# Fexinidazole: Evaluation of the *In Vitro* Intrinsic Clearance and Metabolism with Hepatocytes from African American Donors

Product Name:	Fexinidazole
Study Number:	0327-2008
Study Director:	
Sponsor Reference Study No.:	N.A.
Status:	FINAL

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# SUMMARY

Fexinidazole was incubated with cryopreserved hepatocytes from African American donors, at concentrations of 1 and 10  $\mu$ M. The 1  $\mu$ M samples were used for the intrinsic clearance determination, while the 10  $\mu$ M samples were used for metabolite profile determination. The incubation samples were analyzed by LC-MS/MS.

The intrinsic clearance was calculated using the half-life approach; the half-life and the intrinsic clearance were determined from the concentration of fexinidazole remaining at the sampling time points.

The half-life of fexinidazole was 6.5 minutes, and the corresponding intrinsic clearance value was 257 mL/min/kg.

The metabolite profile of fexinidazole was investigated at t=0 and after 30 and 120 minutes incubation at the concentration of 10  $\mu$ M.

The metabolism was rapid; unchanged fexinidazole accounted for 77% of the drug related material at t=0, and for 1% after 30 minutes incubation.

The main metabolite produced by hepatocytes from African American donors was fexinidazole sulfoxide. This metabolite was present as two enantiomers; the enantiomer with the shorter retention time was detected in a ratio of about 2:1, with respect to the enantiomer with the longer retention time.

Fexinidazole sulfone and fexinidazole sulfoxide des-methylated on the imidazole ring were minor metabolites, accounting for about 1%.

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.

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### TABLES

# **1. ABBREVIATION AND DEFINITIONS OF TERMS**

CID	Collision-induced decomposition
CLint	Intrinsic clearance
Da	Dalton
DMSO	Dimethyl sulfoxide
7-ETC	7-Ethoxycoumarin
ESI	Electrospray ionisation
7-HC	7-Hydroxycoumarin
HPLC	High performance liquid chromatography
LC-MS/MS	Liquid chromatography-Mass spectrometry/Mass spectrometry
Km	Michaelis Menten constant
m/z	Mass to charge
MRM	Multiple reaction monitoring
MS	Mass spectrometry
MS/MS	Mass spectrometry/Mass spectrometry
PDA	Photodiode array
t1/2	Half life
TIS	Turbo ion spray
TOF	Time of flight

### 2. INTRODUCTION AND OBJECTIVES

Fexinidazole is a 5-nitroimidazole derivative biologically active against Trypanosoma parasites (*T.b.rhodesiense* and *T.b. brucei*) under investigation in the treatment of the Human African Trypanosomiasis (HAT), known as sleeping sickness.

The intrinsic hepatic clearance and metabolite profile of fexinidazole has been previously investigated *in vitro* with hepatocytes of some animal species (mouse, rat, dog and cynomolgus monkey) and of Caucasian donors [1].

The purpose of this study (0327-2008) was to evaluate the intrinsic hepatic clearance and metabolite profile of fexinidazole with hepatocytes of African American donors.

The intrinsic hepatic clearance of fexinidazole was determined using the half-life approach, by measuring the substrate disappearance during 120 minutes incubation with hepatocytes of African American donors. LC-MS/MS was used for the detection of the compound during the incubation.

The metabolite profile and metabolite identification of fexinidazole was performed at a concentration of 10  $\mu$ M of fexinidazole. The incubation samples were also analyzed on a chiral column.

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### 3. STUDY SPONSOR

Drugs for Neglected Diseases *initiative* (DND*i*) 15 Chemin Louis-Dunant CH-1202 Geneva Switzerland

### **4. TEST FACILITY**

Accelera

# 5. REGULATORY REQUIREMENTS

This study was conducted for exploratory purposes outside GLP regulations and was not audited by QA. Relevant Standard Operating Procedures of Accelera, Nerviano Medical Sciences, followed during the study were: MET-P/018/00; PCD-S-027; PCD-S-032; PCD-S-134; PCD-S-166.

## 6. SCHEDULE

Experimental Start Date	21 July 2008
Experimental Completion Date	09 September 2008
	1

## 7. MATERIALS AND METHODS

### 7.1. Test Item

Generic Name	Eexinidazole
Chemical name	1H-Imidazole-1-methyl-2-[[4-(methylthio)phenoxy]methyl]-5-
	nitro
Chemical Structure	$O_2 N $
Molecular Formula	$C_{12}\Pi_{13}N_{3}O_{3}S$
Molecular weight	279.31
Lot/Batch Number	3168-82-99/C
D	
Punty	98.5% by HCIO4 assay
Expiry	September 2008
Storage Conditions	-20°C. light protection
Course Courselier	
Source Supplier	The compound, manufactured by Centipharm (France), was
	provided by the Sponsor

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### 7.2. Metabolites

Fexinidazole sulfoxide, lot 1106-I-0170, 99.3% purity, and fexinidazole sulfone, lot 1106-I-0171, 99.9% purity, were provided by the Sponsor.

### 7.3. Test System

Cryopreserved hepatocytes of five African American single donors, lots EHI, REL, KSE, ZIJ and MRS, were purchased from In Vitro Technologies Inc (Baltimore, Maryland, USA). The certificates of analysis are reported in Appendix 1.

### 7.4. Chemicals

Reagents and solvents were of analytical grade and obtained from Sigma-Aldrich (St. Louis, Missouri, USA).

### 7.5. In Vitro Incubations

On the day of the experiment a 10 mM stock solution of fexinidazole in DMSO was prepared and, within 3 hours, aliquots of this freshly prepared solution, after suitable dilution, were added to the incubation matrix, to reach fexinidazole final concentrations of 1 and 10  $\mu$ M. The final percentage of DMSO was 0.01% in the incubation samples for the intrinsic clearance determination and 0.1% in the incubation samples for metabolite profiling.

The cryopreserved hepatocytes were thawed according to the supplier's procedure and the number of viable cells was determined using the Trypan Blue exclusion method. Viabilities were 92% for lot EHI, 89% for lot REL, 82% for lot KSE, 86% for lot ZIJ and 86% for lot MRS in the intrinsic clearance study. In the metabolite profile study viabilities were 91% for lot EHI, 90% for lot REL, 85% for lot KSE, 84% for lot ZIJ and 87% for lot MRS. Afterwards, the same amount of viable cells for each donor were pooled and used for the incubations of fexinidazole.

### 7.5.1. Intrinsic Clearance Study

For the intrinsic clearance determination, fexinidazole was incubated at a concentration of 1  $\mu$ M with pooled human hepatocytes (1 million cells/mL) in a final incubation volume of 1 mL Leibovitz L-15 medium, at 37°C. Incubations were performed in duplicate in a 48-well plate under shaking. Sampling was performed using an automatic liquid handling system (Multiprobe II EX, Packard). At 0, 1, 5, 10, 15, 20, 30, 60 and 120 minutes, 50  $\mu$ L aliquots of the incubates were taken, then 80  $\mu$ L of ice-cold acetonitrile and 20  $\mu$ L of 1  $\mu$ M warfarin in acetonitrile (internal standard) were added, and samples centrifuged at 2000 rpm for 20 min. The supernatant was analyzed by LC-MS/MS.

The chemical stability of fexinidazole was checked by incubating the compound at  $37^{\circ}$ C in the medium alone at t = 0 and 120 minutes.

For the determination of phase I and phase II activities of hepatocytes, 7-ethoxycoumarin (7-ETC) 1  $\mu$ M, and 7-hydroxycoumarin (7-HC) 30  $\mu$ M were used as positive controls and incubated under the same conditions as fexinidazole. Aliquots of incubates were taken at 0, 1, 5, 10, 15, 20, 30, 60 and 120 minutes and processed as fexinidazole samples.

### 7.5.2. Metabolite Profile Study

For metabolite profiling, fexinidazole was incubated at a concentration of 10  $\mu$ M with pooled human hepatocytes (1 million cells/mL) in a final incubation volume of 2 mL Leibovitz L-15 medium, at 37°C. Aliquots of the incubation samples were taken at t = 0, 30 and 120 minutes; the metabolism was stopped by the addition of an equal volume of cold acetonitrile and the samples centrifuged at 1100 rpm for 20 min. The supernatant was stored at -20°C until analysis. Control incubations were performed with the compound in the medium alone for 120 minutes.

### 7.6. Analysis of the Samples of Intrinsic Clearance Study

HPLC Equipment and Conditions							
HPLC system	1100 binary pump (Agilent, Palo Alto, USA)						
Autosampler	2777 (	Waters)					
Analytical column	Guard	Column SB-C8 4.	6 x 12.5 mm, 5 μ	ım (Zor	bax)		
Column temperature	ambier	nt					
Mobile phase A	10 mN	I ammonium form:	ate pH 4.0: aceto	nitrile (	(95:5, v:v)		
Mobile phase B	10 mN	I ammonium form	ate pH 4.0: aceto	nitrile (	(5:95, v:v)		
Injection volume	20 µL						
Gradient conditions	Step	Total time (min)	flow (µL/min)	%A	% B		
	0	0.00	1500	100	0		
	1	0.00	1500	0	100		
	2 0.15 1500		1500	0	100		
	3	0.20	600	0	100		
	4 1.00 600		0	100			
	5	1.35	600	100	0		
	6	1.60	1500	100	0		
Valco Divert Valve	Step	Total time (min)	) Position				
	1	0.30	Waste				
	2	1.50	Source				
	3 1.51 Waste						
MS Equipment and Conditions							

The analysis of the samples for intrinsic clearance determination was performed by an LC-MS/MS method, using the following conditions:

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Mass spectrometer	API 4000 Triple Quadrupole (Applied Biosystems/MDS
	Sciex)
Source	Turbo Ion Spray (TIS)
Ion mode	Positive for fexinidazole and 7-ETC.
	Negative for 7-HC, 7-HC sulphate and 7-HC glucuronide.
	Warfarin was analysed both in positive and negative ion
	mode.
Scan mode	Multiple Reaction Monitoring (MRM)
MRM transitions	$280.0 \rightarrow 140.2$ (fexinidazole)
	$191.0 \rightarrow 163.1 \ (7-\text{ETC})$
	$161.0 \rightarrow 133.1 \ (7-\text{HC})$
	$241.1 \rightarrow 161.1 \text{ (7-HC sulphate)}$
	$336.9 \rightarrow 161.1$ (7-HC glucuronide)
	$309.3 \rightarrow 163.0$ (warfarin positive ion mode)
	$307.3 \rightarrow 57.0$ (warfarin negative ion mode)
Software	Analyst 1.4.1 (Applied Biosystems)

### 7.7. Intrinsic Clearance Determination

The intrinsic clearance (CLint) of fexinidazole and of 7-ethoxycoumarin was calculated using the half-life approach. The half-life and the CLint were determined from the concentration remaining at the different sampling points using the LC-MS/MS method. By plotting the natural logarithmic area of the compound remaining against the time, the slope was calculated by exponential regression analysis, and converted into the half-life (t1/2) and CLint expressed as  $\mu$ L/min/million cells and mL/min/kg.

### 7.8. Analysis of the Samples of Metabolite Profile Study

The Q-TOF 2 mass spectrometer was calibrated with a multi-point calibration in the range 70-1000 Da against the known accurate masses of the fragment ions that resulted from the collision-induced decomposition (CID) of [Glu<sup>1</sup>]-fibrinopeptide B by continuous infusion at 10  $\mu$ L/min of a 10  $\mu$ g/mL solution in a mixture of 1% aqueous formic acid: acetonitrile (1:1, v:v). The tune parameters were those optimized in a previous study with fexinidazole [1]. In order to improve the accuracy of the mass measurements, during all analyses a 10  $\mu$ g/mL solution of Met-Arg-Phe-Ala (m/z 524.2655) in 10 mM ammonium formate, pH 4.5: acetonitrile (1:1, v:v) was infused into the reference source of the LockSpray at a rate of 1-2  $\mu$ L/min and a reference scan obtained every 10 seconds for automatic correction of the accurate masses. All data were acquired in centroid mode.

Before analysis, the incubation samples were evaporated to dryness under a stream of nitrogen (Turbovap), then the residues were reconstituted to the original incubation volumes with 10 mM ammonium formate, pH 4.5: acetonitrile 8:2, v:v.

Nerviano Medical Sciences Page 10 of 24 The analyses were performed using an HPLC system on line with a photodiode array (PDA) detector and a mass spectrometer (MS). Equipment and conditions are given below.

HPLC Equipment and Conditions						
Autosampler	HTC Pal (CTC Analytics, San Jose, USA) equipped with a					
	100 μL sample loop and a 100 μL syringe					
Binary pump	1100 Series (Agilent, Palo Alto, CA, USA)					
Degasser	1100 Series (Agil	ent)				
Column oven	1100 Series (Agil	ent)				
Column	XBridge C8, 2.1	x 150 mm, 3.5 μm	(Waters)			
Guard column	C8, 2 x 4 mm (Ph	enomenex)				
Column temperature	40°C					
Mobile phase A	10 mM ammoniu	m formate, pH 4.5				
Mobile phase B	Acetonitrile					
Flow rate	0.3 mL/min					
Run time	35 minutes					
Injection volume	75 μL					
Gradient conditions	Time (min)	Solvent A (%)	Solvent B (%)			
	0.0	95	5			
	1.0	95	5			
	4.0	75	25			
	21.0	40	60			
	21.5	5	95			
	27.0	5	95			
	27.5	95	5			
	35.0	95	5			
PDA Detector and Condition	ıs					
Diode Array detector	1100 Series (Agil	ent)				
Range	190 to 600 nm					
Resolution	2 nm					
Width	0.1 min					
<b>MS Acquisition Conditions</b>	MS Acquisition Conditions					
Acquisition software	Masslynx 4.1 (Waters)					
Processing software	Metabolynx 4.1 (Waters)					
Scan mode	MS Full Scan and MS/MS Full Scan					
Acquisition polarity	Positive					
Acquisition mode	Centroid					
Acquisition time	27 minutes					
Scan range	75 to 750 Da (MS Full Scan); 50 to 750 Da (MS/MS)					
Scan time	0.9 sec					
Interscan delay	0.1 sec					
Capillary voltage	3 kV					

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Cone voltage	25 eV
Collision energy	10 eV (MS Full Scan); 25 eV (MS/MS)
Extractor voltage	2 eV
Rf Lens	1.5 eV
Ion energy	1.5 eV
Source temperature	120°C
Desolvation gas temperature	250°C

The incubation samples were analyzed in MS positive ion mode and the data searched on the accurate masses of possible metabolites using the Metabolynx software, version 4.1 (Waters), using a mass window of 40 mDa. Samples were then re-run in MS/MS mode by selecting the masses of possible metabolites. The collision energy used to obtain MS/MS data was 25 eV.

# 7.8.1. Separation of Fexinidazole Sulfoxide Enantiomers with a Chiral Column

The separation was performed using the equipment described in 7.8, a chiral HPLC column Chirobiotic TAG 4.6 x 250 mm, 5  $\mu$ m (Astec) and methanol as mobile phase, with isocratic elution at a flow rate of 1 mL/min.

# 8. ARCHIVING

The original protocol, all raw data and supporting documents produced at the Test Facility, and the final report with original signatures were filed in the Archives of Accelera, Nerviano Medical Sciences S.r.l., Nerviano (Italy) for the period of time agreed with the Sponsor (at least 3 years) after which the Sponsor will be contacted for instructions regarding dispatch or disposal of the material.

A copy of the protocol, the report with original signatures and all relevant original documentation of the test item were filed by the Sponsor.

# 9. STUDY DEVIATIONS

No protocol deviations were observed during the study.

## **10. STUDY PERSONNEL**



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# **11. RESULTS AND DISCUSSION**

### 11.1. Phase I and Phase II Activities of Hepatocytes

The hepatocytes used in this study were checked for their activities towards phase I and phase II reactions, using 7-ETC and 7-HC. Table 1 shows the disappearance half-life and intrinsic clearance of 7-ETC incubated at the concentration of 1  $\mu$ M with hepatocytes. Table 2 shows the formation of 7-HC sulphate and 7-HC glucuronide from 7-HC incubated at the concentration of 30  $\mu$ M with hepatocytes. The results confirmed that the hepatocytes used in this study exhibited active metabolism.

### **11.2. Intrinsic Clearance Determination of Fexinidazole**

The intrinsic clearance of fexinidazole was determined at the concentration of 1  $\mu$ M in pooled hepatocytes of five African American donors, using the half-life approach. The starting concentration of 1  $\mu$ M was assumed to be << of Km.

The half-life of fexinidazole was 6.5 minutes, and the corresponding intrinsic clearance value was 257 mL/min/kg (Table 3).

The peak area (counts) of fexinidazole at the different time points are shown in Appendix 2.

Negative controls with fexinidazole incubated for 120 minutes at 37°C in the incubation medium alone show about 40% loss of fexinidazole.

### **11.3. Metabolite Profile and Identification**

The metabolite profile of fexinidazole was determined at the concentration of 10  $\mu$ M at t=0 and after 30 and 120 minutes incubation.

The chromatographic separation of fexinidazole and metabolites was obtained using a reverse phase HPLC column XBridge C8, 2.1 x 150 mm, 3.5  $\mu$ m (Waters), under the same chromatographic conditions as in the previous study, when fexinidazole was incubated with hepatocytes of animal species (mouse, rat, dog and cynomolgus monkey) and of Caucasian donors [1].

The identity of metabolites was proposed based on their accurate mass (using LockSpray correction), with an accuracy of +/-5 mDa. MS/MS analyses were performed to verify that peaks were drug-related and to obtain structural information from the fragment ions formed. The identity of fexinidazole sulfoxide and fexinidazole sulfone in the incubation samples was also confirmed by comparing the retention times of the two metabolites with those of the authentic standard compounds.

The approximate relative amounts were determined from the absolute areas taken from a selected ion chromatogram at the metabolite masses with a mass window of 0.04 Da. The

Nerviano Medical Sciences Page 13 of 24 absolute areas of fexinidazole, fexinidazole sulfoxide and fexinidazole sulfone were normalized based on their relative MS response (factors 1.3, 1 and 8 for fexinidazole, fexinidazole sulfoxide and fexinidazole sulfone, respectively). Nevertheless, these results should be regarded as semi-quantitative (approximate relative amounts of fexinidazole and each metabolite in the samples).

The proposed metabolic pathway of fexinidazole is shown in Figure 1.

The relative amounts of fexinidazole and metabolites detected at t=0, after 30 and 120 minutes incubation with hepatocytes of African American donors are reported in Table 4.

Fexinidazole was rapidly metabolized, as shown by the half-life of the compound.

At t=0, unchanged fexinidazole accounted for 77% of total drug related material; the remaining drug related material was due to the sulfoxide **M1**. Virtually t=0 is approximately 30 seconds, just the time to add fexinidazole to hepatocytes, shake, take an aliquot and stop the metabolism by addition of acetonitrile. This explains the amount of M1 present at this incubation time.

After **30 minutes** incubation, fexinidazole was detected in amounts of 1%. The main component (98%) was M1. Metabolite **M3** (m/z = 282.06), corresponding to M1 desmethylated on the imidazole ring, was detected in traces (less 1%).

After **120 minutes** incubation, M1 accounted for 98%. Traces of the sulfone metabolite **M2** (1%) and of metabolite M3 were also detected.

After 120 minutes incubation in the Leibovitz medium alone (**control sample**), fexinidazole accounted for about 82% of the total drug related material.

The lower loss of fexinidazole in the metabolite profile control sample (18%) compared to the clearance control sample (about 40%) could be explained by the different concentration of fexinidazole used: higher (10  $\mu$ M) in the metabolite profile study and lower (1  $\mu$ M) in the clearance study.

### 11.3.1. Metabolite Profiles with the Chiral Column

A standard solution of fexinidazole sulfoxide and the samples after 30 and 120 minutes incubation with hepatocytes were analyzed on a chiral HPLC column for the chromatographic separation of the two enantiomers of fexinidazole sulfoxide. After both 30 and 120 minutes incubation, the enantiomer with the shorter retention time was detected in a ratio of about 2:1, with respect to the enantiomer with the longer retention time, while in the standard solution the ratio was 1:1. The extracted ion chromatograms of fexinidazole sulfoxide are shown in Figure 2.

# 12. CONCLUSIONS

As previously observed with hepatocytes from Caucasian donors, fexinidazole showed a rapid and high intrinsic clearance with hepatocytes from African American donors. In agreement with the above, unchanged fexinidazole was detected in small amounts (1%) only after 30 minutes incubation. Fexinidazole sulfoxide 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 fexinidazole sulfone and of the sulfoxide des-methylated on the imidazole ring were also detected.

# **13. REFERENCE**

1. Fexinidazole: Evaluation of the *in vitro* cross species intrinsic clearance and metabolism with mouse, rat, dog, monkey and human hepatocytes. Accelera Study Report 0141-2007, November 2007.

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# TABLES AND FIGURES

# Table 1. Intrinsic clearance results for 7-ethoxycoumarin incubated at the concentration of 1 $\mu$ M with hepatocytes from African American donors <sup>(1)</sup>.

Species	t1/2 (min)		CLint <i>in vitro</i> (μL/min/million cells)		CLint <i>in vitro</i> (mL/min/kg)	
	clearance	met profile	clearance	met profile	clearance	met profile
	study	study	study	study	study	study
Human	6.4	6.2	108	111	258	267

(1): Mean values of two determinations

# Table 2. Phase II activity of hepatocytes from African American donors by incubation of 7-hydroxycoumarin at the concentration of 30 $\mu$ M<sup>(1)</sup>.

Species	7-HC si (pmol/min/ r	u <b>lphate</b> nillion cells)	7-HC glucuronide (pmol/min/ million cells)		
	clearance study met profile stu		clearance study	met profile study	
Human	57	38	372	86	

(1): Mean values of two determinations

# Table 3. Intrinsic clearance results for fexinidazole incubated at the concentration of 1 $\mu$ M with hepatocytes from African American donors <sup>(1)</sup>.

Species	t1/2 (min)	CLint <i>in vitro</i> (μL/min/million cells)	CLint <i>in vitro</i> (mL/min/kg)
Human	6.5	107	257

(1): Mean values of two determinations

# Table 4. Metabolites found after 0, 30 and 120 minutes incubation of fexinidazole at the concentration of 10 $\mu$ M with hepatocytes from African American donors.

Motabolito	Molecular	m/ <del>7</del>	RT	% of t	otal drug r	elated mat	erial <sup>(1)</sup>
Welabolite	formula	1172	(min)	t=0	30'	120'	BLK
Р	$C_{12}H_{13}N_3O_3S$	280.08	16.1	77	1		82
M1	$C_{12}H_{13}N_3O_4S$	296.07	9.0	23	98	98	18
M2	$C_{12}H_{13}N_3O_5S$	312.07	10.5			1	
M3	$C_{11}H_{11}N_3O_4S$	282.06	7.5		<1	<1	

(1) The approximate relative amounts were determined from the absolute areas taken from a selected ion chromatogram at the metabolite masses with a mass window of 0.04 Da. The absolute areas of fexinidazole, M1 and M2 were normalized based on their relative MS response. Nevertheless, these results should be regarded as semi-quantitative.

Nerviano Medical Sciences Page 16 of 24 Figure 1. Proposed metabolic pathway of fexinidazole after incubation at the concentration of 10  $\mu$ M with hepatocytes from African American donors.



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## APPENDICES

Appendix 1. Certificates of analysis of hepatocytes from African American donors used in this study.

Product Number: Product: Quantity: Lot Number: storage condition:~	F00995 Cryopreserved Hepatocyte Human, Female 5 million EHI below -150° C	33		RO
Relevant Donor Demo	graphics, **as reported to	o in Vitro Techn		
Age: Cause of Death:	58	Races	African American	
Social History:	Alcohol Use: Yes		Cannabinoid use: (none rep	orted)
Relevant Medical History:			TUNICUS USB: TUB	
Diabates     Hypertansion     High cholestrol     Cancer (specify below)	Depression/Ansiety Asthma Arthritis Other Autoinnnune disease	Hypothyroidiam Kidney disease Gastrointestinal dis	Congestive heart fi Vascular disease Other heart disease	sture e
Relevant Chronic Medicati	ons:	Anti-Inflemmitories	stołytic	
Specification:	Result:			
1) Serology testing:	CMV	POSITIVE	Hepattis B	NEGATIVE
2) ≥ 5 million cells with at lea as determined by Trypan bi	HIV ast 70% post-thaw viability ue exclusion:	NEGATIVE	Hepatitis C	NEGATIVE
Lot Characterization I	<b>Tesuits</b>			
COUM: total rate of formatio	n of 7-hydroxycoumann	88	_pmole/10 <sup>5</sup> cella/min	
DEX: rate of formation of de ECOD: Total rate of formati	xirophan on of 7-bictrownourmedo	3	pmole/10 <sup>6</sup> cella/min	
7-HCG rate of formation of	7-hydroxycoumarin glucuronide	291	pmole/10 <sup>6</sup> cella/min	
7-HCS: rate of formation of	7-hydroxycoumarin sulfate	45	pmole/10 <sup>8</sup> cells/min	
MEPH: rate of formation of 4 TEST: rate of formation of f	V-hydroxymephenytoin	1	pmole/10 <sup>e</sup> cells/min	
TOLB: rate of formation of 4	I-methylhydroxytolbutamide	245	pmole/10 <sup>4</sup> cella/min	
PHEN: rate of formation of	acetaminophen	45	pmola/10 <sup>6</sup> cella/min	
CZA: rate of formation of 6-1 NAT1	lydroxychiorzoxazone -	43	pmole/10° cells/min	
NAT2		0.234	nmol/mg/min	
NAT1 genotype		*4/*10		
2C19 genotype	1788 - 18 <b>1</b> 8 <b>-</b>	*6/*14		
2D6 genotype				
2C9 genotype				
Bicharard Warnings These	nte hana haan mada sala kasaa			
tested negative for HIV and Hepati used in animals or humans. These	tis B and C, caution is advised when has calls have not been approved for any d	and and anound be con ading these products. The legnostic or clinical process	mun de es ponersiel bionezarde. Alt nee products are for research use on turse.	ough me original ties ly and should not be
without interesting to make and the second		ted in actor is matricely gamer a		
Section				
this product has been teste	d by controlled procedures and			

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Product Number:	F00995			
Product:	Cryopreserved Hepatocyte	S		
	Human, Female	-		
Quantity:	5 million			۲O –
ot Number			SECHNOL	OCIES
storage condition:	below -150° C		т., р., т.	
storage condition.		)		
Relevant Donor Demo	ographics, **as reported to	o In Vitro Techno	blogies	
Age:	60	Race:	African American	
Cause of Death	;ICB	BMI	31.6	
Social History	Alcohol Use: Yes		Cannabinoid use: (none repo	orted)
social instory.	Narcotic use: (none reported)		Tobacco use: Yes	
Relevant Medical History:	1	· .		
Diabetes	Depression/Anxlety	Hypothyroidism	Concestive heart fail	lure
Hypertension	🗌 Asthma			
High cholestrol	Arthritis			
Cancer (specify below)	Other Autoimmune disease	Gastrointestinal dis	ease 🔛 Other heart disease	
		,		
Relevant Chronic Medicat	ions:			
Hormone replace therapy	Upid-lowering agents	Anti-Inflammitories	· · · ·	
Anti-hypertensives	Insulin	) I Soll dependent (Se		
			Riblyde	
Specification:	Result:		xioyac	, The second se
Specification: 1) Serology testing:	Result: CMV	NEGATIVE	Hepatitis B	NEGATIVE
Specification: 1) Serology testing:	Result: CMV HIV	NEGATIVE	Hepatitis B Hepatitis C	NEGATIVE
Specification: 1) Serology testing: 2) ≥ 5.0 million cells with at	Result: CMV HIV least 70% post-thaw viability	NEGATIVE	Hepatitis B Hepatitis C	NEGATIVE NEGATIVE
Specification: 1) Serology testing: 2) ≥ 5.0 million cells with at as determined by Trypan bi	Result: CMV HIV least 70% post-thaw viability lue exclusion:	NEGATIVE NEGATIVE NEGATIVE PASS	Hepatitis B Hepatitis C	NEGATIVE NEGATIVE
Specification: 1) Serology testing: 2) ≥ 5.0 million cells with at as determined by Trypan bi Lot Characterization J	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results	NEGATIVE NEGATIVE PASS	Hepatitis B Hepatitis C S	NEGATIVE NEGATIVE
Specification: Secology testing: 2) > 5.0 million cells with at as determined by Trypan bin Lot Characterization I COUM: total rate of formatic	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin	NEGATIVE NEGATIVE PASS	Hepatitis B Hepatitis C S _pmole/10 <sup>6</sup> cells/min	NEGATIVE NEGATIVE
Specification: 1) Serology testing: 2) ≥ 5.0 million cells with at as determined by Trypan bi Lot Characterization I COUM: total rate of formation DEX: rate of formation of determined by the second DEX: rate of formation by the second by the secon	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin extrorphan	NEGATIVE NEGATIVE PAS: 40	Hepatitis B Hepatitis C _pmole/10 <sup>6</sup> cells/min _pmole/10 <sup>6</sup> cells/min	NEGATIVE NEGATIVE
Specification: 1) Serology testing: 2) ≥ 5.0 million cells with at as determined by Trypan bi Lot Characterization I COUM: total rate of formation DEX: rate of formation of de ECOD; Total rate of formation	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin extrorphan ion of 7-hydroxycoumarin	NEGATIVE NEGATIVE PAS: 40 20 15	Hepatitis B Hepatitis C _pmole/10 <sup>6</sup> cells/min _pmole/10 <sup>6</sup> cells/min _pmole/10 <sup>6</sup> cells/min	NEGATIVE NEGATIVE
Specification: 1) Serology testing: 2) ≥ 5.0 million cells with at as determined by Trypan bi Lot Characterization I COUM: total rate of formation DEX: rate of formation of de ECOD; Total rate of formation of 7-HCG: rate of formation of	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin extrorphan ion of 7-hydroxycoumarin 7-hydroxycoumarin glucuronide	NEGATIVE NEGATIVE PAS: 40 20 15 109	Hepatitis B Hepatitis C pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min	NEGATIVE NEGATIVE
Specification: 1) Serology testing: 2) ≥ 5.0 million cells with at as determined by Trypan bi Lot Characterization I COUM: total rate of formation DEX: rate of formation of de ECOD: Total rate of formation of 7-HCG: rate of formation of 7-HCG: rate of formation of	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin extrorphan ion of 7-hydroxycoumarin 7-hydroxycoumarin glucuronide [7-hydroxycoumarin sulfate	NEGATIVE NEGATIVE PAS: 40 20 15 109 9	Hepatitis B Hepatitis C S pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min	NEGATIVE NEGATIVE
Specification: 1) Serology testing: 2) ≥ 5.0 million cells with at as determined by Trypan bi Lot Characterization I COUM: total rate of formation DEX: rate of formation of de ECOD: Total rate of formation of 7-HCG: rate of formation of MEPH: rate of formation of MEPH: rate of formation of	Result: CMV HIV least 70% post-thaw viability fue exclusion: Results on of 7-hydroxycoumarin extrorphan tion of 7-hydroxycoumarin 7-hydroxycoumarin glucuronide f 7-hydroxycoumarin sulfate 4-hydroxymephenytoin	NEGATIVE NEGATIVE PAS: 40 20 15 109 9 65	Hepatitis B Hepatitis C S pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min	NEGATIVE NEGATIVE
Specification: Specification: 1) Serology testing: 2) ≥ 5.0 million cells with at as determined by Trypan bi Lot Characterization I COUM: total rate of formation DEX: rate of formation of de ECOD: Total rate of formation of 7-HCG: rate of formation of MEPH: rate of formation of MEPH: rate of formation of TEST: rate of formation of	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin extrorphan ion of 7-hydroxycoumarin 7-hydroxycoumarin glucuronide f 7-hydroxycoumarin sulfate 4'-hydroxymephenytoin 63-hydroxytestosterone	NEGATIVE NEGATIVE PASS 40 20 15 109 9 65 100	Hepatitis B Hepatitis C S pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min	NEGATIVE NEGATIVE
Specification: Specification: 1) Serology testing: 2) $\geq$ 5.0 million cells with at as determined by Trypan bi- Lot Characterization I COUM: total rate of formation DEX: rate of formation of de ECOD: Total rate of formation of 7-HCG: rate of formation of MEPH: rate of formation of MEPH: rate of formation of TEST: rate of formation of TOLB: rate of formation of	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin extrorphan ion of 7-hydroxycoumarin 7-hydroxycoumarin glucuronide f 7-hydroxycoumarin sulfate 4'-hydroxymephenytoin 63-hydroxytestosterone 4'-methylhydroxytolbutamide	NEGATIVE NEGATIVE PAS: 40 20 15 109 9 65 100 33 27	Hepatitis B Hepatitis C S pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min	NEGATIVE
Specification: Specification: 1) Serology testing: 2) $\geq$ 5.0 million cells with at as determined by Trypan bi- Lot Characterization I COUM: total rate of formation DEX: rate of formation of de ECOD: Total rate of formation of 7-HCG: rate of formation of MEPH: rate of formation of MEPH: rate of formation of TEST: rate of formation of PHEN: rate of formation of PHEN: rate of formation of PHEN: rate of formation of PHEN: rate of formation of	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin extrorphan ion of 7-hydroxycoumarin 7-hydroxycoumarin glucuronide f 7-hydroxycoumarin sulfate 4'-hydroxymephenytoin 63-hydroxytestosterone 4'-methylhydroxytolbutamide acetaminophen butomuthorgoxycoup	NEGATIVE NEGATIVE PASS 40 20 15 109 9 8 65 100 33 75 10	Hepatitis B Hepatitis B Hepatitis C 3 pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min	NEGATIVE
Specification: Specification: 1) Serology testing: 2) $\geq$ 5.0 million cells with at as determined by Trypan bi- Lot Characterization I COUM: total rate of formation DEX: rate of formation of de ECOD: Total rate of formation of COUM: total rate of formation of T-HCG: rate of formation of MEPH: rate of formation of MEPH: rate of formation of COLB: rate of formation of PHEN: rate of formation of CZX: rate of formation of 6- NATI	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin extrorphan ion of 7-hydroxycoumarin 7-hydroxycoumarin glucuronide 17-hydroxycoumarin sulfate 4'-hydroxymephenytoin 6ß-hydroxytestosterone 4'-methylhydroxytolbutamide acetaminophen hydroxychlorzoxazone	NEGATIVE NEGATIVE PASS 40 20 15 109 9 65 100 33 75 10 4 51	Hepatitis B Hepatitis C B pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min	NEGATIVE NEGATIVE
Specification: Specification: 1) Serology testing: 2) $\geq$ 5.0 million cells with at as determined by Trypan bi- Lot Characterization I COUM: total rate of formation DEX: rate of formation of de ECOD: Total rate of formation of 7-HCG: rate of formation of 7-HGS: rate of formation of MEPH: rate of formation of MEPH: rate of formation of FIEST: rate of formation of PHEN: rate of formation of PHEN: rate of formation of CZX: rate of formation of 6- NAT1	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin extrorphan ison of 7-hydroxycoumarin 7-hydroxycoumarin glucuronide f 7-hydroxycoumarin sulfate 4'-hydroxytestosterone 4'-methylhydroxytolbutamide acetaminophen hydroxychlorzoxazone	NEGATIVE NEGATIVE PASS 40 20 15 109 9 55 100 33 75 10 4.51 0 258	Hepatitis B Hepatitis C B pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min	NEGATIVE NEGATIVE
Specification: Specification: Specification: Secology testing: $2) \ge 5.0$ million cells with at as determined by Trypan bi- Lot Characterization I COUM: total rate of formation DEX: rate of formation of de- COUM: total rate of formation of T-HCG: rate of formation of T-HGS: rate of formation of HEPH: rate of formation of TOLB: rate of formation of PHEN: rate of formation of CZX: rate of formation of CZX: rate of formation of 6- NAT1 NAT2 NAT2	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin extrorphan ison of 7-hydroxycoumarin 7-hydroxycoumarin glucuronide (7-hydroxycoumarin sulfate 4'-hydroxytestosterone 4'-methylhydroxytolbutamide acetaminophen hydroxychlorzoxazone	NEGATIVE NEGATIVE PASS 40 20 15 109 9 65 100 33 75 10 4.51 0.268 *4/*10	Hepatitis B Hepatitis B Hepatitis C 3 pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min	NEGATIVE NEGATIVE
Specification: Specification: 1) Serology testing: 2) $\geq$ 5.0 million cells with at as determined by Trypan bi- Lot Characterization I COUM: total rate of formation DEX: rate of formation of definition DEX: rate of formation of 7-HCG: rate of formation of 7-HCS: rate of formation of MEPH: rate of formation of TOLB: rate of formation of PHEN: rate of formation of PHEN: rate of formation of CZX: rate of formation of CZX: rate of formation of CX: rate of formation of CX: rate of formation of CX: rate of formation of CX: rate of formation of 6- NAT1 NAT2 NAT2 genotype NAT2 cenotype	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin extrorphan ion of 7-hydroxycoumarin 7 -hydroxycoumarin glucuronide f7-hydroxycoumarin sulfate 4'-hydroxytestosterone 4'-methylhydroxytoibutamide acetaminophen hydroxychlorzoxazone	NEGATIVE NEGATIVE PASS 40 20 15 100 9 65 100 33 75 10 4.51 0.288 -44*10 wt*5	Hepatitis B Hepatitis C	NEGATIVE NEGATIVE
Specification: Specification: 1) Serology testing: 2) $\geq$ 5.0 million cells with at as determined by Trypan bi- COUM: total rate of formation DEX: rate of formation of determined ECOD: Total rate of formation of 7-HCG: rate of formation of 7-HCS: rate of formation of MEPH: rate of formation of FEST: rate of formation of FOLB: rate of formation of COLB: rate of formation of COL	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin extrorphan ion of 7-hydroxycoumarin 7 7-hydroxycoumarin glucuronide 7-hydroxycoumarin sulfate 4'-hydroxytostosterone 4'-methylhydroxytolbutamide acetaminophen hydroxychlorzoxazone	NEGATIVE NEGATIVE PASS 40 20 15 100 9 55 100 33 75 10 4.51 0.268 *4/10 wt/wt	Hepatitis B Hepatitis C S pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min nmol/mg/min	NEGATIVE NEGATIVE
Specification: Specification: 1) Serology testing: 2) $\geq$ 5.0 million cells with at as determined by Trypan bi- Lot Characterization I COUM: total rate of formation DEX: rate of formation of di- COD: Total rate of formation of 7-HCG: rate of formation of 7-HCG: rate of formation of 7-HCS: rate of formation of 1EST: rate of formation of 1EST: rate of formation of 2HEN: rate of formation of 2-HEN: rate of	Result: CMV HIV least 70% post-thaw viability lue exclusion: Results on of 7-hydroxycoumarin extrorphan ion of 7-hydroxycoumarin glucuronide 7-hydroxycoumarin glucuronide 7-hydroxycoumarin sulfate 4-hydroxytestosterone 4'-methylhydroxytolbutamide acetaminophen hydroxychlorzoxazone	NEGATIVE NEGATIVE PASS 40 20 15 109 9 65 100 33 75 10 4.51 0.268 *4/10 wt/*5 wt/wt wt/*4	Hepatitis B Hepatitis C - pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min nmol/mg/min nmol/mg/min	NEGATIVE

Donor integration is provided as a reference and cannot be confirmed or further substantiated in order to maintain donor confidentiality

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Product Number:	M00995		
Product:	Cryopreserved Hepatocyt	es	and the second
,0 	Human, Male		
Quantity:	5 million		
Lot Number:	KSE		TECHNOLOGIES
storage condition:	below -150° C	1	The state of the s
<b>-</b>			
Relevant Donor Dem	ographics, **as reported to	o In Vitro Techn	ologies
Age	:	Race	: African American
Cause of Deatr	1:CVA	BMI	:32.5
Social History:	Alcohol Use: (none reported)		Cannabinoid use: (none reported)
	Narcotic use: (none reported)		Tobacco use: Yes
Relevant Medical History			
Diabetes	Depression/Anxiety		Concerting basis failure
Hypertension	Asthma		
🔛 High cholestrol	Arthritis		Vascular disease
Cancer (specify below)	Other Autoimmune disease	Gastrointestinal dis	sease Other heart disease
Breast			
Relevant Chronic Medica	tions:		
Hormone replace therapy	Dipid-lowering agents	Anti-Inflammitories	4
Anti-hypertensives	Insulin	Anti-depressants/An	ndalytic
Specification:	Result:		
1) Serology testing:	CMV	POSITIVE	
, enteregy testing.	HIV	NEGATIVE	
2) $\geq$ 5.0 million cells with at	least 70% post-thaw viability		
as determined by Trypan b	iue exclusion:	PAS	S
i of Characterization	Paculte		
OI M. total rate of formati	ncoulto		amaia/t 0 <sup>6</sup> antia/mia
DEX: rate of formation of d	extromban		_priole/ (0 Cells/min priole/10 <sup>8</sup> cells/min
ECOD: Total rate of formal	tion of 7-hydroxycoumarin	82	_privie/10 cells/min
7-HCG: rate of formation of	7-hvdroxycoumarin glucuronide	206	nmole/10 <sup>6</sup> cells/min
7-HGS: rate of formation o	f 7-hydroxycoumarin sulfate	74	pmole/10 <sup>6</sup> cells/min
MEPH: rate of formation of	4'-hydroxymephenytoin	21	pmole/10 <sup>6</sup> cetis/min
TEST: rate of formation of	68-hydroxytestosterone	123	pmole/10 <sup>6</sup> cells/min
TOLB: rate of formation of	4'-methylhydroxytolbutamide	42	pmole/10 <sup>6</sup> cells/min
DESCRIPTION AND AND AND AND AND AND AND AND AND AN	acetaminophen	83	pmole/10 <sup>6</sup> celis/min
PHEN: rate of formation of	hydroxychlorzoxazone	16	pmole/10 <sup>6</sup> cells/min
CZX: rate of formation of 6		4.82	nmol/mg/min
CZX: rate of formation of 6- NAT1			
CZX: rate of formation of 6- NAT1 VAT2	-	0.066	_nmol/mg/min
CIEN: rate of formation of CZX: rate of formation of 6- NAT1 NAT2 NAT1 genotype		0.066	_nmoi/mg/min _
THEN: rate of formation of CZX: rate of formation of 6- NAT1 VAT2 VAT1 genotype VAT2 genotype		0.066 *4/*10 *6/*7	_nmo//mg/min 
Priew: rate of formation of CZX: rate of formation of 6- NAT1 NAT2 NAT1 genotype NAT2 genotype 2019 genotype	-	0.068 *4/*10 *6/*7 wt/wt	nmo//mg/min 
PHEN: rate of formation of CZX: rate of formation of 6- NAT1 VAT2 VAT1 genotype VAT2 genotype 2019 genotype 206 genotype	-	0.066 *4/*10 *8/*7 wt/wt wt/wt	nmo//mg/min 

"Donor information is provided as a reference and cannot be confirmed or further substantiated in order to maintain denor confidentially.

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			010
Product Number: Product: Quantity: Lot Number: storage condition: Relevant Donor Dem	M00995 Cryopreserved Hepatocyt Human, Male <u>ZIJ</u> below -150° C cographics, **as reported to	es o in Vitro Techno Rece	
Cause of Deat	h: HT	BMI	25.1
Social History: Relevant Medical History	Alcohol Use: (none reported) Narcotic use: (none reported)		Cannabinoid use: Marijuana Tobacco use: (none reported)
Diabetes Hypertension High cholestrol Cancer (specify below)	Depression/Anxiety Asthma Asthma Arthritis Other Autoimmune disease	Hypothyroldism	Congestive heart failure
Relevant Chronic Medica	itions:		
Anti-hypertensives	Lipid-lowering agents     Insuin	Anti-Inflammitories	, idolytic
Specification:	Result:		
1) Serology testing: 2) $\geq$ 5.0 million cells with a	CMV HIV t least 70% post-thaw viability	NEGATIVE NEGATIVE	Hepatitis B NEGATIVE Hepatitis C NEGATIVE
as determined by Trypan (	Aue exclusion:	PASS	<b>}</b>
Lot Characterization COUM: total rate of formati DEX: rate of formation of d ECOD: Total rate of formation o 7-HCG: rate of formation o 7-HGS: rate of formation of MEPH: rate of formation of	Dive exclusion: Results ion of 7-hydroxycoumarin extrorphan tion of 7-hydroxycoumarin f 7-hydroxycoumarin glucuronide if 7-hydroxycoumarin sulfate 4'-hydroxymephenytoin	6 33 31 320 29 13	pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min
Lot Characterization COUM: total rate of format DEX: rate of formation of d ECOD: Total rate of formation of 7-HCG: rate of formation of 7-HGS: rate of formation of MEPH: rate of formation of TEST: rate of formation of PHEN: rate of formation of PHEN: rate of formation of CZX: rate of formation of SAT1	Dive exclusion: <b>Results</b> ion of 7-hydroxycoumarin extrorphan tion of 7-hydroxycoumarin f 7-hydroxycoumarin glucuronide f 7-hydroxycoumarin glucuronide f 7-hydroxycoumarin sulfate 4'-hydroxycoumarin sulfate 4'-hydroxytestosterone 4'-methylhydroxytoibutamide acetaminophen -hydroxychlorzoxazone	PASS 6 33 31 320 29 13 25 34 3 13 725	pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min
Lot Characterization COUM: total rate of format DEX: rate of formation of d ECOD: Total rate of formation of 7-HCG: rate of formation of 7-HGS: rate of formation of TEST: rate of formation of TOLB: rate of formation of PHEN: rate of formation of PHEN: rate of formation of CZX: rate of formation of NAT1 NAT2 NAT1 genotype 2C19 genotype 2D6 genotype	Dive exclusion: <b>Results</b> ion of 7-hydroxycoumarin extrorphan f 7-hydroxycoumarin glucuronide f 7-hydroxycoumarin glucuronide f 7-hydroxycoumarin glucuronide 63-hydroxytoumarin sulfate 4'-hydroxytoutarin 63-hydroxytestosterone 4'-methylhydroxytolbutamide acetaminophen -hydroxychlorzoxazone	PASS 6 33 31 320 29 13 25 34 3 13 7.28 0.166 ·4/-4 ·5/-6 wt/th	pmole/10 <sup>6</sup> cells/min pmole/10 <sup>6</sup> cells/min nmol/mg/min

tested negative for HIV and Hepatitis B and C, caution is advised when handling these products are for research use only and should not be used in animals or humans. These cells have not been approved for any diagnostic or clinical procedures.

"Donor information is provided as a reference and served be confirmed or further substantiated in order to melvicule donor confidentiality

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	Certific	ate of Analy	ysis		
Product Number: Product:	M00995 Cryopreserved Hepatocyte Human, Male	35			
Quantity:	5 million			K()	
Lot Number:	MRS		TECHNO	LOGIES	
storage condition:	below -150° C				
Relevant Donor Demo	graphics, **as reported to	In Vitro Techn	ologies		
Age:	42	Race	: African American		
Cause of Death:	ICH	BM	1: 21.3		
Sealed Makes	Alcohol Use: Yes		Cannahinoid use: Mariluana		
Social ristory:	Naroatio usa: Casalan		Takasas was Mar	L	
	Narcouc use: Cocaine	Tobacco use: Yes			
Relevant Medical History:					
Diabetes [	Depression/Anxiety	Hypothyroldism	Congestive heart fa	llure	
U Hypertension [	Asthma	Kidney disease	Vacadas direases		
High cholestrol	Arthritis				
Cancer (specify below)	Other Autoimmune disease				
Relevant Chronic Medicati	ons:				
Hormone replace therapy	Upid-lowering agents	Anti-Inflemmitories			
Anti-hypertensives	🛄 Insulin	Anti-depressants/A	nxiolytic		
Specification:	Result:		4		
1) Serology testing:	CIN		l I Alti		
y controgy topang.	HIV	NEGATIVE	Hepatitis B	NEGATIVE	
$2 \ge 5.0$ million cells with at k	east 70% post-thaw viability		riepauus C	NEGATIVE	
as determined by Trypan blu	ue exclusion:	PAS	S		
ot Characterization F	Results				
OUM: total rate of formatio	n of 7-hydroxycoumarin	446	nmala/10 <sup>8</sup> anti-imia		
DEX: rate of formation of deal	rtrörchan	34	priote/ to cells/min		
COD: Total rate of formatio	on of 7-hydroxycoumarin	110			
-HCG: rate of formation of 7	7-hydroxycoumarin clucuronide	450	_pmole/10 <sup>6</sup> celle/min		
-HGS: rate of formation of	7-hydroxycoumarin sulfate	50	princie/10 <sup>6</sup> cells/min		
EPH: rate of formation of 4	-hydroxymephenytoin	2	pmole/10 <sup>6</sup> celis/min		
EST: rate of formation of 6	B-hydroxytestosterone	675	pmole/10 <sup>6</sup> celis/min		
OLB: rate of formation of 4	-methylhydroxytolbutamide	54	pmole/10 <sup>6</sup> cells/min		
HEN: rate of formation of a	acetaminophen	68	pmole/10 <sup>6</sup> cells/min		
ZX: rate of formation of 6-h	iydroxychlorzoxazone	28	pmole/10 <sup>6</sup> cells/min		
AT1	-	TBD	_nmol/mg/min		
AT2		TED	_nmol/mg/min		
IAT1 genotype		TBD			
		TBD	<u> </u>		
IAT2 genotype	· · · · ·				
NAT2 genotype 2C19 genotype	• • • • • • • • • • • • • • • • • • •	TBD			
VAT2 genotype 2C19 genotype 2D6 genotype		TBDTBD	_		

MPlanas Information In sec. Id. Jac.

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Appendix 2. Peak area (counts) at different time points of fexinidazole incubated at the concentration of 1  $\mu$ M with hepatocytes from African American donors.

Exp	t=0	t=1'	t=5'	t=10'	t=15'	t=20'	t=30'	t=60'	t=120'
1	814000	685000	457000	255000	158000	98900	24800	1400	nd
2	802000	678000	515000	308000	152000	78500	25100	1380	nd

nd: not detectable

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