mean dose was 2.8 gm/kg of DFMO (eflornithine) and 150.2 mg/kg of nifurtimox; consistent with the planned dosing for both components. In study 005, mean fexinidazole duration and cumulative dose were similar to that of study 004. In study 006, fexinidazole treatment was administered for a mean of 10 days with a mean cumulative dose of 9.4 gm; 103/125 (82.4%) pediatric patients in this study received the lower dose of fexinidazole (1200 mg/day Day 1 to 4 then 600 mg/day Day 5 to 10).

Visceral Leishmaniasis

14 subjects were randomized and treated in study VL 001. No subjects discontinued the study.

Chagas Disease (CD)

In Study CH001 (DNDiCHFEXI001) in CD patients, it was initially planned to enroll 140 patients (7 treatment groups of 20 patients each). However, due to safety and tolerability issues identified during the study, from October 17, 2014, all patients who had received greater than 2 weeks of treatment had treatment immediately interrupted and all other patients who had initiated treatment were instructed to complete a 2-week treatment course only. Therefore, 47 patients were randomized and included in the ITT and safety population. Due to the change to study conduct, the duration of treatment was variable and the EOT was defined in accordance to the duration of the actual treatment regimen.

Phase 1/Pharmacokinetic Studies

The Applicant table below highlights the safety database from the early Phase 1/pharmacokinetic studies.

Table 95. Healthy Subjects Disposition in Fexinidazole Pharmacology Studies

Description (N subjects)	FEX001 Part I	FEX001 Part II	FEX001 Part III	FEX002	FEX003	FEX008	INT15307	Total subjects
Screened	129	25	59	23	61	NA	NA	297
Randomized/ safety analysis [a]	72	13	27	12	30	30	12	196
Completed	72	12	23	11	22	30	10	180
Premature withdrawal	0	1	4	1	8	0	2	16
If yes, reasons:								
Withdrew consent	NA	1	3	1	5	0	0	10
SAE	NA	0	1[b]	0	0	0	0	1
AE	NA	0	0	0	3[c]	0	2[d]	5

Abbreviations: AE=adverse event; NA=not applicable; SAE=serious adverse event

a Patients included in safety analysis are recruited patients having received at least 1 dose of study medication

b Subject No. (b) (6) (replacement subject starting later as the other subject was withdrawn on Day 6 after agreement between the Sponsor and the Investigator as he presented an elevation of bilirubin that occurred at the same time as an SAE of transaminases increase was reported for Subject No. (b) (6) on Day 15.

c Subjects No. (b) (6) had TEAE of vomiting, and Subject No. (b) (6) had a panic attack, which led to discontinuation of study treatment.

d Subject (b) (6) had nausea after 2 doses of fexinidazole (Period 2 Day 2) and Subject (b) (6) had vomiting after 4 doses of fexinidazole and the dose of CYP probe cocktail (Period 2 Day 4).

Source: All CSRs in 5.3.1.1, 5.3.1.2 and 5.3.3.1

Source: Summary of Clinical Safety; Table 7

The safety database from these studies is 196 patients, 92% of whom completed the studies. The primary reason for study discontinuation was withdrawal of consent.

Ongoing Studies

The Applicant table below (Table 8, Summary of Clinical Safety) highlights the safety exposure database in the ongoing trials as of the data lock point of Sept. 7th, 2019. While the CD trial has essentially been completed, the majority of subjects in the g-HAT study have not completed the final visit.

Table 96. Disposition of Subjects in Ongoing Studies as of Database Lock, Sept. 7th, 2019

Study	DBL date	Randomized / Treated population	Patients withdrawn	Patients completed to primary endpoint	Patients completed to final study visit	Patients ongoing (07 Sep 19)
DNDi-FEX-09-HAT	NA	174/174	8[a]	45	45	121
DNDiCHFEX012	NA	45/44	0	44	44	44

Abbreviations: DBL=database lock; HAT=human African trypanosomiasis; NA=not applicable
a In DNDi-FEX-09-HAT, 8 patients withdrew (including 1 death, 2 withdrawals of consent and 5 relapses) before completion of the 12-month follow-up visit.
b In DNDiCHFEX012, there were no patient withdrawal and no death during the clinical phase (10-day treatment).
Source: DSUR 5 (May 2019) and Pharmacovigilance databases
Source: Summary of Clinical Safety, Table 8

Adequacy of the Safety Database

The size of the safety database is adequate. Generally, at least 300 patients treated with the proposed dosing regimen is expected in order to assess for adverse event frequencies potentially occurring at a level of less than <1% in the study population ("rule of three"). Given that the fexinidazole safety database includes over 600 subjects with g-HAT disease, including over 300 subjects with late stage 2 disease, the database is adequate to generate a general safety profile for fexinidazole in the labelled indication. Moreover, safety information from the Phase 1 studies, studies in other indications, and ongoing studies in g-HAT, provide supportive data. Though it is difficult to fully assess the applicability of the safety data to patients who may receive treatment in the U.S. (returning immigrants, etc.), the trials were done where the diseases is endemic and could not have been conducted anywhere else; an absence of US patients is expected.

8.2.2. Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

The submission materials were of good quality. An audit of clinical trial data was completed by the Office of Scientific Investigations (see section 4.1 of this review). Three DNDiFEX004 study sites underwent remote (due to the current COVID pandemic) regulatory assessments of the clinical investigator's practices and procedures to determine compliance with applicable regulations in support of this NDA. Based on the results of these investigations, OSI concluded that the study appears to have been conducted adequately, and the data generated by these clinical investigators generally appear reliable in support of the proposed indication.

Categorization of Adverse Events

The safety definitions and monitoring employed in the three g-HAT trials is described in the Applicant tables below (Tables 2 and 3, Summary of Clinical Safety). The measures used are typical of clinical trials and generally considered adequate. Of note, the Applicant conducted analyses of several Adverse Events of Special Interest (psychiatric disorders, neutropenia, vomiting, etc.) retrospectively once particular clinical concerns became apparent during the conduct of these and other clinical trials. TEAEs were primarily recorded during the time from baseline until the end of hospitalization. Similarly, laboratory parameters were primarily recorded during this same period, though a subset of subjects had measurements taken at Week 9 (laboratory measurements at time points beyond this were generally at the discretion of the Investigator).

Table 97. Definitions of Safety Parameters, Studies 004, 005, and 006

Parameter	DNDiFEX004	DNDiFEX005	DNDiFEX006
MedDRA version	16.0	16.1	16.1
CTCAE version	4.03	4.03	4.03
AE [a]	Any untoward and unintended medical occurrence (sign, symptom or disease), including a clinically significant abnormal laboratory or ECG finding, or worsening of any pre-existing condition during the study, whether or not it is considered to be study related. Abnormal laboratory (hematology and biochemistry) results will be reported as AEs if they occur or worsen after the start of the study treatment, and if they are greater than CTCAE Grade 1, unless they are associated with an already reported clinical event.		
AE collection period	First intake of study treatment on D1 until hospital discharge between D13 and D18 or longer if medically necessary.	First intake of the study treatment on D1 until hospital discharge (between D11 and D18), or longer if medically necessary	First intake of study treatment on D1 until hospital discharge between D13 and D18 or longer if medically necessary
	After the AE collection period all AEs the Investigator considers possibly related to study treatment must be reported		
SAE	An AE that results in death, is life-threatening, requires in-patient hospitalization or prolongation of existing hospitalization, results in persistent or significant disability/incapacity, is a congenital anomaly/birth defect, is an important medical event that may not be immediately life-threatening or result in death or hospitalization but may jeopardise the patient or may require intervention to prevent 1 of the other outcomes listed in the definition above, ALT or AST levels higher than 3xULN associated with a total bilirubin level higher than 2xULN		
SAE collection period	From signature of informed consent until the end of follow-up (24 months)	From signature of informed consent until the end of follow-up (18 months)	

Parameter	DNDiFEX004	DNDiFEX005	DNDiFEX006
Recommendations for treatment discontinuation as per protocol instructions	Severe skin reaction, ALT or AST exceeding 8xULN, ALT or AST exceeding 3xULN associated with total bilirubin >2xULN, ALT or AST exceeding 3xULN accompanied by fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%), any condition that, in the opinion of the Investigator, requires treatment discontinuation for medical reasons In DNDiFEX004 and DNDiFEX006, exclusion was also due to pre-dose QTcF measurement ≥500 ms on D2, D3, D4 or D4H23, confirmed by a second ECG recorded after at least a 10-20 minute rest		
Vital signs	Body temperature, BP, HR, RR, height, weight, BMI, Karnofsky score, general health		
Hematology parameters (fasted state)	Hemoglobin, WBC and differentials (neutrophils, eosinophils, basophils, lymphocytes, and monocytes), platelet count		
Biochemistry parameters (fasted state)	ALB, ALP, ALT, AST, BUN, Cl, CRE, GLU, K+, Na+, Ca2+, TBIL, tCO2, TP		
Urinalysis (fasted state)	WBC, pH, protein, urobilinogen, blood, nitrites, glucose, ketone bodies, bilirubin		
Digital ECG recording	CarTouch®: triplicate tracings to assess QT interval	CarTouch®	CarTouch®: triplicate tracings to assess QT interval

Abbreviations: AE=adverse event; ALB=albumin; ALP=alkaline phosphatase; ALT=alanine aminotransferase; AST=aspartate aminotransferase; BMI=body mass index; BP=blood pressure; BUN=blood urea nitrogen; Ca2+=calcium; Cl=chloride; CRE=creatinine; CTCAE=Common Toxicology Criteria for Adverse Events; D=day; ECG=electrocardiogram; GLU=glucose; HR=heart rate; K+=potassium; Na+=sodium; QTcF=QT interval corrected according to Fridericia; RR=respiratory rate; SAE=serious adverse event; TBIL=total bilirubin; tCO_2= total carbon dioxide; TP=total protein; ULN=upper limit of normal; WBC=white blood cell

a Following changes to the CRF, an AE was defined as worsening of any signs or symptoms that were present at inclusion (using a systematic checklist) and that was considered clinically significant

Source: 5.3.5.1 Study DNDiFEX004 Appendix 16.1.1 Amendment 1, 5.3.5.2 Studies DNDiFEX005 Appendix 16.1.1 Protocol and DNDiFEX006 Appendix 16.1.1 Protocol

Source: Summary of Clinical Safety, Table 2

Table 98. General Schedule of Safety Parameters, Studies 004, 005, and 006

Parameter	DNDiFEX004	DNDiFEX005	DNDiFEX006
Karnofsky score	Pre-S and S, D5, D8, D11, D13-18, Week 9, FU to 24 months	Pre-S and S, BL check, D11, FU (6-12-18 months)	Pre-S and S, BL check, D5, D8, D11, D13-18, Week 9, FU (3-6-12-18 months)
Safety ECG (single)	Pre-S and S, BL check, for the fexinidazole group only: D2, D3, D4	BL, D11	Pre-S, S, BL check, D2, D3, D4, D11
ECG for QT/QTcF (triplicate)	BL, D4 (fexinidazole), D7 (NECT), D10	-	BL, D4, D10
Urine pregnancy test	BL (D-4 to D-1) D13-D18, FU at 3 months	BL (D-1), D11-18	BL, D13-18, FU (3 months)
Vital signs	Pre-S and S, BL, D5, D8, D11, D13-18, Week 9, FU to 24 months	Pre-S, S, BL, D5, D8, D11, D11-18, Week 9, FU (6-12-18 months)	Pre-S, S, BL, D5, D8, D11, D13-18, Week 9, FU (3-6-12 months)
Physical examination	BL, D5, D8, D11, D13-18, Week 9, FU to 24 months	BL, D5, D8, D11, D11-18, Week 9, FU (6-12-18 months)	BL, D5, D8, D11, D13-18, Week 9, FU (3-6-12 months)
Neurological/psychiatric examination	BL, D5, D8, D11, D13-18, Week 9, FU to 24 months	BL, D5, D8, D11, D11-18, Week 9, FU (6-12-18 months)	BL, D5, D8, D11, D13-18, Week 9, FU (3-6-12 months)
Hematology and biochemistry	Pre-S and S, BL, D5, D8, D11, Week 9, FU to 24 months	BL, D5, D11, FU (Week 9)	BL, D5, D8, D11, Week 9, FU (3-6-12-18 months)
Urine analysis	Pre-S, S, BL, D11	BL, D11	BL, D11

Source: Summary of Clinical Safety, Table 3
Abbreviations: BL, baseline; ECG, electrocardiogram; D, day; FU, follow-up; QTcF, Fridericia's correction formula; S, screening

Routine Clinical Tests

Please note the tables in the preceding section that highlight the schedule for obtaining safety data in the three g-HAT studies.

8.2.3. Safety Results

Deaths

Deaths in the three g-HAT trials are described below.

Study 004

There was an imbalance in the number of deaths occurring in each arm. There were 9/264 (3.4%) deaths in the fexinidazole arm and 2/130 (1.5%) deaths in the NECT arm. Though most deaths occurred after treatment was finished, two deaths occurred on treatment (Day 5 and Day 8) in the fexinidazole arm while none occurred on treatment in the NECT arm. No deaths were considered to be related to the study drug by the Investigator. Deaths (narratives and CRFs) were examined by this reviewer. Many of the deaths are unlikely to be related to study drug as they occurred much after treatment had been discontinued and other etiologies are more plausible. However, given the remote location of the trial sites, there was a significant lack of clarifying information (for example, what concomitant medications/herbals a patient may have ingested, circumstances surrounding the death, etc.), that made it difficult to come to any certainty regarding the cause of death for some patients.

Below are short narratives describing the deaths in the trial:

- Fexinidazole subject # 1: 45 y/o male who developed a malignancy of mandibular/buccal area 464 days after finishing treatment. The patient deteriorated despite surgery, medical treatment over next 6 months and then eventually died. This patient was considered a responder at 18 months post g-HAT treatment.
- Fexinidazole Subject # 2: 64 y/o male found to have alcohol intoxication then developed coma/shock 665 days after finishing treatment. Had good response to fexinidazole treatment as CSF WBC reduced from 184 cells/µL to 1 cell/µL.
- Fexinidazole subject # 3: 24 y/o male received an unknown traditional treatment orally, rectally for an “exorcism” ceremony on Day 7. Roughly 6 hours later was hypothermic and in shock and died on Day 8. Also received concomitant acetaminophen and metamizole for headache. At baseline, CSF WBC was 886 cells/µL, however patient had normal neurological exam on Day 5 visit. Because of lack of information, difficult to fully rule out fexinidazole role in death.
- Fexinidazole subject # 4: 42 y/o female who died 72 days after finishing treatment. Initially, had symptoms of pneumonia/influenza (fevers, chills, cough) and was treated by traditional healer due to concerns over “bewitchment.” Two weeks later, the patient returned to the village with dyspnea and weight loss and died a day later. In terms of g-HAT response, her CSF WBC was 46 cells/µL at baseline and dropped to 9 cells/µL on

Day 11. From screening to day 13 to 18 there was not much change in the patient's neurologic exam or signs and symptoms, and the patient was depressed on treatment.

- Fexinidazole subject # 5: A 56 y/o male who started having personality changes /hallucinations while on treatment and after treatment was completed. The subject fled the treatment center with hallucinations and behavioral changes but eventually was found and taken back to the treatment center. He was discharged a day later after supposedly improving on chlorpromazine, but the patient discontinued the medication on his own 2 to 3 days post discharge and took traditional medicine. Eventually the patient refused to eat or drink and then developed vomiting and fell into coma. He died 12 days after last dose of treatment. In terms of his g-HAT treatment, his CSF WBC at baseline was 586 cells/µL and it decreased to 33 cells µL by Day 11. As noted above, the patient had changes in his neurologic exam over the course of treatment (behavioral changes, some motor changes). Due to lack of clarity regarding concomitant medications, etc. cannot fully rule out role of fexinidazole in patient's death.
- Fexinidazole subject #6: 56 y/o male had behavior problems, upper limb tremors upon admission for g-HAT treatment. After treatment and discharge the patient still had residual tremors and behavior changes. His baseline CSF WBC was 176 cells µL which dropped to 17 cells/µL at his 6-month visit. At the 6-month visit, the patient's neurological exam and signs/symptoms were much improved from baseline. He had pulmonary TB diagnosed 9 months after g-HAT treatment. He started TB treatment but had tremors/ behavioral problems while on treatment and upon discharge 2 months later. A month after discharge for TB treatment, he eventually stopped eating and taking TB treatment and died (almost a year after fexinidazole treatment).
- Fexinidazole subject #7: 33 y/o female who had asthenia, fevers, tremors at baseline. Had worsening asthenia, tremors and several episodes of vomiting on Day 5 of fexinidazole treatment eventually leading to losing consciousness, dyspnea and death (possible aspiration pneumonia?). The patient's CSF WBC was 435 cells/µL at baseline. Because the death occurred on treatment and given the lack of key details surrounding patient's hospital course, cannot rule out fexinidazole's role in subject's death.
- Fexinidazole subject #8: 49 y/o male with hernia at baseline who died from strangulated hernia/sepsis 584 days after last dose of fexinidazole. CSF WBC dropped from 25 cells/µL at baseline to 1 cell/µL at the 12-month visit. At 18 months, CATT serology test and lymph node sample for trypanosomes were negative (was positive at baseline).
- Fexinidazole subject #9: 35 y/o male who appeared to respond to g-HAT treatment (had an improved neurologic exam with treatment); died 84 days later due to "population displacement due to military/political upheaval."
- NECT subject # 1: 52 y/o female with uncontrolled diabetes. Died from cardiac arrest 187 days after last NECT dose.
- NECT subject # 2: 55 y/o male died 9 days after finishing NECT treatment. Admitted in drowsy, malnourished, bedridden, not eating state. Despite treatment, patient overall remained impaired and would not eat. Discharged in this condition. Died 9 days after finishing treatment due to not eating/hypoglycemia. DSMB review stated patient did not meet eligibility criteria and should not have been enrolled.

Though not included in the official death tally for this study, there was a death of a baby born to a mother who had taken NECT and father who had taken fexinidazole. However, the pregnancy occurred 4 to 5 months after finishing study medication. The baby was born with spina bifida and died 2 months later. Also, one patient committed suicide before randomization into the study and before treatment could be administered.

Taken together, an increased mortality signal with fexinidazole (relative to NECT) cannot be excluded, particularly given the lack of adequate information surrounding several of the deaths, including deaths while on fexinidazole treatment.

Study 005

In this study there were four deaths in the ITT population, 3 in Stage 1 disease and one death in early Stage 2 disease. None were considered by the Investigator to be related to study drug.

As in Study 004, review of the narratives for these deaths are notable for a lack of pertinent diagnostic information, likely due to the resource poor settings of the trial sites. In general, the deaths took place much after the last dose of fexinidazole making any adverse event relation to study drug unlikely (however a lack of fexinidazole efficacy cannot be ruled out in some cases).

Brief narratives are provided below:

- Fexinidazole subject #1: 27 y/o woman who was treated with fexinidazole. The patient developed sepsis, nephropathy, anemia of unclear origin (possible malaria) over a year after finishing treatment and died 422 days after last treatment dose. Her 12-month visit had been unremarkable with no trypanosomes seen in the CSF and CSF WBC of 1 cell/µL.
- Fexinidazole subject #2: 15 y/o male who finished treatment but then died of severe meningeal/encephalitic syndrome of unclear origin 40 days after treatment was finished. No trypanosomes were seen in the CSF when he died but it's unclear if proper specimen was collected.
- Fexinidazole subject #3: 69 y/o male who developed confusion, hallucinations and went to a traditional healer around the time of the 6-month follow-up visit. Did not improve, had septic shock and died 191 days after last dose of fexinidazole.
- Fexinidazole subject #4: 35 y/o male who had underlying Crohn's disease and suffered from peritonitis due to multiple perforations both from underlying Crohn's disease as well as post-surgical perforations. Developed peritonitis 143 days after last fexinidazole dose and died almost a year after last fexinidazole dose.

Three other deaths occurred after completion of the study and were not included in the official death tally. One death occurred in a subject 511 days after last fexinidazole dose (Cardiogenic shock). Two deaths occurred in babies born to mothers who were exposed to fexinidazole though the exposure occurred well in advance of the time of conception.

Study 006

In study 006, a single death occurred in a 12 y/o female with likely underlying nephrotic syndrome who died 172 days after her last dose of fexinidazole. The death was likely a result of underlying renal disease as well as possible trauma around the time of death (hit 5 times with a cane for being late to school).

Serious Adverse Events

Study 004

A total of 73 SAEs were reported during this study, with a similar incidence in both groups: SAEs were reported in 31/264 patients (11.7%) in the fexinidazole arm and in 13/130 patients (10.0%) who received NECT. Most SAEs started after the treatment period (46/51 events and 20/22 events, respectively), and most were considered unrelated to treatment (47/51 events and 22/22 events, respectively). It is instructive to look at SAEs that occurred during treatment and hospitalization because this is when more exposure/direct consequences of the drug are expected. Overall, the SAEs that started during hospitalization included 13 events in 9 patients (3.4%) who received fexinidazole and 3 events in 3 patients (2.3%) who received NECT. A total of 7 of these SAEs were considered possibly related to treatment and were reported in three patients (1.1%) who received fexinidazole: personality change in two cases, acute psychosis, delirium, suicidal ideation, depression, and hyponatremia in the third case. Of note, SAEs occurring in the fexinidazole arm during the treatment/hospitalization time period were dominated by neuropsychiatric events. Examples are below:

- Fexinidazole Subject A: 51 y/o male with history of behavioral disorder, mood instability, hallucinations, aggressiveness prior to receiving medication threatened suicide 6 days after starting fexinidazole. He was treated with chlorpromazine and diazepam and improved.
- Fexinidazole Subject B: 44 y/o male with logorrhea and personality disorders at baseline which became worse on hospitalization including symptoms of crying, agitation, doing flips in bed and trying to assault medical staff. He was treated with diazepam and chlorpromazine and improved. He appeared to be fine on his follow-up visit 2 months later.
- Fexinidazole Subject C: 46 y/o female with no background of mental illness who developed anxiety, agitation, depressive symptoms, delusions, suicidal ideas while on treatment (but also had concomitant severe hyponatremia, gastritis, and hypertension). Her hospitalization was prolonged due to these symptoms though she eventually responded to haloperidol (hyponatremia was improved at this time as well).
- Fexinidazole Subject D: 21 y/o male who had worsening of psychomotor agitation that led to a prolonged hospitalization. He was given chlorpromazine and was better at 6-month follow-up.

Though many of these subjects may have had preexisting neuropsychiatric conditions, the apparent worsening of such symptoms on fexinidazole treatment as well as the lack of similar

findings in the NECT arm, point to the possibility of a neuropsychiatric safety signal with fexinidazole.

The table below shows all study 004 SAEs according to the time of their occurrence.

Table 99. SAEs by Timing of Onset, Study 004

Patient ID	Preferred term	Intensity	Start date – End date	Outcome
ALL SAEs THAT STARTED DURING TREATMENT: from Day 1 to Day 10 (before EOT)				
Fexinidazole group				
(b) (6)	Poisoning [d]	Severe – Grade 5	Day 8 – Day 8	Death
	Suicidal ideation [d]	Severe - Grade 3	Day 7 – Day 8	Recovered
	Personality change [a,b]	Severe – Grade 4	Day 9 – Day 31 [e]	Recovered
	Pneumonia aspiration	Severe – Grade 5	Day 5 – Day 5	Death
	Hyponatremia [a,c]	Severe – Grade 4	Day 5 – Day 55 [e]	Recovered
NECT group				
(b) (6)	Hyperkalemia	Severe – Grade 4	Day 5 – Day 10	Recovered
	Hypoglycemia	Severe	Day 7 – Day 7	Recovered
ALL SAEs THAT STARTED AFTER TREATMENT AND UP TO THE END OF HOSPITALIZATION (EOT to EOH)				
Fexinidazole group				
(b) (6)	Acquired immunodeficiency syndrome	Severe	Day 11 – Day 306	Recovered with sequelae
	Personality change [a,b]	Severe – Grade 4	Day 12 – Unknown [f]	Unknown [f]
	Psychomotor hyperactivity [d]	Moderate	Day 19 – Day 216 [g]	Recovered
	Respiratory tract infection	Moderate	Day 19 – Day 25 [g]	Recovered
	Acute psychosis [a,b]	Severe – Grade 4	Day 19 – Day 24 [g]	Recovered
"	Delirium [a,b]	Severe – Grade 4	Day 19 – Day 24 [g]	Recovered
"	Suicidal ideation [a,b]	Severe – Grade 4	Day 19 – Day 24 [g]	Recovered
"	Depression [a,b]	Severe – Grade 4	Day 19 – Day 24 [g]	Recovered
NECT group				
(b) (6)	Concussion	Moderate	Day 16 – Day 24	Recovered
ALL SAEs THAT STARTED AFTER HOSPITALIZATION AND UP TO THE 3-MONTH VISIT (Post-EOH to 3M)				
Fexinidazole group				
(b) (6)	Pneumonia [d]	Severe – Grade 5	Day 82 – Day 82	Death
	Influenza [d]	Severe – Grade 5	Day 82 – Day 82	Death
	Poisoning	Severe – Grade 5	Day 21 – Day 22	Death
	Gastritis	Severe – Grade 3	Day 47 – Day 51	Recovered
	Headache	Severe – Grade 3	Day 18 – Day 27	Recovered
	Psychotic disorder	Severe – Grade 4	Day 95 – Day 104	Recovered
	Lymphangitis	Moderate	Day 95 – unknown	Recovered
	Acute tonsillitis	Severe – Grade 3	Day 73 – Day 89	Recovered
	Gastritis	Severe – Grade 3	Day 28 – Day 31	Recovered
	Enteritis	Severe – Grade 3	Day 26 – Day 27	Recovered
	Death	Severe – Grade 5	Day 94 – Day 94	Death
NECT group				
(b) (6)	Hypoglycemia [d]	Severe – Grade 5	Day 19 – Day 19	Death
	Diabetes mellitus inadequate control	Severe – Grade 4	Day 46 – Day 46	Recovered
	Forearm fracture	Moderate	Day 46 – Unknown	Not recovered
	Forearm fracture	Moderate	Day 105 – Day 196	Recovered with sequelae
	Forearm fracture	Moderate	Day 106 – Day 182	Recovered with sequelae

Patient ID	Preferred term	Intensity	Start date – End date	Outcome
ALL SAEs THAT STARTED AFTER THE 3-MONTH VISIT AND UP TO THE 6-MONTH VISIT (Post-3M to 6M)				
Fexinidazole group				
(b) (6)	Diabetes mellitus	Severe – Grade 3	Day 106 – Day 194	Recovered with sequelae
NECT group				
(b) (6)	Dissociative disorder	Severe	Day 87 – Day 151	Recovered
	Pyramidal tract syndrome	Severe	Day 87 – Day 151	Recovered
ALL SAEs THAT STARTED AFTER THE 6-MONTH VISIT AND UP TO THE 12-MONTH VISIT (Post-6M to 12M)				
Fexinidazole group				
(b) (6)	Splenomegaly	Mild	Day 375 – Day 1002	Recovered with sequelae
	Gastroenteritis	Severe – Grade 3	Day 378 – Day 386	Recovered
	Pulmonary tuberculosis	Severe – Grade 4	Day 243 – unknown	Not recovered
	Starvation	Severe	Day 352 – Day 352	Death
	Hypoglycemia	Severe	Day 352 – Day 352	Death
	Meningeal disorder	Severe – Grade 3	Day 312 – Day 330	Recovered
	Malaria	Mild	Day 345 – Day 350	Recovered
	Hypertension	Severe – Grade 3	Day 397 – Day 405	Recovered
	Varicella	Moderate	Day 192 – Day 198	Recovered
	Adhesion [i]	Moderate	Day 263 – Day 272	Recovered
	Malaria	Severe	Day 288 – Day 292	Recovered
	Inguinal hernia	Severe – Grade 3	Day 215 – Day 229	Recovered
NECT group				
(b) (6)	Gastroenteritis	Severe	Day 210 – Day 214	Recovered
	Dehydration	Severe	Day 210 – Day 214	Recovered
	Uterovaginal prolapse	Moderate	Day 193 – Day 417	Recovered
	Femoral hernia	Moderate	Day 193 – Day 205	Recovered
	Appendicitis	Moderate	Day 193 – Day 194	Recovered
	Cardio-respiratory arrest	Severe – Grade 5	Day 197 – Day 197	Death
ALL SAEs THAT STARTED AFTER THE 12-MONTH VISIT AND UP TO THE 18-MONTH VISIT (Post-12M to 18M)				
Fexinidazole group				
(b) (6)	Pulmonary sepsis	Severe – Grade 4	Day 529 – Day 535	Recovered
	Septic shock	Severe – Grade 4	Day 529 – Day 535	Recovered
	Anemia	Severe – Grade 4	Day 529 – Day 535	Recovered
	Malaria	Severe – Grade 4	Day 529 – Day 535	Recovered
	Ameloblastoma [j]	Severe – Grade 5	Day 474 – Day 701	Death [j]
	HAT	Severe	Day 549 – Day 626	Recovered with sequelae
	Cerebral malaria	Severe	Day 549 – Day 626	Recovered with sequelae
	Rectocele	Moderate	Day 506 – Day 1077	Recovered
	Inguinal hernia	Moderate	Day 368 – Day 377	Recovered
	Appendicitis	Severe – Grade 3	Day 421 – Day 434	Recovered
	Gastroenteritis	Severe	Day 543 – Day 556	Recovered
"	Hypovolemic shock	Severe	Day 543 – Day 556	Recovered
NECT group				
None				

Patient ID	Preferred term	Intensity	Start date – End date	Outcome
ALL SAEs THAT STARTED AFTER THE 18-MONTH VISIT AND UP TO THE 24-MONTH VISIT (Post-18M to 24M)				
Fexinidazole group				
(b) (6)	Inguinal hernia, obstructive	Moderate	Day 663 – Day 675	Recovered
	Alcohol poisoning	Severe – Grade 5	Day 675 – Day 675	Death
	Inguinal hernia strangulated	Severe – Grade 5	Day 594 – Day 594	Death
	Inguinal hernia	Moderate	Day 769 – Day 781	Recovered
NECT group				
(b) (6)	Tibia fracture	Moderate	Day 962 – Day 1019	Recovered
	Excoriation	Moderate	Day 962 – Day 1019	Recovered
	Convulsion	Moderate	Day 770 – Day 845	Recovered
	Inguinal hernia	Moderate	Day 782 – Day 791	Recovered
	Malaria	Moderate	Day 699 – Day 702	Recovered
	Urinary tract infection	Moderate	Day 699 – Day 702	Recovered

Abbreviations: DBL=database lock; EOH=end of hospitalization; EOT=end of treatment; g-HAT= human African trypanosomiasis due to *T.b. gambiense*; ID=identification; ITT=intention-to-treat; M=month; MedDRA=Medical Dictionary for Regulatory Activities; SAE=serious adverse event; NECT=Nifurtimox-Eflornithine Combination Therapy.

a The event was considered as possibly related to treatment by the Investigator.
b The Sponsor agreed with the Investigator and considered the event as possibly related to treatment.
c The Sponsor disagreed with the Investigator and did not consider the event as possibly related to treatment.
d The event was considered as unrelated to treatment by the Investigator but possibly related to treatment by the Sponsor.
e The event occurred during the treatment period; however, the treatment was not discontinued.
f The outcome was declared as "unknown" in the clinical database; however, the patient died on Day 22 following an SAE of poisoning.
g The EOH visit was performed after Day 18 (minor protocol deviation).
h The SAE of acute psychosis in this patient was split into 4 SAEs of acute psychosis, delirium, suicidal ideation and depression in the follow-up CSR of Study DNDiFEX004.
i The event of adhesion refers to multiple adherences and absence of the left ovary in a patient who underwent exploratory laparoscopy. The laparoscopy followed a gynecological examination during which the uterus had been found to be of normal volume with a closed cervix and sensitivity on mobilisation of ancillaries.
j The AE of ameloblastoma started before the 18-month visit but death occurred after the 18-month visit.

Note: Day 1 was the first day of treatment. According to protocol, the EOT visit was planned on Day 11 or Day 12 and the EOH visit was planned between Day 13 and Day 18. In some patients, this visit was delayed (minor protocol deviation). Follow-up visits were held (relative to EOT) at 3 months (±1 week), 6 months (±2 weeks), 12 months (±4 weeks), 18 months (±4 weeks), and 24 months (±4 weeks).

A same patient can have events in the different timing categories. Causality was assessed by both the Investigator and the Sponsor as planned in the protocol: a "possibly related" AE was any event that was not considered as unrelated to the study treatment and/or for which there was no plausible alternative explanation.

Dictionary used MedDRA version 16.0.

Source: 5.3.5.1 Studies DNDiFEX004 1, 3 to 15 Study report body, Table 55 and DNDiFEX004 FU Appendix 16.2.7 Adverse event data, Listing F-16.2.7.2

Source: Summary of Clinical Safety, Table 28

Study 005

A total of 34 treatment-emergent SAEs were reported in 22/230 patients (9.6%) during the study (17 stage 1 g-HAT patients and 5 early stage 2 g-HAT patients). There were two SAEs that were felt to be related to fexinidazole -psychotic disorder and hyponatremia; both occurred in the time period from treatment initiation up to the EOH. Overall, the majority of preferred terms were reported in one patient only. Those reported in more than one patient were inguinal hernia, appendicitis, ovarian cyst and malaria (including cerebral malaria). However, none of these SAEs occurred during the hospitalization time period; they were spread throughout the period between hospital discharge and the 18-month follow-up. Review of the psychotic disorder event revealed similarities to such cases in study 004 (psychotic symptoms while on study drug which prolonged hospitalization and required treatment). Also of note, there was an SAE event of hyperglycemia that occurred during the hospitalization period that

was also accompanied by symptoms of psychosis for which haloperidol was needed. The hyponatremia event showed a significant decrease in sodium levels (and other electrolytes) from baseline and thus a relationship with fexinidazole could not be excluded.

Study 006

A total of 17 treatment-emergent SAEs were reported in 11/125 patients (8.8%). All preferred terms were reported in 1 patient, except the following: malaria (including cerebral malaria) and anemia. Only one SAE occurred between treatment initiation and end of hospitalization. This was a case of asymptomatic blood potassium increased in a patient which was thought to be possibly related to fexinidazole. The patient had a single potassium value of 7.9 mmol/L measured the day after treatment was finished, however this was not associated with symptoms and had normalized on follow-up 2 months later.

Please note that in the Phase 1 studies three SAEs were recorded that are generally in line with what was seen in the main g-HAT studies - two cases of anxiety and one case of transaminase elevation (discussed in section 8.2.4). Similarly, SAEs noted in ongoing pharmacovigilance studies are in line with what was seen in the completed g-HAT studies.

Dropouts and/or Discontinuations Due to Adverse Effects

Study 004

In the fexinidazole arm, two treatment discontinuations occurred, both due to patient deaths during treatment (on Day 5 and Day 8, respectively; discussed earlier). No permanent treatment discontinuations occurred in the NECT arm.

Study 005

There were no treatment discontinuations.

Study 006

There was one treatment discontinuation on Day 4 due to a mild episode of vomiting. The adverse event resolved that day and treatment finished on Day 11.

Significant Adverse Events

Please see the section below for a full discussion of treatment-emergent adverse events/reactions.

Treatment-Emergent Adverse Events and Adverse Reactions

Study 004

The two treatment arms were very similar in terms of incidence, severity type, and relatedness of TEAEs.

Table 100. Treatment-Emergent Adverse Events Description, Study 004

Description	Fexinidazole (N=264)	NECT (N=130)	All (N=394)
At least one AE	**247 (93.6%) [1528]**	**121 (93.1%) [608]**	**368 (93.4%) [2136]**
At least one TEAE	**247 (93.6%) [1528]**	**121 (93.1%) [608]**	**368 (93.4%) [2136]**
At least one TEAE during the treatment period	235 (89.0%) [1282]	116 (89.2%) [480]	351 (89.1%) [1762]
At least one TEAE after the treatment period	117 (44.3%) [246]	66 (50.8%) [128]	183 (46.4%) [374]
At least one TEAE resulting in treatment discontinuation (temporary or permanent)	2 (0.8%) [2]	3 (2.3%) [3]	5 (1.3%) [5]
At least one TEAE resulting in permanent treatment discontinuation	2 (0.8%) [2]	0 (0.0%) [0]	2 (0.5%) [2]
At least one mild or moderate TEAE	245 (92.8%) [1437]	117 (90.0%) [575]	362 (91.9%) [2012]
At least one severe TEAE	60 (22.7%) [91]	28 (21.5%) [33]	88 (22.3%) [124]
At least one TEAE possibly related to treatment	215 (81.4%) [926]	103 (79.2%) [345]	318 (80.7%) [1271]
At least one SAE	**31 (11.7%) [54]**	**13 (10.0%) [22]**	**44 (11.2%) [76]**
At least one TESAE	31 (11.7%) [54]	13 (10.0%) [22]	44 (11.2%) [76]
At least one TESAE during the treatment period	5 (1.9%) [5]	2 (1.5%) [2]	7 (1.8%) [7]
At least one TESAE after the treatment period	27 (10.2%) [49]	11 (8.5%) [20]	38 (9.6%) [69]
At least one TESAE resulting in treatment discontinuation (temporary or permanent)	2 (0.8%) [2]	1 (0.8%) [1]	3 (0.8%) [3]
At least one TESAE resulting in permanent treatment discontinuation	2 (0.8%) [2]	0 (0.0%) [0]	2 (0.5%) [2]
At least one TESAE possibly related to treatment	3 (1.1%) [7]	0 (0.0%) [0]	3 (0.8%) [7]
At least one TESAE resulting in death	9 (3.4%) [11]	2 (1.5%) [2]	11 (2.8%) [13]

Note: Data are presented as the number of subjects (percent of subjects) [number of events]. Dictionary used MedDRA version 16.0.

Causality was assessed by the Investigator as planned in the protocol: a "possibly related" AE was any event that was not considered as unrelated to the study treatment and/or for which there was no plausible alternative explanation. The Sponsor also assessed AE causality using this definition.

The intensity of adverse event was graded as mild (Grade 1), moderate (Grade 2), or severe (Grade 3-severe, Grade 4-life-threatening, Grade 5-death).

Text highlighted in grey corresponds to updated data.

AE = adverse event; ITT = intention-to-treat; MedDRA = Medical Dictionary for Regulatory Activities; SAE = serious adverse event; TEAE = treatment-emergent adverse event; TESAE = treatment-emergent serious adverse event; NECT = Nifurtimox-Eflornithine Combination Therapy.

Source: Table F-15.3.1.1.a.

Source: Study 004 fu clinical study report; Table 15

Almost all patients in both arms experienced a TEAE, and roughly a fifth of subjects in both arms experienced a severe TEAE. Similarly, roughly 80% of subjects in both arms experienced a TEAE considered to be possibly related to treatment. The sponsor table below highlights all the AEs experienced in at least 3% of either treatment arm in the ITT population by SOC and PT.

Table 101. TEAEs Occurring in at Least 3% of Either Treatment Arm by SOC and PT, Study 004

Description	Fexinidazole (N=264)	NECT (N=130)	All (N=394)
Any TEAEs	**247 (93.6%) [1528]**	**121 (93.1%) [608]**	**368 (93.4%) [2136]**
Nervous system disorders	**158 (59.8%) [308]**	**64 (49.2%) [117]**	**222 (56.3%) [425]**
Headache	92 (34.8%) [134]	32 (24.6%) [46]	124 (31.5%) [180]
Tremor	58 (22.0%) [68]	15 (11.5%) [16]	73 (18.5%) [84]
Dizziness	50 (18.9%) [56]	18 (13.8%) [23]	68 (17.3%) [79]
Convulsion	5 (1.9%) [5]	10 (7.7%) [14]	15 (3.8%) [19]
Extrapyramidal disorder	9 (3.4%) [9]	2 (1.5%) [2]	11 (2.8%) [11]
Gastrointestinal disorders	**157 (59.5%) [355]**	**64 (49.2%) [130]**	**221 (56.1%) [485]**
Vomiting	75 (28.4%) [101]	37 (28.5%) [46]	112 (28.4%) [147]
Nausea	68 (25.8%) [73]	26 (20.0%) [27]	94 (23.9%) [100]
Dyspepsia	34 (12.9%) [43]	10 (7.7%) [11]	44 (11.2%) [54]
Abdominal pain	25 (9.5%) [29]	16 (12.3%) [17]	41 (10.4%) [46]
Abdominal pain upper	27 (10.2%) [32]	6 (4.6%) [8]	33 (8.4%) [40]
Salivary hypersecretion	16 (6.1%) [17]	3 (2.3%) [3]	19 (4.8%) [20]
Constipation	13 (4.9%) [14]	2 (1.5%) [2]	15 (3.8%) [16]
Diarrhoea	8 (3.0%) [8]	5 (3.8%) [5]	13 (3.3%) [13]
Gastritis	8 (3.0%) [13]	2 (1.5%) [3]	10 (2.5%) [16]
Abdominal distension	8 (3.0%) [8]	0 (0.0%) [0]	8 (2.0%) [8]
Metabolism and nutrition disorders	**131 (49.6%) [190]**	**64 (49.2%) [95]**	**195 (49.5%) [285]**
Decreased appetite	56 (21.2%) [58]	24 (18.5%) [29]	80 (20.3%) [87]
Hyperkalaemia	27 (10.2%) [29]	25 (19.2%) [26]	52 (13.2%) [55]
Hypocalcaemia	36 (13.6%) [37]	3 (2.3%) [3]	39 (9.9%) [40]
Hyponatraemia	20 (7.6%) [21]	15 (11.5%) [18]	35 (8.9%) [39]
Hypoalbuminaemia	23 (8.7%) [24]	4 (3.1%) [4]	27 (6.9%) [28]
Hyperglycaemia	9 (3.4%) [9]	9 (6.9%) [9]	18 (4.6%) [18]
General disorders and administration site conditions	**122 (46.2%) [184]**	**51 (39.2%) [85]**	**173 (43.9%) [269]**
Asthenia	60 (22.7%) [73]	19 (14.6%) [23]	79 (20.1%) [96]
Pyrexia	23 (8.7%) [27]	24 (18.5%) [27]	47 (11.9%) [54]
Feeling hot	25 (9.5%) [29]	3 (2.3%) [4]	28 (7.1%) [33]
Chest pain	23 (8.7%) [25]	5 (3.8%) [5]	28 (7.1%) [30]
Chills	4 (1.5%) [4]	12 (9.2%) [12]	16 (4.1%) [16]
Gait disturbance	12 (4.5%) [13]	2 (1.5%) [2]	14 (3.6%) [15]
Psychiatric disorders	**103 (39.0%) [162]**	**23 (17.7%) [31]**	**126 (32.0%) [193]**
Insomnia	74 (28.0%) [83]	15 (11.5%) [17]	89 (22.6%) [100]
Agitation	10 (3.8%) [14]	1 (0.8%) [1]	11 (2.8%) [15]
Psychotic disorder	7 (2.7%) [7]	4 (3.1%) [5]	11 (2.8%) [12]
Anxiety	10 (3.8%) [10]	0 (0.0%) [0]	10 (2.5%) [10]
Musculoskeletal and connective tissue disorders	**58 (22.0%) [92]**	**21 (16.2%) [29]**	**79 (20.1%) [121]**
Back pain	30 (11.4%) [36]	12 (9.2%) [15]	42 (10.7%) [51]
Neck pain	23 (8.7%) [27]	7 (5.4%) [8]	30 (7.6%) [35]
Blood and lymphatic system disorders	**29 (11.0%) [33]**	**18 (13.8%) [19]**	**47 (11.9%) [52]**
Anaemia	24 (9.1%) [25]	14 (10.8%) [14]	38 (9.6%) [39]
Description	**Fexinidazole (N=264)**	**NECT (N=130)**	**All (N=394)**

Respiratory, thoracic and mediastinal disorders	**31 (11.7%) [32]**	**11 (8.5%) [18]**	**42 (10.7%) [50]**
Cough	16 (6.1%) [16]	6 (4.6%) [6]	22 (5.6%) [22]
Vascular disorders	**24 (9.1%) [26]**	**9 (6.9%) [10]**	**33 (8.4%) [36]**
Hot flush	13 (4.9%) [13]	4 (3.1%) [4]	17 (4.3%) [17]
Hypertension	12 (4.5%) [12]	1 (0.8%) [2]	13 (3.3%) [14]
Infections and infestations	**22 (8.3%) [33]**	**8 (6.2%) [10]**	**30 (7.6%) [43]**
Skin and subcutaneous tissue disorders	**22 (8.3%) [23]**	**8 (6.2%) [9]**	**30 (7.6%) [32]**
Pruritus	10 (3.8%) [11]	4 (3.1%) [5]	14 (3.6%) [16]
Injury, poisoning and procedural complications	**15 (5.7%) [18]**	**14 (10.8%) [20]**	**29 (7.4%) [38]**
Procedural pain	7 (2.7%) [7]	9 (6.9%) [9]	16 (4.1%) [16]
Cardiac disorders	**18 (6.8%) [21]**	**7 (5.4%) [10]**	**25 (6.3%) [31]**
Palpitations	13 (4.9%) [16]	5 (3.8%) [5]	18 (4.6%) [21]
Renal and urinary disorders	**13 (4.9%) [16]**	**7 (5.4%) [8]**	**20 (5.1%) [24]**
Eye disorders	**15 (5.7%) [18]**	**3 (2.3%) [3]**	**18 (4.6%) [21]**
Investigations	**7 (2.7%) [8]**	**11 (8.5%) [11]**	**18 (4.6%) [19]**

Note: Data are presented as the number of subjects (percent of subjects) [number of events]. Dictionary used MedDRA version 16.0.
Text highlighted in grey corresponds to updated data.
ITT = intention-to-treat; MedDRA = Medical Dictionary for Regulatory Activities; SOC = system organ class; TEAE = treatment-emergent adverse event; NECT = Nifurtimox-Eflornithine Combination Therapy.
Source: Table F-15.3.1.2.a.

Source: Study 004 fu Clinical Study Report; Table 16

Among SOCs, there was a marked increased incidence of TEAEs in the fexinidazole arm relative to NECT in Psychiatric Disorders, Nervous System Disorders, and Gastrointestinal Disorders. Interestingly, though in Psychiatric Disorders, the difference was driven primarily by the PT insomnia, there was a general tendency toward a higher incidence of AEs in the fexinidazole arm related to PTs referencing mood disorders and psychosis as well (agitation, anxiety, personality change, hallucination, etc.).

The TEAEs occurring in the fexinidazole arm with at least a 10% incidence were: headache, tremor, dizziness, vomiting, nausea, dyspepsia, abdominal pain upper, decreased appetite, hyperkalemia, hypocalcemia, asthenia, insomnia, back pain.

While TEAEs occurring at high incidences are instructive, they may also be confounded by background disease symptoms or may represent AEs common to many medications. In an effort to identify AEs that might be unique to fexinidazole (and thus be useful for labelling), it is instructive to look at AEs occurring both at a high incidence in the fexinidazole arm and at a comparatively lower incidence in the NECT arm. AEs occurring in at least 5% of fexinidazole subjects and at a 3% or greater incidence than the NECT arm are as follows: headache, tremor, dizziness, nausea, dyspepsia, abdominal pain upper, salivary hypersecretion, hypocalcemia, hypoalbuminemia, asthenia, chest pain, feeling hot, insomnia, neck pain.

It is also useful to look at TEAEs which Investigators/Sponsor felt could be possibly related to the study drug. Such TEAEs that occurred in the fexinidazole arm at an incidence ≥ 5% include insomnia, decreased appetite, hyperkalemia, hypocalcemia, chest pain, feeling hot, pyrexia, asthenia, dizziness, tremor, headache, vomiting, nausea, dyspepsia, abdominal pain upper, and salivary hypersecretion.

Study 005

The AE profile in study 005 was similar to that of 004. AEs that occurred at a level ≥ 10 % in ITT subjects were: nausea (43%), vomiting (42.2%), dyspepsia (20.0%) headache (40.4%), dizziness (25.2%), tremor (22.6%), asthenia (30.4%), feeling hot (16.5%), insomnia (24.8%), and decreased appetite (19.1%). Without a control arm, the findings must be interpreted with caution.

Study 006

As with Study 005, the AE profile in study 006 was similar to that of 004 . AEs that occurred at a level ≥ 10 % in ITT subjects were: vomiting (68.8%), nausea (37.6%), salivary hypersecretion (14.4%), abdominal pain (12.0%), headache (38.2%), tremor (19.2%), asthenia (31.2%), decreased appetite (19.2%), and anemia (11.2%). Similarly, without a control arm, the findings must be interpreted with caution.

In terms of a dose response with regard to AEs, comparisons between the different weight-based doses are likely not useful because the exposure in subjects is likely to be similar. There was a small dose finding study in healthy volunteers (study 003, see Table 93, where the larger dose was discontinued due to concerns about tolerability. Also, some dose effects were noticeable in the larger safety database, particularly as relates to hepatotoxicity and neutropenia (see section 8.2.4).

Laboratory Findings

Generally, laboratory parameters such as hematology, biochemistry and urinalysis values were obtained just before and at the end of treatment with a subset of subjects also having repeat labs done at week 9. Mean/median shifts from baseline in laboratory parameters were reviewed for studies 004, 005 and 006. Likewise, pertinent laboratory-related adverse events were reviewed in more detail.

Study 004

In general, no significant mean/median changes from baseline were noted in hematology parameters (please note that neutropenia as an AESI is discussed in more detail in Section 8.2.4); shift tables also did not show any findings of clinical significance. Similarly, no significant changes from baseline were noted for biochemistry parameters (changes in transaminases related to potential hepatotoxicity as an AESI are discussed in more detail in Section 8.2.4); shift tables also did not shown findings of any significance. As regards urinalysis findings, no real differences were noted between the fexinidazole and NECT arms.

Study 005

Similar to Study 004, there was nothing of significance seen in mean/median changes from baseline for hematology and biochemistry parameters. Few clinically significant values (as judged by the Investigator) were noted at EOT or at week 9. Since this was an uncontrolled study, these findings must be interpreted with caution.

Study 006

Similar to Study 005, there was nothing of significance seen in mean/median changes from baseline for hematology and biochemistry parameters. Few clinically significant values (as judged by the Investigator) were noted at EOT or at week 9. Since this was an uncontrolled study these findings must be interpreted with caution.

Adverse Events Related to Laboratory Parameters

In studies 004, 005, 006, there were several AEs (anemia, hyperkalemia, hypocalcemia, hyponatremia, and hypoalbuminemia) related to laboratory parameters that occurred at moderately increased levels and are discussed in more detail below.

Anemia

In study 004, the TEAE "anemia" occurred at an incidence of 24/264 (9.1%) in the fexinidazole arm and at an incidence of 14/130 (10.8%) in the NECT arm; it occurred at an incidence of 3% in study 005 and 11.2% in study 006. In study 004, the median change in hemoglobin (g/dL) from baseline to EOT was -.05 g/dL; in the NECT arm there was no change. When looking at severity classification (mild, moderate, severe, life threatening, resulting in death), there was no real difference between the two arms. When looking at shifts from baseline to the worst possible value in terms of toxicity grade (CTCAE) for hemoglobin, incidences of shifts of two or more grades from baseline were similar between the two arms. Overall, these findings do not suggest a significant difference from current control treatment.

Hyperkalemia

In study 004, the TEAE "hyperkalemia" occurred at an incidence of 27/264 (10.2%) in the fexinidazole arm and at an incidence of 25/130 (19.2%) in the NECT arm; it occurred at an incidence of 4.8% in study 005 and 6.4% in study 006. In study 004, the median change in potassium (mmol/L) from baseline to EOT in the fexinidazole arm was -.40 mmol/L; in the NECT arm the median change was -.30 mmol/L. When looking at severity classification (mild, moderate, severe, life threatening, resulting in death), incidences of higher severity cases were slightly increased in the NECT arm. Overall, these findings do not suggest a significantly worse safety signal from current control treatment.

Hyponatremia

In study 004, the TEAE "hyponatremia" occurred at an incidence of 20/264 (7.6%) in the fexinidazole arm and at an incidence of 15/130 (11.5%) in the NECT arm; it occurred at an incidence of 7.4% in study 005 and 0.8% in study 006. In study 004, the median change in sodium (mmol/L) from baseline to EOT was 1.0 mmol/L; in the NECT arm the median change was also 1.0 mmol/L. When looking at severity classification (mild, moderate, severe, life threatening, resulting in death), incidences of higher severity cases were slightly increased in the fexinidazole arm (severe 2.3% versus 0.8% for fexinidazole versus NECT, respectively), though there were more moderate cases in the NECT arm (moderate 4.9% versus 10.8%,

respectively). Overall, however, these findings do not suggest a significantly worse safety signal from current control treatment.

Hypocalcemia

In study 004, the TEAE "hypocalcemia" occurred at an incidence of 36/264 (13.6%) in the fexinidazole arm and at an incidence of 3/130 (2.3%) in the NECT arm; it occurred at an incidence of 0.4% in study 005 and 2.4% in study 006. In study 004, the median change in calcium (mg/dL) from baseline to EOT was 0 mg/dl in the fexinidazole arm; in the NECT arm the median change was.30 mg/dL. When looking at severity classification (mild, moderate, severe, life threatening, resulting in death), incidences of higher severity cases were increased in the fexinidazole arm, particularly instances of moderate severity (12.5% versus 2.3%, fexinidazole versus NECT, respectively). Overall, these findings suggest that fexinidazole treatment may be associated with hypocalcemia relative to NECT treatment (as assessed by a practitioner).

Hypoalbuminemia

In study 004, the TEAE "hypoalbuminemia" occurred at an incidence of 23/264 (8.7%) in the fexinidazole arm and at an incidence of 4/130 (3.1%) in the NECT arm; it occurred at an incidence of 3.0% in study 005 and 0.8% in study 006. In study 004, the median change in albumin (g/dL) from baseline to EOT in the fexinidazole arm was.20 g/dL; in the NECT arm the median change was.40 g/dL. When looking at severity classification (mild, moderate, severe, life threatening, resulting in death), all cases in both arms were classified as moderate. Overall, these findings suggest that fexinidazole treatment may be associated with hypoalbuminemia relative to NECT treatment (as assessed by a practitioner), however it should be noted that at baseline >40% of subjects in both arms reported hypoalbuminemia in their medical history (47% of subjects in the fexinidazole arm and 41.5% of subjects in the NECT arm).

Vital Signs

Study 004

There were no significant mean/median changes in temperature, heart rate, systolic blood pressure and diastolic blood pressure seen for the fexinidazole arm. There were, however, more AEs for "hypertension" for fexinidazole.

Study 005

There were no significant mean/median changes in temperature, heart rate, systolic blood pressure, and diastolic blood pressure seen with fexinidazole.

Study 006

There were no significant mean/median changes in temperature, heart rate and systolic blood pressure seen with fexinidazole. There was a slight median increase in diastolic blood pressure seen (+10 mm Hg) during and after fexinidazole treatment].

Electrocardiograms (ECGs)

Please note the QT discussion below

QT

QT-IRT consultation was obtained to evaluate fexinidazole's potential for a prolonged QT effect. The consultation concluded that the ECG safety database was adequate to characterize QT prolongation effects and that a TQT study was not needed. Though the consultation noted no effect of fexinidazole on PR or QRS intervals, the drug (and particularly the M2 metabolite) did appear to have a QT prolonging effect as well as increasing chronotropy. The findings are summarized below.

Fexinidazole administration was associated with a dose and concentration-dependent increases in the QT interval over the range of doses evaluated in this QT assessment. The QTc prolongation appears to be associated with concentrations of fexinidazole's M2 metabolite. Based on the exposure-response relationship, significant QT prolongation is expected at the maximum therapeutic dose (under fed condition) with the mean ΔΔQTcF of 17.2 (90% CI upper: 20.0) msec. The expected high clinical exposure scenario has not been identified during the development, and the intrinsic and extrinsic factors that can increase the concentrations of M2 metabolite are expected to further increase the QTc interval.

The effect of fexinidazole was evaluated in healthy subjects using data from 2 clinical studies (Studies # DNDiFEX001 and DNDiFEX003). Study # DNDiFEX001 was a randomized, double-blind, placebo-controlled study evaluating the safety, tolerability, and pharmacokinetics following single- (Part-I) and multiple (Part-III) ascending doses of fexinidazole under fasting condition. Study # DNDiFEX003 was a randomized, double-blind, placebo-controlled study evaluating the safety, tolerability, and pharmacokinetics following multiple ascending doses of fexinidazole under fed condition. The highest dose that was evaluated was 2400 mg once daily (Day 1 to 4), which covers the therapeutic exposures.

The data were analyzed using exposure response analysis as the primary analysis, which suggest that fexinidazole is associated with significant QTc prolonging effect. The reviewer's analysis indicated that QTc prolongation was associated with concentrations of M2 metabolite. This finding is also supported by the sponsor's nonclinical evaluation, which indicated that M2 has higher potential for blocking the hERG channel compared to the parent and its M1 metabolite. The central tendency analysis of QTcF also indicates that the administration of fexinidazole is associated with an increase in QTcF in both healthy subjects. Thus, the findings of exposure response analysis are further supported by the available nonclinical data, by-time analysis, and categorical analysis. Because the highest concentrations are expected on Day 4 during the proposed 10-day dosing regimen, the predicted QT effects are presented for the peak M2 concentrations on Day 4 following maximum therapeutic dose of 1800 mg once daily under fed condition.

Table 102. The Point Estimates and the 90% CIs

ECG Parameter	Treatment	Concentration* (ng/mL)	ΔΔQTcF (msec)	90% CI (msec)
QTc	1800 mg	19700	17.2	14.3 to 20.0

Source: FDA Analysis
Administered as 1800 mg once daily for 4 days followed by 1200 mg once daily for 6 days.
*Peak concentrations of M2 metabolite on Day 4; For further details on the FDA analysis
Abbreviations: CI, confidence interval; ECG, electrocardiogram; QTc, QT interval correct; QTcF, Fridericia's correction formula

In a pooled analysis of trials conducted in the g-HAT population (DNDiFEX004 and DNDiFEX006), 2 patients had QTc >500 msec and 7 patients had an increase from baseline >60 msec. There were no adverse events suggesting arrhythmia per ICH E14 guidelines (i.e., torsades, significant ventricular arrhythmias or sudden cardiac death).

The consultant also noted that there was a concentration dependent increase in heart rate and the magnitude of mean effect on heart rate at the proposed therapeutic doses is below 10 bpm

The consultation proposed labelling changes based on the ECG findings above.

Immunogenicity

As this product is a small molecule and not a monoclonal antibody, immunogenicity findings were not discussed.

8.2.4. Analysis of Submission-Specific Safety Issues

Hepatotoxicity

The sponsor has proposed adding a labelling warning related to fexinidazole's potential for hepatotoxicity. This concern stems primarily from a hepatoxicity episode in a patient receiving 3600 mg of fexinidazole in a Phase 1 study as well as several instances of liver function test elevations in a completed Chagas disease study. It should be noted that though some synthetic nitroimidazoles, such as ornidazole, have been associated hepatotoxicity, currently there is no such labelling in the nifurtimox label).

As regards the Phase 1 study finding, one subject receiving 3600 mg of fexinidazole x 14 days had significant transaminase elevation (30 x ULN for ALT (1666 UL); 38X ULN for AST) 2 days (Day 16) after finishing treatment. No elevation in bilirubin was noted and values returned to normal by Day 45. Hy's Law criteria was not met and there were no signs of hepatic insufficiency (patient was moderately symptomatic initially with nausea, vomiting, and headache but was asymptomatic from Day 16 onwards). Workup for other etiologies of the liver injury was negative including hepatitis A/B/C serologies, autoantibodies, and parasitological exam of the feces, and fexinidazole was suspected as a possible etiology.

In the completed Phase 2 CD trial, several dosing regimens (1200 or 1800 mg fexinidazole /day for 2, 4, or 8 weeks) was compared to placebo. The fexinidazole exposures for CD ranged higher than for g-HAT. 16 subjects were noted to have hepatic injury, with a mix of occurrences during and after treatment. Elevations during treatment were generally mild, with no cases of transaminases > 8X ULN (ALT > 42 for males, ALT > 32 for females, AST > 37 for males, AST > 31

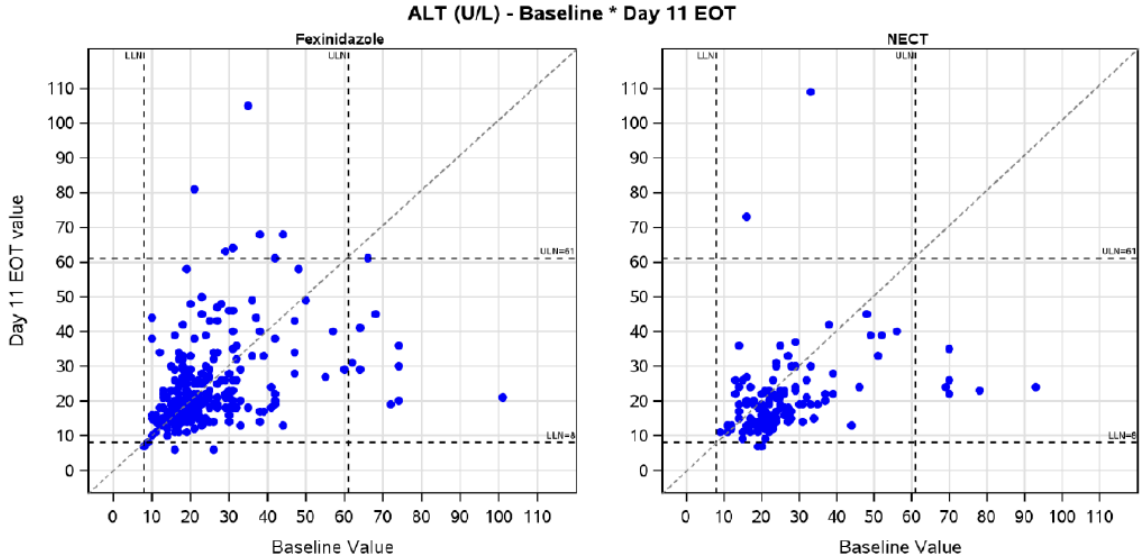
for females) as well as no Hy's Law cases. However, after discontinuation of treatment, ALT elevations up to 15 X ULN were noted and in 8 patients, liver test abnormalities persisted beyond 3 months after the discontinuation of treatment. All eight cases were asymptomatic and exhibited both hepatocellular and mixed/cholestatic patterns. No chronic liver safety finding was observed with cumulative doses <12.6 g.

Exposure/response studies conducted by the sponsor suggested that increased fexinidazole dose and duration correlated with transaminase increases.

For Studies 004, 005, and 006, biochemical laboratory testing was primarily done during treatment. Once the findings from the CD study were noted, a week 9 visit was added (including labs) for patients who had yet to reach this visit date. Thus, week 9 labs were obtained in a subset of patients in all three studies. Labs at further follow-up visits (12 months, 18months, etc.) were rare and at the discretion of the Investigator.

In Study 004, no AEs related to transaminase elevations were noted though 23 subjects were noted to have an AE of hypoalbuminemia (of note, the majority of subjects had low albumin levels at baseline). In general transaminase elevations in the fexinidazole arm were mild, isolated and occurred both during and after treatment. Many of the elevations were reversible though evaluation of reversibility is limited by the limited follow up in most patients. No Grade 2 or higher CTCAE Tox values of ALT, AST, or total bilirubin were obtained in the fexinidazole arm. At the EOT visit, only a single subject had a value > 100 IU/L for ALT (as was the case with AST). No subject had an AST/ALT value > 5 X ULN (ULN being defined as roughly 60 IU/L) and the mean/median change in transaminase values from baseline to EOT were comparable to what was seen in the NECT arm. Similar findings were noted at Week 9. Please note the sponsor scatter plot below.

Figure 4. Scatter Plot From Baseline to EOT, Change in ALT Levels (U/L) During Treatment, Study 004



Note: Baseline was defined as Inclusion visit, or Screening visit if Inclusion visit was missing.

Reference range was 8 – 61 U/L.

ALT = alanine aminotransferase; EOT = end of treatment; ITT = intention-to-treat; LLN = lower limit of normal; ULN = upper limit of normal; NECT = Nifurtimox-Eflornithine Combination Therapy.

Source: Figure 15.3.10.e.

The table below shows a comparison of transaminase changes in the controlled and uncontrolled studies during treatment. As can be noted, in the controlled trial (study 004), generally findings were modest in both arms though the incidence of such changes were slightly more in the fexinidazole arm compared to NECT.

Table 103. Liver Function at Baseline and EOT According to CTCAE Grade for Liver Tests on All Available Data by Study, ITT Population, g-HAT Pooled Analysis

Parameter	Visit	CTCAE grade	DNDiFEX004 NECT (N=130)	DNDiFEX004 Fexi (N=264)	DNDiFEX005 Fexi (N=230)	DNDiFEX006 Fexi (N=125)	All Fexinidazole (N=619)
ALT n (%)	Baseline	]ULN; 3*ULN]	6 (4.6)	10 (3.8)	7 (3.0)	7 (5.6)	24 (3.9)
		]3*ULN; 5*ULN]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Day 11 (EOT)	]ULN; 3*ULN]	2 (1.5)	7 (2.7)	2 (0.9)	1 (0.8)	10 (1.6)
		]3*ULN;5*ULN]	0 (0.0)	0 (0.0)	1 (0.4)	0 (0.0)	1 (0.2)
		]5*ULN;20*ULN]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
AST n (%)	Baseline	]ULN; 3*ULN]	7 (5.4)	13 (4.9)	21 (9.1)	16 (12.8)	50 (8.1)
		]3*ULN; 5*ULN]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	Day 11 (EOT)	ULN; 3*ULN]	2 (1.5)	14 (5.3)	3 (1.3)	3 (2.4)	20 (3.2)
		]3*ULN; 5*ULN]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
ALP n (%)	Baseline	>1.5*ULN	0 (0.0)	2 (0.8)	5 (2.2)	6 (4.8)	13 (2.1)
	Day 11 (EOT)	>1.5*ULN	0 (0.0)	2 (0.8)	5 (2.2)	8 (6.4)	15 (2.4)

Abbreviations: ALP=alkaline phosphatase; ALT=alanine aminotransferase; AST=aspartate aminotransferase; CTCAE = Common Toxicity Criteria for Adverse Events; EOT=end of treatment; Fexi=fexinidazole; g-HAT=human African trypanosomiasis due to *T.b. gambiense*; ITT=intent-to-treat; NECT = nifurtimox-eflornithine combination therapy; ULN=upper limit of normal.
Source: 5.3.5.3 Pooled safety analysis, Table 14.3.2.19.a

Source: Summary of Clinical Safety, Table 39

In the small subgroup of patients in study 004 (around 57 subjects) who had Week 9 data, there were no transaminase abnormalities in either arm. In the uncontrolled studies, of the subset of subjects who had Week 9 evaluations, only a small number had transaminase evaluations and all were between ULN-3XULN range.

In Study 005, there was only a single AE related to transaminase elevation. This was a Grade 2 elevation (ALT 251 IU/L at EOT) in a 37 year old Stage 1 g-HAT patient whose values normalized by week 9. The subject had multiple mild to moderate adverse events during hospitalization including headache, dizziness nausea, vomiting, and epigastric pain. The ALT elevation was considered nonserious but possibly related to study drug; the patient recovered. All other values were below 100 IU/L during treatment. At week 9, the highest ALT/AST value was 101 and 121 IU/L, respectively. No subject had an AST/ALT value > 5 X ULN.

In Study 006, there were no AEs related to transaminase elevations; there was one AE related to increased bilirubin. Through Week 9, the highest ALT value noted was 67 IU/L; for AST the highest value noted was 158 IU/L. No subject had an AST/ALT value > 5 X ULN.

Taken together, though a potential liver safety signal was noted at higher doses and durations in non g-HAT studies, the results from the three g-HAT trials essentially show more benign changes. Though caution is warranted in circumstances where increased concentrations of fexinidazole are possible (hepatic impairment, pertinent drug-drug interactions, etc.), a labelled warning for hepatotoxicity for this indication/proposed treatment regimen is likely not indicated.

Of note, there is no mention of hepatotoxicity in the current labeling for metronidazole and nifurtimox. In the current benzinidazole labeling (indicated for treatment of CD), there is mention of transaminase elevation in section 6.

Neutropenia

The sponsor has proposed labelling that warns of the risk of neutropenia associated with fexinidazole treatment. This risk stems primarily from what was observed in the completed CD study. In this study 8/40 (20%) subjects had an SAE of neutropenia (<1000 cells/µL), 4 patients in the high dose group (1800 mg/day) and 4 patients in the low dose (1200 mg/day) group. The eight events of neutropenia were assessed as related to the study medication by the Investigator and Sponsor and were all resolved without sequelae. These events occurred 8 to 51 days post-treatment, with a consistent nadir between Day 63 to 70, irrespective of date of end of treatment. Eight subjects reached a nadir < 1000 cells/µL and two subjects reached a nadir <500 cells/µL. Patients remained asymptomatic and hematological abnormalities were reversible without specific treatment. The high incidence of neutropenia reported contributed to the premature termination of the study, and the immediate discontinuation of fexinidazole from any patient who had received treatment for over 2 weeks. No case of CTCAE Grade 3 or 4 neutropenia was observed in this study with cumulative dose < 25.2 g or treatment duration <

14 days. Please note the sponsor table below highlighting the eight subjects with an SAE of neutropenia.

Table 104. Serious Neutropenia Events Assessed as Related to Fexinidazole in Patients With Chagas Disease, Study DNDiCHFEXI001

Patient ID	Group	PT	Severity [a]	ANC at Day 0 (cells/µL)	Actual duration of exposure (days)	Study day with ANC nadir	ANC at nadir (cells/µL)	Time to return to Grade 2[a] (days)
(b) (6)	HD-8w	Neutropenia	Grade 4	6732	56	64	200	7
	LD-8w	Neutropenia	Grade 4	3024	28	66	345	9
	HD-4w	Neutropenia	Grade 3	2958	20	64	928	8
	HD-2w	Neutropenia	Grade 3	2750	15	64	744	6
	LD-8w	Neutropenia	Grade 3	3315	30	79	532	5
	HD-4w	Neutropenia	severe	3220	28 [b]	64	530	6
	LD-8w	Leukopenia	Grade 3	-				
		Neutropenia	Grade 3	2110	50	70	720	30
	LD-8w	Neutropenia	Grade 3	2660	23	64	560	6
		Leukopenia	Grade 3	-				

Abbreviations: ANC=absolute neutrophil count; HD=high dose (1800 mg/day); ID=identification; LD=low dose (1200 mg/day); PT=preferred term, w=weeks

a Severity assessed with Common Toxicity Criteria for Adverse Events (CTCAE) grade

b 5 days of interruption

Source: 5.3.5.4 DNDiCHFEXI001, Section 14.3.3.1

Compared to the CD study, the doses and duration of fexinidazole treatment used in the g-HAT studies were significantly lower and shorter. In the g-HAT studies, few neutropenia AEs (12/619; 2%) were observed and no cases resulted in infectious complications; only five cases occurred on treatment. In study 004, the lowest neutrophil value seen while on fexinidazole treatment was 760 cells/µL and changes in absolute neutrophil levels from baseline to EOT did not significantly differ from the NECT arm (please note that current nifurtimox labelling describes neutropenia as a possible AE however it is indicated for CD treatment, the duration of which is recommended to be 60 days). Similar findings were noted in the subset of subjects who had lab measurements taken at Week 9. Only one subject was noted to have an AE of neutropenia in the fexinidazole arm while there were two such subjects in the NECT arm. In Study 005, six subjects had an AE of neutropenia (mostly in Stage 1 subjects) and all recovered. In study 006, neutropenia was reported in five subjects, 4 of which occurred during hospitalization and 4 of which were considered possibly related by investigators. All subjects recovered.

As was noted in the table above, the neutropenia findings in the CD study generally occurred at week 9 and generally involved significant drops from baseline in ANC of > 2000 cells/µL. In study 004, 15 fexinidazole subjects (5.7%) and 4 NECT subjects (3.1%) had an ANC < 1000 cells/µL at any time post baseline. In the three main g-HAT studies there were only a handful of fexinidazole subjects who had both an ANC <1000 cells/µL at week 9 and a drop > 2000 cells/µL from baseline.

In study 004, the mean drop in ANC was negligible for both arms at EOT. In the small subset of subjects with week 9 data, there was a drop of roughly 600-800 cells/µL from baseline to week 9 in both arms; the decrease was slightly more in the fexinidazole arm. Unsurprisingly, starting with a lower baseline ANC was associated with a higher incidence of neutropenia (<1,000 cells/µL) at any time post baseline.

When looking at subjects in study 004,005, and 006, it is instructive to focus on subjects who had ANC < 1000 cells/µL at any point post baseline and for whom baseline and week 9 data were available. Please note the table below highlighting such subjects.

Table 105. Change in Absolute Neutrophil Count in Fexinidazole Subjects With ANC < 1000 Cells/µl at Any Time Post Baseline and Also With Baseline and Week 9 Data, Studies 004, 005, 006

Subject ID	Arm (004) or Stage of Disease (005/006)	Baseline ANC (cells/µL)	EOT ANC (cells/µL)	Week 9 ANC (cells/µL)	Change From Baseline to Week 9 (cells/µL)
Study 004					
FEX004 (b) (6)	Fexinidazole	3250	4000	900	-2350
FEX004	Fexinidazole	1876	4582	980	-896
FEX004	Fexinidazole	1232	1056	819	-413
FEX004	Fexinidazole	3780	1036	2187	-1593
FEX004	NECT	3212	875	2064	-1148
Study 005					
FEX005	Stage 1	1216	1404	715	-501
FEX005	Stage 1	1800	1650	702	-1098
FEX005	Stage 1	1665	270	925	-740
FEX005	Stage 1	2090	2156	731	-1359
FEX005	Early Stage 2	2205	2756	950	-1255
FEX005	Stage 1	1444	2499	900	-544
FEX005	Stage 1	1596	1989	952	-644
FEX005	Stage 1	2805	960	2058	-747
FEX005	Stage 1	1296	735	924	-372
FEX005	Stage 1	2511	560	2340	-171
FEX005	Early Stage 2	1540	4472	4560	3020
FEX005	Stage 1	1520	1643	960	-560
FEX005	Stage 1	697	924	1292	595
Study 006					
FEX006	Stage 1	4539	1980	945	-3594
FEX006	Stage 1	1848	945	1512	-336
FEX006	Early Stage 2	6042	2496	546	-5496
FEX006	Late Stage 2	2730	567	1824	-906
FEX006	Early Stage 2	1200	2262	2236	1036
FEX006	Stage 1	420	1974	1650	1230
FEX006	Stage 1	2871	11748	1640	-1231
FEX006	Early Stage 2	924	1200	1920	996
FEX006	Stage 1	1170	1768	2204	1034

Source: Analysis performed by Statistical Reviewer using sponsor analysis and tabulation datasets
Note: All subjects in table had ANC <1000 µL at some point post baseline and did not have to be at EOT or Week 9
Abbreviations: ANC, absolute neutrophil count; EOT, end of treatment

Though it does appear that neutropenia may occur with the proposed g-HAT fexinidazole regimen, the degree of change in ANC appears to be less severe than what was noted in the CD

study. Given this, it is not clear that a Warning in section 5 of labelling is warranted, listing of the AE in Section 6 of labelling may suffice.

Of note, in the current benzinidazole and metronidazole labeling, neutropenia/lekuopenia is listed as a warning/precaution. As noted earlier, in the nifurtimox labeling, neutropenia is listed in section 6.

Neuropsychiatric Adverse Events

The sponsor has proposed adding labeling language related to fexinidazole's potential for eliciting significant neuropsychiatric events. This is a result of such effects noted in fexinidazole trials for other indications and also due to imbalances in such effects in the pivotal g-HAT trial (study 004). Of note, nifurtimox labelling contains a warning for worsening of neurological and psychiatric conditions.

In the completed CD study, a 36-year-old female with no personal and family history of mental illness was randomized to 1200 mg fexinidazole qd x 4 weeks. Treatment was prematurely discontinued after 18 days and never recommenced (though the patient had missed several days of medication during this period). Two days after discontinuation, the subject was noted to have symptoms of severe depression including thoughts of death, social isolation, lack of appetite, and insomnia. The result of a psychiatric evaluation was a recommendation for hospitalization, but this was not done; the patient was started on an antidepressant and antipsychotic medication. One month later the subject attempted to commit suicide by ingesting lithium. The subject was taken to a hospital and received a gastric lavage, and she was discharged on antidepressant medication. Though again multiple recommendations were made for psychiatric hospitalization, this was not done. 7 days after the initial suicide attempt, the patient died after a second attempt (hanging).

Overall, in this CD study, 70% of subjects reported an AE classified under the Psychiatric Disorders SOC including insomnia, depression, and anxiety. Few of these events were SAEs (such as the case listed above) and most cases were considered by the investigator to be related to the study drug.

Regarding the pivotal trial, study 004, as noted earlier, more AEs were noted in the fexinidazole arm relative to the NECT control for both the Nervous System Disorders and Psychiatric Disorders SOC.

As regards the Nervous System Disorders SOC, the fexinidazole arm had a noticeably increased incidence of such AEs as headache, tremor, dizziness relative to the NECT arm and smaller increases in the fexinidazole arm for such AEs as paresthesia and extrapyramidal disorder. As regards the Psychiatric Disorders SOC, notable increases in the fexinidazole arm were seen for such AEs as insomnia as well as AEs representing mood and psychotic changes such as agitation, anxiety, abnormal behavior, depression, hallucination, and personality change. Two subjects in the fexinidazole arm were also noted to have an SAE suicidal ideation. Only a few of these AEs were considered to be serious and most were considered to be mild/moderate in severity, however most were considered to be related to study drug.

Overall, there does appear to be a safety signal regarding increased neuropsychiatric AEs with fexinidazole and caution should be taken in prescribing the drug to subjects with a history of such AEs. Also, patients, caregivers, and practitioners should be aware that these type of AEs can occur with fexinidazole treatment and act accordingly (discontinuing/switching medication, hospitalization, neuropsychiatric consultation, etc.) should they occur.

8.2.5. Clinical Outcome Assessment (COA) Analyses Informing Safety/Tolerability

This was not applicable to this application.

8.2.6. Safety Analyses by Demographic Subgroups

These studies were conducted in endemic areas of disease in two sub-Saharan countries. Thus, analyses by race and country/site location are not relevant. Moreover, very few subjects in these trials were elderly as defined by the Agency (≥65 years old), and thus a subgroup analysis of this age group is not possible.

Study 004

In the fexinidazole arm, slight differences in gender were noted for the AEs of vomiting (incidence 36% females versus 24% males) and insomnia (22% females versus 32% males). No difference in gender was noted for the overall occurrence of AEs.

In terms of BMI in the fexinidazole arm, asthenia was noted to occur at a higher incidence in those with BMI <18.5 kg/m^2 (28%) versus those with a BMI between 18.5 to 25 kg/m^2 (19%). No difference was noted in the overall occurrence of AEs by BMI.

There was no difference in AE incidence of renal function among groups in the fexinidazole arm (using the Cockcroft Gault classifications of normal (≥80 mL/min); mild (≥50 to <80 mL/min); moderate (≥30 to <50 mL/min); severe renal insufficiency (<30 mL/min).

Interstudy Comparisons

For analyzing incidence of AEs among those with a different g-HAT stage at baseline, it is useful to look at a comparison of AE incidences between study 004 and 005 given that study 004 was comprised of late stage 2 g-HAT patients and study 005 was primarily comprised of stage 1 patients (over 80% of subjects were stage 1). In study 004, the five AEs occurring with the highest incidence in the fexinidazole arm were headache (35%), insomnia (28%), vomiting (28%), nausea (26%), and asthenia (23%). In study 005, the AEs occurring most frequently were nausea (43%), vomiting (42%), headache (40%), asthenia (30%) and dizziness (25%). Therefore, the AE profile was generally similar between the two studies, although the incidence of nausea and vomiting was higher in study 005.

In terms of looking at AE incidence by age it is instructive to look at a comparison between study 005 and study 006, as both were primarily comprised of stage 1/ early stage 2 disease but 005 had primarily adults (over 80% of subjects ≥18 years old) while study 006 had all children <

15 years old. As noted above, in study 005 the AEs occurring with the highest incidence were nausea (43%), vomiting (42%), headache (40%), asthenia (30%) and dizziness (25%). In study 006, the AEs occurring with the highest incidence were vomiting (69%), nausea (38%), headache (33%), asthenia (31%), and tremor and decrease appetite (both 19%). While the adverse event profile is similar between the two studies, it is notable that vomiting occurred at significantly higher incidence in study 006.

8.2.7. Specific Safety Studies/Clinical Trials

The Phase 1/pharmacokinetic studies were reviewed. Study 001 (all three parts), study 002, study 003, study 008, and study INT 15307 had similar adverse event profiles to the three g-HAT studies.

8.2.8. Additional Safety Explorations

Human Carcinogenicity or Tumor Development

Given that the planned fexinidazole dosing regimen is for 10 days only, no effect on tumor development risk is expected. Among the three g-HAT studies, one AE of ameloblastoma was recorded (which developed over a year after treatment was finished) and one AE of uterine leiomyoma was recorded. Both AEs were felt to be unrelated to study drug.

Human Reproduction and Pregnancy

There was no exposure to fexinidazole in pregnant women during treatment in studies 004, 005 and 006. In study 005, there were 11 cases of exposure before pregnancy. Follow-up investigations after delivery revealed that the babies were developing normally except in two cases. In both of these cases, however, exposure to fexinidazole is estimated to have occurred at least 70 days prior to start of pregnancy, thus any complications were not thought to be related. During a CD study (DNDiCHFEXI001), seven cases of maternal exposure before pregnancy were reported . All of these pregnancies were uncomplicated with normal outcome; none of these patients were exposed to treatment during pregnancy; the shortest time between start of pregnancy and last dose was 61 days.

In the ongoing 009 g-HAT study, breastfeeding patients could be enrolled as well as patients who had entered their second trimester of pregnancy. The Applicant's review of the pharmacovigilance database for this trial at the cut-off date of September 7, 2019 retrieved four cases of exposure to fexinidazole during the 2nd and 3rd trimester of pregnancy. All pregnancies were uncomplicated with normal newborns. In an ongoing CD study, three cases of maternal exposure before pregnancy were noted. All were normal except 1 case of induced abortion in a woman whose pregnancy started 287 days after EOT.

In terms of breastfeeding, in the ongoing 009 g-HAT study, at the time of the data cut-off for this submission, 17 case reports of child exposure during breastfeeding were retrieved. Among

them, 2 children exposed via lactation during the 10-day treatment of the mother [patient] were reported with 3 SAEs during the post-treatment follow-up of the mother.

- Patient (b) (6) (mother): the 10-month-old female child was exposed to fexinidazole via breastfeeding during 10 days of study treatment of the mother. During the post-treatment follow-up period, an SAE of malaria complicated by anemia was reported 15 days after mother's last study drug dose, leading to child's death 17 days after EOT. The Investigator and the Sponsor assessed this SAE as not related to fexinidazole.
- Patient (b) (6) (mother): the female breastfed child of 8 months of age was exposed to fexinidazole during the 10 days of study drug administration to the mother. During the follow-up period, two SAEs of malaria and anemia occurred in the child 5 months after mother's last study drug dose, leading to child's death while being transferred to hospital. The Investigator and the Sponsor assessed the both SAEs as not related to fexinidazole.

Taken together, no clear risk to pregnant and breastfeeding women from fexinidazole has been demonstrated, though pregnancy and breastfeeding exposure data, particularly while on treatment, is limited.

Pediatrics and Assessment of Effects on Growth

The 006 study was conducted in pediatric subjects six years of age and older; efficacy and safety of the drug in this population is described earlier in the document.

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

Drug abuse potential with fexinidazole is not expected given its planned controlled distribution. As noted earlier, certain AEs may be dose- and duration-dependent including hepatotoxicity, neutropenia risk, and prolonged QT incidence. Transaminase elevation and worsened tolerability (nausea, vomiting, headache, postural dizziness, panic attack) with higher dosing was noted in Phase 1 trials, including a subject receiving a 3600 mg daily fexinidazole dose (for 14 days) and in a cohort of subjects receiving 2400 mg daily (for 4 days followed by maintenance dosing for 6 days), respectively. A pediatric g-HAT patient in study 006 received the higher dosing regimen(1800/1200 mg) instead of the lower dosing regimen (1200/600 mg) as adjusted for their body weight as per protocol. At inclusion the patient presented with HAT symptoms of headache, pruritus and weight loss; blood potassium and calcium were normal. The patient experienced TEAEs of vomiting over the first 5 days of treatment and increased potassium and decreased calcium levels were observed from Day 11 to Week 9.

8.2.9. Safety in the Postmarket Setting

Safety Concerns Identified Through Postmarket Experience

At the safety cut-off date for the NDA of September 7, 2019, fexinidazole had not yet been launched/distributed on the DRC market. Therefore, information on postmarketing safety is not available.

Expectations on Safety in the Postmarket Setting

The safety concerns noted to date for fexinidazole are not necessarily unique and do not necessitate the development of a REMS. Safety issues can be adequately addressed in labelling and through routine pharmacovigilance. Moreover, the Applicant is planning for a controlled distribution of fexinidazole in the US, and it will not be available in warehouses and pharmacies. Usage of fexinidazole in the United States will be coordinated among the Applicant, CDC, and prescribing physician.

Note: The sponsor provided a 120 Day safety update covering the 6 month period from the cutoff date of the Summary of Clinical Safety (Sept. 8, 2019 to March 31st, 2020). Essentially the data reviewed came from the published literature of fexinidazole and similar class products as well as several ongoing fexinidazole studies:

- Study DNDi-FEX-09-HAT in any stage g-HAT patients ≥6 years old, conducted in DRC and Guinea.
- Study DNDi-FEX-07-HAT in patients ≥6 years old and ≥20 kg with any stage r-HAT, conducted in Uganda and Malawi
- Study DNDi-FEX-12-CH in patients with chronic indeterminate CD, conducted in Spain.

The safety profile generated from the update was similar to that already generated with the original g-HAT trials and discussed above. Of note, follow up of four female subjects from study 009 who had been exposed to fexinidazole during pregnancy found that all four delivered healthy newborns.

8.2.10. Integrated Assessment of Safety

The safety of fexinidazole has been evaluated through a combination of Phase 1 trials, trials in other indications, and through studies done in the proposed g-HAT indication. Although there is

limited controlled data (primarily coming from study 004), the safety database is sufficiently large that an overall safety profile can be generated.

In terms of serious safety issues, with adequate product labeling, there is no particular safety issue that would preclude approval of the drug in the context of this serious, fatal disease. In fact, many of the adverse events noted for fexinidazole are similar to those of currently used treatments for g-HAT. It should be noted that given the serious nature of the proposed indication, with a high likelihood of death/significant morbidity in the absence of treatment for g-HAT, there is a somewhat lower threshold for the acceptability of drug-related adverse events provided they can be adequately described in product labeling.

There is an imbalance in deaths with fexinidazole relative to NECT treatment, however the lack of a clear, direct relationship with the study drug in most cases makes this finding difficult to interpret. Moreover, given the efficacy findings it is likely that fexinidazole will be used as a second line agent for more severe stage 2 g-HAT, puts its safety findings relative to NECT in the proper context. Lastly, though fexinidazole use may have an imbalance in death relative to NECT, this must be viewed in the prism of possible complications associated with IV administration /hospitalization with NECT treatment.

The most serious issue identified to date involves the potential for neuropsychiatric complications with treatment. This may span from insomnia to mood disorders to psychotic events. The Applicant proposed that all subjects presenting with a significant psychiatric medical history or presenting with neuropsychiatric manifestations of g-HAT be monitored as an inpatient and receive fexinidazole as directly observed therapy due to the potential for these complications. In the opinion of this reviewer, however, such monitoring does not need to be explicitly labelled as most g-HAT patients in the United States are likely to be closely monitored by the healthcare providers. It is more important that labelling adequately informs both patient/caregivers and the prescribing physician of the possibilities of these complications so appropriate decisions can be made regarding which patients should be treated with fexinidazole. With adequate labelling, such complications can be anticipated and mitigated as necessary (through regular, daily contact between the practitioner and the patient/caregivers, etc.).

Prolonged QT potential has been identified as a safety issue. The QT-IRT consult provided labeling language both warning of the potential for this risk and avoiding drug exposure in patients particularly at risk for this adverse event. The consultation also noted a potential trend toward increased heart rate with fexinidazole.

While a final agreement regarding product labeling could not be reached, the Applicant proposed warnings for neutropenia and hepatotoxicity based upon findings in other treatment indications and Phase 1 studies that utilized greater fexinidazole exposures than recommended for the treatment of g-HAT. Since these events were not significant in the g-HAT trials, the Agency recommended modified warning language to indicate the potential risk for hepatotoxicity and neutropenia.

In terms of the general tolerability of fexinidazole, AEs such as nausea, abdominal pain, and headache appeared to occur with fexinidazole more frequently than with NECT in the

controlled trial. Moreover, vomiting appears to occur significantly with fexinidazole treatment and may occur soon after administration of the drug; it may also occur more in particular subgroups such as pediatric patients. Patients should be instructed of the possibility for such AEs and managed supportively. Though effects on electrolytes and laboratory parameters are not generally significant with fexinidazole, the possibility for such effects, such as hypocalcemia and hypoalbuminemia, should be recognized and can be monitored through usual laboratory testing before and after treatment.

8.3. Statistical Issues

Trial DNDiFEX004 was unblinded due to the use of oral and IV treatments, oral fexinidazole, oral nifurtimox and intravenous eflornithine for NECT. The two uncontrolled trials were not blinded due to the lack of controls. There were no blinding procedures for technicians to do the laboratory tests. Investigators were not blinded. However, CSF samples were analyzed by two laboratory technicians (and by the head of mobile team or the laboratory supervisor if readings were inconsistent). If the CSF WBC count was between 19 and 22 cells/µL, inclusive, the WBC count had to be confirmed by the supervisor. In February 2015, each site was supplied with a new microscope, equated with a video-captured camera for the detection of trypanosomes and for CSF WBC counting (Protocol Amendment 4). This should have minimized the biases in CSF WBC counting. Lack of blinding was the main limitation of trial DNDiFEX004. The lack of a control arm was the main limitation for trials DNDiFEX005 and DNDiFEX006.

FDA's noninferiority guidance requires that the active control be a drug whose effect is well-defined. We believe the control's efficacy is well-defined through clinical investigations and the noninferiority margin of 13% used for trial DNDiFEX004 is justified. NECT is not FDA-approved; however, it is referenced by the World Health Organization (WHO) as a combination treatment for HAT and adequate information existed on its efficacy in order to justify an NI margin. Therefore, NECT was an acceptable comparator for an NI assessment.

Based on the NI criteria, fexinidazole was noninferior to NECT in DNDiFEX004. However, fexinidazole was significantly less effective than NECT, as the upper limit of the CI for the difference in success proportion excluded 0. In addition, mortality was numerically higher in the fexinidazole group than in the NECT group (3.4% [9/264] versus 1.5% [2/130]). Based on our analyses, the decreased efficacy and increased mortality were limited to subjects who had more severe disease at baseline, as defined by a WBC count in CSF > 100 cells/µL.

8.4. Conclusions and Recommendations

The noninferiority of fexinidazole compared to NECT was demonstrated in an adequate and well-controlled trial. The trial also indicated that fexinidazole's efficacy was inferior to NECT. Supporting data to extend the indication to include stage 1 disease and pediatric patients relied on two historically-controlled single arm trials; while randomized, comparative data are preferred, a comparison to less effective historical regimens was considered a conservative approach since untreated g-HAT is a fatal disease. Furthermore, a drug with efficacy in patients with neurological disease (stage 2) would be expected to be effective for the treatment of

patients with less advanced hemolymphatic disease (stage 1). For pediatric patients, extrapolation of efficacy from an adult population is reasonable since the characterstics of the disease and response to therapy would be expected to be similar.

The decrement in efficacy and increased mortality could be addressed through product labeling; however, at the current time an agreement on labeling could not be reached with the Applicant. A limitation of use statement and a warning are needed in order to avoid the use of fexinidazole in patients with severe second stage g-HAT, unless there are no other treatment options. Product labeling would be sufficient to address the potential safety risks since the product is intended for use in patients with a rare, life-threatening tropical infectious disease that is likely to be treated by specialized healthcare providers (HCPs) in the U.S. such as infectious disease physicians rather than general HCPs, and the severity of disease would be expected to be recognized. Fexinidazole is an orally administered monotherapy for both stages of g-HAT, as compared to the NECT regimen, which contains an injectable (eflornithine). Fexinidazole may provide a treatment option for patients who cannot tolerate NECT, or for patients who lack vascular access.

In general, the safety profile of fexinidazole was similar to NECT for the treatment of g-HAT. Certain adverse reactions were associated with fexindazole use, such as neuropsychiatric adverse reactions, prolonged QT interval, and tolerability issues (nausea and vomiting). Hepatotoxicity and neutropenia were reported with fexinidazole exposures greater than recommended for the treatment of g-HAT. These risks Warnings and Precautions and Adverse Rreactions sections of labeling. Once the product labeling is remediated, emerging safety signals could be assessed through pharmacovigilance activities. Additional safety data and clinical pharmacology data could be collected through postmarketing requirements to address patients with hepatic insufficiency, drug-drug-interations, and use during pregnancy.

Due to lack of agreement with the Applicant on product labeling, the NDA will receive a complete response during this review cycle.

9. Advisory Committee Meeting and Other External Consultations

This product was not discussed at an Advisory Committee meeting as no specific input from the committee or external consultants was needed.

10. Pediatrics

As this drug has orphan designation for this indication, no pediatric studies are required under the Pediatric Research and Equity Act (PREA). However, pediatric patients were studied in all three g-HAT studies, particularly in study 006 which enrolled only pediatric patients. The product is being approved for children 6 years of age and older who weigh at least 20 kg.

11. Labeling Recommendations

11.1. Prescription Drug Labeling

Prescribing Information

The Division has proposed a number of changes to the PI in accordance with federal regulations and the findings of this review. Notable proposals include:

1. Revising section 4 (Contraindications) and section 5 (Warnings and Precautions] of the PI. In particular, language describing fexinidazole's decreased efficacy in patients with severe stage 2 g-HAT disease has been proposed as well as alternative language describing the risk of prolonged QT events (in accordance with recommendations from QT-IRT consultation). Warnings and Precautions for neutropenia and hepatotoxicity have been retained but revised significantly to reflect the limited information available from the g-HAT studies. A contraindication in patients with hepatic impairment was added to section 4 (contraindications).
2. A limitation of use statement was recommended for Section 1 (Indications and Usage) to emphasize the lower efficacy of fexinidazole in patients with severe second stage HAT.
3. The Division has made recommendations to revise section 6 of the PI. In particular, the proposed AE table should be revised to only include information from the controlled trial (study 004) and relevant AE rates should be listed according to incidence alone (regardless of whether the AE was considered to be related to the investigator). Recommendations have been made regarding how best to decide which AEs are relevant to include in the table itself.
4. The Clinical Studies section (Section 14) indicates that the determination of efficacy of fexinidazole was supported by the noninferiority trial DNDiFEX004. Section 14 has been

revised to better describe fexinidazole's efficacy in both less severe and more severe patients with stage 2 g-HAT disease, including that the decreased efficacy and increased mortality appeared to be limited to patients who had higher CSF-WBC at baseline. Language describing the supportive efficacy findings from study DNDiFEX004 and DNDiFEX005 have been revised as well.

5. Sections 12.1 and 12.4 were revised based on the best labeling practice and included pathogens that are relevant to the indication.
6. (b) (4) the Division recommended deleting the following (b) (4)
7. In Section 6.1- Clinical Trials Experience, under the subheadings for Specific Adverse Reactions, Vomiting, the Division recommended including information on the following aspects based on the vomiting experience from the clinical trials:
   The observed vomiting rates along with the information on the timing of vomiting occurrence with respect to the timing of fexinidazole tablets administration.
   Criteria that were used to select patients for redosing in an event of vomiting.
8. The Division recommended presenting the drug-drug interactions information in Section 7 Drug Interactions using a tabular format.
9. The Division recommended presenting the pharmacokinetics information in a tabular format in Section 12.3 Pharmacokinetics.
10. The content and format of the Applicant proposed labeling language in Sections 7 and 12.3 was not aligned with the current Clinical Pharmacology labeling guidance. The Division has recommended multiple revisions to these specific sections so that they are aligned with the Clinical Pharmacology labeling guidance.
11. Nitroimidazole carcinogenicity information was added to section 13.1. Carcinogenicity was not added to section 5 Warnings and Precautions because there were no data to support it and it was not considered to be clinically relevant for this acute treatment indication for a life threatening disease. (see Pharmacology/Toxicology section).

12. Risk Evaluation and Mitigation Strategies (REMS)

No REMS are recommended; risk mitigation could be adequately achieved with labelling, routine pharmacovigilance, as well the planned controlled distribution by the Applicant within the United States (no distribution to warehouses or pharmacies; distribution in conjunction with CDC). The proposed distribution model is as follows:

13. Postmarketing Requirements (PMRs) and Commitment (PMC)

The Applicant agreed to conduct the following studies as PMRs:

1. Conduct a clinical drug-drug interaction (DDI) study to evaluate the effect of repeat doses of Fexinidazole Tablets on the pharmacokinetics of oral midazolam, which is a cytochrome P450 (CYP) 3A4 sensitive drug substrate. Design and conduct this DDI trial in accordance with the FDA Guidance for Industry titled: "Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry." Submit all datasets and the associated bioanalytical reports with the final clinical study report. It is anticipated that the results from this study will inform the Fexinidazole tablets labeling and/or potentially other CYP3A4 drug product labeling regarding the coadministration of Fexinidazole tablets with CP3A4 drug substrates.

The rationale for this PMR is because fexinidazole was shown to be an inhibitor of hepatic CYP3A4 based on findings from in vitro studies and mechanistic model-based analysis. Specifically, the predictions from the mechanistic model-based analysis demonstrated that the PK exposure of CYP3A4 drug substrates may be significantly increased by fexinidazole, and therefore, may lead to increased risk of adverse reactions associated with coadministration of these drugs. Therefore, a clinical drug-drug interaction (DDI) study is needed to evaluate the effect of repeat doses of Fexinidazole tablets on the PK of the sensitive CYP3A4 substrate drug, midazolam. The findings from this DDI study will assess if CYP3A4 drug substrates may be safely coadministered with Fexinidazole tablets and are also expected to inform the Fexinidazole tablets labeling and/or potentially other CYP3A4 drug product labeling regarding the coadministration of Fexinidazole tablets with CP3A4 drug substrates.

2. Conduct a clinical pharmacokinetic (PK) study to evaluate the PK of fexinidazole and the M1 and M2 metabolites in subjects with hepatic impairment who fall in the Child-Pugh (CP) categories of mild (CP-A) and moderate (CP-B) impairment as defined in the FDA Guidance for Industry titled "Pharmacokinetics in Patients with Impaired Hepatic Function: Study Design, Data Analysis, and Impact on Dosing and Labeling." Design and conduct the study in accordance with the abovementioned FDA Guidance for Industry. Submit the datasets and the associated bioanalytical reports with the final clinical study report. The results from this study are expected to inform product labeling regarding the use of and/or need to adjust the dosing regimen of Fexinidazole tablets in patients with hepatic impairment.

The rationale for this PMR is because the PK of fexinidazole and its pharmacologically active M1 and M2 metabolites in subjects with hepatic impairment is unknown. Fexinidazole is extensively metabolized by multiple CYP450 enzymes in the liver and hepatic metabolism and/or excretion likely accounts for a substantial portion of the elimination of parent drug and/or M1 and M2 metabolites. M2 has relatively higher systemic exposure compared to fexinidazole or M1, and therefore, is likely playing a key role in the drug's desired pharmacological activity. The biotransformation pathway of M1 to M2 has not been completely characterized. In addition,

M2 plasma concentrations have also been associated with increased risk of QTc interval prolongation. Therefore, a PK study in subjects with hepatic impairment is warranted to determine if alterations in PK exposure to fexinidazole, M1, and/or M2 are observed and if dosage adjustment of fexinidazole tablets is needed in hepatic impairment.

3. Submit the results of your current worldwide descriptive study that is collecting prospective and retrospective data in women exposed to fexinidazole during pregnancy to assess risk of pregnancy and maternal complications, adverse effects on the developing fetus and neonate, and adverse effects on the infant.

The goal of this study is to evaluate the long-term safety of fexinidazole in women exposed during pregnancy, including assessing risks of pregnancy complications and adverse effects on the developing fetus and neonate. Data are needed on the safe use of Fexinidazole during pregnancy as there is currently no available human data to inform the safety of Fexinidazole during pregnancy.

The Applicant agreed to the following PMC:
Develop a procedure for the (b) (4) and establish it as a first GMP step in the manufacturing process for fexinidazole drug substance.

14. Division Director (Clinical) Comments

I agree with the review team's assessment and recommendations.

15. Office Director Comments

I agree with the review team's assessment and recommendations.

16. Appendices

16.1. References

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16.2. Financial Disclosure

Covered Clinical Studies (Name and/or Number): DNDiFEX004, DNDiHATFEX005, DNDiHATFEX006, DNDi-CH-FEXI-001, and FEXI VL 001

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 50		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 1		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 2		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: 0 Significant payments of other sorts: 0 Proprietary interest in the product tested held by investigator: 0		

Significant equity interest held by investigator in S Sponsor of covered study: 0		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☒	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☒	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) 5		
Is an attachment provided with the reason:	Yes ☒	No ☐ (Request explanation from Applicant)

The Applicant submitted financial disclosure information for 50 clinical investigators covering studies 004, 005, 006 as well as the completed CD study (CH-001) and the completed VL study (VL-001). Only two investigators were found to have disclosable interests.

(b) (6), the study coordinating investigator participating in studies 004, 005, and 006 received financial arrangements (b) (6) The period on which these costs have been calculated is (b) (6)

- The total amount related to the participation to international congresses is **6843 USD**.
- The total amount related to the participation to trainings is **7276 USD**.

(b) (6) the principal investigator participating in the completed (b) (6)

- The total amount related to the (b) (6) is **31219 USD.**

There were 5 investigators for whom financial disclosure information was not available. Form FDA 3454 with box 3 selected was submitted for these individuals. Investigators with missing financial disclosure information were listed with reasons given for the missing information. Two investigators were from the g-HAT studies. One investigator had died and the other investigator could not be found.

Taken together, these disclosures are not expected to have impacted the conduct of the study or integrity of the study findings in a meaningful way.

16.3. OCP Appendices (Technical Documents Supporting OCP Recommendations)

Note: *The following is the summary of individual study reports that supports the OCP review. The conclusions drawn by the Applicant are listed at the end of individual study summary. The Applicant's conclusions are found to be reasonable by the Reviewer unless noted otherwise in a Reviewer's Assessment section.*

16.3.1. Summary of Bioanalytical Method Validation and Performance

Table 106. Bioanalytical Method Validation and Performance Findings

Method/Report	Findings	
Studies: DNDiFEX001 Parts 1, 2, and 3		
Analyte/assessment	Fexinidazole, Fexinidazole sulfoxide (M1), Fexinidazole sulfone (M2)	
Method	LC-MS/MS	
Matrix	Human plasma (Lithium heparin)	
Validation report	Validation report provided: 0341-2008, B081263-1, and B081263-2 Notes: Report 0341-2008 was transferred to a different analytical site, which re-evaluated method under Report B081263-1. Report B0812363-1 expanded analytical range for fexinidazole to 5 to 1000 ng/mL. Methods validated under Report B081263-2 were adapted from Report R0444-2008 and were used to support quantification of analytes in human urine.	☒ Yes ☐ No
	Validation report acceptable Range for plasma samples: 0.5-50 ng/mL (Low range) and 5-1000 ng/mL (High Range) for Fexinidazole, 10-5000 ng/mL for metabolites M1 and M2. Dilution integrity evaluated at factor of 20. Range for urine samples: 5-500 ng/mL for fexinidazole, 100-50000 ng/mL for metabolites M1 and M2	☒ Yes ☐ No
Performance report	Samples analyzed within the established stability period Notes: Exact information on the sample storage duration prior to analysis could not be located. Notes to file under the Clinical Study Reports of all three parts of the study note "All specimens, calibration and QC samples were analyzed within the documented stability period of 105 days at -20"C and at -80"C in human plasma (ref)." The noted stability information is from Study 0058-2009.	☒ Yes ☐ No
	Quality control (QC) samples range acceptable Plasma QC samples: 1.5, 15, 40 ng/mL (Low range) and 15,300,800 ng/mL (High range) for fexinidazole 30, 1500, 4000 ng/mL for metabolites M1 and M2 Urine QC samples: 15, 150, 400 ng/mL for fexinidazole and 300, 15000, 40000 ng/mL for metabolites M1 and M2	☒ Yes ☐ No
	Chromatograms provided Notes: The provided chromatograms are not identified, and notations are not legible.	☒ Yes ☐ No

Method/Report	Findings	
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred sample reanalysis (ISR) acceptable: Notes: Current FDA guidance document on bioanalytical methods validation recommends that at least 10% of the first 1000 samples and the 5% of the remaining samples should be subjected to reanalysis. This report reanalyzed only 42 samples from approximately 3000 samples that were analyzed, i.e., ~1.4% samples. ISR findings noted significant deviation range up to 85% for urine samples with 7/21 (33%) samples with deviation >20% including four samples with no parent compound detected in urine during ISR. It is typically recommended that at least 66% of the investigated ISR sample results should be within 20% based on the respective mean of the first and second result.	☐ Yes ☒ No
	Overall performance reasonable Notes: The provided ISR findings are not acceptable, however, all other findings from the methods performance report are adequate to allow the use of PK findings for the purposes of this review.	☒ Yes ☒ No
Inspection	Will the bioanalytical site be inspected?	☐ Yes ☒ No
Study DNDiFEX002		
Analyte/assessment	Fexinidazole, M1, M2	
Method	LC-MS/MS	
Matrix	Human plasma (Lithium heparin)	
Validation report	Validation reports provided: B081263-1, B1101203-1 Note: Study DNDiFEX002 also evaluated the concentrations of analytes in ultra filtered plasma to evaluate protein binding as per the procedures described in a validation report: B1101203-1.	☒ Yes ☐ No
	Validation report acceptable	☒ Yes ☐ No
Performance report	Samples analyzed within the established stability period	☒ Yes ☐ No
	QC samples range acceptable Plasma QC samples: 15, 300, 800 ng/mL for fexinidazole; 30, 1500, 4000 ng/mL for metabolites M1 and M2	☒ Yes ☐ No
	Chromatograms provided	☒ Yes ☐ No
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred sample reanalysis (ISR) acceptable Note: Information on ISR not provided.	☐ Yes ☒ No
	Overall performance reasonable	☒ Yes ☐ No
Inspection	Will the bioanalytical site be inspected?	☐ Yes ☒ No

Method/Report	Findings	
Study DNDiFEX003		
Analyte/assessment	Fexinidazole, M1, M2	
Method	LC-MS/MS	
Matrix	Human plasma (Lithium heparin)	
Validation report	Same as Study DNDiFEX002	
Performance report	Samples analyzed within the established stability period Notes: Exact information on sample storage duration prior to analysis could not be located. However, based on the study duration/dates and sample analysis timeline, it appears that the majority of samples were analyzed within the established stability duration.	☒ Yes ☐ No
	QC samples range acceptable Plasma QC samples: 15, 300, 800 ng/mL for fexinidazole; 30, 1500, 4000 ng/mL for metabolites M1 and M2	☒ Yes ☐ No
	Chromatograms provided Notes: Only example chromatograms provided.	☒ Yes ☐ No
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred sample reanalysis (ISR) acceptable Note: Information on ISR not provided.	☐ Yes ☒ No
	Overall performance reasonable	☒ Yes ☐ No
Inspection	Will the bioanalytical site be inspected?	☐ Yes ☒ No

Method/Report	Findings	
Study INT15307		
Analyte/assessment	Fexinidazole, M1, M2	
Method	LC-MS/MS	
Matrix	Human plasma (Lithium heparin) for fexinidazole, M1, and M2 Plasma K2EDTA for caffeine and omeprazole	
Validation report	Same as Study DNDiFEX002 for fexinidazole, M1, and M2 Note: For quantification of caffeine and omeprazole, summary of method validation is provided from Reports HB-10-107 and OMEHPC	
	Validation report acceptable Note: As noted above under previous studies, the validation report for fexinidazole, M1, and M2 is acceptable. Complete validation reports for caffeine and omeprazole not provided. The following information is from the provided summary Range: For Caffeine: 25 to 20000 ng/mL For Omeprazole: 0.5 to 1000 ng/mL	☒ Yes ☐ No
Performance report	Samples analyzed within the established stability period	☒ Yes ☐ No
	Quality control samples range acceptable Plasma QC samples: 15, 300, 800 ng/mL for fexinidazole; 30, 1500, 4000 ng/mL for metabolites M1 and M2 75, 1000, 15000 ng/mL for caffeine 1.5, 50, 750 ng/mL for omeprazole	☒ Yes ☐ No
	Chromatograms provided	☒ Yes ☐ No
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred sample reanalysis (ISR) acceptable Note: Information on ISR not provided for fexinidazole, M1, and M2 analysis. ISR information is provided for caffeine and omeprazole.	☒ Yes ☐ No
	Overall performance reasonable	☒ Yes ☐ No
Inspection	Will the bioanalytical site be inspected?	☐ Yes ☒ No
Study DNDiFEX008		
Analyte/assessment	Fexinidazole, M1, M2	
Method	LC-MS/MS	
Matrix	Human plasma (Lithium heparin)	
Validation report	Same as Study DNDiFEX002 for fexinidazole, M1, and M2	
Performance report	Samples analyzed within the established stability period Notes: Exact information on sample storage duration prior to analysis could not be located. Note to file under the Clinical Study Report notes “All specimens, calibration and QC samples were analyzed within the documented stability period of 105 days at -20"C and at -80"C in human plasma (ref)." The noted stability information is from Study 0058-2009.	☒ Yes ☐ No
	Quality control samples range acceptable	☒ Yes ☐ No

Method/Report	Findings	
	QC samples: 15,300, 800 ng/mL for fexinidazole; 30, 1500, 4000 ng/mL for metabolites M1 and M2	
	Chromatograms provided	☒ Yes ☐ No
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred sample reanalysis (ISR) acceptable	☒ Yes ☐ No
	Overall performance reasonable	☒ Yes ☐ No
Inspection	Will the bioanalytical site be inspected? Note: An OSIS inspection was requested. A Remote Record Review (RRR) of bio-analytical site was performed by OSIS and the OSIS summary document notes that the data from the audited studies are reliable.	☒ Yes ☐ No
Study DNDiFEX004		
Analyte/assessment	Fexinidazole, M1, M2	
Method	LC-MS/MS	
Matrix	Dried blood and CSF spots	
Validation report	Validation reports provided: DOH0916 (DBS), B1120550-1 parts 1 and 2 (DBS), B1120550-2 (CSF) Range for plasma samples: 5-1000 ng/mL for fexinidazole, 5-5000 ng/mL for metabolites M1 and M2. Dilution integrity evaluated at factor of 4. Range for CSF samples: 6-5000 ng/mL for metabolites M1 and M2. Dilution integrity evaluated at factor of 10.	☒ Yes ☐ No
	Validation report acceptable Note: Report B1120550-2 notes that the method validation for quantification of fexinidazole in human CSF failed; however, the determination of M1 and M2 in CSF samples was noted as specific, accurate, precise, and reproduceable.	☒ Yes ☐ No
Performance report	Samples analyzed within the established stability period Note: Reported long term stability is 58 weeks at room temperature in DBS and 184 days at room temperature in dried CSF samples.	☒ Yes ☐ No
	Quality control samples range acceptable DBS QC: 15, 400, 800 ng/mL for fexinidazole 15, 2000, 4000 ng/mL for M1 and M2 CSF QC: 12, 2000, 4000 for M1 and M2	☒ Yes ☐ No
	Chromatograms provided Notes: The notations in provided chromatograms are not legible.	☒ Yes ☐ No
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred sample reanalysis (ISR) acceptable	☐ Yes ☒ No

Method/Report	Findings	
	Note: ISR samples were not randomly selected, instead, the project leader selected ISR samples. ~140/1215 DBS and 4/82 dried CSF samples were selected for ISR.	
	Overall performance reasonable	☒ Yes ☐ No
Inspection	Will the bioanalytical site be inspected?	☐ Yes ☒ No
Study DNDiFEX006		
Analyte/assessment	Fexinidazole, M1, M2	
Method	LC-MS/MS	
Matrix	Dried blood and CSF spots	
Validation reports	Same as Study DNDiFEX004	
Performance report	Samples analyzed within the established stability period	☒ Yes ☐ No
	Quality control samples range acceptable DBS QC: 15, 400, 800 ng/mL for fexinidazole 15, 2000, 4000 ng/mL for M1 and M2 CSF QC: 12, 2000, 4000 for M1 and M2	☒ Yes ☐ No
	Chromatograms provided	☒ Yes ☐ No
	Accuracy and precision of the calibration curve acceptable	☒ Yes ☐ No
	Accuracy and precision of the quality control samples acceptable	☒ Yes ☐ No
	Incurred sample reanalysis (ISR) acceptable Note: In total, 62/462 DBS and 0/27 dried CSF samples were selected for ISR.	☐ Yes ☒ No
	Overall performance reasonable	☒ Yes ☐ No
Inspection	Will the bioanalytical site be inspected?	☐ Yes ☒ No

Source: Compiled by the Reviewer.
Abbreviations: CSF, cerebral spinal fluid; FDA, Food and Drug Administration; K_2EDTA, dipotassium ethylenediaminetetraacetic acid; LC-MS/MS, liquid chromatography-mass spectrometry-mass; NADPH, nicotinamide adenine dinucleotide phosphate, OSIS, Office of Study Integrity and Surveillance

16.3.2. In Vitro Studies

Note: The following summary tables are compiled by the Reviewer.

16.3.2.1. Metabolism Characterization Studies

Table 107. Study 6DNDIP4R1

Objectives	To determine the stability of fexinidazole, RO-15-0216-001-004, and RO-15-6547-000-001 in human and mouse liver microsomes.[1]
Methods	
Systems	Human liver microsomes
Methodology	Test conditions: Fexinidazole concentrations: 5 mcM

[1] Data from only human microsomes and for fexinidazole were reviewed.

	Positive control: Testosterone Incubation buffer: Potassium phosphate pH 7.4 plus Magnesium chloride Temperature: 37°C NADPH Incubation time: 60 mins for test analytes and 30 mins for testosterone
Results & Conclusions	

Table 108. Stability of Compounds in Human Liver Microsomes, Study 6DNDIP4R1

Analyte	% Remaining
Fexinidazole	<2
Testosterone	8.2

Source: Adapted from the study report

Fexinidazole was unstable with < 2.0% of remaining after 60 minutes.

Abbreviations: NADPH, nicotinamide adenine dinucleotide phosphate

Table 109. Study 067-10

Title	Metabolic Stability of Fexinidazole, Fexinidazole-Sulfoxide, and Fexinidazole-Sulfone in CDl Mouse and Pooled Human Liver S9 Subcellular Fractions[2]
Methods	
System	Human (pooled mixed gender) liver subcellular S9 fractions
Methods	Fexinidazole, Fexinidazole-Sulfoxide (M1), and Fexinidazole-Sulfone (M2) concentrations: 1 mcM Positive controls: 7-Ethoxycoumarin, propranolol, and verapamil Incubation mixtures: Potassium phosphate buffer (pH 7.4) with β-NADPH, glucose-6-phosphate, uridine 5'-diphosphoglucuronic acid (UDPGA), potassium chloride, and magnesium chloride Temperature: 37°C Sampling times: 0, 15, 30, and 60 minutes Peak areas were measured using LC-MS/MS and based on the percent of compound remaining, the half-lives were estimated
Results & Conclusions	

The metabolic competency of the S9 fraction used in this study was shown based on the stability findings for positive control compounds. For M1 and M2 over 98% parent compound remained in the incubation mixture after 60 mins and degradation half-lives could not be computed. For fexinidazole, approximately 2% parent compound remained in the incubation mixture after 60 mins and degradation half-life was 11 minutes.

Fexinidazole has high potential for metabolic clearance, whereas its metabolites: M1 and M2 have low potential for metabolic clearance.

Abbreviations: LC-MS/MS, liquid chromatography-mass spectrometry-mass; S9, metabolic activation

Table 110. Study 0141-2007

Title	Fexinidazole: Evaluation of the In Vitro Cross Species Intrinsic Clearance and Metabolism with Mouse, Rat, Dog, Monkey and Human Hepatocytes[3]
Methods	
System	Pooled samples of cryopreserved hepatocytes
Methods	Intrinsic Clearance Determination:

[2] Data from only human liver S9 sub-cellular fractions were reviewed.

[3] Data from only human hepatocytes were reviewed.

	Intrinsic hepatic clearance of fexinidazole (1 mcM) was determined by measuring the substrate degradation rate and determining half-life in human hepatocytes. Leibovitz L-15 medium was used for incubation at 37°C. Samples were taken from incubation at 0, 1, 5, 10, 15, 20, 30, 60, and 120 minutes and were analyzed with LC-MS/MS. 7-ethoxycoumarin (7-EC) 1 mcM, and 7-hydroxycoumarin (7-HC) 30 mcM were used as positive controls and incubated under the same conditions as fexinidazole. For negative control, fexinidazole incubated for 120 minutes at 37°C in the incubation medium alone. Metabolite Profile and Identification: The metabolite profile and metabolite identification of fexinidazole was performed also at 10 mcM using human hepatocytes and by analyzing samples with a chiral column. Leibovitz L-15 medium was used for incubation at 37°C. Samples were taken from incubation at 0, 30, and 120 minutes and were analyzed with LC-MS/MS. 7-ethoxycoumarin (7-EC) 1 mcM, and 7-hydroxycoumarin (7-HC) 30 mcM were used as positive controls and incubated under the same conditions as fexinidazole. For negative control, fexinidazole incubated for 120 minutes at 37°C in the incubation medium alone.
Results & Conclusions	
Intrinsic Clearance Determination: Negative controls experiment showed 30-40% loss from incubation medium at 120 min in the absence of hepatocytes. Fexinidazole showed half-life values of 13 minutes with human hepatocytes and estimated hepatic intrinsic clearance was 124 mL/min/kg. Metabolite Profile and Identification: After 2 hours incubation, fexinidazole accounted for about 84% of the total drug-related material in the sample. At t=0, unchanged fexinidazole accounted for 83% of total drug-related material with human hepatocytes. After 30 minutes incubation, fexinidazole was detected in small amounts (2%) only in human hepatocytes and the major part of the drug-related material (90 to 96%) was M1. After 120 minutes incubation, fexinidazole was detected in traces (less than 1%) only in human hepatocytes and M1 was 95% (humans). The remaining part of drug-related material was M2. Fexinidazole showed very rapid and high intrinsic clearance with hepatocytes and high intrinsic clearance with human hepatocytes. The metabolic reactions observed were oxidation of the S atom to M1 and M2. M1 is the major metabolite produced by the hepatocytes in vitro.	

Abbreviations: M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone

Table 111.Study DNDI-13-055

Title	Metabolism of fexinidazole, fexinidazole sulfoxide and fexinidazole sulfone in human, rat and mouse liver microsomes[4]
Methods	
System	Human liver microsomes
Methods	Metabolic stability of fexinidazole and its metabolites M1 and M2 were performed by incubating each test compound (1 mcM) with human at 37°C with 0.4 mg/mL protein concentration and with an NADPH-regenerating system over 60 minutes. Control samples (containing no NADPH) were included (and quenched at 2, 30, and 60 minutes) to monitor for potential degradation in the absence of cofactor. Samples collected from incubations were analyzed by UPLC-MS. Substrate depletion rates were determined from test compound concentration versus time data. Each substrate depletion rate constant was then used to calculate: a degradation half-life, an in vitro intrinsic clearance (CL_{int}, in vitro) value; a predicted in vivo hepatic intrinsic clearance value (CLint); a predicted in vivo blood clearance (CL_{blood}) value; and a predicted in vivo hepatic extraction ratio (EH).
Results & Conclusions	

Table 112. Metabolic Stability Parameters for Fexinidazole, M1, and M2, Study DNDI-13-055

Compound	Degradation Half-Life (min)	In Vitro CL_{int} (mcL/min/mg Protein)	CL_{blood} (mL/min/kg)	Microsome-Predicted E_H
Fexinidazole	14	127	17	0.83
M1	147	12	7	0.32
M2	>247	<7	<5	<0.22

Source: Adapted from the study report

Fexinidazole exhibited a high rate of degradation in human liver microsomes, whereas, M1 and M2 were more stable, exhibiting a minimal to low rate of degradation.

Abbreviations: M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; NADPH, nicotinamide adenine dinucleotide phosphate; UPLC-MS, ultra-performance liquid chromatography - tandem mass spectrometer

Table 113. Study 14925

Objective	The purpose of this study was to test compounds fexinidazole, M1, and M2 in various metabolic stability assays with human recombinant CYP450s
Methods	
System	Human recombinant CYP450s
Methods	Metabolic stability of fexinidazole, M1, and M2 were evaluated by incubating each test compound (1 mcM) with human recombinant CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, and 3A5. Incubations were performed at 37°C in phosphate buffer, pH 7.4 with NADPH-generating system. Reference compounds were also incubated under similar conditions. Samples were collected at t=0 and t=60 mins from incubations were analyzed by LC-MS/MS.

[4] Data from only human liver microsomes were reviewed.

Results & Conclusions

Table 114. Metabolic Stability Parameters for Fexinidazole, M1, and M2, Study 14925

	Mean Parent Substrates Remaining (n=2)		
Recombinant CYP450	**Fexinidazole**	**M1**	**M2**
CYP1A2	28	79	101
CYP2B6	12	99	98
CYP2C8	91	96	97
CYP2C9	97	93	97
CYP2C19	0	102	101
CYP2D6	73	103	100
CYP3A4	13	102	97
CYP3A5	21	103	105

Source: Adapted from the study report

Table 115. Metabolic Stability Parameters for Control Substrates, Study 14925

Recombinant CYP450	Substrate	Mean Parent Substrates Remaining (n=2)
CYP1A2	Ethoxyresorufin	1
	Propranolol	44
CYP2B6	Benaphetamine	4
	Propranolol	100
CYP2C8	Paclitaxel	2
	Propranolol	98
CYP2C9	Diclofenac	0
	Propranolol	101
CYP2C19	Promazine	57
	Propranolol	91
CYP2D6	Terfenadine	122
	Propranolol	11
CYP3A4	Terfenadine	12
	Propranolol	102
CYP3A5	Terfenadine	7
	Propranolol	102

Source: Adapted from the study report

Fexinidazole was highly metabolized by CYP1A2, -2B6, -2C19, -3A4 and -3A5 isoforms, whereas the M1 and M2 metabolites showed little or no metabolism by the isoforms tested.

Abbreviations: LC-MS/MS, liquid chromatography-mass spectrometry-mass; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; NADPH, nicotinamide adenine dinucleotide phosphate

Table 116. Study 0327-2008

Title	Fexinidazole: Evaluation of the In Vitro Intrinsic Clearance and Metabolism with Hepatocytes from African American Donors
Methods	
System	Cryopreserved hepatocytes from African American donors
Methods	Similar to methods described in Study 0141-2007, however, only fexinidazole was evaluated.

Results & Conclusions
The half-life of fexinidazole was 6.5 minutes, and the corresponding intrinsic clearance value was 257 mL/min/kg. M1 was the main metabolite produced by human hepatocytes; this metabolite was present as two enantiomers in a ratio of about 2:1. Small amounts of M2 and of the sulfoxide des-methylated on the imidazole ring were also detected. As previously observed with hepatocytes from Caucasian donors, fexinidazole showed a rapid and high intrinsic clearance with hepatocytes from African American donors.
Reviewer's Assessment: Conclusion drawn in 2.7.2 Summary of Clinical Pharmacology studies is that both the intrinsic clearance and the metabolite profile of fexinidazole after incubation with hepatocytes from African American donors were similar to those previously observed with hepatocytes from Caucasian donors in Study 0141-2007. The estimated t1/2 and Clint in human hepatocytes from African American donors were 49% and 207% of estimates obtained from hepatocytes from Caucasian (Study 0141-2007) donors. Therefore, the Reviewer does not agree with the Applicant's conclusion that both the intrinsic clearance of fexinidazole after incubation with hepatocytes from African American donors were similar to those previously observed with hepatocytes from Caucasian donors.

Abbreviations: CL_{int}, intrinsic clearance; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; $t_{1/2}$, half-life

Table 117. Study 0291-2011

Title	Fexinidazole: Evaluation of the In Vitro Intrinsic Clearance and Metabolism with Hepatocytes from Young Pediatric Donors
Methods	
System	Cryopreserved hepatocytes from young pediatric donors of different ages: 3 months old, 1 year old, and 2 years old
Methods	Similar to methods described in Study 0141-2007, however, only fexinidazole was evaluated
Results & Conclusions	
The half-lives of fexinidazole with hepatocytes from a male 3 months, a male 1 year, and a male 2 year were 61, 37, and 33 minutes, respectively, and the corresponding intrinsic clearance values were 46, 79, and 95 mL/min/kg, respectively. M1 was detected and was present as two enantiomers in a ratio of about 2:1 to 4:3. Fexinidazole sulfoxide des-methylated on the imidazole ring was detected in traces, while M2 was not detected Fexinidazole showed a moderate intrinsic clearance with hepatocytes from young pediatric donors.	
Reviewer's Assessment: The estimated t1/2 and CLint in human hepatocytes from young donors, i.e., ≤2 years, appear to suggest that the estimate for fexinidazole clearance is slower. However, it is noteworthy that the proposed indication for Fexinidazole tablets is for the treatment indication is first-stage (hemolymphatic) and second-stage (meningoencephalitic) of human African trypanosomiasis (HAT) due to Trypanosoma brucei gambiense in adult and pediatric patients who are 6 years and older.	

Abbreviations: CL_{int}, intrinsic clearance; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; $t_{1/2}$, half-life

Table 118. Study 11DNDIP1

Title	FMO Reaction Phenotyping of Fexinidazole and Fexinidazole Sulfoxide Using Human Recombinant FMO Enzymes
Methods	
System	Human recombinant FMO-3 enzyme
Methods	Fexinidazole and M1 (1µM each) were incubated with human recombinant FMO-3 enzyme at 37°C in the presence of phosphate buffer (pH 8.4), magnesium chloride, and NADPH. During incubation period, samples were withdrawn at 0, 5, 15, 30, and 60 minutes. Within the collected samples, the disappearance of fexinidazole and M1, and the formation of related metabolites were analyzed by LC-MS/MS. The half-life and intrinsic clearance were estimated by a substrate depletion approach. The FMO enzyme activities were verified in parallel by

	determining the formation of FMO probe metabolite (methyl p-tolyl sulfoxide) after 30 minutes of incubation with FMO probe substrate (methyl p-tolyl sulfide) by LC-MS/MS.
Results & Conclusions	
The estimated in vitro half-life ($t_{1/2}$) and intrinsic clearance (CL_{int}) values of fexinidazole for recombinant human FMO-3 enzyme were 15 minutes and 0.93 mL/min/mg protein, respectively. The major metabolite detected was fM1, while M2 was not detectable. M1 was not metabolized by human FMO-3 under the current experimental conditions. Fexinidazole was metabolized by human FMO-3, however, M1 was not metabolized by FMO-3.	
Reviewer's Assessment: The study report does not include information on the concentration or reference metabolism rate for Methyl p-tolyl sulfide, which was used as a positive control. Therefore, the Reviewer could not verify the relative FMO enzyme activities within the experimental setup used in the study.	

Abbreviations: FMO, flavin-containing monooxygenase; LC-MS/MS, liquid chromatography-mass spectrometry-mass; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; NADPH, nicotinamide adenine dinucleotide phosphate

Table 119. Study EIH0019

Title	In vitro identification of the major enzymes involved in the metabolism of Fexinidazole, Fexinidazole-sulfoxide, Fexinidazole-sulfoxide enantiomers and Fexinidazole-sulfone using human recombinant enzymes, human liver, kidney, lung and intestinal microsomes and human cryopreserved hepatocytes
Methods	
System	Metabolic stability: Human liver, kidney, lung and intestinal microsomes as well as cryopreserved human hepatocytes Reaction phenotyping: rhCYPs 1A1, 1A2, 2A6, 2B6, 2C8, 2C9, 2C18, 2C19, 2D6, 2E1, 2J2, 3A4, 3A5, and 4F2 rhFMOs 1, 3, and 5
Methods	Metabolic stability in microsomes: Fexinidazole, M1, and M2 (Concentrations: 0.5, 5, 20 and 100 mcM) were incubated in a pH 7.4 buffer at 37°C and metabolic reactions were initiated by adding NADPH. Mixtures without microsomes were used as controls. Fexinidazole, M1, and M2 metabolism were quantified using substrate depletion approach by determining the difference between reactions mixtures incubated with or without cofactor (NADPH). Reaction phenotyping: Fexinidazole, M1, and M2 (Concentrations: 0.5, 5, 20 and 100 mcM) were incubated in individual rhCYP and rhFMO preparations at 37°C and metabolic reactions were initiated by adding NADPH. Mixtures without rhCYP or rhFMO were used as controls. Fexinidazole, M1, and M2 metabolism were quantified using substrate depletion approach by determining the difference between reactions mixtures incubated with or without cofactor (NADPH). Metabolic stability in Cryopreserved human hepatocytes: The metabolic capacity of the two hepatocyte preparations was assessed with standard in vitro probes: Midazolam (CYP3A4-mediated Midazolam 1'-hydroxylase activity) and Benzydamine (for FMO-mediated N-oxidation activity). Midazolam and Benzydamine were incubated with and without 10 mcM Azamulin (CYP3A4 inhibitor), with and without 1mM 1-aminobenzotriazole (ABT, pan-CYP inhibitor) and with and without 1mM Methimazole (FMO inhibitor). Fexinidazole, M1, and M2 (Concentrations:10 mcM) were incubated in in the presence of 1mM Methimazole, 10 mcM Azamulin or 1mM ABT, were conducted in order to determine the contribution of FMO, CYP3A and overall CYPs,

	respectively, to the in vitro intrinsic clearance of fexinidazole, M1, M1 enantiomers, and M2. Samples were removed at 0.5, 1, 3, 5, 8 and 24 hours. Fexinidazole, M1, M1 enantiomers, and M2 metabolism were quantified using a substrate depletion approach and metabolites' formation were also monitored.

Results & Conclusions

Metabolic stability in microsomes:
Fexinidazole was metabolized in human liver, kidney, lung, and intestinal microsomes and M1 (fexinidazole sulfoxide) was quantified in all matrixes. M2 (fexinidazole sulfone) was quantified only in human liver and intestinal microsomes experiments.

M1 was not markedly metabolized in human liver, kidney, lung, or intestinal microsomes but M2 was quantified in all matrixes, mainly in human liver and kidney microsomes.

M2 was not metabolized in human liver, kidney, lung, or intestinal microsomes.

Reaction phenotyping:
Fexinidazole was mainly metabolized by CYP1A1, -1A2, -2A6, -2B6, -2C9, -2C19, -2D6, -3A4, -3A5, -4F2 and FMO1, FMO3 and FMO5, main isoforms exhibiting more than 15% of metabolism. In CYP1A1, -1A2, -2A6, -2B6, -2C8, -2C9, -2C19, -2D6, -2J2, -3A4, -3A5, -4F2 and FMO1, FMO3 and FMO5 experiments, M1 was quantified. M2 was quantified only in CYP1A1, -1A2, -2B6, -2D6, -2J2, -3A4, -3A5 and FMO1 experiments.

M1 was mainly metabolized by FMO1 but M2 was quantified in CYP1A1, -1A2, -2B6, -2D6, -2E1, - 2J2, -3A4, -3A5, -4F2 and FMO1, FMO3 and FMO5 experiments.
M1 enantiomer 1 (RA15664588) was mainly metabolized by CYP1A1 but M2 was quantified in CYP1A1, -1A2, 2D6, -3A4 and FMO5.
M1 enantiomer 2 (RA15664590) was mainly metabolized by FMO1 but M2 was quantified in CYP1A1, -2D6, -3A4 and FMO1.

M2 was found metabolized neither by CYPs nor by FMOs isoforms.

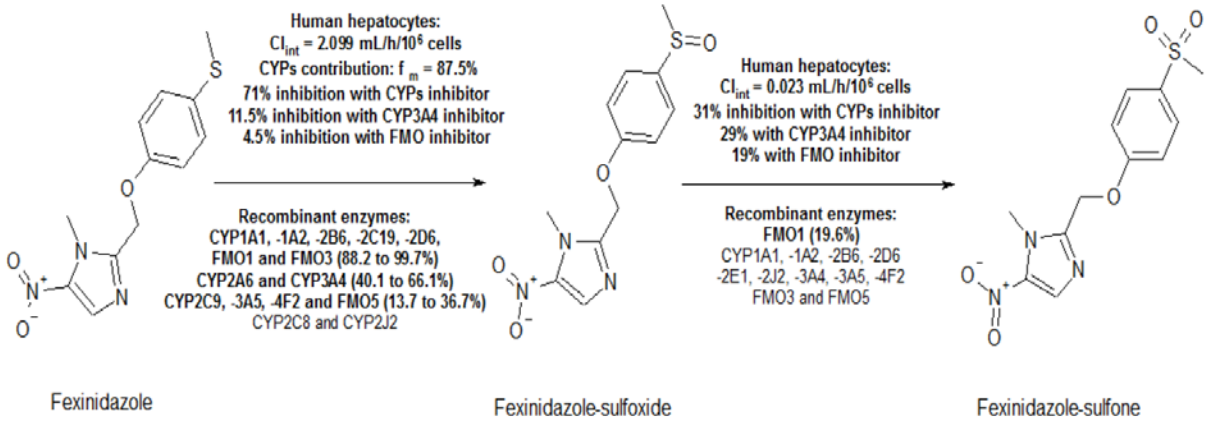
Metabolic stability in Cryopreserved human hepatocytes:
Metabolism of CYP and FMO probe substrates, used as controls, demonstrated that the cryopreserved human hepatocytes were metabolically active.

Overall metabolism summary and conclusions are provided in Figure 5 below.

Fexinidazole was found extensively metabolized by human hepatocytes. M1 was the main metabolite formed while M2 was sequentially formed but in lesser amounts.

M2 was not markedly metabolized in human hepatocytes and no significant effect of FMOs and CYPs inhibitors could be evidenced.

Figure 5. Overall Metabolism Summary/Conclusion, Study EIH0019



Source: From the study report
Abbreviation: CL_{int}, intrinsic clearance

Reviewer's Assessment: Findings from experiments that incubated fexinidazole without cofactor (NADPH) in microsomes is not provided.

It is noteworthy that transformation of fexinidazole to M1 is well characterized and appears mainly driven by CYP450s (CYP1A2, -2B6, -2C9, -2C19, -2D6, and -3A4) and FMOs. However, the biotransformation pathway for conversion of M1 to M2 is not completely characterized. Specifically, the report notes that due to the very slow metabolism and lack of turnover of M1 and M1 enantiomers, the estimates of fraction metabolized in cryopreserved human hepatocytes by CYP3A (fm CYP3A4), FMO (fm FMO) and total CYPs (fm Total CYPs) could not be determined accurately. Therefore, it appears to this reviewer that the available information on the biotransformation pathway of formation of M1 to M2 is not complete.

Abbreviations: FMO, flavin-containing monooxygenase; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; NADPH, nicotinamide adenine dinucleotide phosphate

16.3.2.2. Transporters and Distribution Characterization Studies

Table 120. Study 6DNDIP2

Objective	To determine the bidirectional Caco-2 permeability and efflux limited absorption potential of three test compounds: Fexinidazole, RO-15-6547-000-001, and RO-15-0216-001-004[5]
Methods	
System	Caco-2 monolayers
Methods	The mass transfer of Lucifer Yellow from the donor to the receiver compartment was evaluated to assess the integrity of monolayer. Substrate (Concentration): Fexinidazole (5 mcM) Reference compounds: Digoxin, atenolol, propranolol, digoxin

[5] Information related to fexinidazole only was reviewed.

Results & Conclusions

Table 121. Apparent Permeability (10-6 cm/s) of Test Compounds, Study 6DNDIP2

Test Compound Identification	Mean Papp, A-B (n=2) in 10^{-6} cm/s	Mean Papp, B-A (n=2) in 10^{-6} cm/s	Papp B-A to Papp A-B Ratio
Fexinidazole	57.2	50.9	0.9

Source: Information compiled by the Reviewer from study report

Absorption Potential Classification:

- High if Papp (A-to-B) ≥ 1.0 x 10-6 cm/s and low if Papp (A-to-B) < 0.5 x 10-6 cm/s: Low

Efflux considered significant if:

- Papp (B-to-A) ≥ 1.0 x 10-6 cm/s and Ratio Papp (B-to-A) / Papp (A-to-B) > 3.0

All three test compounds were all classified as having a high absorption potential and having no significant efflux; their intestinal permeability is not expected to be a limiting factor for their absorption in humans.

Table 122. Study 9DNDIP2

Objective	To determine the extent of binding to human plasma proteins, Caco-2 permeability, and MDR1-MDCK permeability for M1 and M2
Methods	
System	Equilibrium dialysis for protein binding Caco-2 and MDR1-MDCK monolayers for permeability
Methods	Permeability Analysis: The mass transfer of Lucifer Yellow from the donor to the receiver compartment was evaluated to assess the integrity of monolayer.

Table 123. Experiment Conditions, Study 9DNDIP2

Cell Line	Substrate: Concentration
CACO-2	M1 & M2: 5 mcM
	Reference compounds: Digoxin, atenolol, propranolol, digoxin.
MDR1-MDCK	M1 & M2: 5 mcM
	Reference compounds: Digoxin, atenolol, propranolol, digoxin.

Source: Information compiled by the Reviewer from study report

Protein Binding Analysis:
Equilibrium dialysis method was used. Dialysis chambers were separated with a membrane with molecular weight cutoff of 5,000 Daltons. PBS buffer and plasma containing the test compounds and control compound were added each side of the membrane. The chambers were incubated overnight at 37°C and both chambers were sampled after ~ 22 hours. Samples were analyzed via LC/MS/MS. M1 and M2 concentrations used were 5 mcM and warfarin at a concentration of 10 mcM.

Results & Conclusions

Table 124. Apparent Permeability (10-6 cm/s) of Test Compounds, Study 9DNDIP2

Test Compound Identification	Mean Papp, A-B (n=2) in 10^{-6} cm/s	Mean Papp, B-A (n=2) in 10^{-6} cm/s	Papp B-A to Papp A-B Ratio
CACO-2 Cell Line[A]			
M1	23.4	31.5	1.3
M2	49.8	35.6	0.7
MDR1-MDCK Cell Line[B]			
M1	7.19	62.5	8
M2	20.7	62.5	3

Source: Information compiled by the Reviewer from study report
[A]Efflux considered significant by the Applicant if: Papp (B-to-A) ≥ 1.0 x 10-6 cm/s and Ratio Papp (B-to-A) / Papp (A-to-B) > 3.0
[B]Brain Penetration Classification used by the Applicant:

- Papp (A-to-B) ≥ 3.0 x 10-6 cm/s and Efflux < 3.0: High
- Papp (A-to-B) ≥ 3.0 x 10-6 cm/s and 10.0 > Efflux > 3.0: Moderate
- Papp (A-to-B) ≥ 3.0 x 10-6 cm/s and Efflux > 10.0: Low
- Papp (A-to-B) < 3.0 x 10-6 cm/s: Low

Absorption Potential Classification used by the Applicant:
High if Papp (A-to-B) ≥ 1.0 x 10-6 cm/s and low if Papp (A-to-B) < 0.5 x 10-6 cm/s: Low

The observed protein binding estimates for M1 and M2 in human plasma were 25.9% and 41.6%

The membrane permeability of the metabolites M1 and M2 is expected to be efficient and the study findings indicate a moderate brain penetration potential for M1 and M2 metabolites [From 2.7.2 Summary of Clinical Pharmacology studies (human African trypanosomiasis)].

Abbreviations: M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; P-gp, p-glycoprotein; LC/MS/MS, liquid chromatography-mass spectrometry-mass; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; MDCK, Madin Darby canine kidney; PBS, phosphate-buffered saline

Table 125. Study 6DNDIP3

Objective	The objective of this study was to determine the bidirectional MDR1-MDCK permeability and efflux limited absorption potential of three test compounds.: Fexinidazole, RO-15-6547-000-001, and RO-15-0216-001-004[6]
Methods	
System	MDR1-MDCK monolayers
Methods	The mass transfer of Lucifer Yellow from the donor to the receiver compartment was evaluated to assess the integrity of monolayer. Substrate(Concentration): Fexinidazole (5 mcM) Reference compounds: Digoxin, atenolol, propranolol, digoxin.

[6] Information related to fexinidazole only was reviewed.

Results & Conclusions

Table 126. Apparent Permeability (10-6 cm/s) of Test Compounds, Study 6DNDIP3

Test Compound Identification	Mean Papp, A-B (n=2) in 10^{-6} cm/s	Mean Papp, B-A (n=2) in 10^{-6} cm/s	Papp B-A to Papp A-B Ratio
Fexinidazole	60.6	55.1	0.9

Source: Information compiled by the Reviewer from study report
Note: Brain penetration classification:

- Papp (A-to-B) ≥ 3.0 x 10-6 cm/s and efflux < 3.0: high;
- Papp (A-to-B) ≥ 3.0 x 10-6 cm/s and 10.0 > Efflux > 3.0: moderate;
- Papp (A-to-B) ≥ 3.0 x 10-6 cm/s and efflux > 10.0: low;
- Papp (A-to-B) < 3.0 x 10-6 cm/s: low

Fexinidazole was classified as having a high brain penetration potential.

Table 127.Study 068-10

Title	Determination of In Vitro Permeability and P-gp Interaction Potential in MDCK-MDRI Cells for Fexinidazole, Fexinidazole-Sulfoxide, and Fexinidazole-Sulfone
Methods	
System	MDCK-MDRI cell line
Methods	The mass transfer of Lucifer Yellow from the donor to the receiver compartment was evaluated to assess the integrity of monolayer.

Table 128. Experiment Conditions, Study 068-10

Transporter	Substrate: Concentration	Inhibitor: Concentration
P-gp	Fexinidazole, M1, and M2: 3 mcM	GF-918: 2mcM
	Reference compounds: Amprenavir and propranolol	

Source: Information compiled by the Reviewer from study report
Abbreviations: M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; P-gp, p-glycoprotein;

Results & Conclusions

Table 129. Apparent Permeability (10-6 cm/s) of Test Compounds, Study 068-10

Test Compound Identification	Mean Papp, A-B (n=3) In nm/s	Absorption Quotient (AQ)
Fexinidazole	730	0.01
Fexinidazole plus GF-918	735	-
M1	671	0.06
M1 plus GF-918	711	-
M2	762	0.05
M2 plus GF-918	802	-

Source: Information compiled by the Reviewer from study report
Abbreviations: M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone

Gut Penetration Potential Classification:

- P app (A-to-B) ≥ 50 nm/s: High
- P app (A-to-B) 5 nm/s < Papp< 50 mn/s: Moderate
- Papp (A-to-B) ≤ 5 nm/s: Low

P-gp Interaction Potential Classification:

- AQ= (Papp (A-to-B) with inhibitor – Papp (A-to-B) without inhibitor)/ Papp (A-to-B) with inhibitor
- AQ < 0.1: Low
- < AQ < 0.3: Moderate
- AQ > 0.3: High

Brain Penetration Potential Classification:

- Papp (A-to-B) 2: 150 nm/s; AQ < 0.1: High
- Papp (A-to-B) 2: 150 nm/s; 0.1 < AQ < 0.3: Moderate
- Papp (A-to-B) 2; 150 nm/s; AQ > 0.3: Low
- P app (A-to-B):= 150 nm/s: Low

Fexinidazole, M1, and M2 all demonstrated high permeability in the apical to basolateral direction, with an apparent permeability values (Papp A-to-B) of approximately 730,671, and 762 nm/s, respectively. The Papp (A-to-B) plus GF918 values were approximately 735, 711, and 802 nm/s, respectively. The minor differences in Papp values in the presence of an inhibitor (GF-918) indicate that test compounds are not a strong substrate for the P-gp efflux transporter. The Absorption quotient values for Fexinidazole, M1, and M2 were 0.01, 0.06, and 0.05, respectively, all indicating a low propensity for efflux transporter by P-gp.

All three compounds are permeable and should be readily absorbed through the gut wall with little or no hindrance by P-gp. Fexinidazole, M1, and M2 may also cross the blood-brain barrier due to their high permeability and low affinity for P-gp.

Abbreviations: M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; MDCK, Madin Darby canine kidney; P-gp, p-glycoprotein

16.3.2.3. In Vitro Drug-Drug Interaction & Drug-Transporter Assessments

Table 130. Study TRI0039

Title	Evaluation of HOE239, Fexinidazole-sulfoxide and Fexinidazole-sulfone as inhibitors of the human uptake transporters OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1 and MATE2-K and of the human efflux transporters P-gp and BCRP
Methods	
System	Transfected cell lines (TransportoCells™), Sf9-BCRP vesicles and Caco-2/TC7 cell line
Methods	Cell monolayer functionality (i.e., tight junction integrity) was evaluated by measuring the monolayer's "Apical to Basal" permeability to 20 mcM [^{14}C]-Mannitol in the absence of a concentration gradient.

Fexinidazole concentration range: 0-317 mcM.
M1 concentration range: 0-251 mcM.
M2 concentration range: 0-353 mcM.

Table 131. Experiment Conditions, Study TRI0039

Test System	Probe Substrate [Concentration]	Probe Inhibitor [Concentration]	Incubation Time
TransportoCells™ OATP1B1	3H-Estradiol-17-β-Glucuronide [1 mcM]	Rifampicin [100 mcM]	10 min (with and without 30 min preincubation)
TransportoCells™ OATP1B3	3H-Cholecystokinine-8 [0.5 mcM]	Rifampicin [100 mcM]	10 min (with and without 30 min preincubation)
TransportoCells™ OCT1	14C-TEA [25 mcM]	Cimetidine [1500 mcM]	15 min
TransportoCells™ OCT2	14C-Metformin [25 mcM]	Cimetidine [1000 mcM]	5 min
TransportoCells™ OAT1	3H-p-Aminohippuric acid [5 mcM]	Diclofenac [100 mcM]	2 min
TransportoCells™ OAT3	3H-Estrone-3-sulfate [1 mcM]	Probenecid [200 mcM]	3 min
TransportoCells™ MATE1	14C-TEA [5 mcM]	Cimetidine [100 mcM]	5 min
TransportoCells™ MATE2-K	14C-TEA [3 mcM]	Cimetidine [1000 mcM]	5 min
Caco-2/TC7 – P-gp	3H-Digoxine [5 mcM]	PSC833 [5 mcM]	120 min
Sf9-P-gp vesicles	N-Methyl-quinidine [2 mcM]	PSC833 [0.1, 0.3, 1 and 3 mcM]	3 min
Sf9-BCRP vesicles	Estrone-3-sulfate [1 mcM]	Fumitromorgin C [0.3, 1, 3 and 10 mcM]	1 min

Source: Information compiled by the Reviewer from study *report*
The IC_{50} was obtained using the 4-parameter logistic model according to Ratkowsky and Reedy (DOI: 10.2307/2531207 PMID: 3567290).
Abbreviations: BCRP, Breast Cancer Resistance Protein; OAT, Organic Anion Transporters; OATP, organic-anion-transporting polypeptides; OCT, organic cation transporter; P-gp, p-glycoprotein

Results & Conclusions

Table 132. IC_{50} Mean Value (CV %) of Test Compounds, Study TRI0039

Transporter	Fexinidazole	M1	M2
OATP1B1	69.8 mcM (17.3%) without preincubation and 72.0 mcM (18.5%) with preincubation	No inhibition	> 353 mcM
OATP1B3	121 M (13.8%) without preincubation and 59.1 mcM (17.7%) with preincubation	No inhibition	No inhibition
OCT1	68.7 mcM (11.0%)	No inhibition	282 mcM (15.8%)
OCT2	38.9 mcM (13.4%)	> 251 mcM	202 mcM (16.4%)
OAT1	267 mcM (19.1%)	> 251 mcM	71.8 mcM (12.5%)
OAT3	33.5 mcM (12.5%)	113 mcM (9.3%)	44.3 mcM (12.0%)
MATE1	2.08 mcM (18.3%)	53.9 mcM (16.2%)	18.1 mcM (17.9%)
MATE2-K	4.38 mcM (15.3%)	44.9 mcM (13.8%)	39.3 mcM (19.6%)
P-gp	> 100 mcM	No inhibition	> 300 mcM
Sf9-P-gp vesicles	No inhibition	No inhibition	No inhibition
Sf9-BCRP vesicles	No inhibition	281 mcM (14.1%)	203 mcM (4.1%)

Source: Information compiled by the Reviewer from study *report*
Abbreviations: BCRP, Breast Cancer Resistance Protein; CV, coefficient of variation; IC_{50}, half maximal inhibitory concentration; OAT, Organic Anion Transporters; OATP, organic-anion-transporting polypeptides; OCT, organic cation transporter; P-gp, p-glycoprotein
Fexinidazole was found to be inhibitor of OATP1B1, OATP1B3, OCT1, OCT2, OAT1, OAT3, MATE1, and MATE2-K.

M1 was found to be inhibitor of OAT3, MATE1, and MATE2-K.

M2 was found to be inhibitor of OCT1, OCT2, OAT1, OAT3, MATE1, and MATE2-K.

None of the tested compound was identified as a time dependent inhibitor of OATP1B1 or OATP1B3.

Abbreviations: BCRP, Breast Cancer Resistance Protein; CV, coefficient of variation; IC_{50}, half maximal inhibitory concentration; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; MATE, multidrug and toxic compound extrusion; MDCK, Madin Darby canine kidney; OAT, Organic Anion Transporters; OATP, organic-anion-transporting polypeptides; OCT, organic cation transporter; P-gp, p-glycoprotein

Table 133. Study TRS0018

Title	Evaluation of HOE239, M1 and Fexinidazole-sulfone as a substrate of the human OATP1B1 and OATP1B3 uptake transporters and of human P-gp and BCRP efflux transporters
Methods	
System	HEK, HEK-hOATP1B1 and HEK-hOATP1B3 recombinant cell lines and Caco-2/TC7 cell line
Methods	Cell monolayer functionality (i.e., tight junction integrity) was evaluated by measuring the monolayer's "Apical to Basal" permeability to 20 mcM [^{14}C]-Mannitol in the absence of a concentration gradient.

Table 134. Experiment Conditions, Study TRS0018

Test System	Probe Substrate [Concentration]	Probe Inhibitor [Concentration]	Incubation Time
HEK-hOATP1B1	[3H]-E17βG [1 mcM] Fexinidazole concentrations: 0.5, 1, 5 and 50 mcM M1 and M2 concentrations: 1, 5, 20 and 100 mcM	Rifampicin [100 mcM]	10 min

HEK-hOATP1B3	[3H]-CCK8 [0.5 mcM] Fexinidazole concentrations: 0.5, 1, 5 and 50 mcM M1 and M2 concentrations: 1, 5, 20 and 100 mcM	Rifampicin [100 mcM]	10 min
Caco-2/TC7 (P-gp)	[3H]-Digoxin [5 mcM] Fexinidazole, M1 and M2 concentrations: 0.5, 1, 5, 20 and 100 mcM	PSC833 [5 mcM]	120 min
Caco-2/TC7 (BCRP)	[3H]-E3S [5 mcM] Fexinidazole, M1 and M2 concentrations: 0.5, 1, 5, 20 and 100 mcM	Fumitromorgin C [10 mcM]	120 min

Source: Information compiled by the Reviewer from study report
Abbreviations: BCRP, Breast Cancer Resistance Protein; HEK, human embryonic kidney; OATP, organic-anion-transporting polypeptides

Results & Conclusions
All reference compounds worked as expected, validating the experimental approach. None of the test compound was found to be a substrate for human OATP1B1 and OATP1B3 uptake transporters, nor is it a substrate for human P-gp and BCRP efflux transporters in vitro.

Abbreviations: BCRP, Breast Cancer Resistance Protein; HEK, human embryonic kidney; OATP, organic-anion-transporting polypeptides; P-gp, p-glycoprotein

Table 135. Study 063-10

Title	Comparison of In Vitro Inhibition of CYP450 by Fexinidazole, Fexinidazole-Sulfoxide (M1), and Fexinidazole-Sulfone (M2)
Methods	
System	Human recombinant CYP450s
Methods	The assays were performed using a commercially available test system. Test compounds were incubated with microsomes at concentrations ranging from 1-100 mcM. Metabolic competency of the microsomal preparations was evaluated using positive control compounds as indicated by the manufacturer for each isoform.

Results & Applicant's conclusion

Table 136. IC_{50} Values and Positive Control Inhibition, Study 063-10

Isoform	Fexinidazole	M1	M2	Positive Control Inhibitor: % Inhibition
CYP3A4	>100 mcM	>100 mcM	>100 mcM	Ketoconazole: 8.9%
CYP1A2	2.6 mcM	37.2 mcM	>100 mcM	α-Napthoflavone:8.7%
CYP2C9	>100 mcM	>100 mcM	>100 mcM	Sulfaphenazole: 7.9%
CYP2C19	0.4 mcM	4.1 mcM	>100 mcM	Troglitazone: 15%
CYP2D6	>100 mcM	>100 mcM	>100 mcM	Quinidine: 9.7%

Source: Information compiled by the Reviewer from study report

In Vitro Inhibition Classification(Applicant's classification):
IC_{50} < 1.0 mcM; Strong Inhibitor (High DDI Risk)
1 < IC_{50} < 10 mcM; Moderate Inhibitor (Med DDI Risk)
IC_{50} ≥ 10 mcM: Non Inhibitor (Low DDI Risk)

Fexinidazole has a high and moderate potential for CYP450-based drug-drug interactions towards CYP2C19 and CYP1A2, respectively. M1 appears to be a moderate potential for inhibiting CYP2C19.

Reviewer's Assessments: The rationale for how in vitro classification was defined is not provided. Also, the control inhibitor does not appear to show significant inhibition compared to control. The maximum inhibition observed for troglitazone was 15%, whereas, for all other inhibitor was less than 10%. Therefore, the results from this study/report were not used for the purposes of this review nor was used for the review of the Applicant proposed labeling language.

Abbreviations: DDI, drug-drug interaction; IC_{50}, half maximal inhibitory concentration;

Table 137. Study ICH0106

Title	In Vitro Inhibition of Human Cytochrome P450 Enzymes by Hoe239, Fexinidazole-Sulfoxide and Fexinidazole-Sulfone in Human Liver Microsomes
Methods	
System	Human liver microsomes
Methods	Human liver microsomal reaction mixtures contained microsomes and fexinidazole, M1, or M2 in potassium phosphate buffer at pH 7.4, $MgCl_2$, EDTA, and a CYP450 enzyme selective probe substrate. Microsomal reaction mixtures were kept at 37°C and the enzymatic reactions were initiated by addition of NADPH. Incubation periods were 10 min. The following controls were included with each experiment: • Reaction mixtures prepared without any of the three test compounds • Reaction mixtures prepared with a CYP450 enzyme selective inhibitors (positive control) (Listed in table below) Time-dependent inhibition was assessed by comparing IC_{50} values with pre-incubation (30 min) with added NADPH and compared to condition with pre-incubation without added NADPH and concurrent incubation. Fexinidazole concentration range: 0-169 mcM (0-9.67 mcM for CYP2C19) M1 concentration range: 0-283 mcM. M2 concentration range: 0-302 mcM

Table 138. Experiment Conditions, Study ICH0106

Isoform	Selective Substrate (Concentration)	Marker Metabolite (Analyte)	Selective Inhibitor for Concurrent Incubation/ pre-Incubation Assays
CYP1A2	Phenacetin (80 mcM)	4-Acetamidophenol	α-Naphthoflavone/Furafylline
CYP2B6	Bupropion (100 mcM)	Hydroxybupropion	Thio-TEPA/Bergamottin
CYP2C8	Amodiaquine (3 mcM)	Desethylamodiaquine	Quercetin/Gemfibrozil O-glucuronide
CYP2C9	Diclofenac (8 mcM)	4'-Hydroxydiclofenac	Sulfaphenazole/Tienilic acid
CYP2C19	S-Mephenytoin (45 mcM)	4'-Hydroxymephenytoin	Tranylcypromine/Ticlopidine
CYP2D6	Dextromethorphan (7 mcM)	Dextrorphan	Quinidine/Paroxetine
CYP3A4/5	Midazolam (3 mcM)	1'-Hydroxymidazolam	Ketoconazole/Troleandomycin
CYP3A4/5	Testosterone (70 mcM)	6β-Hydroxytestosterone	Ketoconazole/Troleandomycin

Source: Information compiled by the Reviewer from study report

The IC_{50} estimates were obtained by fitting the following nonlinear equation.

$$y = \min + \frac{\max - \min}{1 + \left(x / IC_{50}\right)^{Hillslope}}$$

min: zero concentration response (relative rate at zero concentration of inhibitor)

max: infinite concentration response (relative rate at infinite concentration of inhibitor)

Results & Conclusions

Table 139. IC_{50} Mean Value in mcM (CV %) of Test Compounds From Concurrent Inhibition, Study ICH0106

Isoform	Fexinidazole	M1	M2
CYP1A2	22.3 (1.6%)	142 (2.6%)	No inhibition
CYP2B6	26.5 (2.5%)	No inhibition	No inhibition
CYP2C8	147 (8.7%)	No inhibition	No inhibition
CYP2C9	118 (4.4%)	No inhibition	No inhibition
CYP2C19	0.863 (3.4%)	10.8 (3.1%)	No inhibition
CYP2D6	98.5 (2.8%)	278 (5.1%)	No inhibition
CYP3A4/5	35.4 (1.5%)	No inhibition	No inhibition
CYP3A4/5	45.1 (2.7%)	No inhibition	>302

Source: Information compiled by the Reviewer from study report
Abbreviations: CV, coefficient of variation; IC_{50}, half maximal inh bitory concentration; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone;

Table 140. IC_{50} Shift (in Fold) for Assessing Time-Dependent Inhibition, Study ICH0106

Isoform	Fexinidazole	M1	M2
CYP1A2	2	0.663	NA
CYP2B6	1.73	NA	NA
CYP2C8	NA	NA	NA
CYP2C9	0.98	NA	NA
CYP2C19	0.271	0.859	NA
CYP2D6	NA	NA	NA
CYP3A4/5	1.61	NA	NA
CYP3A4/5	1.62	NA	NA

Source: Information compiled by the Reviewer from study report
Abbreviations: IC_{50}, half maximal inhibitory concentration; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; NA, not applicable

Fexinidazole was found to be inhibitor of human CYP1A2, -2B6, -2C8, -2C9, -2C19, -2D6 and -3A4/5 isoforms with IC_{50} values of 22.3, 26.5, 147, 118, 0.863, 98.5, 35.4, and 45.1 mcM, respectively.

M1 was found to be inhibitor of human CYP1A2, -2C19 and -2D6 isoforms with IC_{50} values of 142, 10.8 and 278 mcM, respectively.

M2 was found to be inhibitor of human CYP3A4/5 (with Testosterone as substrate) isoforms, with IC_{50} value higher than 302 mcM (27.8% at 302 mcM).

None of the tested compound was identified as a time dependent inhibitor of CYPs.

Reviewer's Assessment: The report notes that an IC_{50} shift of 0.271 was observed for CYP2C19 for fexinidazole. This higher IC_{50} value obtained after pre-incubation with NADPH is attributed to the fact that fexinidazole is rapidly converted into M1, the latter being a less strong inhibitor of CYP2C19 isoform.

Abbreviations: CV, coefficient of variation; IC_{50}, half maximal inhibitory concentration; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; NADPH, nicotinamide adenine dinucleotide phosphate

Table 141. Study XT093051

Title	In Vitro Evaluation of Fexinidazole and Metabolites as Inducers of Cytochrome P450 Expression in Cultured Human Hepatocytes.
Methods	
System	Cultured human hepatocytes
Methods	Human hepatocytes were treated with vehicle (0.1 % DMSO), fexinidazole (1, 10, and 100 mcM), M1 (1, 10, and 100 mcM), M2 (1, 10, and 100 mcM) or known CYP450 inducers (omeprazole 100 mcM, phenobarbital 750 mcM or

	rifampin 100 mcM). After treatment, the level of mRNA expression was measured specifically for CYP1A2, CYP2B6, and CYP3A4 by qRT-PCR.
Results & Conclusions	
On average, treatment with omeprazole, phenobarbital, and rifampin caused a 159-, 21.2- and 10.0-fold increase, in CYP1A2, CYP2B6 and CYP3A4 mRNA, respectively. Fexinidazole at concentrations up to 100 mcM caused concentration-dependent increases (up to 3.15-fold) in CYP1A2 mRNA expression. Treatment with M1 and M2 at concentrations up to 100 mcM caused little or no increase in corresponding CYP1A2 mRNA expression. Fexinidazole, M1, and M2 at concentrations up to 100 mcM caused concentration-dependent increases (up to 10.2-, 9.16-, and 2.69-fold, respectively) in CYP2B6 mRNA expression. These increases were 45.5%, 40.4%, and 8.37% of the increase caused by the positive control, phenobarbital, respectively. Fexinidazole and its two metabolites M1 and M2, caused concentration-dependent decreases in CYP3A4 mRNA expression (down to 44.2%, 51.8%, and 65.7%, respectively, compared to hepatocytes treated with the vehicle control (0.1 % DMSO). Fexinidazole and M1 may warrant in vivo investigation for the potential to induce CYP2B6.	

Abbreviations: DMSO, dimethyl sulfoxide; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; mRNA, messenger ribonucleic acid

Table 142. Study IHH0074

Title	In vitro evaluation of inducibility of cytochrome P450s by Fexinidazole, Fexinidazole Sulfoxide (M1) and Fexinidazole Sulfone (M2) using three batches of human hepatocytes
Methods	
System	Cryopreserved cultured human hepatocytes from three donors
Methods	Human hepatocytes were treated with vehicle (0.1 % DMSO), fexinidazole (1-300 mcM), M1 (1-400 mcM), M2 (1-400 mcM) or known CYP inducers (omeprazole 50 mcM, phenobarbital 2000 mcM, CITCO 0.1 mcM, flumazenil 25 mcM, or rifampin (0.1-20 mcM). After treatment, the level of mRNA expression was measured specifically for CYP1A2, CYP2B6, and CYP3A4 by qRT-PCR.

Results & Conclusions
Viability of hepatocytes from the three donors was not affected by fexinidazole, M1, and M2 up to 400 mcM. CYP1A2: Fexinidazole induced CYP1A2 mRNA expression in 3/3 primary human hepatocytes cultured in vitro, modeled E_{max} being respectively 100.7-, 14.8- and 15.7-fold of control, corresponding to calculated 9.8%, 7.0% and 10.6% of positive control (PC) in donors 1, 2 and 3; EC_{50} ranged between 138.6µM and 199.7 mcM. M1 induced CYP1A2 mRNA expression in 3/3 primary human hepatocytes cultured in vitro, modeled E_{max} being respectively 16.7-, 16.2- and 5.2-fold of control, corresponding to calculated 1.6%, 7.7% and 3.0% of PC in donors 1, 2 and 3; EC_{50} ranged between 11.5 mcM and 50.0 mcM. M2 did not induce CYP1A2 up to 400 mcM in 3/3 primary human hepatocytes cultured in vitro. A concentration-dependent decrease in CYP1A2 mRNA expression by M2 was observed in 2/3 primary human hepatocytes cultured in vitro (donors 2 and 3). CYP2B6: Fexinidazole induced CYP2B6 mRNA expression at the two highest tested concentrations in 3/3 primary human hepatocytes cultured in vitro, modeled E_{max} being respectively 2.0-, 2.0- and 1.9-fold of control, corresponding to calculated 57.1%, 22.7% and 29.7% of PC in donors 1, 2 and 3; EC_{50} could not be determined. M1 also induced CYP2B6 mRNA expression at the two highest tested concentrations in 3/3 primary human hepatocytes cultured in vitro, modeled E_{max} being respectively 3.3-, 2.2- and 1.8-fold of control, corresponding to calculated 94.3%, 25.0% and 28.1% of PC in donors 1, 2 and 3; EC_{50} was 98.7 mcM and 10.5 mcM in donor 1 and 2 respectively and could not be determined in donor 3. M2 did not induce CYP2B6 up to 200 mcM in 3/3 primary human hepatocytes cultured in vitro. A 2.0-fold increase in CYP2B6 mRNA expression at 400 mcM, corresponding to 18% of PC was seen in only 1/3 primary human hepatocyte culture in vitro (donor 3). CYP3A4: Fexinidazole induced CYP3A4 mRNA expression in only 1/3 primary human hepatocyte culture in vitro at 200 mcM and 300 mcM (donor 1), with a maximum of 2.3-fold (200 mcM), corresponding to 7% of PC. M1 did not induce CYP3A4 mRNA expression in 3/3 primary human hepatocytes cultured in vitro. M2 did not induce CYP3A4 mRNA expression up to 400 mcM in 3/3 primary human hepatocytes cultured in vitro. Fexinidazole and M1 should be considered as potential CYP1A2 and CYP2B6 inducers and M2 as repressing CYP1A expression. None of the compounds consistently affected CYP3A4 mRNA expression.
Reviewer's Assessment: The study findings above show that fexinidazole and M1 induces CYP1A2 mRNA expression. However, the findings from an in vivo DDI cocktail study that included caffeine as a probe substrate showed that the net effect of fexinidazole treatment on CYP1A2 would be inhibition.

Abbreviations: DMSO, dimethyl sulfoxide; $_{Emax}$; maximal effect at high drug concentrations when all the receptors are occupied by the drug; EC_{50}, drug concentration to give the half-maximal effect; mRNA, messenger ribonucleic acid; PC, qRT-PCR, real-time quantitative reverse transcription polymerase chain reaction

Table 143. Static Mechanistic Drug-Drug Interaction Output File:Ddi-Input-Output-Data

File	An excel file containing output from DDI Risk Calculator (DDIRC) Version: 2017.1
Methods	
System	Drug-Drug Interaction Risk Calculator (DDIRC), Pharmapendium®
Methods	DDIRC was used for the predictions of the in vivo drug interactions using a similar mechanistic static model as in the FDA Guidance on in vitro drug interaction studies. Specifically, static mechanistic models of DDIRC were used to predict the effect ratio on CYP2D6 and CYP3A4 substrates. The comparison of the mechanistic models from the 2020 FDA guidance on in vitro drug interaction studies and the DDIRC (from the Drug-Drug Interaction Risk Calculator User Guide) is presented in the table below:

Table 144. Comparison of Mechanistic Models

	FDA Guidance 2020	DDIRC
MSM	$\frac{AUCi}{AUC}=\frac{1}{Fg+(1-Fg)\times(A_g\times B_g\times C_g)}\times\frac{1}{(A_h\times B_h\times C_h)\times fm(E)_h+1-fm(E)_h}$	
Reversible inhibition $A_{h/g}$	$\frac{1}{1+\frac{[I]_{h/g}}{K_i}}$	
Time-dependent inhibition $B_{h/g}$	$\frac{1}{1+\frac{k_{inact}\times[I]_{h/g}}{k_{deg,H}\times([I]_{h/g}+K_i)}}$	
Induction $C_{h/g}$	$1+\frac{d\times Emax\times[I]_{h/g}}{[I]_{h/g}+EC_{50}}$	$1+\frac{Emax\times[I]_{h/g}^{n}}{[I]_{h/g}^{n}+EC_{50}^{n}}$
Perpetrator concentration, liver [I]h	$[I]_h=fb\times\left(Cmax,b+\frac{Fabs\times Fg\times kabs\times D}{Qh}\right)$	
Perpetrator concentration, gut [I]g	$[I]_g=\frac{Fabs\times kabs\times D}{Qg}$	

Source: From DDIRC User Guide provided by the Applicant

For glossary of terminologies above, refer to the FDA guidance document: In Vitro Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions Guidance for Industry, January 2020.
A difference between the two models is how the contribution of induction is incorporated in the predictions. The Calculator's User Guide provided by the Applicant notes that the two models remain exactly the same when the E_{max} value used in the DDIRC takes into account the in vitro to in vivo scaling factor d and the hill coefficient n value is equal to one. Also, the User Guide notes the default values of hepatic (Q_h = 1.61 L/min) and gut blood flow (Q_g = 18 L/h).
The Applicant has also provided following information related to the concentrations and parameter estimates that were used in the predictions:
1. C_{max} of fexinidazole: 777 ng/mL corresponds to the mean C_{max} at the end of the loading dose regimen of 1800 mg fexinidazole for 4 days in fed conditions from study DNDIFEX003.
2. FaFg is set to 1
3. kabs is the absorption rate constant of fexinidazole calculated from fexinidazole t_{max} and $t_{1/2}$ ($1/k_{el}$) using a first order absorption rate according to following equation:

$$Tmax=\frac{\ln\left(\frac{kabs}{kel}\right)}{kabs-kel}$$

4. For perpetrator 2 (M1 metabolite): the concentration at the enzyme site is extrapolated as the unbound blood mean C_{max}. The input data are the mean total C_{max} of M1 i.e., 7768 ng/mL, at the end of the 1800 mg loading dose regimen from study DNDIFEX003.
5. Because fexinidazole and its metabolite M1 are competitive inhibitors acting via the same mechanism of inhibition on CYP1A2, 2C19, and 2D6, consequently the reduction of intrinsic clearance of the victim drug was incorporated as additive (sum of [I]/Ki for fexinidazole and M1 in the equation below).

$$\text{Fold reduction } Clu_{int_k}=1+\sum_{j=1}^{p}\left(\frac{[Iu]_{H,j}}{Ki,u_j}\right)$$

Input parameters that were used in the predictions are summarized in the table below:

Table 145. Input Parameter Estimates

Name	M1	Fexinidazole
MW (g/mol)	295	270
Dose(mg)	0.0	1800
Dosing regimen	Repeated	Repeated
$[I]_{in}$ estimation	(C_{max})	(Optimized [I])
C_{max}/C_{avg} (ng/ml)	7768	777
Q_h (L/min)	1.61	1.61
FaFg	1	1
kabs(min-1)	0.1	0.012
$t_{1/2}$(h)		13.4
T_{max}(h)		4
fu plasma	0.647	0.063
Rb	0.98	0.61
fu(mic)	0.855	0.796
$[C]_{prot}$ (g/L)	1	1
Method	Hallifax	Hallifax
LogP/LogD	1.8(LogD)	2.3(LogD)
pKa	2.1	1.8

Source: Information compiled from the Applicant's file

Results & Conclusions
For CYP2D6 metabolized drugs, the predictions showed that fexinidazole would not notably increase the exposure of CYP2D6 probe or sensitive substrate drugs such as metoprolol (mean predicted interaction ratio of 1.1-fold), desipramine (1.1-fold), nebivolol (1.1-fold), propafenone (1.1-fold), risperidone (1.1-fold), and venlafaxine (1.3-fold). For CYP3A4 metabolized drugs, the findings showed that fexinidazole would increase the exposure of sensitive or probe CYP3A4 drugs, having a notable intestinal first pass metabolism such as lovastatin (mean predicted interaction ratio of 8-fold), simvastatin (4-fold), midazolam (2-fold), nisoldipine (6-fold), and saquinavir (2.5-fold). Fexinidazole treatment would not notably increase the exposure of CYP2D6 probe or sensitive drug substrates and would increase the exposure of sensitive or probe CYP3A4 drugs.
Reviewer's Assessment: The Reviewer did not have access to above-mentioned DDIRC. Therefore, the Applicant's exact analysis could not be repeated, nor the prediction findings submitted in the file could be verified. In an effort to predict the potential DDI from fexinidazole treatment with CYP2D6 and CYP3A4 substrates, the Reviewer used an internal FDA/OCP Microsoft Excel-based tool that assesses the DDI prediction using static mechanistic models. The internal DDI prediction tool utilizes the models as described in the above- mentioned FDA guidance document. The input parameter estimates for fexinidazole for the Reviewer's analysis were mostly kept same except for the molecular weight for fexinidazole and Cmax concentration values of fexinidazole. The Applicant used molecular weight of fexinidazole as 270 g/mol, which is incorrect. The correct fexinidazole molecular weight of 279 g/mol, was used by the Reviewer. In addition, in the Reviewer's analysis, the geometric mean concentration of fexinidazole from Day 1 of the start of therapy from Study DNDIFEX003 was used, i.e., 1519 ng/mL. The Applicant used the Cmax estimates from the end of the 1800 mg loading dose regimen (Day 4), i.e., 777 ng/mL for fexinidazole. For CYP2D6 metabolized drugs, the predictions from Reviewer's analysis showed that fexinidazole would not notably increase the exposure of the CYP2D6 substrate, dextromethorphan (mean predicted interaction ratio of 1.1-fold), which is similar to the findings from the output file from DDIRC for dextromethorphan (1.12-fold). For CYP3A4 metabolized drugs, the predictions from Reviewer's analysis showed that fexinidazole would notably increase the exposure of the CYP3A4 substrate, midazolam (2.37-fold), which is similar to the findings from the output file from DDIRC for midazolam (2.2-fold).

Abbreviations: AUC, area under the curve; C_{avg}, average serum concentration; C_{max}, maximum serum concentration DDI, drug-drug interaction; E_{max}, maximal effect at high drug concentrations when all the receptors are occupied by the drug; FDA, Food and Drug Administration; M1, Fexinidazole sulfoxide; MW, molecular weight; OCP, Office of Clinical Pharmacology; T_{max}, time at which the Cmax is observed; $T_{1/2}$, half-life

16.3.3. Pharmacokinetics in Healthy Adults

All Phase 1 PK studies, bioavailability (BA) studies, and a clinical drug interaction study were conducted in healthy Sub-Saharan African adults. The PK of fexinidazole in HAT patients was characterized using a population PK analysis approach. Please see Section 16.3.4 for information on the PK in HAT patients.

16.3.3.1. Study DNDiFEX001 Part II

Overview

This was a double-blind, randomized, single dose, cross-over study that assessed and compared bioavailability (BA) of fexinidazole and its active metabolites: fexinidazole sulfoxide (M1) and fexinidazole sulfone (M2), following the administration of fexinidazole as oral suspension and as tablets in sub-Saharan African healthy male subjects. The study also assessed the effect of food on fexinidazole BA and systemic exposure to its metabolites. In total, 12 subjects were planned to be enrolled to receive the following three treatments in a cross-over manner separated by a wash-out period of at least 14 days:

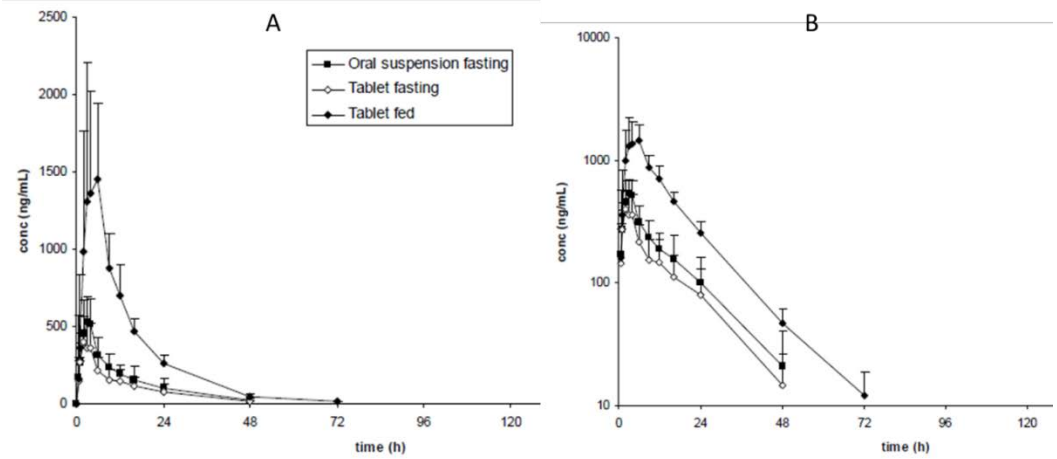
- Treatment A: 1200 mg fexinidazole as an oral suspension in fasting condition
- Treatment B: 1200 mg fexinidazole as tablets in fasting condition
- Treatment C: 1200 mg fexinidazole as tablets in fed condition

Prior to administering each treatment, subjects fasted overnight for a minimum of 10 hours except for Treatment C. For Treatment C, a high-fat standard breakfast was administered at 30 minutes before dosing. The composition of breakfast contained total 963 Kcal (62% from fat, 17% from protein, and 21% from carbohydrate).

From 12 randomized subjects, one subject withdrew consent during the course of the study, after receiving one treatment (Treatment C). Therefore, the one subject was replaced and 12 subjects completed the study. In total, 13 subjects were included in the safety analysis and 12 were included in the PK analysis. The age and weight of enrolled patients ranged between 19 to 43 years and between to 65 to 81 kg. The enrolled patients received the assigned 1200 mg single dose as a suspension (600 mg/20 mL) or 2x 600 mg fexinidazole tablets.

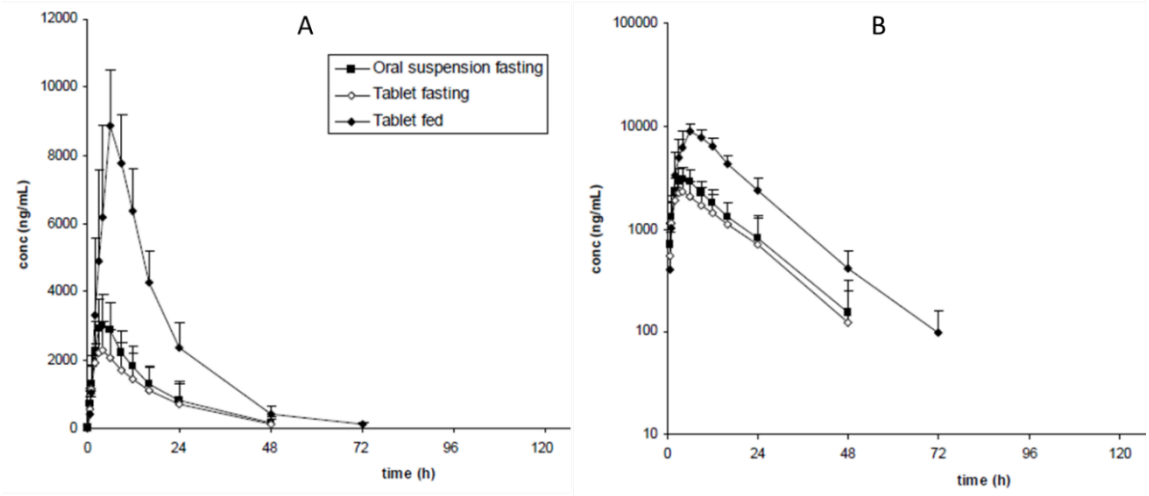
The patients remained at the study center up to 168 hours postdose. For PK assessments, 17 blood samples were collected between predose to 168 hours postdose. Fexinidazole, M1, and M2 concentrations in plasma were measured using validated LC-MS/MS methods. The reported mean plasma concentration-time profiles for fexinidazole, M1, and M2 from all cohorts are presented in Figure 6, Figure 7, and Figure 8 respectively. Plasma concentrations of all three analytes were subjected to noncompartmental analysis to derive PK parameter estimates, which are reported in Table 146.

Figure 6. Mean (SD) Concentrations of Fexinidazole in Plasma



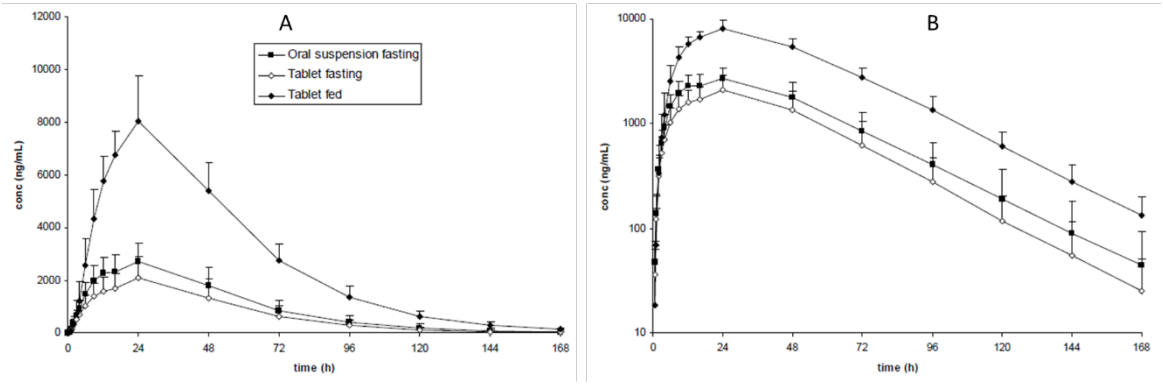
Source: Adapted from study report
Note: A: Linear scale, B: semilogarithmic scale
Abbreviations: h, hours; SD, standard deviation

Figure 7. Mean (SD) Concentrations of M1 in Plasma



Source: Adapted from study report
Note: A: linear scale, B: semilogarithmic scale
Abbreviations: h, hours; M1, Fexinidazole sulfoxide; SD, standard deviation

Figure 8. Mean (SD) Concentrations of M2 in Plasma



Source: Adapted from study report
Note: A: linear scale, B: semilogarithmic scale

Table 146. Geometric Mean (CV%) for Exposure and Pharmacokinetic Parameter Estimates for Fexinidazole, M1, and M2

Treatment (Formulation-Meal condition)	$AUC_{0\text{-}t.last}$ (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)[a]	$T_{1/2}$ (h)
Fexinidazole				
A (suspension-fasting)	6671.49 (41.59)	559.47 (31.65)	3 (1-4)	9.90 (28.47)
B (tablet-fasting)	4610.94 (50.36)	417.92 (38.48)	2 (1-4)	11.11 (29.95)
C (tablet-fed)	21473.48 (14.56)	1789.55 (28.77)	5 (2-6)	11.20 (34.10)
M1				
A (suspension-fasting)	52751.11 (41.46)	3066.89 (26.70)	4 (3-6)	10.50 (39.49)
B (tablet-fasting)	40678.66 (54.50)	2282.87 (33.33)	3 (2-6)	9.70 (34.24)
C (tablet-fed)	159522.24 (19.72)	9026.11 (15.28)	6 (4-9)	12.33 (36.26)
M2				
A (suspension-fasting)	151372.24 (31.11)	2709.11 (24.74)	24 (12-48)	19.53 (18.13)
B (tablet-fasting)	107465.74 (44.70)	1980.12 (37.37)	24 (16-24)	19.39 (15.11)
C (tablet-fed)	458090.77 (13.96)	7914.93 (21.53)	24 (16-24)	20.93 (13.05)

Source: Study report
[a] Median [Range]
Abbreviations: $AUC_{0\text{-}t.last}$, area under the concentration versus time curve up to last concentration timepoint; C_{max}, maximum serum concentration; h, hours, M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; T_{max}, time at which the C_{max} is observed; $T_{1/2}$, half-life

No notable difference between T_{max} was observed when comparing tablet versus suspension; however, food intake was found to significantly ($p<0.05$) delay T_{max} by about 2 to 3 hours for fexinidazole and M1. Such a delay was not observed for M2. The mean estimates for C_{max} and $AUC_{0\text{-}t}$ for fexinidazole, M1, and M2 following dosing with tablet formulation were 73 to 75% and 69 to 77%, respectively, of the estimates following dosing with suspension formulation (Table 147). The mean estimates for C_{max} and $AUC_{0\text{-}t}$ for fexinidazole, M1, and M2 following dosing with tablet under fed condition were 395 to 400 % and 392 to 466% of the estimates following dosing with tablet under fasted condition (Table 147).

Table 147. Summary of the Bioavailability Assessment

	% Point Estimates	
Parameters	**B (Tablet-Fasting)/A (Suspension-Fasting)**	**C (Tablet-Fed)/ B (Tablet-Fasting)**
Fexinidazole		
$AUC_{0\text{-}t.last}$	69 (58-83)	466 (365-595)
C_{max}	75 (60-94)	428 (327-561)

	% Point Estimates	
Parameters	**B (Tablet-Fasting)/A (Suspension-Fasting)**	**C (Tablet-Fed)/ B (Tablet-Fasting)**
M1		
$AUC_{0\text{-}t.last}$	77 (67-89)	392 (333-462)
C_{max}	74 (65-87)	395 (351-445)
M2		
$AUC_{0\text{-}t.last}$	71 (62-81)	426 (354-513)
C_{max}	73 (64-83)	400 (335-476)

Source: Adapted from study report
Abbreviations: $AUC_{0\text{-}t.last}$, area under the concentration versus time curve up to last concentration timepoint; C_{max}, maximum (or peak) serum concentration; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone

Comparisons of ratios of C_{max} and $AUC_{0\text{-}t}$ between fexinidazole, M1, and M2 did not show any difference between treatments suggesting that the increase or decrease in relative BA across the treatments did not lead to change on the rate of biotransformation of fexinidazole into its metabolites M1 and M2 (Table 148).

Table 148. Mean Ratios of PK Parameters for Fexinidazole, M1, and M2

Treatment	Ratio M1:Fexinidazole	Ratio M2: Fexinidazole	Ratio M2: M1
$AUC_{0\text{-}t.last}$			
A (suspension-fasting)	7.91	22.69	2.87
B (tablet-fasting)	8.82	23.31	2.64
C (tablet-fed)	7.43	21.33	2.87
C_{max}			
A (suspension-fasting)	5.48	4.84	0.88
B (tablet-fasting)	5.46	4.74	0.87
C (tablet-fed)	5.04	4.42	0.88

Source: Adapted from study report
Abbreviations: $AUC_{0\text{-}t.last}$, area under the concentration versus time curve up to last concentration timepoint; C_{max}, maximum serum concentration; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone

Applicant's Conclusions

After a single administration of 1200 mg fexinidazole as an oral suspension or a tablet in healthy subjects:

- The tablet exhibited comparable dissolution and distribution profiles than the oral suspension with a decrease in relative BA of about 25%
- No change on metabolic ratio was observed for both biotransformation steps (fexinidazole into M1, M1 into M2)
- Concomitant food intake significantly increased the bioavailability of the tablet formulation (+200% for fexinidazole) but did not impact the overall PK pattern

***Reviewer's Assessment**: The Applicant's conclusion notes that food intake led to an increase in the relative bioavailability of fexinidazole from the tablet formulation by 200%. The reviewer finds this conclusion inaccurate. The AUC and C_{max} estimates of fexinidazole following tablet administration under fed conditions were 466% and 428% of the estimates following tablet administration under fasted conditions (Table 149), respectively. Therefore, the increase in the relative bioavailability (AUC) of fexinidazole from the tablet formulation under fed conditions*

was approximately 4-fold when compared to fasted conditions. The BA (AUC) of the M1 and M2 metabolites also increased significantly by the same order of magnitude as parent fexinidazole.

16.3.3.2. Study DNDiFEX001 Part III

Overview

This was a double-blind, randomized, multiple ascending dose study that assessed BA of fexinidazole and the systemic exposure to its active metabolites: fexinidazole sulfoxide (M1) and fexinidazole sulfone (M2), following the administration of multiple doses of fexinidazole 600 mg tablets in healthy sub-Saharan African male subjects. The enrolled subjects were randomized to three different dose cohorts (Table 149). In total, 27 subjects were randomized, however, three subjects withdrew consent and were replaced. One subject was discontinued from the highest dose cohort by the Applicant due to the observed serious adverse event (sudden raise in transaminases). Consequently, 23 subjects completed the study and were part of the PK analysis.

Table 149. Description of Cohorts in Study DNDiFEX001 Part III

Cohort	Treatment and Dose (Subjects)
1	1200 mg fexinidazole (6) or placebo (2) from Day 1 to Day 14 (tablet)
2	2400 mg fexinidazole (6) or placebo (2) from Day 1 to Day 14 (tablet)
3	3600 mg fexinidazole (5) or placebo (2) from Day 1 to Day 14 (tablet)

Source: Adapted from study report

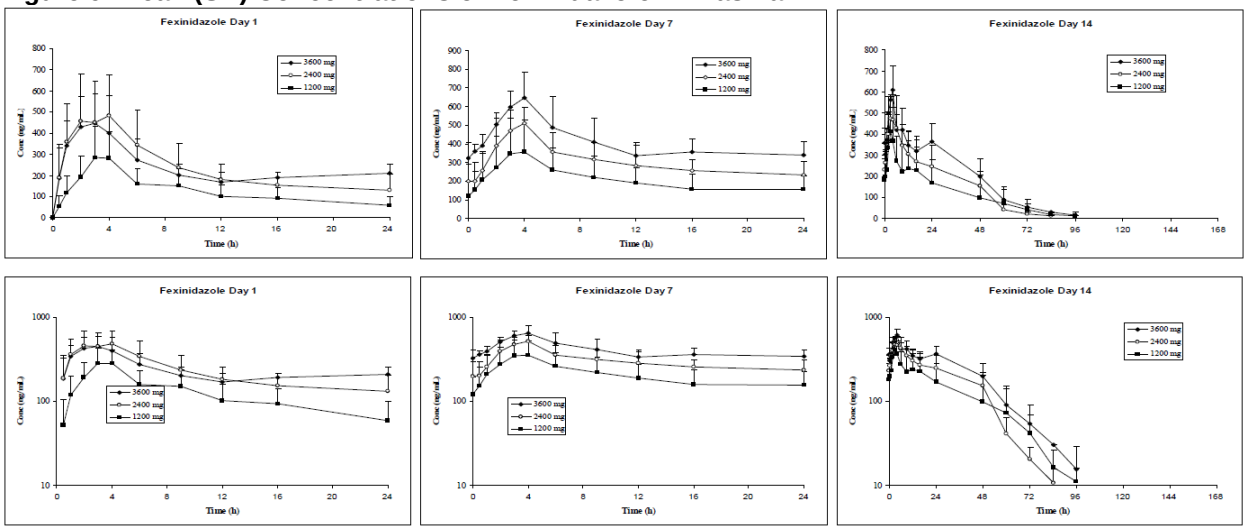
Across all cohorts, the age and weight of enrolled patients ranged between 18 to 40 years and between to 55 to 96 kg, respectively. The enrolled patients received the assigned tablet dose in fasting state (at least 10 hours) at approximately same time in the morning for 14 days.

The patients remained at the study center up to 168 hours post-last dose. For PK assessments, blood samples were collected as outlined below:

- Day 1: 11 samples from predose to 24 hours postdose.
- Days 4 and 6: one predose sample
- Day 7: 11 samples from predose to 24 hours postdose.
- Days 9, 11 and 13: one predose sample
- Day 14: 19 samples from predose to 168 hours postdose

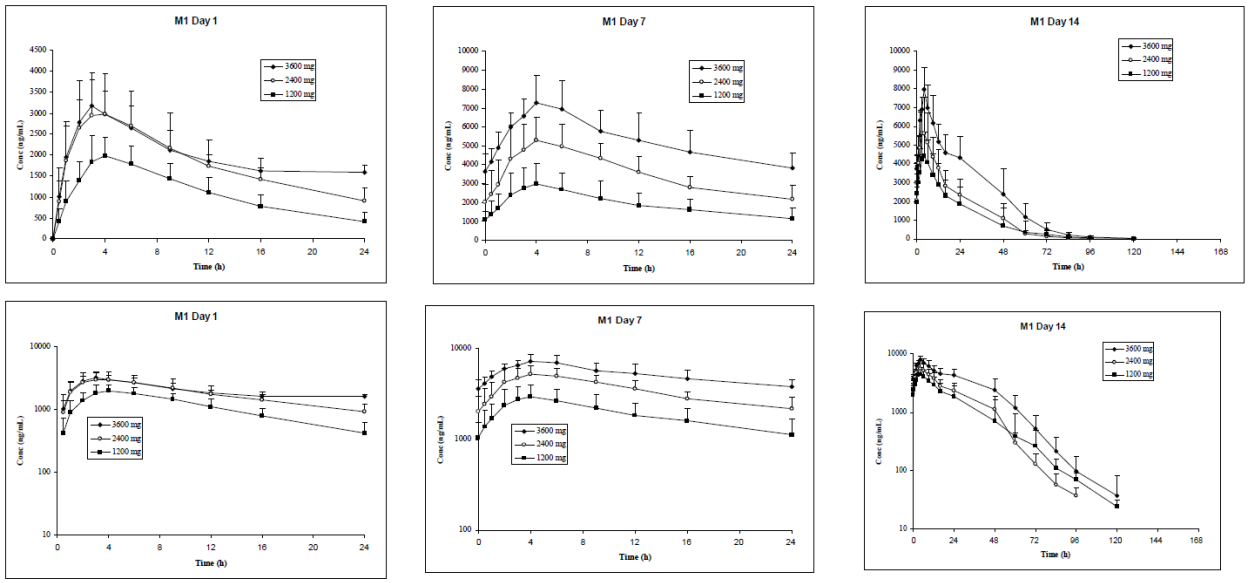
Fexinidazole, M1, and M2 concentrations in plasma were measured using validated LC-MS/MS methods. Urine was also collected over the 24-hour period postdose on days 1, 7, and 14, to estimate renal excretion. The reported mean plasma concentration-time profiles for fexinidazole, M1, and M2 from all cohorts are presented in Figure 9, Figure 10, and Figure 11, respectively. Plasma concentrations of all three analytes were subjected to noncompartmental analysis to derive PK parameter estimates, which are reported in Table 150.

Figure 9. Mean (SD) Concentrations of Fexinidazole in Plasma



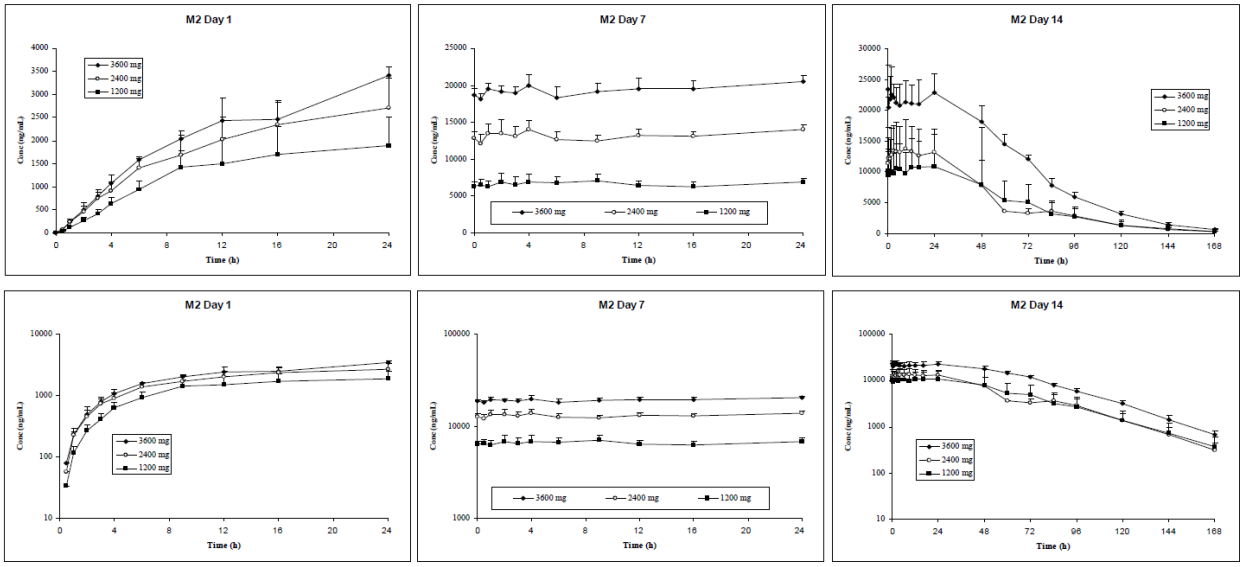
Source: From the study report
Note: Linear: Top panel, log-linear scale: Bottom panel
Abbreviations: h, hours; SD, standard deviation

Figure 10. Mean (SD) Concentrations of M1 in Plasma



Source: From the study report
Note: Linear: Top panel, log-linear scale: Bottom panel
Abbreviations: h, hours; M1, Fexinidazole sulfoxide; SD, standard deviation

Figure 11. Mean (SD) Concentrations of M2 in Plasma



Source: From the study report
Note: Linear: Top panel, log-linear scale: Bottom panel
Abbreviations: h, hours; M2 Fexinidazole sulfone; SD, standard deviation

Table 150. Geometric Mean (CV%) for Exposure and Pharmacokinetic Parameter Estimates for Fexinidazole, M1, and M2

Treatment Day	Cohort (QD DoseX14days, n)	$AUC_{0\text{-}t.last}$ (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)[a]	$T_{1/2}$(h)[b]
Fexinidazole					
Day 1	1 (1200,6)	-	264.70 (45)	3 (3-4)	12.3 (50)
	2 (2400,6)		511.66 (34)	3.5 (1-4)	13.3 (50)
	3 (3600,5)		452.92 (29)	3 (0.5-4)	-
Day 7	1 (1200,6)	-	302.95 (50)	4 (3-4)	20.9 (111)
	2 (2400,6)		516.06 (16)	4 (3-6)	42.5 (176)
	3 (3600,5)		653.44 (19)	4 (3-4)	68.3 (190)
Day 14	1 (1200,6)	7448.56 (72)	385.40 (39)	3 (3-12)	14.2 (49)
	2 (2400,6)	12602.31 (35)	485.69 (24)	3 (0-6)	12 (26)
	3 (3600,5)	18956.46 (25)	635.05 (12)	4 (3-4)	14.8 (21)
M1					
Day 1	1 (1200,6)	-	2020.44 (21)	4 (2-4)	8.0 (22)
	2 (2400,6)		3097.38 (27)	3 (2-6)	12.5 (29)
	3 (3600,5)		3215.90 (17)	3 (2-4)	28.5 (64)
Day 7	1 (1200,6)	-	2891.85 (34)	4 (2-6)	14.4 (34)
	2 (2400,6)		5385.14 (23)	4 (3-6)	17.1 (79)
	3 (3600,5)		7236.67 (19)	4 (3-6)	27.7 (56)
Day 14	1 (1200,6)	102163.85 (53)	4468.99 (29)	3 (3-4)	12.7 (42)
	2 (2400,6)	138243.51 (29)	5602.67 (32)	3 (3-4)	13.1 (14)
	3 (3600,5)	244110.19 (29)	7912.16 (15)	4 (4-4)	12.8 (26)

Treatment Day	Cohort (QD DoseX14days, n)	$AUC_{0\text{-}t.last}$ (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)[a]	$T_{1/2}$(h)[b]
M2					
Day 1	1 (1200,6)	-	1826.63 (36)	24 (16-24)	-
	2 (2400,6)		2696.59 (32)	24 (16-24)	-
	3 (3600,5)		3405.04 (9)	24 (24-24)	-
Day 7	1 (1200,6)	-	7374.89 (29)	4 (0.5-9)	-
	2 (2400,6)		14677.69 (31)	2.5 (1-12)	-
	3 (3600,5)		21257.22 (8)	16 (0-24)	-
Day 14	1 (1200,6)	708847.38 (49)	10956.81 (43)	5.5 (0-24)	24.8 (14)
	2 (2400,6)	904734.98 (32)	14758.27 (33)	9 (0-48)	22.2 (13)
	3 (3600,5)	1750815.38 (9)	24428.65 (16)	2 (0-24)	23.0 (9)

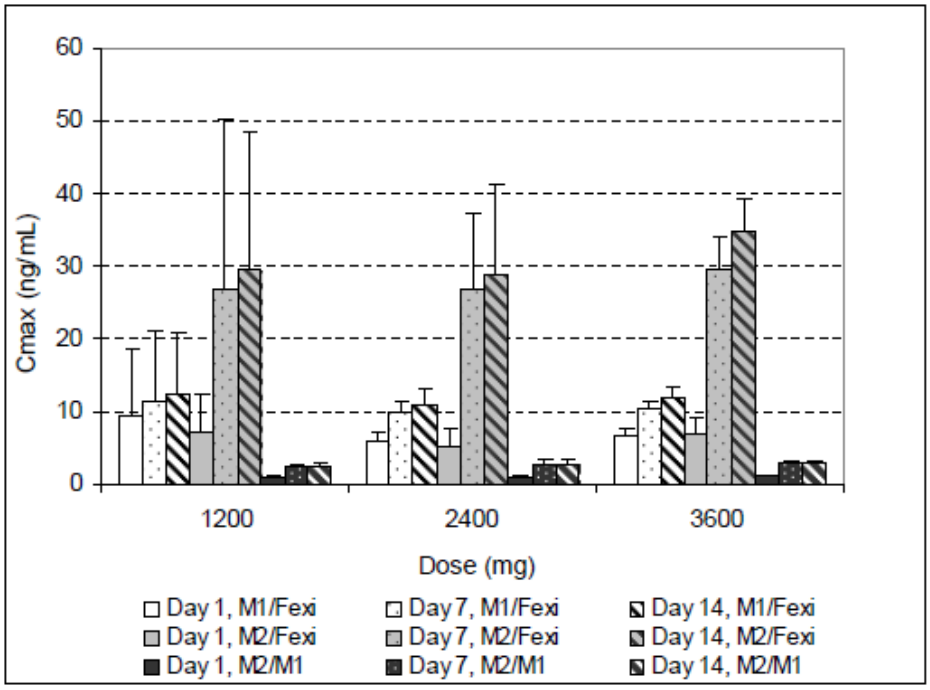
Source: Adapted from study report
[a] Median (Range)
[b] From Reviewer's Analysis
Abbreviations: $AUC_{0\text{-}t.last}$, area under the concentration versus time curve up to last concentration timepoint; C_{max}, maximum serum concentration; CV, coefficient of variation; h, hours, M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; QD, once daily; T_{max}, time at which the C_{max} is observed; $T_{1/2}$, half-life

Relationships between dose and exposure parameter estimates for fexinidazole and its metabolites showed that their exposure increased with dose; however, in a less than dose proportional manner.

Relative exposure of fexinidazole's metabolites M1 and M2 were measured without adjusting the concentrations for molecular weight. On Day 1, the observed mean ratio for C_{max} ranged between 6 and 9 for M1/fexinidazole and between 5 and 7 for M2/fexinidazole. M2/M1 ratio ranged between 0.9 and 1 (Figure 12). The observed mean ratio for AUC values ranged between 8 and 14 for M1/fexinidazole and between 7 and 15 for M2/fexinidazole (Figure 13). M2/M1 ratio ranged between 1 and 1.1.

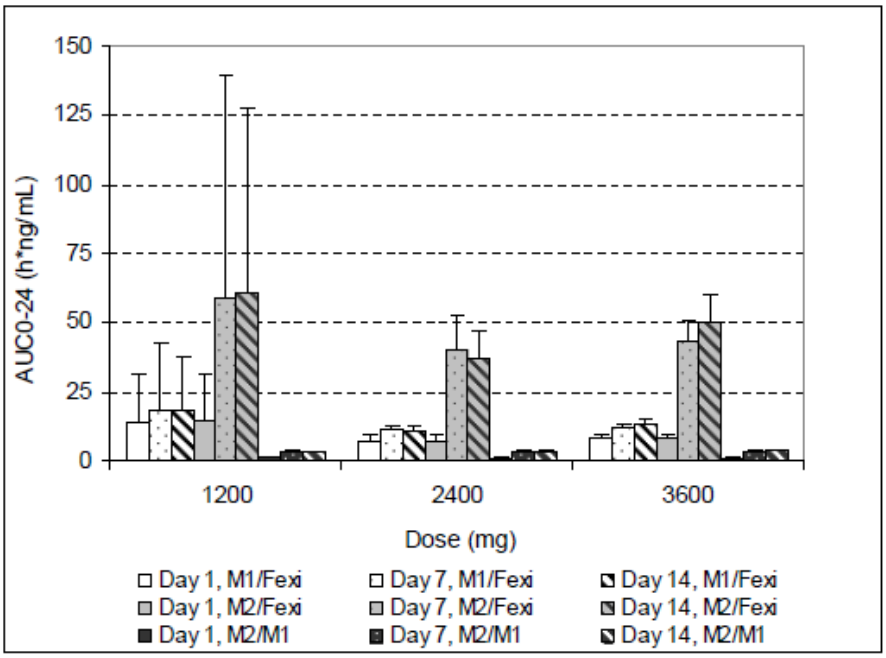
When comparing Day 7 and Day 14, all metabolic ratios (C_{max} and AUC) investigated were in the same order of magnitude. The mean M2/fexinidazole ratios C_{max} were always markedly higher (mean values ranging between 27 to 30 on Day 7 and 29 to 35 on Day 14) than the M1/fexinidazole ratios (mean values ranging between 10 to 11 on Day 7 and 11 to 12 on Day 14). Similarly, the observed mean ratio of AUC for M2/fexinidazole ranged between 40 to 59 on Day 7 and 37 to 61 on Day 14 versus 11-18 on Day 7 and 11 to 18 on Day 14 for M1/fexinidazole ratio. M2/M1 ratio C_{max} and AUC were very relatively close between Day 7 and Day 14 for C_{max} and AUC. Overall, these data suggested that after repeated oral administration for 7 or 14 days there was no saturation of the metabolism of fexinidazole either at the first step leading to generation of M1 or for the biotransformation of M1 into M2. The high exposure value of M2 could be explained by the longer $T_{1/2}$ (of about 20 hours) associated with a possible saturation of M2 elimination after repeated administration of fexinidazole.

Figure 12. Mean Metabolic Ratios M1/Fexinidazole, M2/Fexinidazole and M2/M1 of C_{max} Versus Dose



Source: Study report
Abbreviations: C_{max}, maximum serum concentration; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone;

Figure 13. Mean Metabolic Ratio M1/Fexinidazole, M2/Fexinidazole and M2/M1 of $AUC_{0-\infty}$ Versus Dose



Source: Study report
Abbreviations: AUC_{0-24}, area under the plasma concentration-time curve from time zero to 24 hours; $AUC_{0-\infty}$, total drug exposure across time; C_{max}; maximum serum concentration; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone;

PK assessments in urine samples showed that negligible amount (<60mcg) of unchanged fexinidazole was excreted in urine. For both metabolites M1 and M2, total amounts of M1 and M2 excreted in urine were 100 to 700 fold higher than the parent drug, however, amounts of M1 and M2 excreted in urine were reported to be low, i.e., <32 mg and <46 mg, respectively.

During the study, the observed biochemistry parameters were within their respective normal limits except Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and creatine phosphokinase. Mean AST and ALT values above the normal upper limit occurred at the 2 last assessments (Day 21 and end of study) in the 2400 mg dose group and on Day 15 and Day 21 in the 3600 mg dose group. However, increases of AST and ALT were considered non clinically significant except for one subject whose maximum elevation of AST and ALT was 38 and 30 folds the upper normal limit, respectively. The observed creatine phosphokinase levels were always above the normal range but remained constant across the study duration. The postdose values were always close to those measured at baseline. These increases could be ascribed to the study population itself (as observed in subjects receiving placebo) and the mild increase in creatinine could be related to inhibition of renal tubular secretion of creatinine

Applicant's Conclusions:

After a single and repeated oral administration once daily, 7 and 14 consecutive days of increasing doses of fexinidazole (1200 to 3600 mg) in healthy male subjects:

- Liver function enzymes were detected as target parameters over 14 days administration. Shorter duration of exposition and complementary Holter explorations should be considered.
- Within the dose range 1200 mg to 3600 mg, the dose proportionality was not demonstrated for C_{max} and AUC neither for fexinidazole nor its metabolites (M1/M2), under single (first oral administration) and repeated condition. Both parameters tended to increase in a less than dose proportional manner over the 1200 to 3600 mg dose range.
- After repeated administration on Day 7, based on AUC, there was an accumulation of 1.5, 2.1 and 7.3 for fexinidazole, M1 and M2, respectively. On Day 14 limited accumulation was observed compared to Day 7 with mean factor of accumulation 1.7, 2.5 and 8.4 for fexinidazole, M1 and M2, respectively.
- After single (first oral administration) or repeat administration, M1 and M2 metabolites were circulating with higher levels as compared to parent compound. On Day 1, following first oral administration of fexinidazole, AUC ratio of M1/fexinidazole and M2/fexinidazole were comparable. On Day 7, after repeated dosing AUC ratio of M2/fexinidazole were higher than M1/fexinidazole AUC ratio. On Day 14, AUC ratios were comparable to those observed on Day 7.
- Steady-state was reached on Day 4 for fexinidazole and M1 and on Day 9 for M2 for the two highest doses 2400 mg and 3600 mg. For the lowest dose 1200 mg, steady-state was not completely demonstrated.

Reviewer's assessment: *The study report notes increase in liver function enzymes as well as serum creatinine levels. Specifically, the report notes "Some liver enzymes (ASAT and ALAT) variations were observed in all dose groups mostly less than 3x [upper limit] UNL as often observed in long multiple dose studies in healthy subjects and were considered as not clinically significant by the investigator." The Applicant has not conducted any dedicated exposure-response analysis neither has submitted raw lab data. Therefore, further analysis on these observations was not pursued. It is noteworthy that the proposed fexinidazole dosage regimen is different than the dosing regimen that was evaluated in this study. Specifically, the total fexinidazole doses evaluated in Cohort 1 and Cohorts 2 & 3 were lower and higher than the proposed fexinidazole dosing, respectively.*

The study report also notes that creatinine blood levels increased with the level of circulating fexinidazole and/or metabolites, however, remained within normal limits. This observation is attributed to an inhibition of renal tubular secretion. This observation is in line with the findings from Study TRI0039 that showed that fexinidazole treatment has potential to inhibit renal transporters.

16.3.3.3. Study DNDiFEX002

Overview

This was an open-label, randomized, single dose study that assessed effect of different food types on the BA of fexinidazole and on the systemic exposure to its active metabolites: fexinidazole sulfoxide (M1) and fexinidazole sulfone (M2), following the administration of 1200 mg fexinidazole as oral tablets in healthy adult subjects. The study evaluated and compared BA of these analytes following administration of a fexinidazole tablet given in fasting (Treatment A) and fed conditions (2 different types of meal Treatment B= Plumpy Nuts®, Treatment C = Rice plus beans) in a three-way cross-over design. In total, 12 sub-Saharan African male subjects were randomized to three different dose cohorts as described in Table 151 with a wash out period of at least 14 days. From 12 subjects, one subject withdrew consent and was not replaced. Consequently, 11 subjects completed the study and were part of the PK analysis.

Table 151. Description of Cohorts in Study DNDiFEX002

Treatment	Treatment and Dose (Subjects)
A	1200 mg fexinidazole under fasting condition
B	1200 mg fexinidazole with Plumpy nuts® meal (~500 Kcal including ~60% from fat)
C	1200 mg fexinidazole with Rice plus Beans meal (~393 Kcal including ~1% from fat)

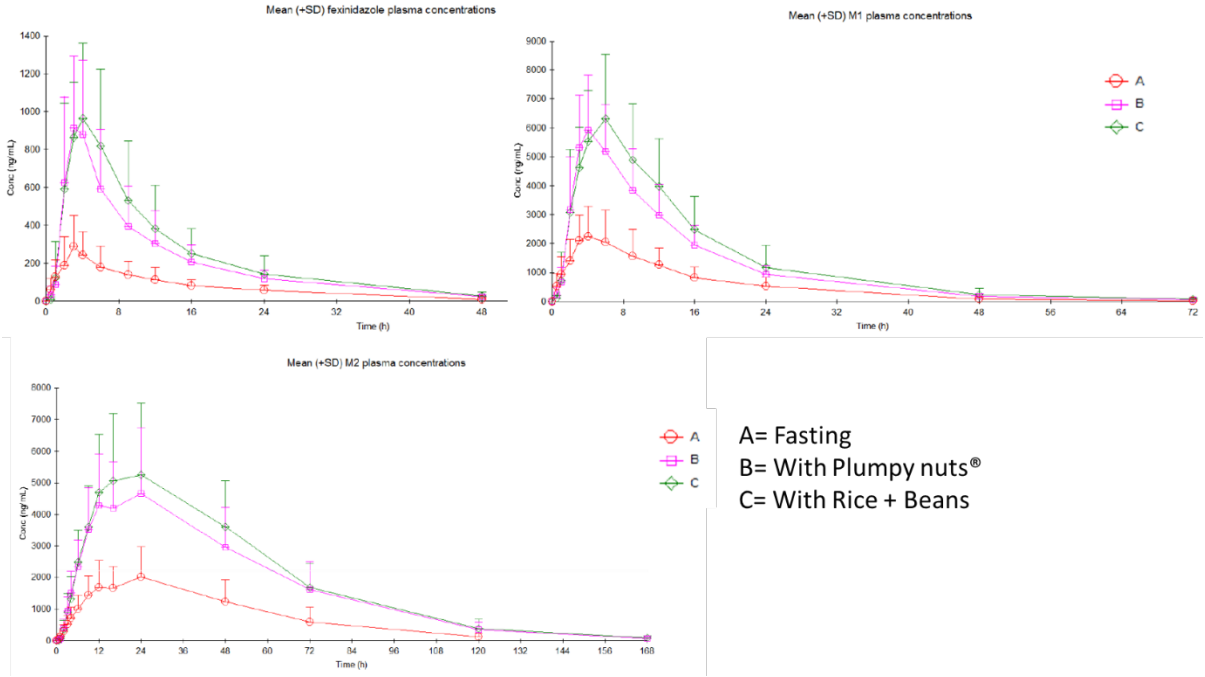
Source: Adapted from study report

Across all the cohorts, the age and weight of enrolled patients ranged between 19 to 44 years and between 59 to 88 kg. The enrolled patients received the assigned single dose in a form of tablet (600 mg) following overnight fast of at least 10 hours except when the study drug was given in fed conditions.

The subjects remained at the study center up to 48 hours postdose. The subjects came back on the morning of Day 4 (72 h postdose), Day 6 (120 h postdose) and Day 8 (168 h postdose). For

PK assessments, 17 blood samples were collected between predose to 168 hours postdose. In Treatment B cohort, additional blood samples were collected at 1, 4, 12, 24, and 72 hours postdose for the assessment of the free fraction of fexinidazole and its metabolites. Fexinidazole, M1, and M2 concentrations in plasma were measured using validated LC-MS/MS methods. The reported mean plasma concentration-time profiles for fexinidazole, M1, and M2 from all cohorts are presented in Figure 14. Plasma concentrations of all three analytes were subjected to noncompartmental analysis to derive PK parameter estimates, which are reported in Table 152.

Figure 14. Mean (SD) Concentrations of Fexinidazole, M1, and M2



Source: Adapted from the study report
Abbreviations: h, hours; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; SD, standard deviation

Table 152. Geometric Mean (CV%) for Exposure and Pharmacokinetic Parameter Estimates for Fexinidazole, M1, and M2

Treatment (Formulation-Meal condition)	$AUC_{0\text{-}t.last}$ (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)[a]	$T_{1/2}$ (h)
Fexinidazole				
A (Fasting)	3295.3 (49)	261.8 (55)	3 (1-4)	11.3 (27)
B (With Plumpy nuts®)	9718.7 (39)	1005.7 (42)	3 (2-4)	10.7 (25)
C (With Rice plus Beans)	11435.5 (45)	1025.8 (36)	4 (2-6)	11.3 (38)
M1				
A (Fasting)	33707.0 (50)	2114.3 (45)	4 (1-6)	10.1 (33)
B (With Plumpy nuts®)	81726.5 (31)	5977.8 (30)	4 (3-6)	12.2 (41)
C (With Rice plus Beans)	94588.6 (42)	6222.8 (32)	6 (2-6)	12.1 (39)

Treatment (Formulation-Meal condition)	$AUC_{0\text{-}t.last}$ (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)[a]	$T_{1/2}$ (h)
M2				
A (Fasting)	102533.3 (53)	1854.2 (46)	24 (12-24)	19.2 (17)
B (With Plumpy nuts®)	263832.1 (43)	4482.6 (43)	16 (9-24)	21.0 (13)
C (With Rice plus Beans)	300263.3 (39)	5333.0 (39)	24 (12-24)	20.0 (13)

Source: Study Report
[a] Median (Range)
Abbreviations: $AUC_{0\text{-}t.last}$, area under the concentration versus time curve up to last concentration timepoint; C_{max}, maximum serum concentration h, hours, M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; T_{max}, time at which the C_{max} is observed; $T_{1/2}$, half-life

No notable difference in T_{max} was observed across different cohorts. The mean estimates for C_{max} and $AUC_{0\text{-}t}$ for fexinidazole, M1, and M2 following dosing with Plumpy nuts® were approximately 240 to 380% and 240 to 290% of the estimates following dosing under fasted conditions (Table 153), respectively. The mean estimates for C_{max} and $AUC_{0\text{-}t}$ for fexinidazole, M1, and M2 following dosing with tablet with Rice plus Beans were approximately 290 to 390% and 280 to 350% of the estimates following dosing with tablet under fasted condition (Table 153), respectively. The mean estimates for C_{max} and $AUC_{0\text{-}t}$ for fexinidazole, M1, and M2 following dosing with Rice plus Beans were approximately 100 to 120% and 120% of the estimates following dosing with Plumpy nuts® tablet under fasted condition (Table 153), respectively.

Table 153. Summary of the Bioavailability Assessment

	Point Estimates 90%CI		
Parameters	B (Plumpy Nuts®)/A (Fasting)	C (Rice plus Beans)/ A (Fasting)	C (Rice plus Beans)/ B (Plumpy Nuts®)
Fexinidazole			
$AUC_{0\text{-}t.last}$	2.9 (2.4; 3.5)	3.5 (2.9; 4.2)	1.2 (1.0; 1.4)
C_{max}	3.8 (3.2; 4.5)	3.9 (3.3; 4.7)	1.0 (0.9; 1.2)
M1			
$AUC_{0\text{-}t.last}$	2.4 (2.0; 2.8)	2.8 (2.4; 3.3)	1.2 (1.0; 1.4)
C_{max}	2.8 (2.4; 3.3)	3.0 (2.5; 3.5)	1.1 (0.9; 1.3)
M2			
$AUC_{0\text{-}t.last}$	2.5 (2.2; 2.9)	2.9 (2.6; 3.3)	1.2 (1.0; 1.3)
C_{max}	2.4 (2.1; 2.7)	2.9 (2.5; 3.3)	1.2 (1.1; 1.4)

Source: Adapted from study report
Abbreviations: $AUC_{0\text{-}t.last}$, area under the concentration versus time curve up to last concentration timepoint; CI, confidence interval; C_{max}, maximum serum concentration; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone

The protein binding estimates were 98% for fexinidazole, 41% for M1 and 57% for M2. The bound and unbound fraction of fexinidazole, M1, and M2 was constant regardless of sampling time, suggesting concentration-invariant protein binding.

Applicant's Conclusions:

- Overall exposure to fexinidazole, M1, and M2 was increased after dosing concomitantly with meal type 2 (Rice plus Beans) in a slightly higher extent to that observed after dosing with meal type 1 (Plumpy nuts®), on average +20%, +19%, +16% (based on AUC) for fexinidazole, metabolite M1, and metabolite M2, respectively.
- Free fractions were about 2% for fexinidazole, 59% for M1, and 43% for M2.

Reviewer's assessment: *The Applicant proposed to administer fexinidazole tablets in HAT patients with food. Study DNDiFEX001 Part II evaluated the effect of FDA-recommended meal, i.e., high-calorie, high-fat breakfast on the PK of fexinidazole and reported increase in fexinidazole BA of about +328-366%. The increase reported in this study ranges from +190-290% following administration with meals with different levels of fat content, i.e., Plumpy nuts® meal with ~60% calories from fat and Rice plus Beans meal with ~1% calories from fat. Similar findings were observed for M1 and M2. Therefore, all three types of meals increased fexinidazole BA and the systemic exposure to metabolites. Nonetheless, for the purposes of the product labeling, a recommendation to include the findings only from Study DNDiFEX001 Part II will be sent to the Applicant.*

16.3.3.4. Study DNDiFEX003

Overview

This was a double-blind, randomized, multiple dose, randomized multiple ascending dose study that assessed safety and tolerability of two different fexinidazole dosing regimens under fed conditions for 10 days in healthy male sub-Saharan volunteers. The study planned to enroll 36 subjects to receive the following two 10-day treatments:

- Cohort 1: 1800 mg fexinidazole or placebo tablets from Day 1 to Day 4 (12 subjects) or placebo (6 subjects), then 1200 mg from Day 5 to Day 10 (12 subjects) or placebo (6 subjects)
- Cohort 2: 2400 mg fexinidazole or placebo tablets from Day 1 to Day 4 (12 subjects) or placebo (6 subjects), then 1200 mg from Day 5 to Day 10 or placebo (12 subjects) or placebo (6 subjects)

The assigned treatment was administered 30 minutes following lunch as described hereafter. On the days of PK evaluations, i.e., Day 1, Day 4, Day 7, and Day 10, a standard lunch was served that comprised of rice and red beans (plus olive oil). On other days, variation of seasoning composition included rice plus beans plus olive oil plus chicken or fish or hamburger and /or tomato sauce etc.

From the planned 18 subjects in Cohort 1, 6 subjects dropped out. The investigator suspected a group effect and therefore all subjects were replaced and consequently, all 12 subjects completed the study as planned. In Cohort 2, the study was stopped due to unacceptable tolerability after the inclusion of six subjects. The six subjects in Cohort 2 included two subjects who received the active treatment as planned, two subjects received placebo, and the remainder of the subjects did not receive complete treatment. Overall, PK analysis included 14 subjects, i.e., 12 from Cohort 1 and two from Cohort 2.

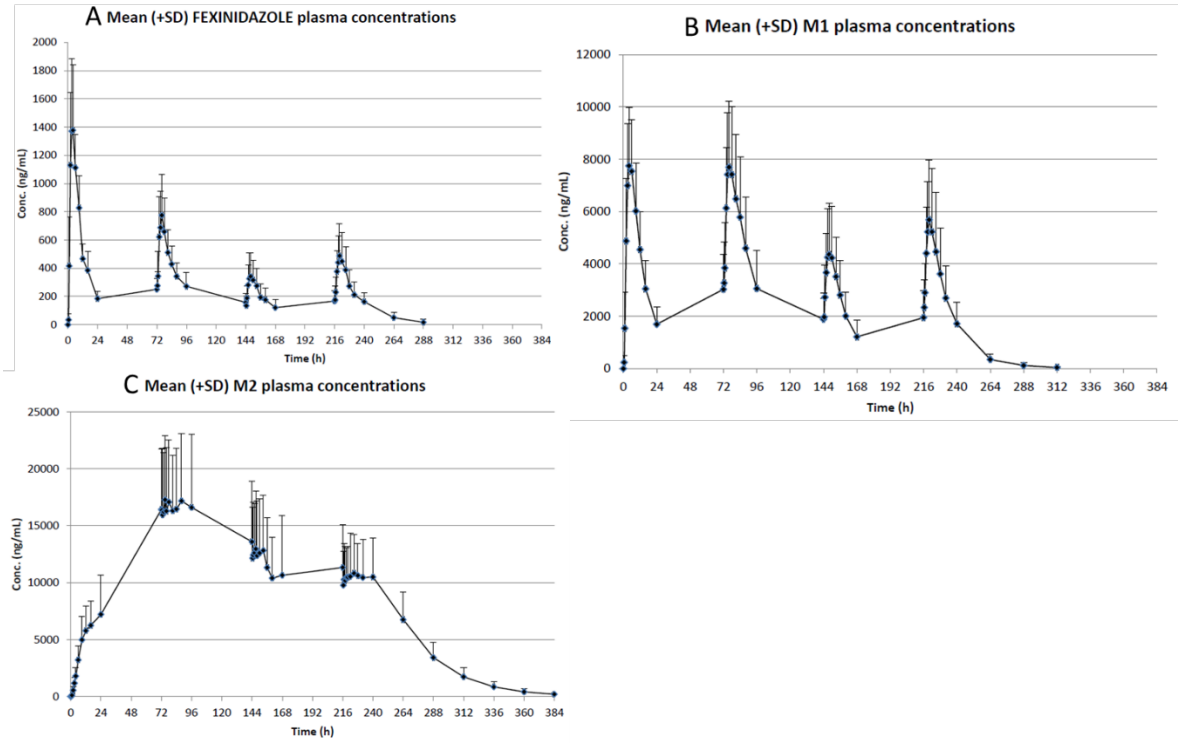
The age and weight of enrolled patients ranged between 19 to 40 years and between to 61 to 93 kg. The enrolled patients received the assigned single dose in a form of 600 mg fexinidazole tablet. The patients remained at the study center up to 168 hours postdose hours after receiving the last dose of the assigned treatment. For PK assessments, On Day 1, Day 4, and Day 7, 11 blood samples were collected from predose to 24 hours postdose. On Day 10, 17 blood

samples were collected from predose to 168 hours postdose. Fexinidazole, M1, and M2 concentrations in plasma were measured using validated LC-MS/MS methods. The reported mean plasma concentration-time profiles for fexinidazole, M1, and M2 from Cohort 1 are presented in Figure 15. The mean exposure parameter estimates from Cohort 1 were compared against estimates from two subjects in Figure 16. Plasma concentrations of all three analytes were subjected to noncompartmental analysis to derive PK parameter estimates, which are reported in Table 154.

When compared to the mean value reported in Cohort 1, C_{max} and AUC of fexinidazole and metabolites in Cohort 2 were higher regardless of the treatment day for one subject; by contrast this was only true on Days 7 and 10 for the other subject. This suggests that a higher loading dose for 4 days provides higher exposure.

The study report also included exposure and PK parameter estimates from subjects who had dropped out from Cohorts 1 and 2. The observed differences in estimates for C_{max} and AUC in drop out subjects were <14%, <11%, and <27%, for fexinidazole, M1, and M2, respectively. For Cohort 2, the reported differences in GM estimates for C_{max} and AUC were approximately similar to the estimates for patients who did not drop-out. However, for M2, C_{max} and AUC were slightly higher in drop out subjects than in the subject who completed the study.

Figure 15. Mean (SD) Concentrations of Fexinidazole



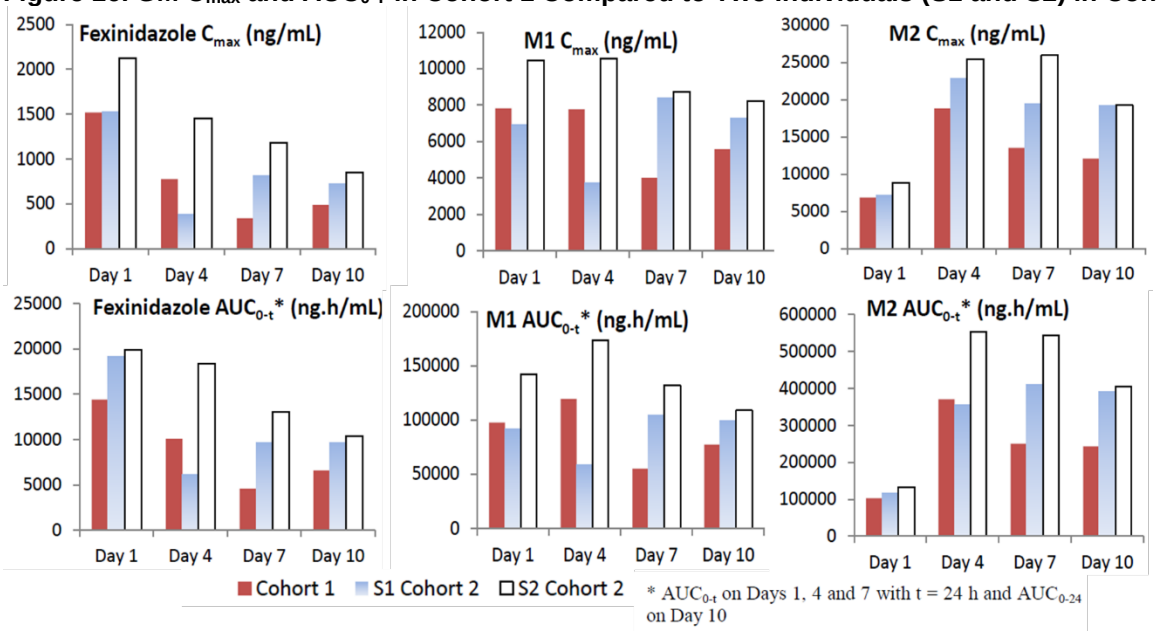
Source: Adapted from the study report
Note: (A), M1(B), and M2(C)
Abbreviations: h, hours; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; SD, standard deviation

Table 154. Geometric Mean (CV%) for Exposure and Pharmacokinetic Parameter Estimates for Fexinidazole, M1, and M2 From Cohort 1

Treatment Day	$AUC_{0\text{-}t.last}$ (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)[a]	$T_{1/2}$ (h)
Fexinidazole				
Day 1	14404 (20)	1519 (24)	4 (2-9)	8.84 (22)
Day 4	10094 (27)	777.3 (35)	4 (0-9)	13.38 (32)
Day 7	4593 (41)	335.5 (50)	4 (0-6)	12.81 (51)
Day 10	9846 (43)	485.5 (40)	4 (2-9)	14.36 (39)
M1				
Day 1	97617 (28)	7818 (27)	4 (3-6)	8.01 (16)
Day 4	119141 (37)	7768 (29)	4 (0-6)	11.97 (40)
Day 7	55141 (42)	3999 (43)	4 (0-6)	12.00 (55)
Day 10	106825 (44)	5574 (36)	3.5 (2-6)	15.20 (38)
M2				
Day 1	103089 (37)	6837 (44)	23 (16-24)	-
Day 4	370415 (32)	18790 (28)	6 (0-23.5)	-
Day 7	250411 (36)	13520 (36)	3 (0-12)	-
Day 10	665963 (31)	12060 (28)	4 (0-24)	22.28 (17)

Source: Study Report
[a] Median [Range]
Abbreviations: $AUC_{0\text{-}t.last}$, area under the concentration versus time curve up to last concentration timepoint; C_{max}, maximum serum concentration h, hours, M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; T_{max}, time at which the C_{max} is observed; $T_{1/2}$, half-life

Figure 16. GM C_{max} and $AUC_{0\text{-}T}$ in Cohort 1 Compared to Two Individuals (S1 and S2) in Cohort 2



Source: Adapted from study report
Abbreviations: $AUC_{0\text{-}t}$, area under the plasma concentration-time curve from time zero to the last timepoint with measurable analyte concentrations; C_{max}, maximum serum concentration; GM, geometric mean; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; S, subject

Applicant's Conclusions

- The tolerability was considered as poor in the dose group 2 and led to a decision to not expose more healthy subjects to this dose level and to stop the study progression.

- The present study has demonstrated that the best regimen should include a loading dose (1800 mg) for 4 days followed by a treatment dose (1200 mg) from Day 5 to Day 10, those allowing to reach high M2 levels quite early after the start of the treatment (Day 2), to maintain high concentrations until Day 11 with a peak of 3 x MIC from Day 4 to Day 6.
- This regimen should lead to have a significant percentage of subjects with plasma M2 concentration above 2 x MIC for 10 days (Day 2 to Day 11).

Reviewer's assessment: *The safety findings from Cohort 2 of this study noted tolerability issues in healthy subjects leading to discontinuation of the study. Cohort 2 evaluated the fexinidazole dosage that is higher (2400 mg loading dose, 1200 mg maintenance dose) than the Applicant proposed fexinidazole 1800 mg loading and 1200 mg maintenance doses for HAT patients. However, it is noteworthy that for most of the study duration, the systemic exposure to fexinidazole, M1, and M2 in two subjects from Cohort 2 were higher compared to mean exposures from Cohort 1 (Figure 16). Therefore, although these findings are from a limited number of subjects, they suggest that higher systemic exposures to fexinidazole and its active metabolites may increase the risk of poor tolerability to fexinidazole treatment.*

16.3.3.5. Study DNDiHATFEX008

Overview

This was an open-label, randomized, 2-treatment, 2-sequence, 4-period, single-dose, replicate cross-over study that assessed bioequivalence (BE) between the clinical trial 600 mg fexinidazole tablet formulation versus the proposed to-be-marketed 600 mg fexinidazole tablet formulation. The study planned to enroll 30 sub-Saharan African male subjects to receive the following two single dose treatment two times in a cross-over manner (Total four treatments over two periods) under fed condition:

- Reference Treatment (R): 2 x 600 mg clinical trial tablets
- Test Treatment (T): 2 x 600 mg proposed to-be-marketed tablets

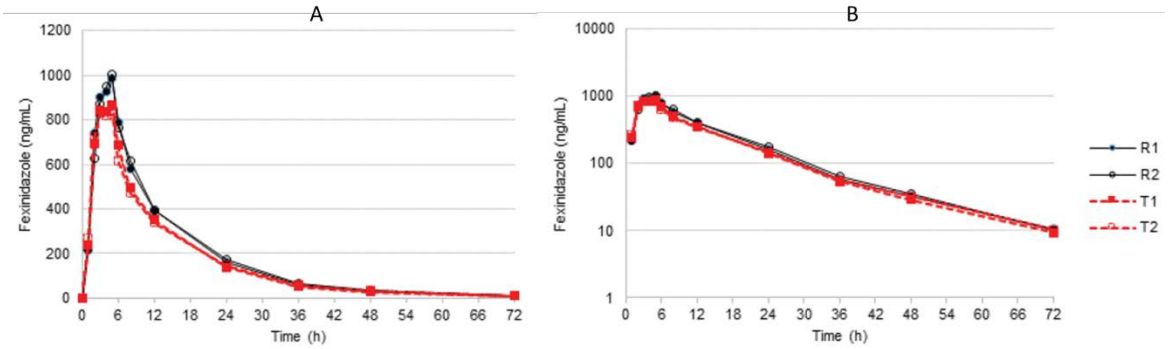
All 30 subjects received the assigned treatment and completed the study. Across all the cohorts, the age and weight of enrolled patients ranged between 19 to 40 years and between to 55 to 103 kg.

The enrolled subjects received the assigned single dose following overnight fasting (on the preceding day) and after a meal that was given about 30 minutes before study drug administration. The meal was as close as possible to field conditions, i.e., Rice plus beans plus chicken 150 g with 2 spoons of fat for cooking (~700 Kcal including ~17% from fat).

During each treatment period, the subjects were hospitalized from Day -1 evening to 48 h postdose. The subjects came back to the clinical unit at 72 hours postdose, 120 hours postdose, and at 168 hours postdose for PK sampling purpose. Each treatment was separated by a wash out period of at least 14 days. For PK assessments, 15 blood samples were collected from predose to 168 hours post dose. Fexinidazole, M1, and M2 concentrations in plasma were measured using validated LC-MS/MS methods.

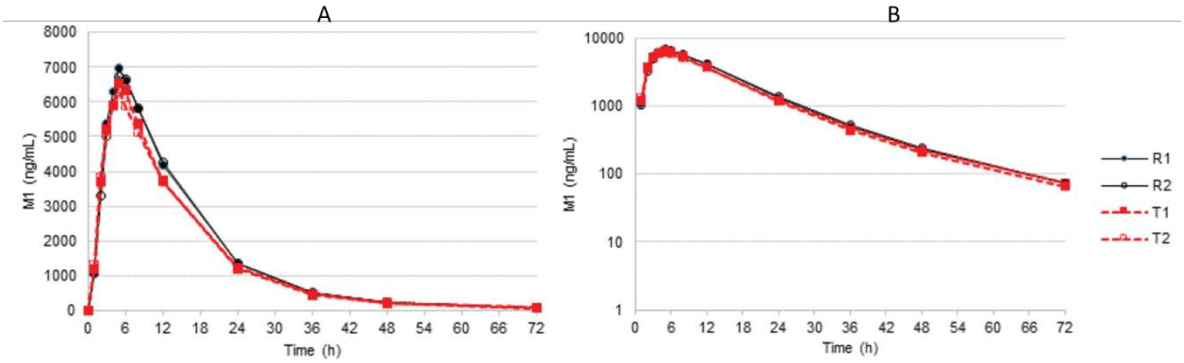
The reported mean plasma concentration-time profiles for fexinidazole, M1, and M2 from all cohorts are presented in Figure 17, Figure 18, and Figure 19, respectively. Plasma concentrations of all three analytes were subjected to noncompartmental analysis to derive PK parameter estimates, which are reported in Table 155.

Figure 17. Mean (SD) Concentrations of Fexinidazole in Plasma



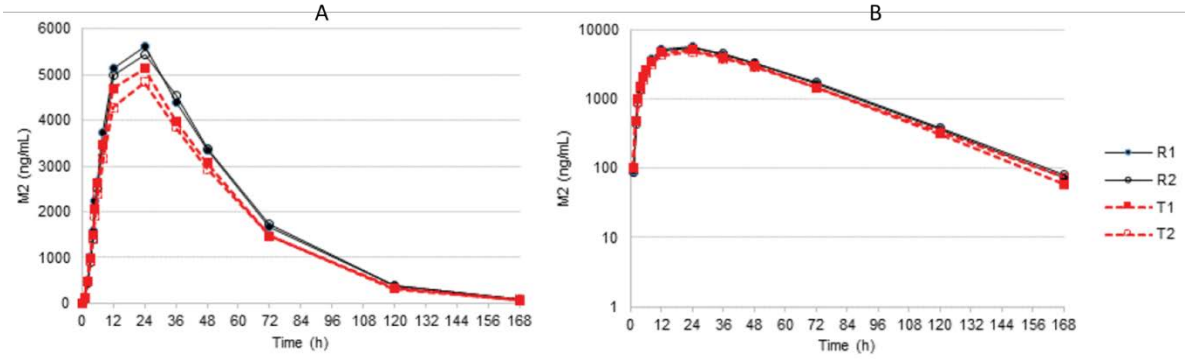
Source: Adapted from study report
Note: A: linear scale, B: semilogarithmic scale
Abbreviations: h, hour; M1, Fexinidazole sulfoxide; R, reference treatment; SD, standard deviation; T, test treatment

Figure 18. Mean (SD) Concentrations of M1 in Plasma



Source: Adapted from study report
Note: A: linear scale, B: semilogarithmic scale
Abbreviations: h, hour; M1, Fexinidazole sulfoxide; R, reference treatment; SD, standard deviation; T, test treatment

Figure 19. Mean (SD) Concentrations of M2 in Plasma



Source: Adapted from study report
Note: A: linear scale, B: semilogarithmic scale
Abbreviations: h, hour; M2, Fexinidazole sulfone; R, reference treatment; SD, standard deviation; T, test treatment

Table 155. Geometric Mean (CV%) for Exposure and Pharmacokinetic Parameter Estimates for Fexinidazole, M1, and M2

Treatment (Formulation, Period)	$AUC_{0\text{-}t.last}$ (ng·h/mL)	C_{max} (ng/mL)	T_{max} (h)[a]	$T_{1/2}$ (h)
Fexinidazole				
R1 (Reference, 1)	11427 (36)	1056 (30)	4 (2-8)	10.9 (44)
R2 (Reference, 2)	11081 (46)	1014 (44)	4 (2-8)	11.2 (44)
T1 (Test, 1)	10292 (38)	941 (32)	4 (2-6)	10.7 (49)
T2 (Test, 2)	9543 (45)	929 (39)	4 (2-6)	11.2 (47)
M1				
R1 (Reference, 1)	110196 (22)	7148 (15)	5 (3-12)	12.7 (39)
R2 (Reference, 2)	105386 (33)	6802 (26)	5 (3-8)	13.0 (42)
T1 (Test, 1)	101024 (23)	6649 (17)	5 (3-6)	12.8 (42)
T2 (Test, 2)	97674 (32)	6319 (24)	5 (3-8)	13.2 (42)
M2				
R1 (Reference, 1)	312118 (24)	5573 (23)	24 (12-24)	20.9 (15)
R2 (Reference, 2)	297826 (36)	5241 (34)	24 (12-36)	20.7 (19)
T1 (Test, 1)	283426 (21)	5087 (22)	24 (12-24)	20.7 (14)
T2 (Test, 2)	269120 (26)	4701 (25)	24 (12-24)	21.4 (17)

Source: Study Report
[a] Median [Range]
Abbreviations: $AUC_{0\text{-}t.last}$, area under the concentration versus time curve up to last concentration timepoint; C_{max}, maximum serum concentration h, hours, M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; T_{max}, time at which the C_{max} is observed; $T_{1/2}$, half-life

No notable difference between T_{max} was observed when comparing test versus reference tablet formulation. The mean estimates for C_{max} and $AUC_{0\text{-}t}$ for fexinidazole, M1, and M2 following dosing with test tablet formulation were ~90% of the estimates following dosing with reference formulation (Table 156). Similar decrease in exposure, i.e., ~10% lower, exposures were also observed for the metabolites (Table 156).

Table 156. Summary of the Bioequivalence Assessment

Parameters	% Point Estimates (Test Tablets/Reference Tablet)
Fexinidazole	
$AUC_{0\text{-}t.last}$	88 (82-95)
C_{max}	90 (83-98)
M1	
$AUC_{0\text{-}t.last}$	92 (87-98)
C_{max}	93 (88-98)
M2	
$AUC_{0\text{-}t.last}$	90 (85-96)
C_{max}	91 (85-96)

Source: Adapted from study report
Abbreviations: $AUC_{0\text{-}t.last}$, area under the concentration versus time curve up to last concentration timepoint; C_{max}, maximum serum concentration; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone

Applicant's Conclusions:

- PK results shows that in the fed state, the 600 mg to-be-marketed tablet test formulation is bioequivalent to the 600 mg clinical trial reference formulation as indicated by 90% CI included within the interval 0.80 to 1.25 for fexinidazole C_{max} and AUC.

- The shape of the PK profile of the 2 metabolites were also similar between the two formulations. The 90% CIs were also within the 0.80 to 1.25 BE criteria for fexinidazole sulfoxide (M1) as well as for fexinidazole sulfone (M2).

Reviewer's assessment: *The findings reported in Table 156 suggest that the BA of fexinidazole and systemic exposure to its active metabolites from the to-be-marketed formulation is ~10% lower than the exposures of respective analysis from the reference formulation. The Applicant notes that a replicate design was selected for this study because of the observed intrasubject variability of ~25% in fexinidazole PK parameter estimates in Study DNDiFEX002, which was deemed close to 30% by the Applicant. The Reviewer performed an independent analysis of the PK findings from two periods separately and compared the PK of all analytes from both the treatment arms. The findings from the Reviewer's analysis (not shown) were similar to the findings reported above, i.e., the BA of fexinidazole from the to-be-marketed formulation is ~10% lower than from the reference formulation that was used in the clinical trials. Nonetheless, given that the point estimates and associated 90% CIs were contained within 80-125%, the Applicant's conclusion appears reasonable, i.e., the to-be-marketed 600 mg tablet formulation is bioequivalent to the reference formulation that was used in the clinical trials.*

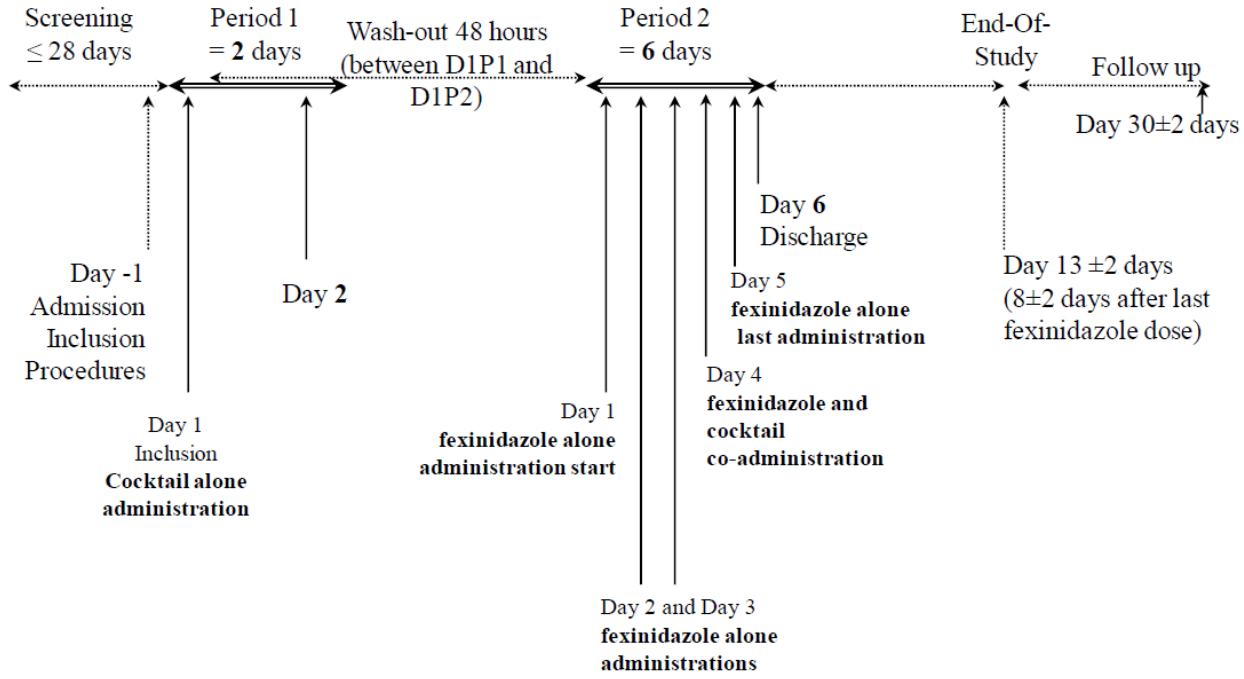
16.3.3.6. Study INT15307

Overview

This was an open-label, nonrandomized PK drug-drug interaction study that evaluated effect of 5-day fexinidazole treatment on a single dose cocktail of caffeine and omeprazole used as probe substrates for CYP1A2 and CYP2C19 activities, respectively. The study enrolled 12 African sub-Saharan healthy subjects who were randomized to receive the following two treatments in one sequence over two periods separated by at least 48 hours of wash-out as illustrated in Figure 20:

- Period 1: Single dose of 100 mg caffeine and 20 mg omeprazole (cocktail probes)
- Period 2: 1800 mg fexinidazole QD for 4 days plus 1200 mg fexinidazole on Day 5 with co-administration of single dose cocktail probes (at same dose as in Period 1) on Day 4

Figure 20. Study INT15307 Design



Source: Study report
Abbreviations: D, day; P, period

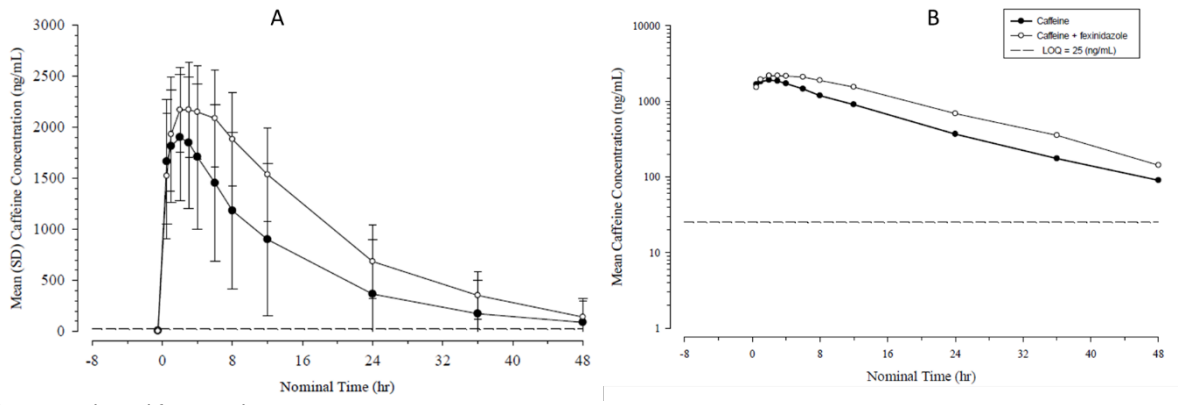
All treatments were administered with field adapted breakfast, i.e., Rice plus beans plus chicken 150 g with 2 spoons of fat for cooking (~700 Kcal including ~17% from fat).

The study enrolled 12 subjects including three female subjects. The age and weight of enrolled patients ranged between 21 to 48 years and between to 55 to 96 kg. From the subjects who were randomized, two subjects were discontinued due to treatment-emergent adverse events of nausea (male subject)and vomiting (female subject). In addition, one subject had omeprazole concentrations in all samples from Period 2 below LLOQ (confirmed by repeat bioanalysis of the full PK profile) for an unknown reason. All 12 subjects were included in the safety analysis; however, 10 subjects were included for PK analysis of caffeine, fexinidazole, M1, and M2 whereas only nine subjects were included for PK analysis of omeprazole.

The patients remained at the study center up to Day 6, i.e., 24 hours after the last fexinidazole dose on Day 5. For cocktail substrates' PK assessments, 11 blood samples were collected between predose to 36 hours postdose from Period 1 and 12 blood samples were collected between predose to 24 hours postdose from Period 2. For fexinidazole, M1, and M2 PK assessments, 14 blood samples were collected from predose to 24 hours postdose.

Concentrations of all analytes in plasma were measured using validated LC-MS/MS methods for all analytes. The reported mean plasma concentration-time profiles for caffeine and omeprazole from both treatment periods are reported in Figure 21 and Figure 22, respectively. Fexinidazole, M1, and M2 PK profiles on Day 4 are presented in Figure 23. Plasma concentrations of all analytes were subjected to noncompartmental analysis to derive PK parameter estimates, which are reported in Table 157.

Figure 21. Mean Plasma Concentration of Caffeine Following a Single 100 mg Dose Alone (Day 1) and With 1800 mg Fexinidazole QD Dosing (Day 4)



Source: Adapted from study report
Note: A: linear scale, B: semilogarithmic scale
Abbreviations: hr, hour; QD, once daily; SD, standard deviation

Table 157. Geometric Mean (CV%) Exposure and Pharmacokinetic Parameter Estimates for Caffeine, Omeprazole, Fexinidazole, M1, and M2

Day	$AUC_{0\text{-t.last}}$ (ng·h/mL)	C_{max} (ng/mL)	T_{max}(h)[a]	$T_{1/2}$ (h)
Caffeine				
Day 1 (n=12)	22700 (82)	1960 (28)	2 (0.5-6)	6.61 (59)
Day 4 (n=10)	42700 (35)	2220 (20)	3 (1-6)	9.90 (29)
Omeprazole				
Day 1 (n=12)	612 (85)	186 (70)	4 (2-6)	1.1 (37)
Day 4 (n=9)	1210 (14)	350 (35)	4 (2-6)	1.44 (68)
Fexinidazole				
Day 4 (n=10)	7610 (46)[#]	610 (46)	4 (1.5-8)	-
M1				
Day 4 (n=10)	131000 (40)[#]	8390 (38)	5 (2-8)	-
M2				
Day 4 (n=10)	381000 (29)[#]	17300 (29)	10 (6-24)	-

Source: Study Report
AUC0-24
a Median [Range]
Abbreviations: $AUC_{0\text{-t.last}}$, area under the concentration versus time curve up to last concentration timepoint; C_{max}, maximum serum concentration; h, hours, M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; T_{max}, time at which the C_{max} is observed; $T_{1/2}$, half-life

The observed increase in mean caffeine C_{max} was ~19% after single 100 mg dose with (Day 4) or without (Day 1) co-administered fexinidazole; however, the mean estimates for $AUC_{0\text{-}24}$ of caffeine were ~200% of the estimates following dosing with fexinidazole (Day 4) compared to when administered alone (Day 1) (Table 158). For omeprazole, both C_{max} and $AUC_{0\text{-}t}$ estimates were ~200% of the estimates following co-administration with fexinidazole (Day 4) compared to when administered alone (Day 1) (Table 158).

Table 158. Summary of the Bioavailability Assessment

Parameters	% Point Estimates Day 4 (With Fexinidazole)/ Day 1 (Without Fexinidazole)
Caffeine	
$AUC_{0\text{-}t.last}$	205 (166-252)
C_{max}	119 (114-124)
Omeprazole	
$AUC_{0\text{-}t.last}$	205 (142-295)
C_{max}	195 (131-291)

Source: Adapted from study report
Abbreviations: $AUC_{0\text{-}t.last}$, area under the concentration versus time curve up to last concentration timepoint; C_{max}, maximum serum concentration

Applicant's Conclusions

Repeated oral co-administration of fexinidazole (1800 mg QD for 4 days followed by 1200 mg for 1 day, in fed conditions) with single dose administration of 100 mg of caffeine and 20 mg of omeprazole in healthy male and female subjects of African sub- Saharan origin increased mean caffeine (probe substrate of CYP1A2) AUC by 2.1-fold with no significant increase in caffeine C_{max}. Mean C_{max} and AUC of omeprazole (probe substrate of CYP2C19) were increased by approximately 2-fold.

Reviewer's assessment: *The Applicant's conclusions appear reasonable and the findings from this study will be used for reviewing the Applicant proposed drug-drug interaction related information for the fexinidazole tablet product labeling.*

16.3.4. Pharmacometrics Review

Results of Applicant's Analysis

Population Pharmacokinetic Modeling Review

The Applicant submitted Population Pharmacokinetic (PPK) Report 16109 to support the approval of Fexinidazole tablets in patients with human African trypanosomiasis (HAT). The studies assessed in this PPK report are shown in Table 159.

Table 159. Studies Included in PPK Report 16018.

Study type	Study code	Dose or dose range and treatment duration	Tested formulation	Number enrolled
Pharmacokinetics/pharmacodynamics (PK/PD) and initial tolerability in g-HAT patients				
Efficacy and safety study in adult g-HAT patients	DNDiFEX004	1800 mg/d for 4 days and 1200 mg/d for 6 days	600-mg tablets	394 [g]
Efficacy and safety study in pediatric g-HAT patients	DNDiFEX006	BW ≥35 kg: 1800 mg/d for 4 days and 1200 mg/d for 6 days 20 ≤ BW <35 kg: 1200 mg/d for 4 days and 600 mg/d for 6 days	600-mg tablets	125 [h]

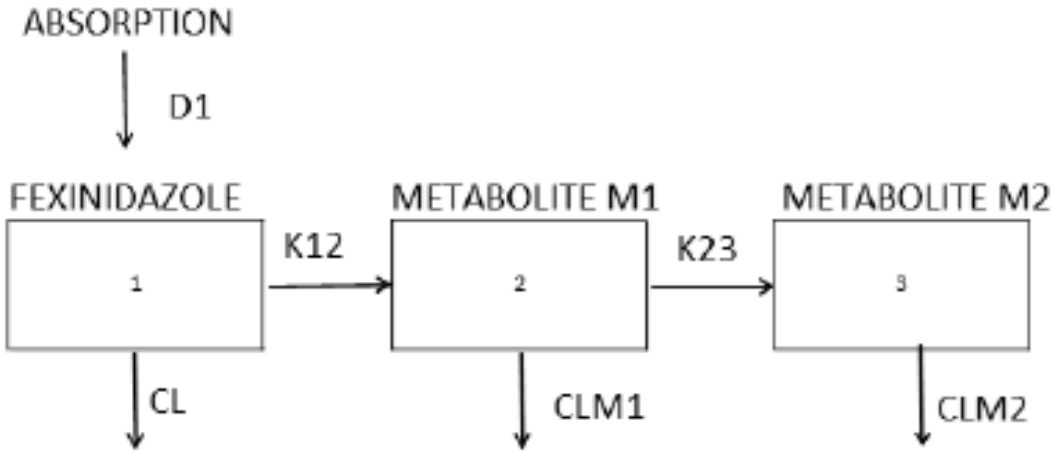
Source: Applicant Population PK Report 16109.
Abbreviations: BW, body weight; g-HAT, human African trypanosomiasis due to *T. b. gambiense;* PD, pharmacodynamics; PK, pharmacokinetics; PPK, population pharmacokinetics

Studies DNDiFEX004 and DNDiFEX006 were Phase 3 efficacy studies conducted in adult and pediatric patients administered the proposed clinical dose regimen.

Reviewer's comments: The population PK analysis did not include Phase 1 studies, which had richer sampling than the than Studies 004 and 006 (4-6 samples per patient). The Applicant's approach appears to be reasonable because patient PK is more relevant for the analysis than healthy volunteer PK given that the disease may affect fexinidazole PK.

The structure of the Applicant's final model is shown in Figure 22.

Figure 22. Applicant's Final PPK Model Schematic.



Source: Applicant Population PK Report 16109
Abbreviations: CL/CLM1/CLM2: clearance of fexinidazole/M1/M2; D1, duration of absorption; ka,b: rate constant describing formation from one moiety (a) to another moiety (b) of fexinidazole; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; PPK, population pharmacokinetics

Each moiety of fexinidazole was modeled as 1 compartment in a composite 3-compartment structure to describe fexinidazole and its M1 and M2 metabolites. The absorption of fexinidazole was modeled as a zero-order process with D1 representing the total duration of absorption.

Reviewer's comments: Zero-order processes are more commonly used to describe intravenous infusion of drugs while first-order processes are more typically used for oral absorption, which would be more relevant for fexinidazole. However, it may not be feasible to characterize absorption fully because sparse sampling was used in this study with few samples taken close to the drug administration. Additionally, the main PK parameters of interest are minimum concentration (C_{min}) and AUC; thus, the Applicant's approach appears to be reasonable, contingent on the model's performance in diagnostic plots.

Parameter estimates for the final model are shown in the Table 160.

Table 160. Parameter Estimates of Applicant's Final PPK Model (Model 10a).

Description	Parameter	RSE	Shrinkage
Fixed Effects			
CL	489	4.30%	
V	6450	3.80%	
D1	3.89	1.90%	
V2	1*		
V3	1*		
k12	0.0148	10.90%	
CLM1	2.16	9.20%	
k23	0.173	5.10%	
CLM2	0.054	2.40%	
WT-CL	0.75*	-	
WT-CLM1	0.75*	-	
WT-CLM2	0.75*	-	
WT-V1	1*		
WT-k12	-0.191	29.20%	
WT-k23	0.844	6.30%	
IIV			
CL	0.365	9.30%	2.8%
$_{cov}$CL,V1	0.284	9.9%	
V1	0.275	9.70%	4.9%
k12	0.0672	8.70%	12.8%
k23	0.0651	9.90%	11.5%
Residual Error			
[Proportional] Fexinidazole	0.124	4.50%	10.2%
[Proportional] M1	0.0737	4.40%	11.8%
[Proportional] M2	0.0103	10.40%	9.5%
[Additive] M2	2.96	8.40%	9.5%

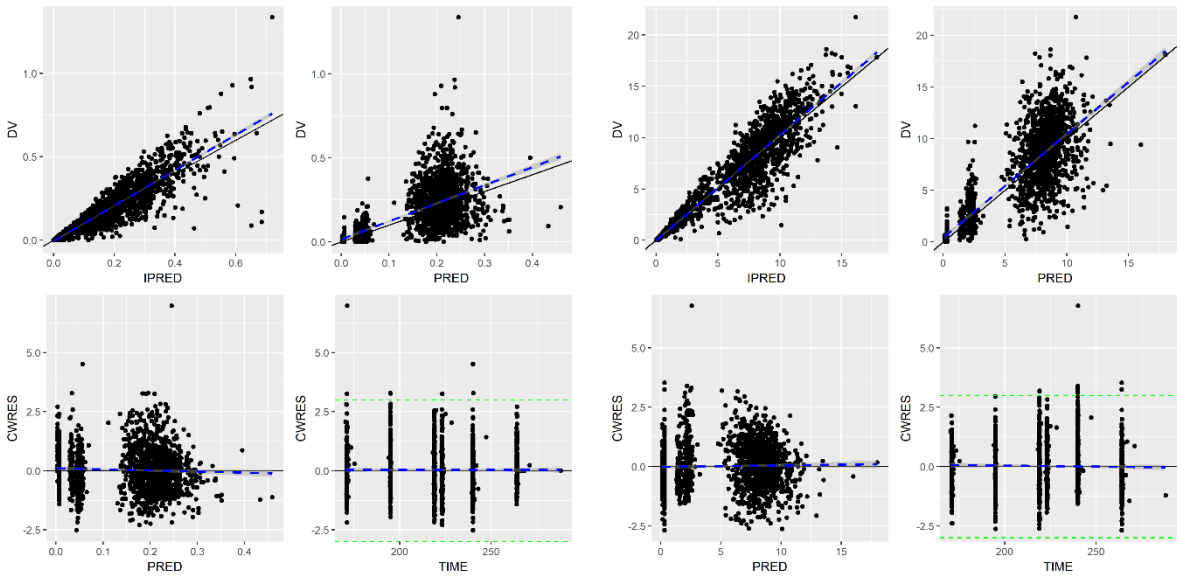
Source: Adapted from the Applicant's Model.
*indicates that the parameter was fixed to the value shown
Abbreviations: CL/CLM1/CLM2, clearance of fexinidazole/M1/M2; D1, duration of absorption; $k_{a,b}$, rate constant describing formation from one moiety (a) to another moiety (b) of fexinidazole; IIV, interindividual variability; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; RSE, residual standard error; V, volume; WT, weight; X-Y, effect of covariate X on PK parameter Y

Reviewer's comments: Note that compartments 1, 2, and 3 refer to compartments for fexinidazole, the M1 metabolite, and the M2 metabolite, respectively. To avoid identifiability issues, V2 and V3 (volumes of the fexinidazole metabolites) were fixed to 1 while the rates of metabolite formation (k12 and k23) were estimated. This approach appears to be reasonable; however, it alters the interpretation of the parameters. k12 appears to be more accurately described as k12/V2, CLM1 as CLM1/V2, k23 as k23*V2/V3, and CLM2 as CLM2/V3. This also alters the expected allometric scaling exponents (i.e., not -0.25 for k), which the Applicant did not estimate.

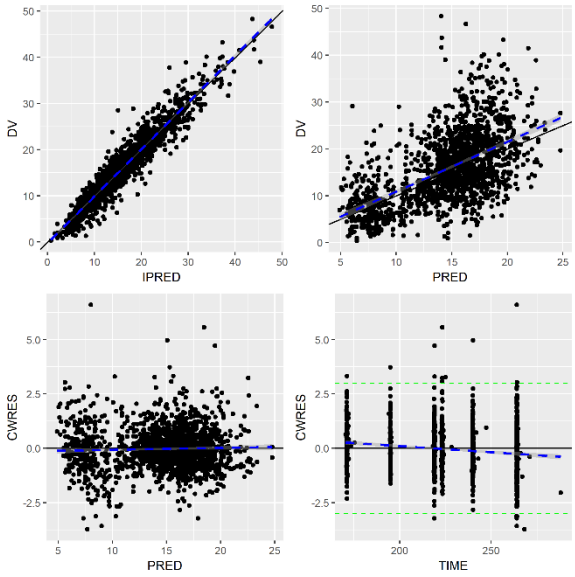
Goodness-of-fit (GOF) plots for fexinidazole and its M1 and M2 metabolites are shown in Figure 23.

Figure 23. GOF Plots for Applicant's PPK Model 10a for Fexinidazole and Its M1 and M2 Metabolites.

Fexinidazole **M1 Metabolite**



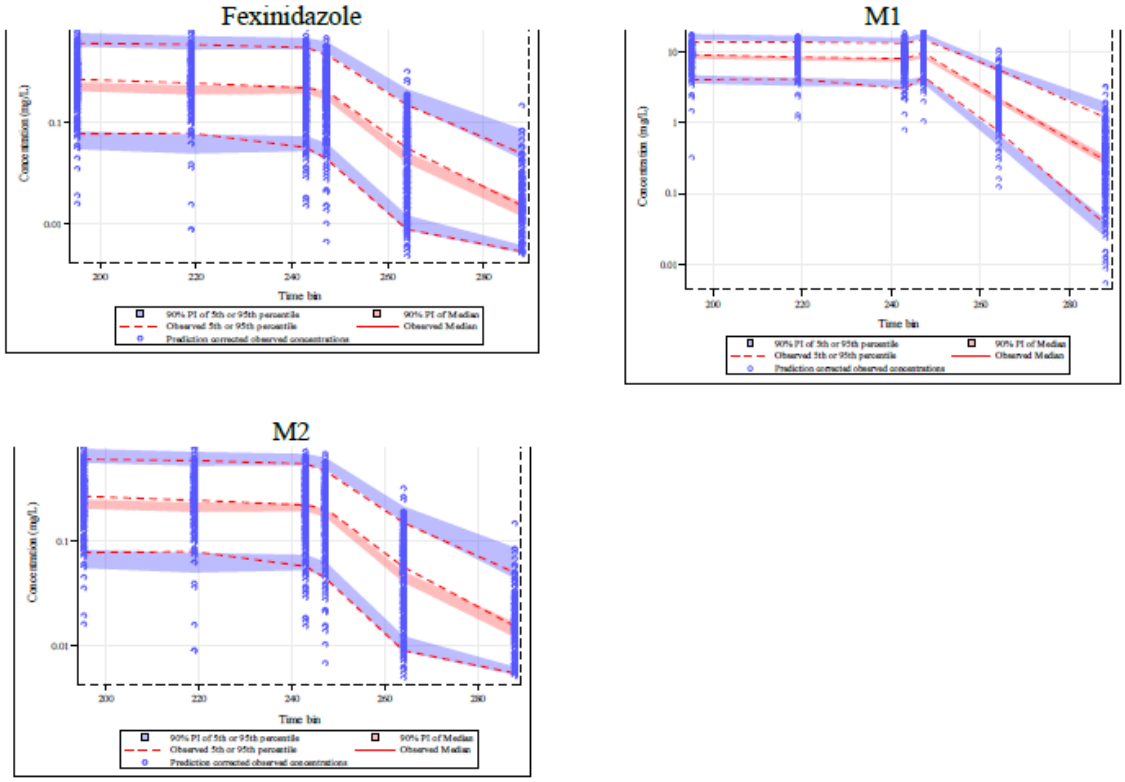
M2 Metabolite



Source: Adapted from the Applicant's Model.
Note: The blue line represents the trend of the data relative to the line of unity or x-axis (black line).
Abbreviations: CWRES, conditional weighted residuals; DV, dependent variable, concentrations of fexinidazole and its metabolites; GOF, goodness-of-fit; IPRED, individual prediction; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; PPK, population pharmacokinetics; PRED, population prediction,

Prediction-corrected visual predictive checks (VPC) for fexinidazole and its M1 and M2 metabolites are shown in Figure 24.

Figure 24. Prediction-Corrected VPC for Applicant's PPK Model 10a for Fexinidazole and Its M1 and M2 Metabolites.



Source: Applicant Population PK Report 16109.
The figures are displayed with concentration of fexinidazole and its metabolites on the y-axis in units of mg/L with time after the first dose on the x-axis in units of hr. The blue shaded area represents the 90% prediction interval of the 5th and 95th percentiles. The red shaded area represents the 90% prediction interval of the median. The circles represent the prediction-corrected observations, and the lines represent the observed median and 5th and 95th percentiles.
Abbreviations: M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; PI, prediction interval; PPK, population pharmacokinetics; VPC, visual predictive checks

Reviewer's comments: The GOF and VPC plots demonstrate reasonable agreement between the observed concentrations and model predictions. There do not appear to be any significant discrepancies due to estimating absorption with a zero-order process or by fixing V2 and V3 to 1.

Reviewer's Analysis

Introduction

The OCP Pharmacometrics review team conducted an independent analysis of fexinidazole PK data to address concerns regarding the allometric scaling exponents. The team also used the model to evaluate labeling claims made by the Applicant.

Methods

Data Sets

Data sets used are summarized in Table 161.

Table 161. Analysis Data Sets

Study Number	Name	Link to EDR
PH16109	Pooled Population PK Analysis of Fexinidazole and Metabolites in Patient (Adults and Children) with HAT from Studies: DNDI-FEX-004-HAT and DNDI-FEX-006-HAT	\\CDSESUB1\evsprod\NDA214429\0001\m5\datasets\ph16109\analysis\legacy\datasets\FEX0046.xpt

Source: Adapted from Applicant's submission.
Abbreviations: HAT, human African Trypanosomiasis; PK, pharmacokinetic

Software

NONMEM v7.4 was used for population pharmacokinetics modeling. Rv3.6.1 was used for data visualization and statistical analyses.

PPK Modeling

The Pharmacometrics review team assessed different methods to improve the Applicant's PPK model. Each concentration was corrected to a molar mass equivalent weight for modeling (*i.e.*, by dividing by the molecular weight of the given moiety and multiplying by the molecular weight of fexinidazole). Exponents for covariate relationships between weight and each PK parameter were estimated. Covariate relationships were assessed for significance in a backwards selection process. The contribution of each covariate was considered in terms of change of objective function value, change in associated ETA, and the graphical relationship between the covariate and the associated ETA.

The model was used to calculate derived PK parameters, i.e., steady-state AUC and steady-state C_{min}. This calculation was done using the amounts of fexinidazole and its metabolites converted back to real mass units, instead of molar mass equivalents. The calculated parameters were used to assess the Applicant's labeling claims regarding effects of covariates on fexinidazole PK.

Results

PPK Modeling

The Pharmacometrics review team revised the Applicant's PPK Model 10a to generate Model 12a0. A comparison of the PPK parameter estimates for Models10a and 12a0 are shown in Table 162. Model 12a0 improves upon Model 10a by significantly reducing objective function value.

Table 162. Parameter Estimates of PPK Models 10a and 12a0.

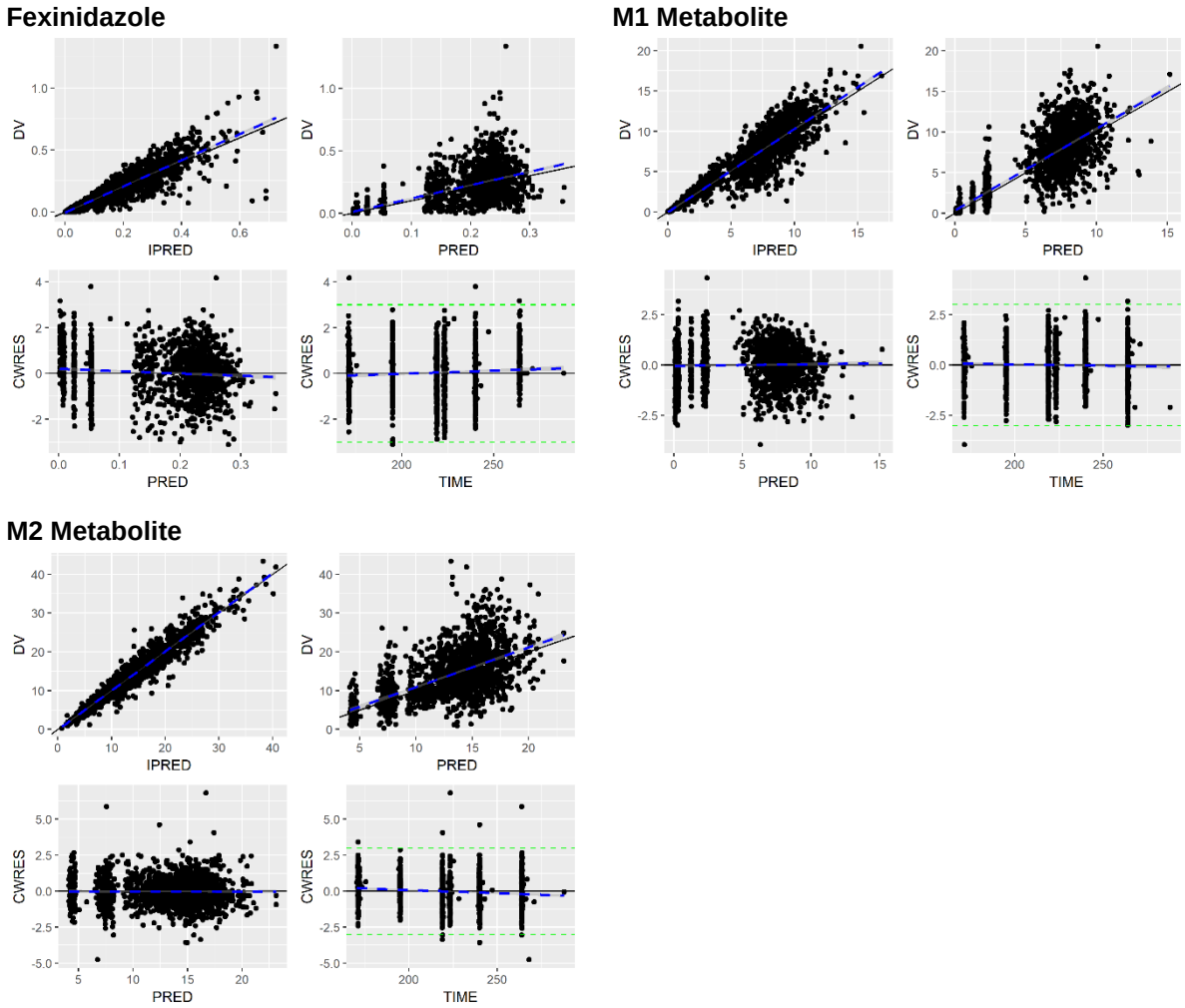
	Run10a (Applicant's Final Model)			Run12a0 (Reviewer's Final Model)		
Description	**Parameter**	**RSE**	**Shrinkage**	**Parameter**	**RSE**	**Shrinkage**
OFV			1002			115
Fixed Effects						
CL	489	4.3%		393	8%	
V	6450	3.8%		5360	7.8%	
D1	3.89	1.9%		3.82	2.3%	
V2	1*			1*		
V3	1*			1*		
K12	0.0148	10.9%		0.00852	11.6%	
CLM1	2.16	9.2%		1.23	10.4%	
K23	0.173	5.1%		0.0963	5.8%	
CLM2	0.054	2.4%		0.035	4.1%	
WT-k12	-0.191	29.2%		-0.835	7.2%	
WT-k23	0.844	6.3%		-0.353	21.9%	
WT-CL1	0.75*			0.336	31.3%	
WT-V1	1*			0.65	15.7%	
WT-CLM1	0.75*			1*		
WT-CLM2	0.75*			-0.246	22.7%	
IIV						
CL	0.365	9.30%	2.8%	0.35	9.20%	2.8%
V	0.275	9.70%	4.9%	0.269	9.70%	4.9%
K12	0.0672	8.70%	12.8%	0.0713	8.50%	12%
K23	0.0651	9.90%	11.5%	0.0512	15.70%	21.7%
CLM2	0 (FIX)			0.0139	31.70%	43.5%
Residual Error						
[Proportional] Fexinidazole	0.124	4.50%	10.2%	0.121	4.60%	10.2%
[Proportional] M1	0.0737	4.40%	11.8%	0.0731	4.40%	11.9%
[Proportional] M2	0.0103	10.40%	9.5%	0.0154	4.70%	11%
[Additive] M2	2.96	8.40%	9.5%	0.665	16.40%	11%

Source: The parameters for Model 10a were adapted from the Applicant's model. The parameters for Model 12a0 were calculated form the Reviewer's analysis.
*indicates that the parameter was fixed to the value shown
Abbreviations: CL/CLM1/CLM2, clearance of fexinidazole/M1/M2; D1, duration of absorption; IIV, interindividual variability; $k_{a,b}$, rate constant describing formation from one moiety (a) to another moiety (b) of fexinidazole; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; OFV, objective function value; PPK, population pharmacokinetics; RSE, residual standard error; V, volume; WT, weight; X-Y, effect of covariate X on PK parameter Y

GOF Plots

GOF plots for the reviewer's final PPK model for fexinidazole, 12a0, are shown in Figure 25.

Figure 25. GOF Plots for Reviewer's PPK Model 12a0 for Fexinidazole and Its M1 and M2 Metabolites.



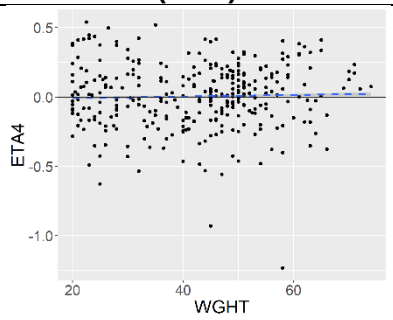
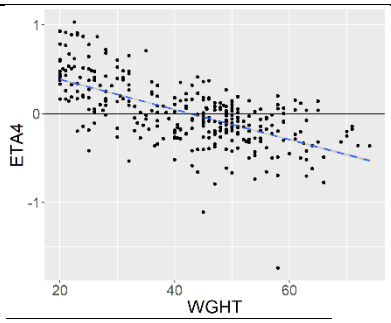
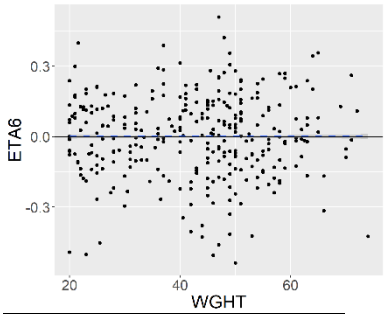
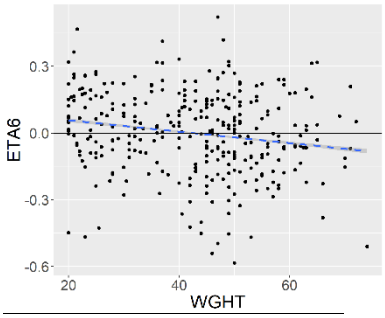
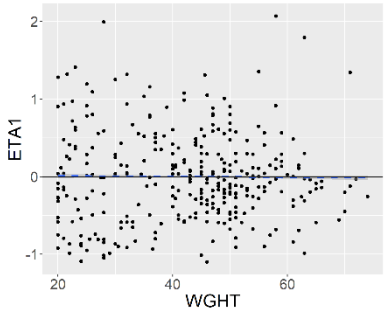
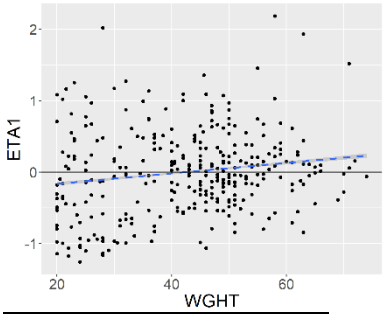
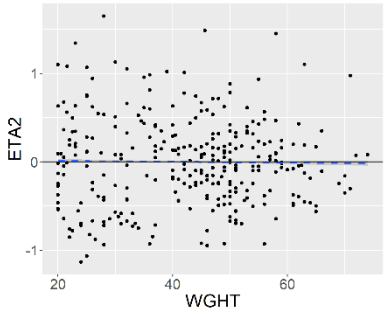
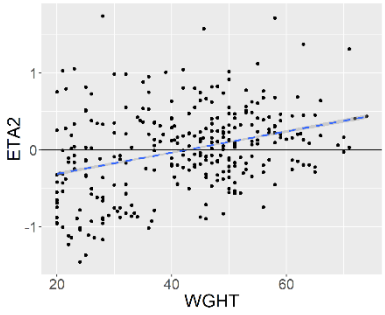
Source: Reviewer's Analysis.
Note: The blue line represents the trend of the data relative to the line of unity or x-axis (black line)
Abbreviations: CWRES, conditional weighted residuals; DV, dependent variable, concentrations of fexinidazole and its metabolites; GOF, goodness-of-fit; IPRED, individual prediction; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; PPK, population pharmacokinetics; PRED, population prediction

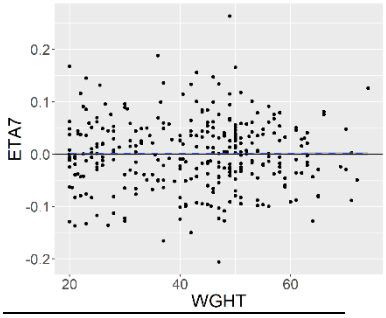
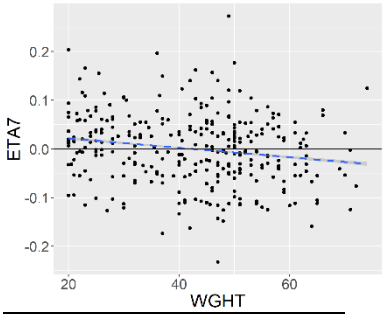
Similar to the Applicant's model, the Reviewer's model appears to describe the PK data reasonably well.

The covariates from the final model were all evaluated by backwards selection. Each covariate was removed to assess whether they significantly affected the PPK model by comparing the change in objective function value (OFV), corresponding interindividual variability (ETA, η) term, and graphical fit of the ETA term and the corresponding covariate. As shown in Table 163, each of the five covariate relationships, i.e., the effect of body weight on k12, k23, CL, V1 and CLM2, had a significant impact on the final model. Removal of each increased the OFV by at least 3.84, corresponding to a p-value of less than 0.05, and increased the corresponding ETA value. Graphically, removal of the body weight covariate relationships resulted in a linear trend in the

residuals. Based on these results, the body weight covariate relationships were kept in the final model.

Table 163. Assessment of Impact of PPK Model Covariates.

Covariate Relationship	Model 12a0 (Final)	Backwards Selection
Effect of WGHT on k12	 OFV η Shrinkage 115.534 0.0713 12%	 OFV η Shrinkage 305.734 0.148 6.3%
Effect of WGHT on k23	 OFV η Shrinkage 115.534 0.0512 21.7%	 OFV η Shrinkage 140.448 0.0552 19.9%
Effect of WGHT on CL	 OFV η Shrinkage 115.534 0.35 2.8%	 OFV η Shrinkage 126.174 0.368 2.6%
Effect of WGHT on V1		

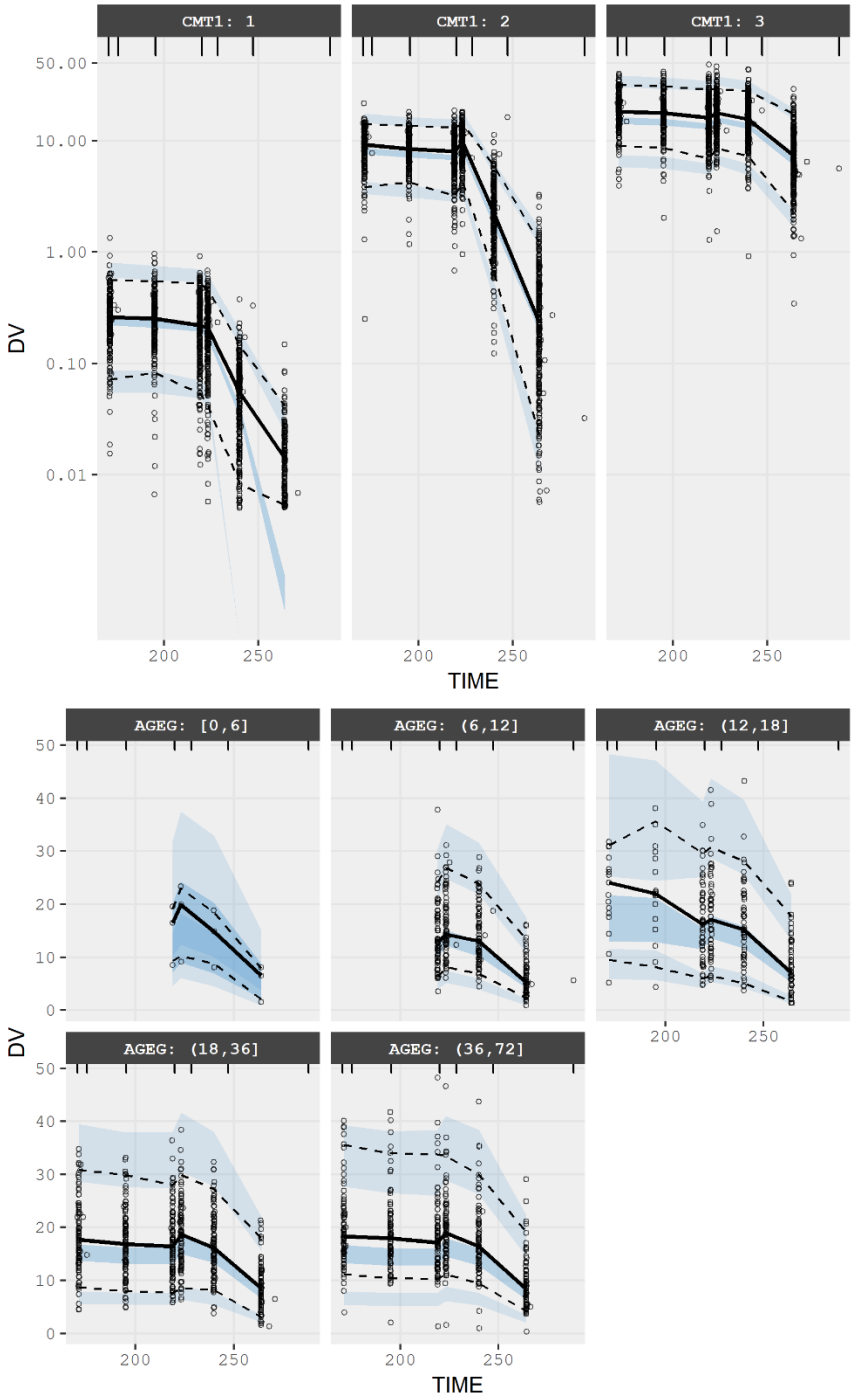
Covariate Relationship	Model 12a0 (Final)			Backwards Selection		
	OFV	η	Shrinkage	OFV	η	Shrinkage
	115.534	0.269	4.9%	160.783	0.326	4%
Effect of WGHT on CLM2						
	OFV	η	Shrinkage	OFV	η	Shrinkage
	115.534	0.0139	43.5%	139.845	0.0155	41.6%

Source: Reviewer's Analysis
The first column shows covariate relationship in the final model with the associated graph and parameters. The second column shows the covariate relationship in the final after having the covariate removed (backwards selection) with the associated graph and parameters.
Abbreviations: ETAs: CL (ETA1), V1 (ETA2), K12 (ETA4), K23 (ETA6), CLM2 (ETA7), CL/CLM1/CLM2: clearance of fexinidazole/M1/M2; $k_{a,b}$: rate constant describing formation from one moiety (a) to another moiety (b) of fexinidazole; OFV, objective function value; PPK, population pharmacokinetics; WGHT; body weight

Visual Predictive Check

A VPC of Model 10a with and without stratification by age is shown in Figure 26. The model predictions appear to capture the general trends in the observed PK profile in patients.

Figure 26. VPC for Fexinidazole (CMT1:1), M1 Metabolite (CMT1:2), and M2 Metabolite (CMT1:3)



Source: Reviewer's Analysis.
Note: 10a [Top] and VPC for M2 Metabolite with Stratification by Age (Bottom).
The black lines represent the median (solid) and 5th and 95th percentiles (dashed) of observed concentrations. The shaded areas represent the 90% prediction interval of the median (blue) and 5th and 95th percentiles (light blue).
Abbreviations: AGEG, age group in years; DV, dependent variable, concentrations of fexinidazole and its M1 and M2 metabolites; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; VPC, visual predictive checks

Model Assessment

Overall, the reviewer's PPK model appears to reasonably describe the PK of fexinidazole

Assessment of Labeling Claims

The pharmacometrics review team assessed the Applicant's labeling claims regarding the effect of intrinsic factors on fexinidazole PK.

Effect of Age and Body Weight on PK

The Applicant claimed that the AUC values of fexinidazole administered to pediatric patients ≥35 kg are similar or higher than those in adult patients when administered the proposed Fexinidazole tablet dose. They also claimed that AUC values in pediatric patients <35 kg are lower than those in adult patients when administered the proposed dose. The Fexinidazole tablet dose regimens that were evaluated for this analysis are as follows:

- Adults and pediatric patients >35 kg: 1800 mg QD for 4 days, then 1200 mg QD for 6 days
- Pediatric patients <35 kg: 1200 mg QD for 4 days, then 600 mg QD for 6 days

Using the model, AUC was calculated for each patient in the fexinidazole clinical trials from whom PK was sampled. The results of the PK analysis are shown in Table 164. Pediatric patients ≥35 kg had similar AUCs to those in adults, especially for the M2 moiety (within 5%). Pediatric patients <35 kg had 22% lower AUCs relative to those in adults. The lower AUCs in pediatric patients <35 kg relative to adults is likely because those pediatric patients are administered a lower dose regimen of Fexinidazole tablets. Overall, the Applicant's labeling claims regarding the effect of age on PK are acceptable.

Table 164. Mean (CV%) AUC of Patients Receiving Fexinidazole Stratified by Age and Weight

			AUC (mcg*h/mL)		
Age (yr)	**Weight (kg)**	**n**	**Fexinidazole**	**M1**	**M2**
<18	<35	95	2.85 (60%)	109 (27%)	362 (37%)
	≥35	29	4.19 (58%)	146 (25%)	485 (32%)
≥18	<35	2	3 (36%)	170 (43%)	496 (17%)
	≥35	191	3.74 (45%)	151 (27%)	465 (35%)

Source: Reviewer's Analysis
Abbreviations: AUC, area under the time concentration curve; CV, coefficient of variation; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; n, number of subjects in subgroup; yr, year

The effect of age on PK was further assessed by the Pharmacometrics reviewer into more categories beyond what the Applicant proposed. This analysis compared each fexinidazole moiety's AUC and M2 trough, which the Applicant proposes is most relevant for effectiveness. Over 90% of patients in each category of age and weight have free M2 troughs greater than the MIC (3 mcg/mL). See Table 165 below.

Table 165. Mean (CV%) Fexinidazole and Metabolites AUC and M2 Trough in Patients Receiving Fexinidazole Stratified by Age and Weight

Weight (kg)	Age Group (yr)	n	AUC (mcg*h/mL) Fexinidazole	M1	M2	Free M2 Trough Concentration (mcg/mL)	Percent With Free M2 Trough>MIC
<35	6-<12	62	2.8 (59%)	115 (26%)	382 (35%)	5.99 (37%)	100%
	12-<18	33	2.94 (60%)	99.5 (28%)	325 (39%)	5.22 (41%)	94%
	18-<75	2	3 (36%)	170 (43%)	496 (17%)	8.18 (19%)	100%
≥35	12-<18	29	4.19 (58%)	146 (25%)	485 (32%)	7.88 (33%)	97%
	18-75	191	3.74 (45%)	151 (27%)	465 (35%)	7.68 (36%)	98%

Source: Reviewer's Analysis.
Abbreviations: AUC, area under the time concentration curve; CV, coefficient of variation; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; MIC, minimal inhibitory concentration; n, number of subjects in subgroup; yr, year

Effect of Sex on PK

Table 166 shows that there is no consistent relationship between sex and fexinidazole moiety AUCs.

Table 166. Mean (CV%) Fexinidazole and Metabolites AUC in Patients Receiving Fexinidazole Stratified by Age, Weight, and Sex.

Age (yr)	Weight (kg)	Sex	n	AUC (mcg*h/mL) Fexinidazole	M1	M2
<18	<35	Male	54	2.85 (61%)	106 (28%)	352 (37%)
		Female	41	2.84 (58%)	114 (26%)	375 (37%)
	≥35	Male	13	4.11 (66%)	157 (25%)	530 (27%)
		Female	16	4.25 (53%)	137 (24%)	449 (35%)
≥18	<35	Male	1	3.75	222	556
		Female	1	2.24	119	436
	≥35	Male	121	3.8 (40%)	151 (25%)	467 (33%)
		Female	70	3.64 (53%)	152 (31%)	463 (38%)

Source: Reviewer's Analysis.
Abbreviations: AUC, area under the time concentration curve; CV, coefficient of variation; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; n, number of subjects in subgroup; yr, year

Effect of Hepatic Impairment on PK

Data were insufficient to assess the effect of hepatic impairment on fexinidazole PK. In the subset of patients from whom PK samples were collected, only 5% had abnormal liver function, and 1.3 to 1.6% each had AST, ALT, or ALP greater than or equal to 3 times the upper limit of normal. Therefore, no conclusions can be made regarding the effect of hepatic impairment on fexinidazole PK.

Effect of *Renal Impairment on PK*

There does not appear to be a clear relationship between renal function status and fexinidazole PK. The addition of glomerular filtration rate did not affect the PPK model and thus was not utilized as a covariate. Additionally, there did not appear to be any significant trends in fexinidazole moiety AUC by renal function as shown in Table 167. Glomerular filtration rate was estimated using the MDRD equation in adult patients and the bedside Schwartz equation in pediatric patients.

Table 167. Mean (CV%) Fexinidazole and Metabolites AUC in Patients Receiving Fexinidazole Stratified by Age, Weight, and Renal Impairment

Age (yr)	Weight (kg)	Renal Function	n	AUC (mcg*h/mL) Fexinidazole	M1	M2
<18	<35	Normal	68	3.03 (58%)	111 (28%)	371 (37%)
		Mild	24	2.33 (62%)	105 (27%)	335 (36%)
		Moderate	1	1.49	85.9	348
		Severe	2	3.42 (63%)	117 (5%)	379 (37%)
	≥35	Normal	21	4.12 (56%)	145 (16%)	494 (28%)
		Mild	7	4.51 (68%)	150 (44%)	464 (48%)
		Moderate	1	3.24	143	448
≥18	<35	Normal	2	3 (36%)	170 (43%)	496 (17%)
	≥35	Normal	121	3.79 (44%)	154 (27%)	485 (36%)
		Mild	58	3.67 (48%)	148 (27%)	428 (32%)
		Moderate	12	3.6 (37%)	141 (30%)	454 (36%)

Source: Reviewer's Analysis.
Abbreviations: AUC, area under the time concentration curve; CV, coefficient of variation; M1, Fexinidazole sulfoxide; M2 Fexinidazole sulfone; n, number of subjects in subgroup; yr, year

Assessment of Covariate Effects on Fexinidazole PK

There were differences in fexinidazole moiety AUC by age and weight; however, these AUC differences are likely caused by the different dose regimens administered to those patient groups. Sex and renal function do not appear to affect the PK of fexinidazole. Not enough data are present to evaluate the effect of liver dysfunction on AUC.

16.4. Clinical Microbiology Appendix

16.4.1. Nonclinical Studies

16.4.1.1. Mechanism of Action

The mechanism by which fexinidazole, a nitroimidazole, and its metabolites (fexinidazole sulfoxide and fexinidazole sulfone) exhibit activity against *T. brucei* has not been investigated. Fexinidazole as well as the sulfoxide and sulfone metabolites have low redox potentials of -511, -493 and -488 mV, respectively (Torreele et al. 2010; Tweats et al. 2012). The low redox electron transfer systems of trypanosomes, like some of the other protozoans and bacteria, may allow for fexinidazole to act as electron acceptors resulting in inhibition of the electron transport mechanism, formation of reactive intermediates, and cell death.

Studies with *T. brucei* and other protozoans such as *Leishmania donovani* and *Trypanosoma cruzi* suggest that nitroreductase (NTR) enzyme plays an important role in the bioactivation of fexinidazole; this is similar to other nitro-containing drugs such as nifurtimox and benznidazole (Patterson and Wyllie 2014). For example, (Wilkinson et al. 2008) and (Hall et al. 2011) reported that modulation of NTR levels within the *T. brucei brucei* and *T. cruzi* parasites affected their sensitivity to nitro-containing drugs; increased enzyme levels were associated with increased drug sensitivity in vitro. Another study (Wyllie et al. 2012) reported that the amastigotes of *Leishmania* species, over expressing the NTR enzyme, were more sensitive to fexinidazole (19-fold) as well as nifurtimox (27-fold), in vitro, compared to the wild-type strain (for details see

Section 16.4.1.2). Additionally, cross-resistance studies using fexinidazole- and nifurtimox-resistant clones of *T. brucei brucei* suggest NTR as a common mechanism of action among the nitro-containing drugs (Sokolova et al. 2010) for details see Section 16.4.1.6).

Overall, studies suggest that the activation of fexinidazole, like other nitro-containing drugs, by the NTR enzyme results in generation of reactive amines that can damage DNA and proteins of the parasite.

16.4.1.2. in Vitro Activity

Activity Against Different Strains/Isolates of *T. brucei*

The studies supporting the activity of fexinidazole and its metabolites (fexinidazole sulfoxide and fexinidazole sulfone) against different strains of *T. brucei brucei, T. brucei gambiense* and *T. brucei rhodesiense* are summarized in Table 168. Briefly, the axenic cultures of the blood stream trypanosomes were used for testing. The methods for measurement of in vitro sensitivity of *T. brucei* are not standardized and limited to testing in research laboratories. The experimental design used in different studies varied; some of the variations include strains of the parasite, inoculum concentration, duration of incubation and the methods used to measure activity such as ATP content or use of vital dye to assess parasite growth or viability.

The results show that the 50% inhibitory concentration (IC_{50}) values of fexinidazole, based on inhibition of growth, against different strains of *T. brucei brucei, T. brucei gambiense* and *T. brucei rhodesiense*, were ≤ 2.86, 0.93 and 0.94 µg/mL, respectively (for details see Table 168).

One study (Nare 2010) reported complete inhibition of visible parasite growth (MIC) of *T. brucei brucei*, at fexinidazole, fexinidazole sulfoxide and fexinidazole sulfone concentrations of 5.0, 4.7 and 2.2 µg/mL, respectively. Against strain STIB900 of *T. brucei rhodesiense*, one study (Kaiser 2010) reported 90% inhibitory concentration (IC_{90}) values of fexinidazole, fexinidazole sulfoxide and fexinidazole sulfone were 1.55, 0.94 and 0.99 µg/mL, respectively.

Overall, the activity of fexinidazole, fexinidazole sulfoxide and fexinidazole sulfone appears to be similar based on the small number of strains tested; the cytotoxicity of the mammalian cells (rat skeletal myoblast L6 cells) occurred at >30-fold higher concentration of fexinidazole (CC_{50} value was >90 µg/mL).

One study (Nare 2010) reported that transient/pulse exposure of trypanosomes of *T. brucei brucei* to fexinidazole or its metabolites, for 6 to 24 hours, followed by incubation in drug free medium for 72 hours did not reverse the viability of the parasites suggesting cidal activity of fexinidazole (Figure 27).

Table 168. In Vitro Activity of Fexinidazole and the Two Metabolites (M1 and M2) Against the Blood Stream Trypanosomes of Different Subspecies of *Trypanosoma Brucei.*

Experimental Design (Reference)	Strain	IC_{50} µM (µg/mL)		
			Fexinidazole Metabolites	
		Fexinidazole	Sulfoxide (M1)	Sulfone (M2)
Trypanosoma brucei brucei (n=4 or 5)				
Parasites (concentration not specified) were incubated with different concentrations of fexinidazole and/or its metabolites for 30 h and activity determined by measurement of ATP content conducted using a luciferase based assay kit (Cell Titer-glo from Promega). MICs, defined as the lowest concentrations that completely inhibited visible parasite growth, for fexinidazole, fexinidazole sulfoxide and fexinidazole sulfone were 5.00, 4.74 and 2.20 µg/mL, respectively. Time kill studies showed that transient exposure to fexinidazole or its metabolites, for 6 to 24 h, followed by incubation in drug free medium for 72 h did not reverse the viability of the parasites suggesting cidal activity. The activity of all 3 test compounds was concentration- and time-dependent. The activity of the two metabolites of fexinidazole was apparent at ≥6 to 8 h of incubation when exposed to ≥3X the MIC value whereas that of fexinidazole was reported at ≥12 h. (Nare 2010)	Not specified	10.24 (2.86)	6.40 (1.89)	2.86 (0.89)
Blood stream trypanosomes were cultured with different concentrations of fexinidazole and/or its metabolites for 3 days; resazurin was added and cultures incubated for additional 4 h and fluorescence measured at 528 nm excitation and 590 nm emission. (Sokolova et al. 2010)	S427	1.0 (0.28)	1.4 (0.41)	0.06 (0.18)
Blood stage parasites (2000) were cultured for 70 h with or without fexinidazole and/or its metabolites; resazurin was added and cultures incubated for additional 2 to 4 h and plates read using SpectraMax Gemini XS	BS221*	2.38 (0.66)	1.49 (0.44)	1.63 (0.51)

Experimental Design (Reference)	Strain	IC_{50} µM (µg/mL)		
			Fexinidazole Metabolites	
		Fexinidazole	Sulfoxide (M1)	Sulfone (M2)
microplate fluorescence scanner (Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588 nm. (Kaiser et al. 2011)	AT1KO**	1.33 (0.37)	0.85 (0.25)	0.56 (0.17)
*BS221 is a derivative of S427 isolated in Uganda in 1960 **AT1KO is a P2 transporter knockout strain of the BS221 strain ***STIB950mdr, is a derivative of the CP 2469 strain isolated in 1985 from a cow in Hakaka, Soakow District, Somalia.	STIB950 mdr***	2.44 (0.68)	1.21 (0.36)	0.99 (0.31)
Trypanosoma brucei gambiense (n=14)				
Parasites ($3x10^4$/mL) were cultured with and without different concentration of fexinidazole or its metabolites for 72 h and Alamar blue added for the final 3 h of incubation. Viability, based on fluorescence, was assessed at an excitation wavelength of 588 nm. L-6 rat skeletal myoblast cells were used to determine CC; the fexinidazole, M1 and M2 CC50 values were >322µM (>90 µg/mL), >305µM (>90 µg/mL), and >289µM (>90 µg/mL), respectively. (Torreele et al. 2010)	STIB930	0.58-1.29 (0.16-0.36)	0.61-1.22 (0.18-0.36)	0.48-1.25 (0.15-0.39)
	Clinical isolates (n=6)	1.07-3.33 (0.30-0.93)	ND	ND
Blood stage parasites (10^4) were cultured for 70 h with or without test compounds and resazurin added; cultures were incubated for additional 6 to 8 h and plates read using SpectraMax Gemini XS microplate fluorescence scanner (Molecular Devices) at an excitation wavelength of 536 nm and an emission wavelength of 588 nm. (Kaiser et al. 2011) !The STIB930 strain is a derivative of the TH1/78E (031) strain isolated from a patient in Côte d'Ivoire in 1978. It is melarsoprol sensitive. !!The DAL 898R strain was isolated from a patient in Côte d'Ivoire in 1985. !!!K03048 strain was isolated from a patient in South Sudan in 2003. !!!!Strains 40R, 45R, 130R, 349Pi, and 349R were isolated from patients in the Democratic Republic of Congo in 2003 to 2004.	STIB930!	1.84 (0.51)	0.94 (0.28)	0.91 (0.28)
	DAL 898R!!	1.01 (0.28)	1.03 (0.30)	0.76 (0.24)
	K3048!!!	0.95 (0.27)	ND	ND
	45R!!!!	2.47 (0.69)	1.24 (0.37)	0.95 (0.30)
	40R!!!!	2.61 (0.73)	0.95 (0.28)	0.67 (0.21)
	349Pi!!!!	1.07 (0.30)	ND	ND
	349R!!!!	3.31 (0.92)	ND	ND
	130R!!!!	2.37 (0.66)	ND	ND

Experimental Design (Reference)	Strain	IC_{50} µM (µg/mL)		
			Fexinidazole Metabolites	
		Fexinidazole	Sulfoxide (M1)	Sulfone (M2)
Trypanosoma brucei rhodesiense (n=3)				
Trypanosomes (10^4) were incubated with different concentrations of either fexinidazole, the two metabolites, and other drugs for 72 h. Alamar blue was added and cultures incubated for 2 to 4 h. The activity was measured using in a SpectraMax Gemini XS microplate fluorometer at an excitation wavelength of 536 nm and an emission wave length of 588 nm. The fexinidazole, M1 and M2 IC90 values were 5.56, 3.2 and 3.2µM (i.e., 1.55, 0.94 and 0.99 µg/mL), respectively. (Kaiser 2010) #From a patient in Tanzania, maintained in mice and in vitro in axenic cultures	STIB900#	3.37 (0.94)	1.89 (0.56)	2.02 (0.63)
Parasites ($3x10^4$/mL) were cultured with and without different concentration of fexinidazole or the metabolites for 72 h and Alamar blue added for the final 3 h of incubation. Viability, based on fluorescence, was assessed at an excitation wavelength of 588 nm. L-6 rat skeletal myoblast cells were used to determine CC; the fexinidazole, M1 and M2 CC50 values were >322µM (>90 µg/mL), >305µM (>90 µg/mL), and >289µM (>90 µg/mL), respectively. (Torreele et al. 2010)	STIB900	1.71-2.93 (0.48-0.82)	1.33-1.65 (0.39-0.49)	1.14-1.30 (0.35-0.40)
Blood stage parasites ($2x10^3$) were cultured for 70 h with or without test drugs or its metabolites and resazurin added; cultures were incubated for additional 2 to 4 h and plates read using SpectraMax Gemini XS microplate fluorescence scanner (Molecular Devices) using an excitation wavelength of 536 nm and an emission wavelength of 588	STIB900 ^	2.17 (0.61) 5.56 (1.55)	1.64 (0.48) 3.20 (0.94)	1.44 (0.45) 3.20 (1.00)
	STIB900 mel^^	2.66 (0.74)	1.16 (0.34)	1.26 (0.39)

Experimental Design (Reference)	Strain	IC_{50} µM (µg/mL)		
			Fexinidazole Metabolites	
		Fexinidazole	Sulfoxide (M1)	Sulfone (M2)
nm. Time kill studies show that 48-hour period of exposure to fexinidazole or its metabolites is required to exhibit activity similar to those in the 72-hour assay (Figure 28). (Kaiser et al. 2011) ^STIB900 strain is a derivative of the STIB704 strain isolated from a patient in Ifakara, Tanzania, in 1982. ^^STIB900mel and STIB900pent are melarsoprol and pentamidine-resistant lines, respectively, which were generated by growing STIB900 in increasing subcurative drug concentrations.	STIB900 pent^^	2.71 (0.76)	1.48 (0.44)	1.16 (0.36)

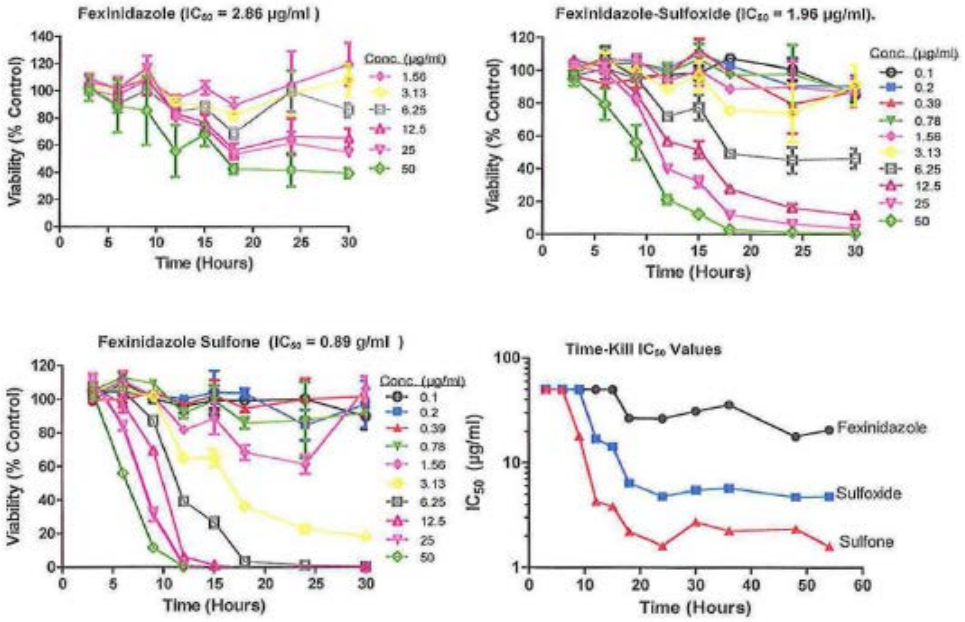
Source: Table prepared by the Reviewer
Note: Some of the *T. brucei* strains were stated to be resistant to different drugs. The criteria used for characterizing resistance were not specified.
Abbreviations: ATP, adenosine triphosphate; CC, cell cytotoxicity; h, hours; MIC, minimal inhibitory concentration; n, represents number of strains/isolates; ND, not done

Time Kill Studies

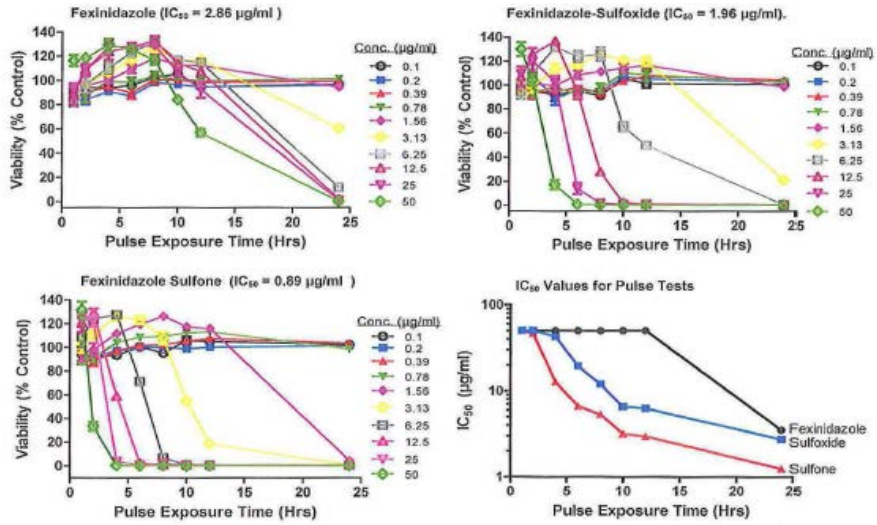
Studies with *T. brucei brucei* (Nare 2010) and *T. brucei rhodesiense* (Kaiser et al. 2011) suggest that the activity of fexinidazole and its metabolites to be concentration- and time-dependent (Figure 27 and Figure 28). The activity of the two metabolites of fexinidazole was apparent at ≥6 h of incubation when exposed to ≥3X the MIC value whereas that of fexinidazole was reported at ≥12 hours (Nare 2010). The maximum killing activity required up to 48 hours of exposure to the drug; this was similar to that observed in a standard 72-hour assay (Kaiser et al. 2011).

Figure 27. Time Kill Plots for *T. brucei brucei* Exposed to Fexinidazole, Fexinidazole Sulfoxide and Fexinidazole Sulfone

(A) Concentration Dependent Effects of Fexinidazole and Its Metabolites on the Viability of the Parasites



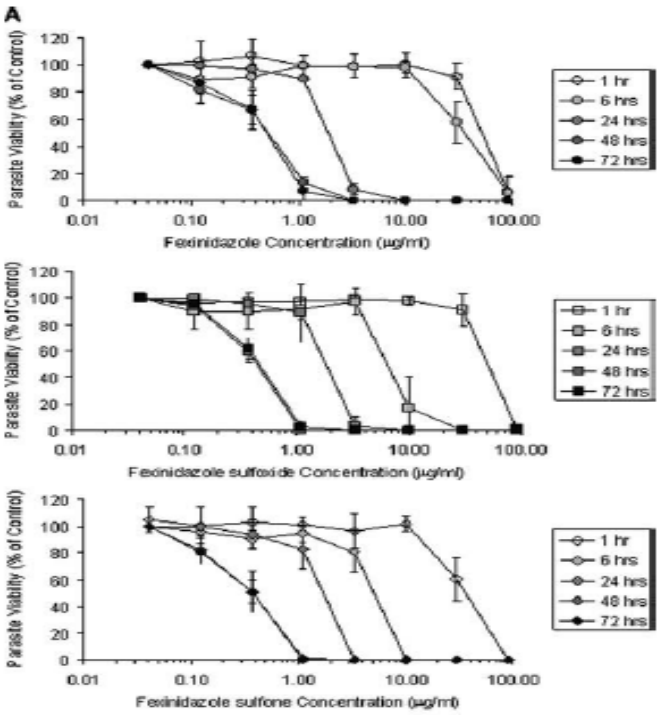
(B) Reversibility of Trypanocidal Effects Following Transient Exposure to Fexinidazole and Its Metabolites



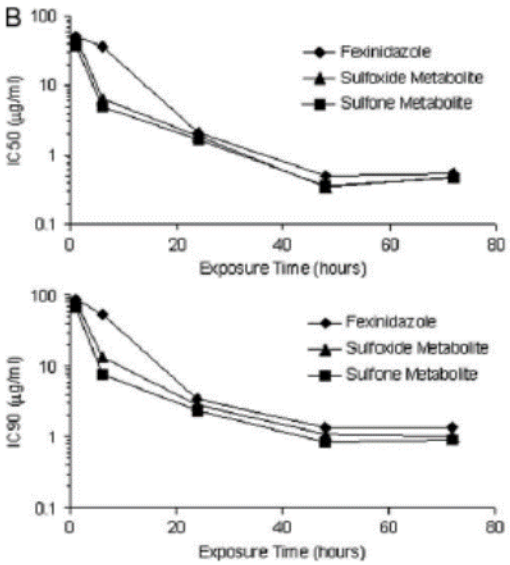
Source: NDA
ATP measurement was used as an indicator of real time parasite viability. The test compound were serially diluted into 96 well plates starting at concentration >10-times the MIC and incubated with *T. brucei brucei* parasites for 0 to 30 hrs. Viability was determined at each time point using a fast measurement of ATP content conducted with the luciferase based assay kit (Cell Titer-glo) from Promega. Time-kill data were plotted as viability signal versus time for each concentration tested in order to determine the key parameter that drives killing e.g., concentration and/or time above MIC. To establish the time required to cause persistent or irreversible effects, the parasites were assessed for their ability to recover from transient exposure to test compounds and viability was assessed after 72 h growth in drug free media.
Abbreviations: hr, hour; IC_{50}, half maximal inhibitory concentration

Figure 28. In Vitro Dynamic Assays With *T. brucei rhodesiense*, STIB900 Strain

(A) Growth Inhibition Curves After Compound Washout at Specified Times and Viability Assessment at 72 hours.



(B) IC_{50} and IC_{90} Values Calculated From the Compound Washout Procedure.



Source: NDA
Note: Values are the means and standard deviations for 4 experiments (n = 4)
Trypanosomes of *T. brucei rhodesiense* (STIB900) were seeded in clear 96-well V-bottom plates at a density of 10,000 parasites per well in 100 µL medium and incubated for 1, 6, and 24 hours with serially diluted test compounds. One plate was prepared for each time point. At the designated time point, a plate was spun at 650 relative centrifugal force (RCF) for 5 min to sediment the parasites. The supernatant was removed, and 100 µL of warm MEM added to each well to resuspend the parasites. The wash process was repeated 4X times. After the washing procedure, the parasites were resuspended in 100 µL medium and transferred into new culture plates and further incubated. After a total of 70 hours of incubation, resazurin was added and trypanocidal activities (IC_{50} and IC90 values) were determined as described for the in vitro growth inhibition assays. The maximum killing activity required up to 48 hours of exposure to the drug; this was similar to that observed in a 72-hour assay.
Abbreviations: hr, hour; IC_{50}, half maximal inhibitory concentration; IC_{90}, 90% of the maximum inhibitory concentration

16.4.1.3. In Vivo Activity (Animal Studies)

The studies supporting the in vivo activity of fexinidazole and its metabolites in acute and chronic murine infection models of HAT are summarized in Table 169. The experimental design for different studies varied. Briefly, for the acute infection model, mice were infected intraperitoneally, with trypanosomes of either *T. brucei brucei* (S427 strain), *T. brucei gambiense* (130R strain), or *T. brucei rhodesiens*e (STIB900 and LSH/TM 180 strains). Treatment was initiated, either orally or intraperitoneally, on Days 1, 4, or 6 postinfection for 1 to 7 days. Cure was based on evidence of parasites in blood and/or brain in surviving mice. Overall, the studies show that fexinidazole is effective in curing mice infected with either *T. brucei brucei*, *T. brucei gambiense*, or *T. brucei rhodesiens*e. No relapse was reported in any study under the experimental conditions tested.

One study (Kaiser et al. 2011) reported the activity of the metabolites of fexinidazole in mice infected with *T. brucei rhodesiens*e. The activity of the fexinidazole sulfoxide and fexinidazole sulfone metabolites was lower than that of fexinidazole: 50% and 25% of mice treated with fexinidazole sulfoxide and fexinidazole sulfone, respectively, survived; the relapse day was >38.5 days and >31.5 days, respectively. All fexinidazole treated mice survived and no relapse was observed until Day 60.

For the chronic infection model, mice were infected, intraperitoneally, with either different clones of the GVR35 strain of *T. brucei brucei* or the LSH/TM 180 strain of *T. brucei rhodesiense* and treatment was initiated ≥21 days postinfection. Other than that, the experimental design was similar to that for the studies in acute infection model. A majority of the studies reported that fexinidazole was effective in curing mice infected with *T. brucei brucei* or *T. brucei rhodesiense* as well as reducing relapse rate (for details see Table 169). The activity of metabolites in the chronic infection model was not measured.

One study (Jennings et al. 1996) reported that the fexinidazole gel applied topically was not effective in curing mice under the experimental conditions tested.

Table 169. Activity of Fexinidazole and/or Its Metabolites in Mice Infected With the Blood Stream Forms of the Three Subspecies of *T. Brucei.*

Strain of *T. brucei Sub*species (Reference)	**Study Summary**
T. brucei (subspecies not specified)	
Not specified (Winkelmann and Raether 1978)	Structure-activity relationship of several 1-methyl-5-nitroimidazoles substituted at position 2 was assessed in infected NMRI mice. The details of the experimental design were not included in the publication. Fexinidazole was not effective whereas the fexinidazole sulfoxide and fexinidazole sulfone metabolites were effective in curing mice.

Strain of *T. brucei Sub*species (Reference)	**Study Summary**
T. brucei brucei – acute (stage 1) and chronic (stage 2) infection models	
S427 (Sokolova et al. 2010)	**Acute infection model:** Mice were infected with 10^4 trypanosomes, IP, and treated with oral fexinidazole 24 h PI. Animals were examined daily for clinical signs of infection as well as microscopic evidence of parasites in blood on wet smears. Cure was based on the absence of parasites in blood in mice surviving up to 60 days PI. Fexinidazole at a dose of 50 mg/kg cured all mice; ED_{50} was 21±0.3 mg/kg.
GVR35/1* (Jennings and Urquhart 1983)	**Chronic infection model:** CFLP or CD-1 mice were infected IP with 10^4 trypanosomes and treated IP with 250 mg/kg fexinidazole on Day 24, for 4 days. A group of 5 mice in each group were necropsied 3-6 days after the last dose and blood (~1.0 mL) as well as homogenized brain were inoculated into experimentally naïve mice; the recipient mice were then examined weekly for a maximum of 30 days for the appearance of circulating parasitemia. The results show that fexinidazole was effective in curing all 5 mice euthanized 3 to 6 days post-treatment; no relapse was observed until Day 30 PI of experimentally naïve mice. Another group of mice (n=14) were allowed to relapse naturally and examined weekly for circulating parasitemia for a period of 120-213 days. At the end of study period, brain homogenates were also subinoculated into experimentally naïve mice to assess cure. Of the 14 mice followed until Day 200, 11 (78.6%) remained cured. None of the 11 suramin treated mice were cured. There was no placebo group included in the study. In another experiment, the activity of fexinidazole was measured in mice previously treated with a single dose (20 mg/kg) of suramin on Day 21 PI followed by different doses (25-250 mg/kg) of fexinidazole on Day 24 PI for 4 days; cure was based on subinoculation of blood and brain homogenates on Days 3 to 6 post-treatment to experimentally naïve mice; fexinidazole doses of ≥30 mg/kg, after initial treatment with suramin, cured all mice for the 200-day observation period whereas a single dose of suramin was ineffective in curing mice.
GVR35*/C1.2 (Jennings 1991))	**Chronic infection model:** CD-1 mice were infected IP with $2x10^4$ trypanosomes and treated with 100 mg/kg dose of fexinidazole, by the IP route, on Days 28, 31, 33 and 36 PI. Parasitemia was assessed, on a drop of blood, examined weekly for at least 240 days after the last dose; 2 of the 6 mice were cured in one experiment and none in another experiment. However, fexinidazole (100 mg/kg) in combination with trimelarsan (≥5 mg/kg) cured all mice. Lower doses of fexinidazole (30, 60, and 80 mg/kg) in combination with trimelarsan (≥2.5 mg/kg) were also effective in curing all mice (n=5 or 6 per group).
GVR35/C1.5* (Jennings et al. 1996)	**Chronic infection model:** CD-1 mice were infected IP with $2x10^4$ trypanosomes and topical treatment was initiated on Day 21 PI with fexinidazole gel (2.5 and 5 mg/mL; 0.1 mL - equivalent to 7.2 and 14.3 mg/kg) was applied over the shoulder or back area and gently massaged into the skin daily for 3 or 4 days. Mice were followed for parasitemia by microscopic examination up to 180 to 240 days. The results show that 4 days of treatment was ineffective in curing mice; parasitemia was cleared transiently in only 2 of the 6 mice for 3 days. However, a combination of fexinidazole with melarsoprol was effective in curing mice and reducing relapse rate.
GVR35 (Torreele et al. 2010)	**Chronic infection model:** NMRI mice were infected IP with blood stream forms (parasite inoculum concentration used for infection not specified); fexinidazole treatment was initiated on Day 21 PI, for 5 days. Mice were followed for survival and parasitemia assessed until Day 180. Cure rates in untreated mice were not provided. The results show a dose-dependent activity of fexinidazole; a dose of 200 mg/kg/day for 5 days was most effective and cured 7/8 (87.5%) mice. MRD was >164 days. The authors state that in two other experiments, 100% cure was obtained in groups of 5 mice administered fexinidazole oral dose of 100 mg/kg, b.i.d. for 5 days (data not shown).

Strain of *T. brucei Sub*species (Reference)	**Study Summary**
GVR35 (Kaiser et al. 2011)	**Chronic infection model:** NMRI mice were infected IP with 2 x10^4 bloodstream forms and treatment with fexinidazole (oral or IP) initiated on Day 21 PI, once a day for 5 days. Diminazene aceturate (40 mg/kg) was used as a control; it is subcurative since it clears the trypanosomes only in the hemo-lymphatic system and not in the CNS, leading to a subsequent reappearance of trypanosomes in the blood. Parasitemia was assessed two times a week for the first 5 weeks post-treatment followed by once a week for up to 180 days PI. Surviving and aparasitemic mice at Day 180 were considered cured and were euthanized. The day of relapse of the animals (including the cured mice) was recorded (as >180) to calculate the MRD. The results show a dose-dependent activity of fexinidazole; 200 mg/kg fexinidazole, administered orally, was most effective (7/8 mice were cured and MRD was 163.8 days). A group of mice treated with fexinidazole 50 mg/kg bid, IP was most effective; all 5 mice were cured and no relapse observed until Day 180.
T. brucei gambiense – acute infection (stage 1) model	
130R (Torreele et al. 2010)	NMRI mice were infected IP with blood stream forms (parasite inoculum concentration not specified) and oral treatment with fexinidazole (100 mg/kg) was initiated on Day 7 PI, for 4 days. Mice were followed, for survival and parasitemia, until Day 180. Untreated mice were not cured and mean relapse time was 10 days. The results show that fexinidazole treatment cured all 3 mice; MRD was >90 days.
T. brucei rhodesiense – acute (stage 1) and chronic (stage 2) infection models	
LSH & TM 180^ (Raseroka and Ormerod 1986)	**Acute and chronic infection models:** Mice (TO strain) were infected with 5x10^3 trypanosomes and treated with fexinidazole (200 mg/kg) daily for 6 days (route of treatment not specified). Treatment was initiated at different time points (Days 3, 7, 14, 21, and 28) PI and mice euthanized after parasites were not detectable in the blood; blood and 3 parts of the brain (choroid plexus, ventricular wall or cerebrum) were separated, homogenized and injected IP into 4 groups of uninfected experimentally naïve mice. Control mice were infected and dosed in the same way but were not euthanized. The results show that fexinidazole was active against trypanosomes in blood and all 3 parts of the brain; especially when treatment was initiated early in the acute phase of infection.
STIB900 (Torreele et al. 2010)	**Acute infection model:** NMRI mice were infected IP with blood stream forms (parasite inoculum concentration not specified) and oral treatment initiated on Day 3 PI, for 4 days. Mice were followed for survival and parasitemia assessed until Day 180. Untreated mice were not cured with mean relapse time of 7 days. The results show a dose-dependent activity of fexinidazole; 100 mg/kg fexinidazole was most effective and cured all 4 mice; MRD was >60 days.
STIB900 (Kaiser et al. 2011)	**Acute mouse model**: NMRI mice were infected IP with 10^4 bloodstream forms and treatment with fexinidazole or its metabolites (oral or IP) initiated on Day 3 PI, once a day for 4 days. Parasitemia was assessed up to 60 days PI. Surviving and aparasitemic mice at Day 60 were considered cured and were euthanized. The day of relapse of the animals (including the cured mice) was recorded (>60) to calculate the MRD. The untreated mice died with a mean survival of 8.75 days. All mice treated with fexinidazole, administered orally (100 mg/kg) or IP (50 mg/kg), survived and no relapse was reported up to Day 60. However, the two metabolites appear to be less effective; 50% and 25% of mice treated with fexinidazole sulfoxide and fexinidazole sulfone, respectively, survived and the MRD was >38.5 days and >31.5 days respectively.

Source: Table prepared by the Reviewer
* The *T. brucei brucei* GVR35 strain was isolated from a wildebeest in the Serengeti in 1966 (primary isolate S10); the line obtained by mouse passage was named *T. brucei* GVR 35/1.
^ *T. brucei rhodesiense* 180A, isolated from a patient in Botswana.
Abbreviations: CFLP, Carworth Farms and Lane-Petter; CNS, central nervous system; ED_{50}, 50% effective dose; h, hours; IP, intraperitoneally; MRD, maximum day of relapse; n, number of subjects in subgroup; NMRI, Naval Medical Research Institute; PI, postinfection

16.4.1.4. Drug Combination

In Vitro

The activity of fexinidazole and its metabolites was measured in combination with other antiparasitic drugs, pentamidine, melarsoprol, and eflomithine (Kaiser 2010). The strain STIB900 of *T. brucei rhodesiense* was used for testing. The experimental design was similar to that summarized in Table 168, except that different drugs were combined and tested in the ratio of 1:3, 1:1, and 3:1. The IC_{50} values of the individual drugs as well as the fractional inhibitory concentrations (FIC) for different drugs tested in combination were determined. An interaction with sum FICs (ΣFICs) <0.75 was characterized as synergistic, ΣFICs between 0.8 and 1.4 as additive, and ΣFICs >1.5 as antagonistic. The results showed that the combination of fexinidazole or its metabolites with pentamidine, melarsoprol or eflomithine were additive. No antagonism was observed under the experimental conditions tested.

Similar observations were reported in another study (Kaiser et al. 2011). The experimental design was similar to that summarized above (Kaiser 2010) except that an interaction with ΣFICs <0.5 was characterized as synergistic, ΣFICs >4.0 as antagonistic, and ΣFICs between 0.5 and 4.0 as indifferent. The authors state that a combination of fexinidazole with its sulfoxide and sulfone metabolites or other drugs (melarsoprol, eflornithine, or pentamidine) resulted in an indifferent activity. Similarly, a combination of fexinidazole sulfoxide or fexinidazole sulfone with other drugs showed indifferent activity. Overall, the study suggests no antagonism between fexinidazole and its metabolites with melarsoprol, eflornithine, or pentamidine.

In Vivo

Low doses of fexinidazole, administered intraperitoneally, in combination with trimelarsan (≥5 mg/kg) cured all mice infected with the GVR35/C1.2 strain of *T. brucei brucei* (Jennings 1991).

Topical application of a combination of fexinidazole gel and melarsoprol gel was more effective in curing mice and reducing relapse rate compared to monotherapy with either of the drugs (Jennings et al. 1996).

For details see Table 169.

A combination of fexinidazole or its metabolites in combination with nitro-containing drugs such as nifurtimox was not tested in vitro or in vivo.

16.4.1.5. Drug Resistance

In Vitro

A potential for development of resistance to fexinidazole by *T. brucei* was tested in vitro (Sokolova et al. 2010). Briefly, blood stage trypanosomes of *T. brucei brucei* (strain 427) were incubated with step-wise increasing concentrations of fexinidazole (1.5 to 50 µM i.e., 0.42 to 13.97 µg/mL). The fexinidazole IC_{50} values increased by about 10-fold suggesting a decrease in

sensitivity of the resistant clones compared to the parental strain. The growth rate of the fexinidazole resistant clones was not measured. It is possible that the doubling time of fexinidazole resistant clones, similar to nifurtimox resistant clones, is slower than the parasites of the parental strain.

In Vivo

No studies supporting the development of resistance to fexinidazole, in animal models of HAT, were available for review.

Mechanism of Drug Resistance

A single Type I *NTR*-like gene has been reported in *T. brucei* (*TbNTR*; GenBank accession no. XP_846343) as well as *T. cruzi* (*TcNTR*; GenBank accession no. XP_810645). An 800-kbp band, representing *Type I NTR,* present in the parent strain was missing in the resistant clone of *T. cruzi* generated in vitro (Wilkinson et al. 2008).

Trypanosomes of *T. brucei* and *T. cruzi* with reduced NTR enzyme levels were resistant to nifurtimox, whereas cells overexpressing the enzyme were hypersensitive (Wilkinson et al. 2008)(Hall et al. 2010; Hall et al. 2011). Similar observations were reported using *L. donovani* parasites; the amastigotes of *L. donovani*, over expressing the NTR enzyme, were more sensitive in vitro to fexinidazole (19-fold) as well as nifurtimox (27-fold) compared to the wild-type strain (Wyllie et al. 2012).

Overall, the studies suggest that the mechanism of resistance for fexinidazole is likely to be similar to other nitro-containing drugs such as nifurtimox; this may be due to reduced expression of *Type I NTR,* either by mutation or by deprivation of the necessary cofactor (World Health Organization 2013).

16.4.1.6. Cross-Resistance

Fexinidazole resistant clones of *T. brucei brucei*, generated in vitro, were resistant to nifurtimox (10-fold increase in IC_{50} value); additionally, nifurtimox resistant clones, with 8-fold higher nifurtimox IC_{50} values compared to the wild-type strain, were resistant to fexinidazole (27-fold increase in IC_{50} value) and its metabolites (13- to 15-fold increase in IC_{50} values) in vitro. Although the in vitro growth rate of the nifurtimox resistant clones was slower compared to the wild-type parasites, the virulence of the resistant clones was not compromised in mice. Fexinidazole or nifurtimox were not effective in curing mice infected with the parasites of the nifurtimox resistant clone of *T. brucei brucei* (Sokolova et al. 2010).

The activity of fexinidazole against *T. brucei rhodesiense* strains resistant to non–nitro-containing compounds such as pentamidine or melarsoprol was not altered.

Overall, the nonclinical studies suggest that the activity of fexinidazole is not altered by current non–nitro-containing HAT therapies. However, the potential for cross-resistance between fexinidazole and other nitro-containing drugs exists; therefore, resistance to fexinidazole in patients previously treated with NECT cannot be ruled out.

16.4.2. Parasitological Assessments in Clinical Studies

T. brucei gambiense infects only humans and is prevalent in western and Central Africa. *T. brucei rhodesiense* can infect animals and humans and is prevalent in eastern and southern Africa; the two species cannot be distinguished morphologically. Subspecies were based on epidemiological findings.

The Applicant conducted the following three clinical studies, in patients from two regions in Central Africa, known to be endemic for *T. brucei gambiense*:

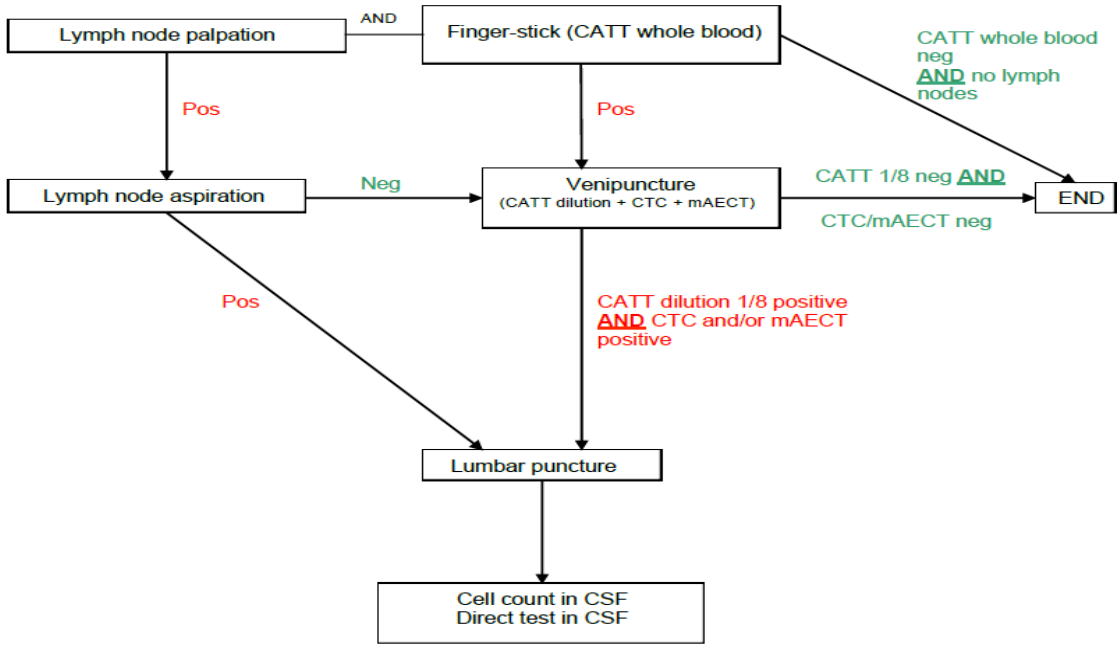
- Study DNDiFEX004 in patients, ≥ 15 years of age, with late Stage 2 HAT: Study was conducted at 9 sites in DRC and one site in CAR.
- Study DNDiFEX005 in patients, ≥ 15 years of age, with Stage 1 or early Stage 2 HAT: Study was conducted at 8 sites in DRC.
- Study DNDiFEX006, in children 6 to 14 years of age, with Stage 1, early Stage 2 and late Stage 2 HAT: Study was conducted at 8 sites in DRC.

The parasitological assessments in the 3 clinical studies included detection of

- anti-*T. brucei gambiense* antibodies by CATT, as well as
- parasites in blood, lymph node aspirate and/or CSF.

Initial screening of the participants was performed by CATT. The confirmation of diagnosis, for enrollment, was based on microscopic evidence of parasites in blood, lymph node aspirate and/or CSF (Figure 29).

Figure 29. Schematic of Parasitological Examinations Required to Diagnose HAT and the Type of Specimens



Source: NDA
Abbreviations: CSF, cerebrospinal fluid; CATT, card agglutination test for trypanosomiasis; CTC, capillary tube centrifugation technique; mAECT, mini-anion exchange column and centrifugation; Pos, positive; Neg, negative

The criteria for characterizing patients as Stage 1 and early Stage 2 included the presence of parasites in blood and/or lymph node aspirate and CSF WBC count of ≤5 cells/µL and 6 to 20 cells/µL, respectively. For the late Stage 2 HAT patients, the criteria included evidence of parasites in the CSF, blood and/or lymph node aspirate; if the parasites were undetectable in the CSF, CSF WBCs were >20 cells/µL (Table 170).

Table 170. Summary of Criteria Used for Characterizing Patients With Different Stages of HAT

Parameter	Stage 1^	Early Stage 2 HAT[a]	Late Stage 2 HAT[b]
Parasites	In blood and/or lymph node*	In blood and/or lymph node*	In blood, lymph node and/or CSF
CSF WBC	≤5 cells/µL	6 to 20 cells/µL	>20 cells/µL**

Source: Table prepared by the Reviewer
[a] Studies DNDiFEX005 and DNDiFEX006
[b] Studies DNDiFEX004 and DNDiFEX006
*Absence of parasites in CSF.
**Patients with parasites in blood or lymph node aspirate but absence of parasites in CSF had CSF WBC >20 cells/µL.
Abbreviations: CSF, cerebrospinal fluid; HAT, human African Trypanosomiasis; WBC, white blood cells

Follow-up, for clinical and parasitological response to treatment, was at Day 11 (end of treatment) and Months 3, 6, 12, 18 and/or Month 24 after the last dose; Month 24 samples were tested if relapse was suspected or required according to the local standard of care. CSF sampling was performed at Month 3 if the patient presented with clinical signs or symptoms of HAT, and at Month 24 if relapse was suspected, or if required according to the local standard of care. Overall assessments were in accordance with the recommendations by the World Health Organization (World Health Organization 2007; World Health Organization 2017).

For details of Study protocol see Section 8.

The parasitological tests used for patient enrollment and for assessment of efficacy as well as the parasitological findings in the clinical trials are summarized below in Sections 16.4.2.1 and 16.4.2.2, respectively.

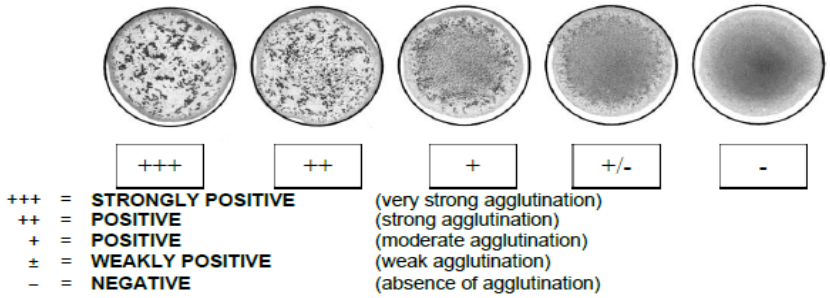
16.4.2.1. Description of the Parasitological Tests

Parasitological testing, at different visits, was performed at the local laboratories, either at the investigational sites or by mobile teams at the village level. Adequate oversight, on-site visits and training of laboratory staff were implemented.

Detection of Anti-*T. brucei gambiense* Antibodies by Card Agglutination Test for Trypanosomiasis

The CATT, a screening test for detecting agglutinating antibodies against the lyophilized variable antigen type LiTat 1.3 of the blood stream trypanosomes of *T. brucei gambiense*, is not cleared by the FDA. The test is available in the endemic areas in Africa. Briefly, a drop of undiluted and diluted (1/4, 1/8, and 1/16) blood was added on top of the CATT reagent (on the circle of the card), mixed, spread on the circle and shaken for 5 minutes. For quality control (QC), positive and negative controls were included for testing each day and each time a new reagent vial was used. The agglutination reaction was read immediately and the results expressed as negative, weakly positive to strongly positive (Figure 30). The antibody titer was the last dilution that tested positive.

Figure 30. CATT Test, Read Out



Source: NDA
Abbreviations: CATT, Card Agglutination Test for Trypanosomiasis;

Reviewer's comments: *The Applicant used whole blood for testing. Note that the CATT is more specific for testing of serum and plasma samples compared to whole blood (Chappuis et al. 2005; Bonnet et al. 2015). False-positive results were reported for patients with other parasitic diseases, such as malaria and filariasis, or a transient infection with trypanosomes of T. brucei brucei (Table 171). As this is a direct agglutination test, prozone phenomenon can occur leading to false negative findings; therefore, testing of serial dilutions of specimens is recommended. The test is adequate for initial screening of patients with T. brucei gambiense HAT; however, diagnosis of HAT is based on microscopic evidence of parasites.*

Table 171. CATT Test, Summary of Assay Performance

Reference	Sensitivity	Specificity	NPV	PPV	Comments
(Chappuis et al. 2005)	87-98%	95%	High during mass population screening. False negative results can occur	Not specified	False negative results affect the sensitivity of the assay; this may be due to prozone phenomenon especially when undiluted samples are tested. Addition of EDTA to the dilution buffer may improve the sensitivity of the assay. The PPV in whole blood is limited. The test is used for mass screening in populations where the prevalence of HAT is usually below 5%.
(Bonnet et al. 2015)	91% (78-99.8%)	97%	99-100%	Not specified	False-negative results and lower sensitivity for patients infected with strains of trypanosomes that do not express the LiTat 1.3 gene in some endemic areas. Despite a specificity of about 97%, the PPV of the CATT remains limited when the test is used for mass screening in populations in which the overall prevalence of *T. brucei gambiense* HAT is low.

Source: Table prepared by the Reviewer
Abbreviations: CATT, Card Agglutination Test for Trypanosomiasis; EDTA, Ethylenediaminetetraacetic acid; HAT, human African Trypanosomiasis; NPV, negative predictive value; PPV, positive predictive value

Detection of Parasites in Blood by Concentration Tests

The parasites were detected in blood by concentration tests that include

- Capillary tube centrifugation (CTC) test also known as Woo test and microhematocrit capillary centrifugation test (mHCT),
- Mini-anion exchange column test (mAECT), and/or
- mAECT on buffy coat (mAECT-BC).

Testing was completed within 30 minutes of blood collection. It is important to keep the time between sampling and examination as short as possible to avoid immobilization and lysis of trypanosomes in the specimens. Trypanosomes are rapidly killed by direct sunlight but can survive for a few hours when the sample is kept in a cool dark place.

Centrifugation of Whole Blood

The CTC test, also known as Woo test or mHCT, was performed on blood samples collected in lithium heparin tubes; 8 capillary tubes per patient were filled with blood, sealed at ends with dry clay sealant, and centrifuged at high speed (12000 rpm) for 5 minutes in a hematocrit centrifuge. The capillaries were mounted between the slide and coverslip and read, preferably using a Woo viewing chamber, to identify the presence of mobile trypanosomes between the white blood cells (WBCs) and the plasma (buffy coat) at low magnification (10x10 or 10x20). Quality control parameters included confirmation of the reading by a second reader for each positive test.

The sensitivity of mHCT/CTC test increases with the number of tubes examined; estimated detection threshold is 500 trypanosomes/mL.

Testing by the CTC test or mHCT was performed, if the mAECT was not available.

Mini-Anion Exchange Column Test on Whole Blood

Parasites were concentrated using a mini-anion exchange column test (mAECT) on heparinized whole blood samples. As the host blood cells are negatively charged, the trypanosomes can be concentrated by anion exchange chromatography followed by low-speed centrifugation; the sensitivity is <100 trypanosomes/mL. Briefly, 350 µL of heparinized blood was run through the anion-exchange column (kit of the Institut National de Recherche Biomédicale in Kinshasa, RD Congo) and elute collected; parasites in the elute were concentrated by low-speed centrifugation. The motile trypanosomes were examined in sediments, using a viewing chamber, by light microscopy. Results were expressed as the presence or absence of trypanosomes. The QC measures included confirmation of the reading by a second reader.

Mini-Anion Exchange Column Test on Buffy Coat

Parasites were concentrated using a mini-anion exchange column test on buffy coat (mAECT-BC) samples, obtained by centrifuging heparinized blood at 2000 rpm for 10 minutes; the buffy coat contains a portion of the plasma, red blood cells, WBCs, and parasites. The buffy coat was run through the anion-exchange column and processed as summarized above for the whole blood.

Detection of Parasites in Lymph Nodes

The *T. brucei* parasites were detected in lymph node aspirate by pushing the aspirate with a syringe plunger on to the slide; the aspirate on slide was covered with a coverslip and examined for the presence or absence of trypanosomes under the microscope (10X objective). The QC included confirmation of the reading by a second person.

Time within which specimens were processed and examined was not specified.

Detection of Parasites in Cerebrospinal Fluid

The detection of trypanosomes in CSF was performed by a modified single centrifugation method. Briefly, 4 mL CSF sample was centrifuged for 10 minutes at 3500 rpm or 15 minutes at 3000 rpm. The trypanosomes were observed, microscopically, as small mobile organisms in the pellet among the WBCs and the results recorded as the presence or absence of trypanosomes. The QC included confirmation of the reading by a second person.

The maximum duration between CSF collection and the test was 15 minutes.

Reviewer comments: *The parasitological tests used by the Applicant were adequate for diagnosis and follow-up. All patients enrolled based on antibody positive findings by CATT had evidence of parasites in either blood, lymph node aspirate, or CSF. Early and late Stage 2 HAT patients with evidence of parasites in blood or lymph node aspirate, but not in the CSF, had WBC*

count of 6 to 20/ µL and >20 /µL, respectively, in the CSF; Stage 1 HAT patients had no parasites in the CSF and WBC count was <6/µL.

16.4.2.2. Parasitological Findings at Follow-Up Visits

Study DNDiFEX004: Late Stage 2 HAT

A majority of the patients in the mITT population (comprised of all ITT patients, except those who fled an area of armed conflict and for whom no post-treatment data were available) showed no evidence of parasites, at the end of treatment (Day 11) or any of the follow-up visits. At the end of treatment, only one patient, in the fexinidazole treatment group tested positive by the mAECT test; at Month 3, no parasites were detectable in any patient in the two treatment groups. By Month 18, 8.8% (23/262) and 2.4% (3/127) of the patients failed treatment in the fexinidazole and NECT treatment groups, respectively (Table 70).

Of the 26 patients who failed treatment, based on the prespecified multifactorial criteria (either evidence of trypanosomes in any body fluid (confirmed relapses), WBC >20 /µL of CSF, death, or lost to follow-up), 11 patients in the fexinidazole group, were confirmed relapses with parasites in either blood (n=2), CSF (n=8), or CSF plus blood (n=1); none of the 3 patients in the NECT group had any evidence of parasites (Table 172).

Table 172. Study DNDiFEX004: Parasitological Findings in the 26 Patients, in the mITT Population, That Failed Treatment by Month 18

Subject ID	Failure Visit Time	Parasitological Findings	Comments
Fexinidazole			
FEX004-(b) (4)	EOT Day 11	No test information.	Premature death (Day 8).
FEX004-(b) (4)	EOT Day 11	No test information.	Premature death (Day 5).
FEX004-(b) (4)	FU M3	Day 11: CTC, mAECT, mAECT-BC, LN, CSF negative.	Died on Day 82.
FEX004-(b) (4)	FU M3	Day 11: CTC, mAECT-BC, CSF negative.	Died on Day 22.
FEX004-(b) (4)	FU M3	Day 11: CTC and CSF negative.	Died on Day 94.
FEX004-(b) (6)	FU M6	Day 11, M6, M12 and M18: CTC, mAECT, and/or CSF negative. M24: No testing results.	No parasites detected at any visit until Month 18. WBC/µL: 75 (M6), 65 (M12), and 60 (M18). Rescue treatment: Day 554 onwards.
FEX004-(b) (4)	FU M6	Day 11: CTC, LN mAECT, mAECT-BC, CSF negative. M3: mAECT and mAECT-BC negative. M6: CTC, mAECT-BC, CSF negative. M12: CTC, mAECT-BC, and CSF negative. M18: CSF positive. M24: No test results.	**Relapse** at Month 18. WBC/µL: 165 (M6), 56 (M12), 300 (M18). Rescue treatment at Month 18.

Subject ID	Failure Visit Time	Parasitological Findings	Comments
FEX004-(b) (4)	FU M12	Day 11: CTC and CSF negative. M3 and M6: CTC and CSF negative.	No parasites detected at any visit until Month 6. Died on Day 352.
FEX004-(b) (4)	FU M12	Day 11: CTC and CSF negative. M3: CTC and mAECT-BC negative. M12 CTC and CSF negative. Unscheduled visit 2 (Day 292 i.e., Month 9.7): CTC and mAECT negative; CSF positive.	**Relapse** at Month 9/10 and became negative at Month 12. WBC/µL: 52 (M6). Rescue treatment: Day 299 onwards.
FEX004-(b) (4)	FU M12	Day 11: CTC, mAECT, mAECT-BC, CSF negative. M3: CTC and mAECT-BC negative. M6: CTC, mAECT-BC negative, CSF positive.	**Relapse**. WBC/µL: 782 (M6). Rescue treatment: Day 193 onwards.
FEX004-(b) (4)	FU M12	Day 11: CTC, mAECT-BC, CSF negative. M3: CTC, mAECT-BC negative. M6: CTC, mAECT-BC, CSF negative. Unscheduled Visit 1 (Day 281 i.e., Month 9.4): CTC, mAECT-BC negative but CSF positive.	**Relapse**. WBC/µL: 115 (M6). Rescue treatment: Day 283 onwards.
FEX004-(b) (4)	FU M12	Day 11: CTC, LN, mAECT-BC, CSF negative. M3: CTC, LN, mAECT-BC negative. M6: CTC, LN, mAECT-BC, negative. M12: CTC, mAECT-BC, CSF negative. M18: CTC, mAECT-BC negative and CSF positive.	**Relapse**. WBC/µL: 40 (M6), 59 (M12), 55 (M18). Rescue treatment at Day 551.
FEX004-(b) (4)	FU M18	Day 11, Months 6, and 12: Negative. M12: mAECT-BC positive; mAECT, CTC and CSF negative Unscheduled visit 1 (Day 403 i.e., Month 13.4): mAECT-BC positive; CSF negative.	**Relapse**. Rescue treatment: Day 406 onwards.
FEX004-(b) (4)	FU M18	Day 11: CTC mAECT, mAECT-BC, CSF negative. M3: mAECT, mAECT-BC negative. M6: mAECT-BC and CSF negative. M12: CTC and mAECT-BC negative; CSF positive.	**Relapse**. WBC/µL: 85 (M6) and 406 (M12). Rescue treatment: Day 371 onwards.
FEX004-(b) (4)	FU M18	Day 11: CTC, mAECT, mAECT-BC, LN, and CSF negative. M3: mAECT and mAECT-BC negative. M6: CTC, mAECT BC and CSF negative. M12: CTC, MAECT-BC and LN negative; CSF positive. M18 or M24: No test results.	**Relapse**. WBC/µL: 466 (M6) and 586 (M12). Rescue treatment: Day 368 onwards.

Subject ID	Failure Visit Time	Parasitological Findings	Comments
FEX004-(b) (4)	FU M18	Day 11: CSF negative; other tests (CTC LN mAECT, mAECT-BC) not done. M3: CTC and mAECT negative. M6: CTC, mAECT and CSF negative. M12: CTC and CSF negative but mAECT positive. M18 or M24 visit: No test results.	**Relapse**. Rescue treatment: Day 443 onwards.
FEX004-(b) (4)	FU M18	Day 11: CTC, mAECT, and CSF negative. M3: CTC and mAECT negative. M6: CTC, mAECT, mAECT-BC and CSF negative. M12: No test results included. M18: CTC negative; mAECT and CSF positive.	**Relapse**. WBC/µL: 110 (M18). Rescue treatment: Day 637 onwards.
FEX004-(b) (4)	FU M18	Day 11: CTC, mAECT-BC, and CSF negative. M3: CTC and mAECT-BC negative. M6: CTC and mAECT-BC negative. M12: CTC and MAECT-BC negative; CSF positive.	**Relapse**. WBC/µL: 339 (M12) Rescue treatment on Day 402.
FEX004-(b) (4)	FU M24	Negative at Day 11, Months 6, 12, 18 or 24: CTC, mAECT and CSF.	No parasites detected at any visit. WBC/µL: 105 (M6), 40 (M12), 6 (M18).
FEX004-(b) (4)	FU M24	Day 11, Months 6, 12 and 18: CTC, mAECT, or CSF negative. M24: No test results	No parasites detected at any visit. Died after M18.
FEX004-(b) (4)	FU M24	Day 11, Months 6, 12 and 18: CTC, mAECT, or CSF negative. M24: No test results	No parasites detected at any visit. Died after M18; the Applicant states that the death unrelated to treatment or disease evolution.
FEX004-(b) (4)	FU M24	Day 11, Months 6, 12, 18 and 24: CTC, mAECT, and/or CSF negative.	No parasites detected at any visit. WBC/µL: <20 at M4, M12, M18. Rescue treatment: M18 onwards.
FEX004-(b) (4)	FU M24	Day 11: CTC, mAECT-BC and CSF negative. M3: CTC and mAECT-BC negative. M6: CTC, mAECT-BC and CSF negative. M12: CTC, mAECT-BC and CSF negative. M18, CTC and mAECT-BC, negative; CSF not done. M24: No test results.	No parasites detected at any visit. Died after M18; the Applicant states that the death unrelated to treatment or disease evolution
NECT			
FEX004-(b) (4)	FU M3	Day 11: CTC, mAECT, and CSF negative.	No parasites detected at any visit. Died on Day 11.

Subject ID	Failure Visit Time	Parasitological Findings	Comments
FEX004-[redacted] (b) (4)	FU M12	Day 11: CTC and mAECT-BC negative. M3: CTC and mAECT-BC negative. M6: CTC, mAECT-BC and CSF negative.	No parasites detected at any visit. Died on Day 197.
FEX004-[redacted] (b) (4)	FU M12	M11: CTC, mAECT-BC, LN, and CSF negative. M3: CTC, mAECT and LN negative. M6: CTC, mAECT-BC, LN, and CSF negative. M12: CTC, mAECT-BC, and CSF negative.	No lumber puncture at M18 and M24.

Source: Table prepared by the Reviewer
Note: WBC/µL, represent cell count in CSF.
Abbreviations: CSF, cerebrospinal fluid; CTC, capillary tube centrifugation technique; EOT, end of treatment; FU, follow-up; LN, lymph node; M, Month; mAECT, mini-anion exchange column and centrifugation on whole blood; mAECT-BC, mini-anion exchange column and centrifugation on buffy coat; WBC, white blood cell

Study DNDIFEX005: Stage 1 and Early Stage 2 HAT

The majority of the patients (≥98%) with Stage 1 or early Stage 2 HAT (Table 83 and Table 84) were a treatment success based on the prespecified multifactorial criteria that were same as for the Study DNDIFEX004. None of the patients showed any evidence of parasites in blood, lymph node aspirate and/or CSF, at the end of treatment (Day 11) or any of the follow-up visits; none of the failures were confirmed relapses.

Study DNDiFEX006: Stage 1, Early Stage 2 and Late Stage 2 HAT

The majority of the patients (≥98%) with Stage 1, early Stage 2 and late Stage 2 HAT were a treatment success (Table 87 and Table 88) based on the prespecified multifactorial criteria that were same as for the Study DNDIFEX004. None of the patients showed any evidence of parasites, in blood, lymph node aspirate, and/or CSF, at the end of treatment (Day 11) or any of the follow-up visits; none of the failures were confirmed relapses.

Reviewer comments: *In a majority of the patients, in all three studies, there was no evidence of parasites in any of the body fluid tested (blood, lymph node aspirate, and/or CSF) at the end of treatment. Relapse, based on evidence of parasites, in any body fluid, was observed in 11 of the 23 late stage 2 HAT patients who failed fexinidazole treatment in Study DNDiFEX004; of the 11 patients, 9 had evidence of parasites in the CSF.*

A study (Study DNDi-FEX-07-HAT) in patients with *T. brucei rhodesiense* is ongoing in Malawi and Uganda.

16.4.2.3. Interpretive Criteria

The Applicant has not requested any susceptibility test interpretive criteria. This is appropriate as the tests to measure in vitro sensitivity of the *T. brucei* parasites are not standardized and their use is limited to research laboratories.

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