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A Multi-Peptide Hybrid LC-MS/MS Assay for the Determination of CTI-1601 in Monkey Tissues Provides Insight into its Disposition and Processing

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INTRODUCTION

Friedreich's Ataxia (FRDA) is a rare genetic disease caused by a deficiency of the mitochondrial protein Frataxin (FXN). CTI-1601 is a 24.9kDa fusion protein currently under investigation as a protein replacement therapy to restore functional levels of FXN in the mitochondria of patients.

CTI-1601 consists of the transactivator of transcription (TAT) transduction domain linked to the N-terminus of the full-length human FXN protein (**Figure 2**). CTI-1601 mechanism of action relies on the cell-penetrating ability of the TAT peptide and the subsequent processing into mature FXN after translocation into the mitochondria.

To understand the disposition and processing of CTI-1601, a multi-peptide hybrid LC-MS/MS assay was developed to quantify CTI-1601 in cynomolgus monkey buccal cells, skin biopsies, and platelets, following repeated administration of CTI-1601 for 14 days.

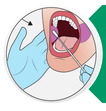
METHODS

BIOLOGICAL SAMPLES

Calibration standards and QCs were prepared in proxy matrix fortified with CTI-1601 from 0.250 to 25.000 ng/mL. Matrix QCs were prepared in pooled tissue homogenates at low and high QC concentrations.

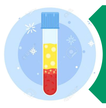
Skin Biopsies

8 mm skin punches were finely minced with a scalpel and homogenized in RIPA buffer using a FastPrep-96 and sonication.



Buccal Cells

Cells scraped with a buccal swab (Isohelix SK-2S) were extracted and lysed by incubation in RIPA buffer, vortexing, and sonication.



Platelets

Blood samples were collected in 8.5 mL ACD tubes; the platelets were isolated by centrifugation and lysed in RIPA buffer by vortexing and sonication.

Figure 1: Biological sample preparation

TOTAL PROTEIN DETERMINATION

Total protein concentration of each sample was determined using the Pierce™ BCA Protein Assay Kit, and used for data normalization.

SAMPLE PROCESSING

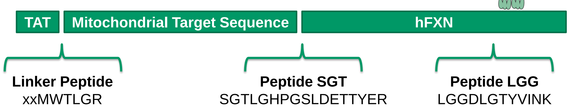


Figure 2: Hybrid LC-MS/MS assay design. CTI-1601 is immunopurified using a biotinylated anti-FXN antibody. A ^{15}N-labeled SILAC CTI-1601 is used as internal standard. Following trypsin digestion, 3 peptides and corresponding ^{15}N-labeled peptides are monitored by LC-MS/MS on a SCIEX 6500+.

Table 1. Peptides monitored for CTI-1601 quantitation

Peptide	Characteristics
Linker Peptide	Unique to CTI-1601 Linker between TAT and MTS sequences Not present in CTI-1601 mature form Monitored for informative purpose
Peptide SGT	N-terminal peptide of the mature CTI-1601 Not found in the monkey proteome Surrogate peptide for CTI-1601 quantitation
Peptide LGG	Peptide from conserved region of FXN protein Common to both CTI-1601 and cynomolgus monkey FXN Can be used to quantify the endogenous cynomolgus monkey FXN Monitored for informative purpose

PROCESSING OF RESULTS

Peak area ratios of CTI-1601 derived tryptic peptides and their corresponding ^{15}N-labeled peptides were used to construct calibration curves (weighted 1/x^2 linear regression).

The concentration of CTI-1601 in samples was determined (in ng/mL) using the calibration curve equation. After normalization using total protein concentration (µg protein per mL of homogenate), the CTI-1601 concentrations were reported as pg of CTI-1601 per µg of total protein.

RESULTS

Table 2. CTI-1601 precision and accuracy (multi-peptide) in buccal cells, skin biopsies, and platelets.

Peptide	Sample Type		Nominal Concentration (ng/mL)	Accuracy	%CV (N =3)	Code
Linker	Buccal Cells	Low QC	0.750	97.4	2.3	
		High QC	18.750	101.9	1.3	
	Skin Biopsies	Low QC	0.750	99.7	8.0	
		High QC	18.750	104.5	4.6	
	Platelets	Low QC	0.750	97.5	11.1	
		High QC	18.750	106.4	4.4	
Peptide SGT	Buccal Cells	Low QC	0.750	84.4	4.3	
		High QC	18.750	102.6	1.2	
	Skin Biopsies	Low QC	0.750	87.8	9.0	
		High QC	18.750	107.1	3.1	
	Platelets	Low QC	0.750	92.7	7.1	
		High QC	18.750	107.3	2.5	
Peptide LGG	Buccal Cells	Endogenous	4.869*	100.0	3.2	
		Low QC	5.619**	95.6	3.7	
		High QC	23.619**	102.0	2.2	
	Skin Biopsies	Endogenous	10.153*	100.0	5.0	
		Low QC	10.903**	99.4	2.8	
		High QC	28.903**	104.4	2.3	>ULOQ
	Platelets	Endogenous	19.867*	100.0	2.2	
		Low QC	20.617**	94.5	3.8	
		High QC	38.617**	104.1	1.1	>ULOQ

> ULOQ: Above upper limit of quantitation. Extrapolated values are presented.
* Back-calculated endogenous level. Average of 3 replicates.
** Endogenous level + spiked-in CTI-1601 at low and high QC levels.

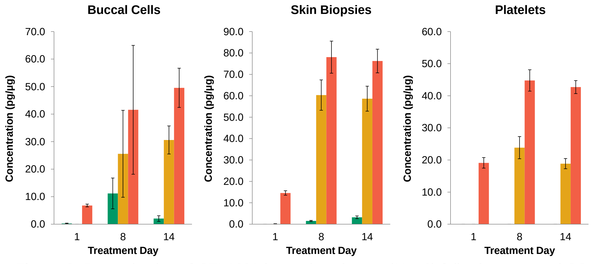


Figure 3: Determination of CTI-1601 in monkey tissues (N = 6) following repeated SC administration at 15 mg/kg BID for 14 days. Data is expressed as pg of CTI-1601 per µg of total protein. Linker Peptide (■), Peptide SGT (■) and Peptide LGG (■).

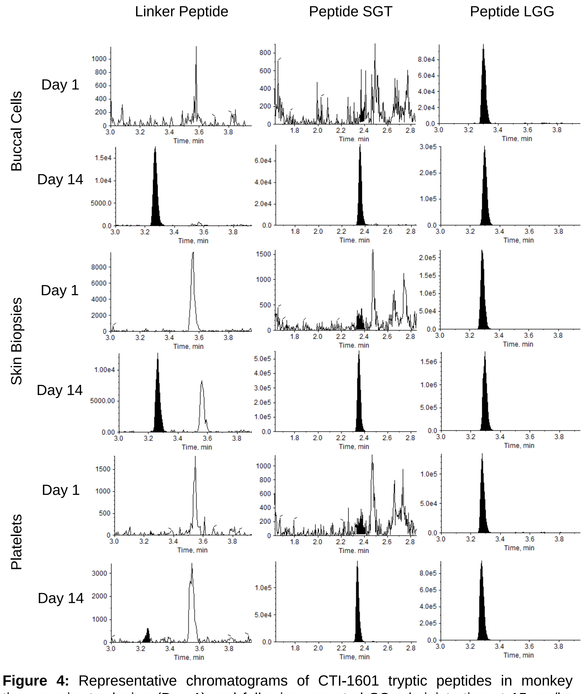


Figure 4: Representative chromatograms of CTI-1601 tryptic peptides in monkey tissues prior to dosing (Day 1) and following repeated SC administration at 15 mg/kg BID for 14 days.

CONCLUSION

The data suggests that, following repeated administration of CTI-1601 for 14 days, CTI-1601 not only accumulates in the tissues outside of the systemic circulation but is also predominantly present as the mature FXN protein.

This study exemplifies how hybrid LC-MS/MS assays can be used to simultaneously gain insight into the concentration, disposition, and processing of biotherapeutics.

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