Author/s:
van Diemen, MPJ;Hart, EP;Abbruscato, A;Mead, L;van Beelen, I;Bergheanu, SC;Hameeteman, PW;Coppen, E;Winder, JY;Moerland, M;Kan, H;van der Grond, J;Webb, A;Roos, RAC;Groeneveld, GJ

Title:
Safety, pharmacokinetics and pharmacodynamics of SBT-020 in patients with early stage Huntington's disease, a 2-part study

Date:
2021-05-01

Citation:
van Diemen, M. P. J., Hart, E. P., Abbruscato, A., Mead, L., van Beelen, I., Bergheanu, S. C., Hameeteman, P. W., Coppen, E., Winder, J. Y., Moerland, M., Kan, H., van der Grond, J., Webb, A., Roos, R. A. C. & Groeneveld, G. J. (2021). Safety, pharmacokinetics and pharmacodynamics of SBT-020 in patients with early stage Huntington's disease, a 2-part study. British Journal of Clinical Pharmacology, 87 (5), pp.2290-2302. https://doi.org/10.1111/bcp.14656.

Persistent Link:
https://hdl.handle.net/11343/280587

Received: 31 March 2020 | Revised: 29 October 2020 | Accepted: 30 October 2020

DOI: 10.1111/bcp.14656

ORIGINAL ARTICLE

Safety, pharmacokinetics and pharmacodynamics of SBT-020 in patients with early stage Huntington's disease, a 2-part study

Marcus P.J. van Diemen[1] | Ellen P. Hart[1] | Anthony Abbruscato[4] | Liz Mead[4] | Ilse van Beelen[1] | Sandrin C. Bergheanu[4] | Pieter W. Hameeteman[1] | Emma Coppen[5] | Jessica Y. Winder[5] | Matthijs Moerland[1] | Hermien Kan[2] | Jeroen van der Grond[3] | Andrew Webb[2] | Raymund A.C. Roos[5] | Geert Jan Groeneveld[1]

[1]Centre for Human Drug Research, Leiden, The Netherlands

[2]Gorter Centre for high-field MRI, Leiden University Medical Center, Leiden, The Netherlands

[3]Radiology Research Center, Department of Radiology, Leiden University Medical Center, Leiden, The Netherlands

[4]Stealth BioTherapeutics Inc, Newton, Massachusetts, USA

[5]Department of Neurology, Leiden University Medical Center, Leiden, The Netherlands

Correspondence
Jan Groeneveld and Marcus PJ van Diemen, Centre for Human Drug Research, Zernikedreef 8, 2333CL, Leiden, The Netherlands.
Email: ggroeneveld@chdr.nl; marcus.vandiemen@gmail.com

Aims: Huntington's disease (HD) is a neurodegenerative disease with cognitive, motor and psychiatric symptoms. Toxic accumulation of misfolded mutant huntingtin protein induces mitochondrial dysfunction, leading to a bioenergetic insufficiency in neuronal and muscle cells. We evaluated the safety, pharmacokinetics and pharmacodynamics of SBT-020, a novel compound to improve mitochondrial function, in a 2-part study in early stage HD patients.

Methods: Part 1 consisted of 7-day multiple ascending dose study to select the highest tolerable dose for Part 2, a 28-day multiple dose study. Mitochondrial function was measured in the visual cortex and calf muscle, using phosphorous magnetic resonance spectroscopy, and in circulating peripheral blood mononuclear cells.

Results: Treatment-emergent adverse events were mild and more present in the SBT-020 group. Injection site reactions occurred in 91% in Part 1 and 97% in Part 2. Mitochondrial function in calf muscle, peripheral blood mononuclear cells or visual cortex was not changed overall due to treatment with SBT-020. In a *posthoc* analysis, patients with a higher degree of mitochondrial dysfunction (below the median [$\Delta\Psi_m$ < 3412 and τPCr > 42.5 s]) showed more improvement than patients with a relatively lower level of mitochondrial dysfunction.

Conclusion: SBT-020 was safe at all doses, but no significant differences in any of the pharmacodynamic measurements between the treatment groups and placebo group could be demonstrated. The data suggest that the better than expected mitochondrial function in our patient population at baseline might explain the lack of effect of SBT-020.

The authors confirm that the PI for this paper is Dr G.J. Groeneveld and that he had direct clinical responsibility for patients.

KEYWORDS

clinical toxicology, magnetic resonance imaging, neurodegeneration, neuropharmacology, patient safety

1 | INTRODUCTION

Huntington's disease (HD) is a hereditary, progressive neurodegenerative disorder, characterized by motor, cognitive and psychiatric deficits. It is caused by an elongated CAG (glutamine) expansion in the gene coding for the huntingtin protein[1] and there is currently no disease-modifying treatment. Its prevalence in Caucasians is approximately 10 per 100 000.[2] Mitochondrial dysfunction plays a central role in the pathogenesis of HD through toxic accumulation of misfolded/mutant huntingtin protein (Htt).[3] *In vivo* assessment of phosphorous metabolism, using phosphorous magnetic resonance spectroscopy (31P-MRS), has previously shown a decreased bioenergetic profile in muscle and brain of (pre)manifest HD gene carriers when compared to healthy volunteers.[4,5] Cardiolipin plays a central role in oxidative phosphorylation by organizing the complexes of the mitochondrial electron transport chain, thereby improving the electron flow between complexes. SBT-020 (aka SS-20, H-Phe-D-Arg-Phe-Lys-NH_2) is 1 of the Szeto–Schiller (SS) proteins, a novel class of tetrapeptides of which SS-31 (also known as elamipretide) is furthest in clinical development.[6,7] SS-31 and SBT-020 both improve mitochondrial respiration by binding to cardiolipin, a phospholipid which is uniquely expressed on the inner mitochondrial membrane.[8] Cardiolipin peroxidation through cytochrome c in the early stage of apoptosis is essential for the transduction of apoptotic signals and formation of the mitochondrial permeability transition pore, a key element to cell apoptosis.[9–11] SBT-020-bound cardiolipin is protected from peroxidation, which optimizes mitochondrial bioenergetics and prevents triggering apoptosis.[11,12] In a 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-induced mouse model of Parkinson's disease, SBT-020 was effective in attenuating injury and improving neurotransmitter release when given systemically,[13] protecting against loss of dopaminergic neurons and causing normalization of dopamine and its metabolites. Additionally, SBT-020 improved cell viability and reduced apoptosis in cultured SN4741 cells (dopaminergic neurons derived from the substantia nigra of transgenic mouse embryos) when exposed to MPTP.[13] The efficacy of SS-31, the predecessor of SBT-020, was shown in a preclinical HD model of cultured mutant Htt expressing nigrostriatal neurons (STHDhQ111/Q111) by normalizing mitochondrial structure and function.[14]

The primary objectives in the current study were assessment of safety, tolerability and pharmacokinetics (PK) of SBT-020 in early-stage HD. The secondary objectives were to assess the effect of SBT-020 on central and peripheral mitochondrial function through *in vivo* 31P-MRS measurements, and on mitochondrial membrane potential ($\Delta\Psi_m$) measurements in peripheral blood mononuclear cells (PBMCs). Finally, effects on motor and neurocognitive functioning were assessed through the unified Huntington's disease rating scale (UHDRS) and a battery of neurocognitive tests.

What do we now know as a result of this study that we did not know before?

- Daily treatment with 25 mg SBT-020 for 28 days is safe.
- No effects on mitochondrial function were observed.

What take-home message do you want to impart to readers?

- Mitochondrial dysfunction is a common phenomenon in neurodegenerative diseases and a druggable target.
- To demonstrate improvement of mitochondrial function in patients with Huntington's disease due to treatment with a compound enhancing mitochondrial function it may be necessary to only enrol patients with a relatively high degree of mitochondrial dysfunction.

2 | METHODS

2.1 | Trial design

This phase II study was conducted at the Centre for Human Drug Research (CHDR, Leiden, The Netherlands) as a single-centre, randomized, double-blinded, placebo-controlled trial in patients with early stage HD. It consisted of a 7-day multiple ascending dose-determination part (Part 1) followed by a 28-day multiple dose part (Part 2). In Part 1, 24 patients were randomized into 1 of 3 dose cohorts (5, 15 or 25 mg) of 8 patients each (6 active, 2 placebo). For Part 2, the same patients were re-randomized into 12 placebo and 12 active, to receive the dose selected from Part 1.

2.2 | Dosing rationale

The dose of SBT-020 was chosen based on preclinical and clinical studies. In a pharmacodynamic (PD), preclinical study on the neuroprotective effects of SBT-020 in MPTP-treated mice, a single dose of 4 mg/kg of SBT-020 attenuated 40% of the MPTP-induced dopamine depletion.[13] In a preclinical study on ischaemia/reperfusion damage in

rats, a single dose of 4 mg/kg SBT-020 significantly reduced infarct size and myocardial lipid peroxidation.[15] This corresponds to a human equivalent dose of 0.76 mg/kg. For a 60-kg human this corresponds to a starting dose of 4.5 mg.

Doses of 5, 10, 20 and 30 mg were assessed in a subcutaneously administered single and multiple ascending dose study in healthy volunteers, which proved safe and tolerable, but with dose related occurrence of injection site reactions. In this patient study, 25 mg was hypothesized to be the effective and safe dose for a 28-day multiple dose study. For safety assessment, a 7-day multiple ascending dose study with 5, 15 and 25 mg single and multiple dose part was performed prior to the 28-day multiple dose part.

2.3 | Study schedule

Part 1 of the study consisted of a screening period for eligibility, a 7-day treatment period and a follow-up visit. Patients were screened for medical status (interview, physical examination, vitals, laboratory and electrocardiogram [ECG]), motor and functional status (UHDRS assessment) and peripheral mitochondrial function (31P-MRS scan of the calf muscles). The 31P-MRS scans were performed at the Leiden University Medical Center (Leiden, The Netherlands). After randomization, SBT-020 or placebo was subcutaneously administered once daily for 7 days. For the administrations on days 1, 2 and 7, patients were admitted at the Clinical Research Unit (CRU) of CHDR in order to perform PK and PD measurements. The administrations on days 3, 4, 5, and 6 were performed at the patient's home by trained staff. Safety (including blood samples for plasma histamine concentrations) and PK measurements were performed continuously on days 1, 2, 7 and 8. PD measurements, 31P-MRS of the calf muscle and blood sampling for measurement of $\Delta\Psi_m$, were performed during screening (31P-MRS), 1 hour before dosing on day 1 ($\Delta\Psi_m$) and 1.5 hours after the final dose administration on day 7 (31P-MRS and $\Delta\Psi_m$). There was a wash-out period of at least 1 month between the end of Part 1 and the start of Part 2 for each patient. Dose escalation in Part 1 was evaluated after completing each dose cohort based on PD, PK and safety.

Part 2 of the study consisted of a re-assessment of eligibility, a 28-day treatment period and a follow-up visit. The set of PD measurements in Part 2 was expanded with central mitochondrial function assessment (31P-MRS scan of the brain) and neurocognitive testing, in addition to the PD assessments included in Part 1. PD measurements 31P-MRS of skeletal muscle and visual cortex were performed on day −1 (before the first dose administration of Part 2), 1.5 hours after dose administration on day 27 (31P-MRS of the calf muscles and brain and $\Delta\Psi_m$ measurements in PBMCs) and 1.5 hours after final dose administration on day 28 (neurocognitive and motor testing). Patients were admitted to the CRU at day 1, 2, 27, and 28 and visited at home on days 7, 14 and 21 for safety assessments (vitals, laboratory and ECG) and trough PK sampling. On the days that the patients were not scheduled to visit the CRU, the daily drug administration was performed at home.

2.4 | Participants

Patients with mild to moderate HD were included. The main inclusion criteria were: a genetically confirmed CAG repeat expansion of 36 or more repeats in the Htt gene; total motor score (TMS) of 5 or more and total functional capacity score (TFC) of 7 or more as assessed by the UHDRS; and a time constant of phosphocreatine recovery (τPCr) after a bout of exercise of at least 40 seconds, measured by dynamic 31P-MRS of the calf muscles. This threshold was based on earlier work with 31P-MRS in HD patients, to ensure sufficient mitochondrial dysfunction.[5] The threshold was later lowered to 32.4 seconds to better reflect the early stage HD patient population (see the section on sample size calculation).

The main exclusion criteria were: positive test for drugs of abuse; history (within 3 months of screening) of alcohol consumption exceeding 2 standard drinks per day on average; smoking more than half a pack of cigarettes daily; history of active malignancy within the last 5 years, with the exception of localized or in situ carcinoma (e.g., skin basal or squamous cell carcinoma); positive hepatitis B surface antigen, hepatitis C antibody or human immunodeficiency virus antibody; aspartate transaminase, alanine transaminase, γ-glutamyl transferase or total bilirubin levels >1.5 times the upper limit of normal; renal insufficiency (defined as eGFR < 60 mL/min); history of photosensitive epilepsy; any contraindication to have magnetic resonance imaging (MRI) scans performed; significant cardiac abnormalities on the resting ECG (QTcF >450 or <300 ms, evidence of atrial fibrillation, atrial flutter, complete branch block, Wolf–Parkinson–White Syndrome or cardiac pacemaker); any confirmed significant allergic reactions (urticaria or anaphylaxis) against any drug, or multiple drug allergies (nonactive hay fever was acceptable).

2.5 | Concomitant medications

Paracetamol (up to 4 g/d) and ibuprofen (1 g/d) were allowed before and during the study period. Medications with an effect on cognitive functioning (e.g. antidepressants) were allowed on a stable dose, but medications with known mitochondrial toxicity (e.g. statins and metformin) were not allowed until the end of the study period and needed to be discontinued 21 days before study enrolment if applicable. Use of other medications were allowed under scrutiny of the investigator. The use of hormonal contraceptives was allowed during the study.

2.6 | Plasma sample collection

During Part 1 and Part 2, blood and urine samples were collected at various time points to measure plasma concentrations of SBT-020 (Table S9). In Part 1 a 24-hour profile at day 1 and at steady state was performed; in Part 2 only trough samples at steady state were

measured to assess potential accumulation. Urine was collected for 24 hours during days 1 and 7 of Part 1 to assess renal clearance of SBT-020. Aliquots for plasma and containers for urine were spiked with a 5% formic acid aqueous solution to prevent the compound from binding to the collection materials.

2.7 | SBT-020 concentration measurement

Concentrations of SBT-020 were measured by a validated liquid chromatography–mass spectrometry method for both plasma and urine. Sample analysis was performed for patients receiving SBT-020 and not for patients receiving placebo. The lower levels of quantification were 2.5 ng/mL in plasma and 50 ng/mL in urine.

2.8 | Pharmacodynamics

During Part 1 and 2, measurements for PD were performed at various time points to assess effects on mitochondrial and clinical functioning (Table S10). Mitochondrial function measurements were performed prior to initiation of drug treatment and at the end of drug treatment in each part of the study. Measurements for central mitochondrial function and motor and neurocognitive function were performed at the start and end of Part 2 only.

2.9 | Skeletal muscle

Dynamic 31P-MRS in skeletal muscle before, during and after exercise was performed in a 7-Tesla MRI scanner (Phillips, Best, The Netherlands) with surface coil and custom-built MRI-compatible pedal ergometer. The ergometer was designed to allow the patients to perform isometric plantar flexion exercise by pressing against a foot pedal while supine. The foot was strapped firmly to the ergometer and the subject's lower extremity was secured to the MRI table with straps across the mid-thigh and mid-lower leg in order to isolate usage of the posterior calf muscles. The scanning protocol consisted of localizer sequences and the acquisition of a field map for shimming purposes. Thereafter, 31P-MRS data were acquired before, during and after exercise using a pulse-acquire sequence with a time resolution of 2 seconds (flip angle 45°, surface coil localization, 1 signal average). Peak integrals of inorganic phosphate (Pi), PCr and ATP signals were obtained using jMRUI software (version 5.0, jMRUI Consortium) and the τPCr was determined by mono-exponential fit using a custom made MatLab script (version 2012b). The frequency difference between PCr and Pi was used to calculate tissue pH. The τPCr is considered unreliable when tissue pH is <6.8 and rescanning after a 10-minute break was allowed to reach an end-exercise pH >6.8. Outlying data (up to 10% of total), deviating >5% from the plotted curve overall data points, resulting from noise due to a high amount of overlying subcutaneous fat were removed using the MatLab script.

2.10 | Visual cortex

31P-MRS of the brain was performed on a 3-Tesla MRI scanner (Philips, Best, The Netherlands). A custom-made 6-cm 31P transmit/receive surface coil was used to detect signals from the visual cortex while limiting muscle contamination. A small sphere (⌀ 10 mm) filled with water was placed below the coil along the coil axis to verify and adjust the positioning of the 31P RF coil on 1H images. An adiabitic pulse-acquire sequence (TR 2 s, flip angle 90°) was used to collect free induction decays for 4 minutes at rest (128 signals averaged), 8 minutes during visual activation (256 signals averaged) and 8 minutes after visual stimulation (256 signals averaged). Analysis of the 31P spectra using jMRUI allowed quantification of the following resonances: βATP, αATP, γATP, PCr and Pi, from which the ratios of PCr/ATP, Pi/PCr and Pi/ATP were calculated as well as the pH. The spectra were analysed in the time domain using AMARES in the jMRUI software. AMARES allowed the inclusion of prior knowledge about relations between peaks (derived from the method of Mochel *et al.*[4]).

2.11 | Mitochondrial membrane potential

The $\Delta\Psi_m$ can be used as a general outcome for mitochondrial health, because most mitochondrial inhibition or damage results in a decrease of $\Delta\Psi_m$.[16] The $\Delta\Psi_m$ of live PBMCs was assessed using the JC-1 dye and flowcytometry (method described elsewhere[17]). Healthy mitochondria emit different fluorescent (FL-2) from dysfunctional mitochondria (FL-1). Treating a small fraction of cells with the uncoupling agent carbonyl cyanide m-chlorophenyl hydrazine (CCCP) to act as positive control, the $\Delta\Psi_m$ was calculated:

$$\Delta\Psi_m = \left(\frac{FL2}{FL1} \Big/ \frac{FL2_{CCCP}}{FL1_{CCCP}}\right) x100.$$

In Part 2 of the study, the *stressability* of the $\Delta\Psi_m$ was additionally assessed by *ex vivo* titration of mitotoxic medications verapamil and carvedilol.[18] Verapamil decreases the calcium fluctuation under stress by increasing sensitivity to H_2O_2, and enhances oxidative stress by increasing reactive oxygen species levels.[19] Carvedilol has an adverse effect on mitochondrial complex I, resulting in a decreasing activity of this complex and, therefore, an increase in reactive oxygen species production.[20] When challenged with cyanide (an inhibitor of complex IV), the $\Delta\Psi_m$ of HD patients collapsed to a much greater extent than in heathy controls.[21] The same was found in a study using Ca^{2+} as a stressor.[22] Freshly isolated PBMCs were incubated with a concentration range (0, 0.125, 0.25, 0.5, 1 and 2mM) of verapamil and carvedilol, at predose baseline and after 27 days of SBT-020 administration. With the titration curve, we calculated the half maximal inhibitory concentration values per timepoint, per mitotoxic compound.

2.12 | Motor and neurocognitive assessments

To assess the neurocognitive and motor functioning, we used a comprehensive set of tests (Table S10). The assessments were selected as they have been proven sensitive to detect cognitive and motor deterioration in HD.[22–29] The single digit modalities task, Stroop, and trail making test were paper-and-pencil tasks, the sustained attention to response task, adaptive tracking, and visual verbal learning test were computerized and were administered using the CHDR's NeuroCart.

2.13 | Unified Huntington Disease Rating Scale

The UHDRS is a clinical rating scale, which is used to assess the TMS (range 0–124) and the TFC (range 0–13; described in detail elsewhere[30,31]). The UHDRS was performed by certified physicians. A higher TMS indicates increased motor symptoms and a lower TFC indicates increased functional disability.

2.14 | Sample size calculation

Sample size was calculated based on τPCr data in the literature. The effect size (9.8 s) was set on the difference between the means of asymptomatic HD patients (43.0 s)[5] and heathy controls (33.2 s), because the goal of the treatment was to normalize mitochondrial function in HD patients. The variability was set on the standard deviation (8.2 s) of prefrail sedentary elderly.[32] This meant that a sample size of 12 in each group would have a power of 0.80 using a 2-sample *t*-test with a .05 2-sided significance level.

The threshold for inclusion in the study was set on a τPCr of 40s, enabling at least half of the screened patients to be eligible. However, data from the literature did not reflect our patient population. After the first 11 patients were screened, the median τPCr was 32.4 s instead of the earlier reported mean value of 43 s, which led to exclusions of most of the screened patients. However, the standard deviation was considerably lower in our measurements (4.0 s instead of the reported 8.2 s[5]). Therefore, we amended the study protocol to set the inclusion threshold on at least 32.4 s, in order to include the 50% of patients with a τPCr above average (median) and re-performed the sample size calculation. Based on these new data, the sample size of 12 patients per treatment arm would have a power of 0.833 to detect a difference in means of 5.0 s, using a 2-sample *t*-test with a .05 2-sided significance level. It was subsequently decided to not change the sample size.

2.15 | Statistical methods

Statistics were performed using SAS, version 9.4, by a study-independent CHDR statistician. To establish whether significant treatment effects could be detected on the repeatedly measured biomarker parameters (mitochondrial function), each parameter was analysed with a mixed model analysis of covariance (ANCOVA) with treatment, time and treatment by time as fixed factors and subject as random factor and the baseline measurement as covariate. To establish whether significant treatment effects could be detected on the single measured efficacy and PD endpoints (neurocognitive and motor function) each parameter was analysed with a mixed model ANCOVA with treatment as fixed factor and the baseline measurement as covariate. There was no adjustment for multiplicity due to the exploratory nature of the study.

2.16 | *Posthoc* analysis

A *posthoc* analysis was performed on the PD data from Part 2 in order to assess the effect of SBT-020 on the patients with relatively low *vs* relatively high mitochondrial function. To divide the active cohort (n = 11), the median values of the τPCr (42.4 s) and $\Delta\Psi_m$ (3412) prior to drug administration in Part 2 were used as cutoff values. Patients with a low mitochondrial function were defined as a τPCr > 42.4 s and $\Delta\Psi_m$ < 3412 and patients with a high mitochondrial function as a τPCr < 42.4 s and $\Delta\Psi_m$ > 3412.

2.17 | Randomization procedure

The randomization code was generated by a study independent CHDR statistician using SAS v9.4. The randomization code could be broken and made available for data analysis only after study closure, i.e. when the study was completed, the protocol deviations determined, and the clinical database declared complete, accurate and locked. The randomization code was kept strictly confidential. Individual randomization codes, per subject and per treatment, were placed in a sealed envelope containing the labelled *emergency decoding envelopes* and kept in a locked cabinet.

2.18 | Pharmacokinetics analysis

PK analysis was performed, using SAS v9.4, by a study independent CHDR statistician. Plasma PK parameters were derived by noncompartmental analysis of the plasma concentration data. Data below the limit of quantification before reaching maximum plasma concentration (Cmax) were replaced with zero, data after reaching Cmax were excluded from the analysis. No outlying data were removed.

3 | RESULTS

3.1 | Demographics

Figure 1 summarizes the disposition of patients. A total of 24 patients enrolled in the study (mean age 47.5 years, range 20–64; mean CAG repeat number 44.3, range 39–60). At baseline, patients had a mean

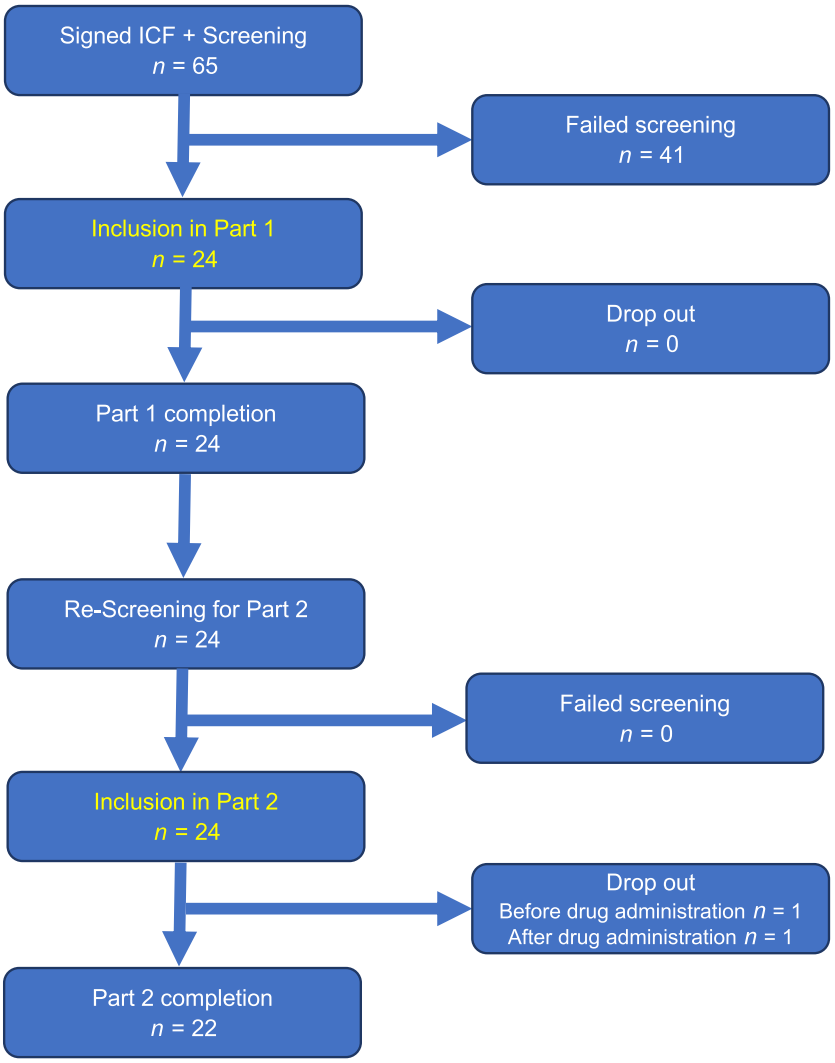

FIGURE 1 Flow chart patient disposition parts 1 and 2. ICF = informed consent form

TMS of 18.9 (range 6–47), mean TFC of 9.9 (range 7–13) and mean τPCr of 40.2 s (range 33.3–57.5). Demographics and baseline values are listed in Table 1. All 24 patients successfully passed rescreening for Part 2, but 1 patient dropped out due to severe adverse events (SAEs) before drug administration and 1 patient withdrew consent after inclusion due to the perceived study burden. Hence 22 patients completed Part 2.

There were 2 SAEs in 1 patient, after the follow-up visit of Part 1 (54 days), but prior to the drug administration in Part 2: a pneumonia followed by a pulmonary embolism. Both SAEs were deemed unrelated to the study treatment, due to the extended time between receiving the last dose of SBT-020 and the start of symptoms. There were no clinically significant findings in any laboratory assessments, (including plasma histamine), vital signs, ECGs or physical examinations.

3.2 | Safety

3.2.1 | Adverse events

The frequency of treatment-emergent adverse events (TEAEs) per treatment is listed in Table 2 (with a detailed overview in Table S5). The grand majority of TEAEs (91% in Part 1 and 97% in Part 2) were injection site reactions (ISR; erythema, swelling, pain and pruritus). All ISRs were mild, generally developed within a few minutes of dosing and resolved within the hour. Injection site pruritus and pain were most often seen in the 25 mg dose cohort.

3.3 | Pharmacokinetics

A noncompartmental PK analysis (Table S6 for plasma and Table S7 for urine) was performed for SBT-020 concentration (plasma PK is depicted in Figure 2). In Part 1, concentrations were measured for 24 hours after the first and last administration. SBT-020 was rapidly absorbed and an early Cmax (around 1 h postdose) was observed in all subjects, independent of dose. Plasma concentration *vs* time profiles were consistent with extravascular dosing and the variability was <26% for both Cmax and AUC(0–last). At day 7, the median percentage of extrapolated AUC was <8% (max 12.5%) in the 5-mg cohort

TABLE 1 Demographics and baseline values for the UHDRS sub-scores and the PCr recovery time of 31P-MRS of the calf muscle. UHDRS = unified Huntington's disease rating scale; TMS = total motor score; TFC = total functional capacity; τPCr = PCr recovery time; SD = standard deviation

	Mean	SD	Min	Max
Number of patients (*n*)	24			
Age (y)	47.5	9.3	20	64
Sex (% female)	50%			
BMI (kg/m^2)	25.9 (4.8)	24.7	18.6	39.7
CAG repeat (*n*)	44.3	4.4	39	60
Age of disease onset (y)	40.6	9.7	19	59
Time since HD-related complaints (y)	28.5	21	1	60
UHDRS (score)				
TMS	18.9	10.4	6	47
TFC	9.9	1.8	7	13
τPCr (calf muscle, in s)	40.2	6.4	33.3	57.5

TABLE 2 Occurrence of treatment emergent adverse events (TEAEs)

Treatment	Number of TEAEs	Number of patients that reported TEAEs (%)
Part 1		
5 mg (*n* = 6)	41	6 (100)
15 mg (*n* = 6)	64	6 (100)
25 mg (*n* = 6)	99	6 (100)
Placebo (*n* = 6)	15	5 (83)
Part 2		
25 mg (*n* = 11)	423	11 (100)
Placebo (*n* = 12)	67	11 (92)

and less than 2% in the 15-mg and 25-mg cohorts. Exposures between day 1 and day 7 were approximately 10% higher in the 25-mg dose cohort, but within reasonable variability. The apparent elimination half-life at day 7 appeared to be fairly independent of dose (3.13 h in the 5-mg cohort, 3.94 in the 15-mg cohort and 4.14 h in the 25-mg cohort). The apparent volume of distribution at day 7 was consistent across. Based on the trough samples taken weekly in Part 2, SBT-020 did not accumulate over a period of 28 days of daily drug administration.

3.4 | Pharmacodynamics

A summary of the statistical analysis (results of the ANCOVA analysis and the least square means change from baseline) of the mitochondrial function tests has been listed in Table 3 for Part 1 and in Table 4 for Part 2. The results of the neurocognitive and motor function tests can be found in Table S8.

3.4.1 | Part 1

No overall or dose-related effects were noted on τPCr, $\Delta\Psi_m$ and the percentage of dysfunctional PBMCs after 7 days of treatment. The mean τPCr changed from 38.8 to 33.6 s (placebo) 41.9 to 42.5 seconds (5-mg cohort), 40.0 to 43.1 seconds (15-mg cohort) and 39.2 to

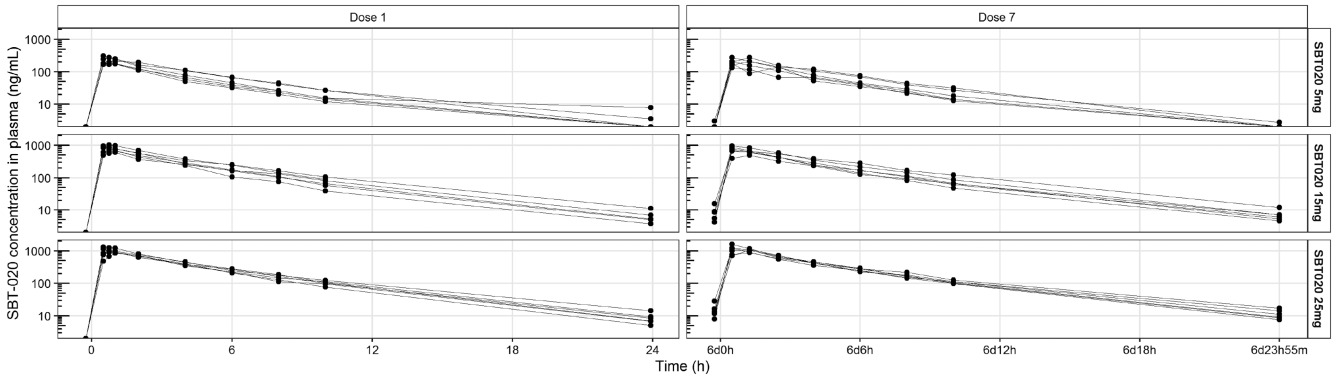

FIGURE 2 Plasma SBT-020 concentrations on (A) day 1 and (B) day 7 of part 1 for the 3 different dose cohorts. Concentrations of individual patients are depicted

TABLE 3 Summary of pharmacodynamic results in P1. PCr = phosphocreatine; pi = inorganic phosphate; ^{31P}-MRS = phosphorous magnetic resonance spectroscopy; LS = least square; PBMCs = peripheral blood mononuclear cells; CI = confidence interval; ATP = adenosine triphosphate, τPCr = PCr recovery time

	LS means					Contrasts (95% CI)			LS means change from baseline			
Parameter	Placebo	5 mg SBT-020	15 mg SBT-020	25 mg SBT-020	Treatment P-value	5 mg SBT-020 placebo	15 mg SBT-020 placebo	25 mg SBT-020 placebo	Placebo	5 mg SBT-020	15 mg SBT-020	25 mg SBT-020
τPCr with 31P-MRS (s)	34.3969	40.9016	43.2040	39.8743	.3104	6.50472 (−3.4601, 16.4696) *P* = .1844	8.80711 (−1.0294, 18.6436) *P* = .0757	5.47741 (−5.5072, 16.4620) *P* = .3047	−5.40847	1.09625	3.39864	0.06894
PCr/ATP resting phase 31P-MRS scan	3.8055	3.7838	3.8400	3.8510	.9045	−.02172 (−.22404, 0.18060) *P* = .8235	0.03449 (−.17248, 0.24146) *P* = .7295	0.04551 (−.17953, 0.27054) *P* = .6750	0.04631	0.02459	0.08080	0.09181
PCr/pi resting phase 31P-MRS scan	10.4685	9.9289	11.5607	11.2289	.2035	−.53955 (−2.2149, 1.13580) *P* = .5060	1.09226 (−.68069, 2.86521) *P* = .2110	0.76042 (−1.0773, 2.59818) *P* = .3948	−0.30014	−0.83969	0.79212	0.46028
Percentage of dysfunctional PMBCs (%)	5.80	3.82	2.80	3.75	.0337	−1.980 (−3.819, −0.141) *P* = .0363	−3.002 (−4.980, −1.025) *P* = .0051	−2.052 (−3.894, −0.210) *P* = .0310	2.410	0.430	−0.593	0.358
Mitochondrial membrane potential (Delta psi)	2898.74	3162.27	2882.36	4411.95	.0919	263.528 (−1151.3, 1678.31) *P* = .7001	−16.375 (−1411.2, 1378.48) *P* = .9806	1513.21 (114.067, 2912.36) *P* = .0356	−457.692	−194.164	−474.067	1055.521

TABLE 4 Summary of mitochondrial function PD results of part 2. PCr = phosphocreatine; pi = inorganic phosphate; 31P-MRS = phosphorous magnetic resonance spectroscopy; LS = least square; PBMCs = peripheral blood mononuclear cells; CI = confidence interval; ATP = adenosine triphosphate; τPCr = PCr recovery time

	LS means			Contrasts (95% CI)	LS means change from baseline	
Parameter	Placebo	25 mg SBT-020	Treatment *P*-value	25 mg SBT-020 placebo	Placebo	25 mg SBT-020
τPCr with 31P-MRS (sec)	38.0	40.8	.63	2.8 (−9.3, 15.0) *P* = .63	−1.7	1.2
PCr/ATP resting phase 31P-MRS scan	3.8	3.7	.54	−.07 (−0.3, 0.2) *P* = .54	0.03	−0.04
PCr/pi resting phase 31P-MRS scan	10.1	10.2	.9	0.1 (−2.0, 2.1) *P* = .94	0.2	0.3
Percentage of dysfunctional PMBCs (%)	4.83	4.08	.62	−0.75 (−3.93, 2.42) *P* = .62	1.062	0.308
Mitochondrial membrane potential (Delta psi)	3025.07	4090.47	.17	1065.4 (−495.2, 2626.0) *P* = .17	−422.405	642.997
PCr/ATP after visual stimulation (central 31P-MRS)	0.9	0.9	.57	−0.02 (−0.1, 0.05) *P* = .57	−0.003	−0.02
PCr/ATP before visual stimulation (central 31P-MRS)	0.8	0.8	.71	−0.01 (−0.09, 0.06) *P* = .71	−0.04	−0.05
PCr/ATP during visual stimulation (central 31P-MRS)	0.8	0.8	.41	−0.03 (−0.1, 0.04) *P* = .41	−0.03	−0.06
Pi/ATP after visual stimulation (central 31P-MRS)	0.2	0.2	.66	0.0073 (−0.0270, 0.0415) *P* = .6613	−0.01	0.0001
Pi/ATP before visual stimulation (central 31P-MRS)	0.2	0.2	.80	−0.002 (−0.02, 0.02) *P* = .80	0.008	0.005
Pi/ATP during visual stimulation (central 31P-MRS)	0.2	0.2	.57	−0.004 (−0.02, 0.01) *P* = .57	−0.007	−0.01
Pi/PCr after visual stimulation (central 31P-MRS)	0.2	0.2	.32	0.02 (−0.02, 0.06) *P* = .32	−0.01	0.01
Pi/PCr before visual stimulation (central 31P-MRS)	0.2	0.2	.94	0.0007 (−0.02, 0.02) *P* = .93	0.02	0.02
Pi/PCr during visual stimulation (central 31P-MRS)	0.2	0.2	.81	0.004 (−0.03, 0.03) *P* = .81	0.003	0.006
PCr/ATP during-before visual stimulation (central 31P-MRS)	0.02	−0.03	.22	−0.04 (−0.11, 0.03) *P* = .23	0.02	−0.02
PCr/ATP during-after visual stimulation (central 31P-MRS)	−0.05	−0.05	.92	−0.004 (−0.07, 0.06) *P* = .92	−0.03	−0.03
Pi/ATP during-before visual stimulation (central 31P-MRS)	−0.002	−0.01	.42	−0.009 (−0.03, 0.01) *P* = .42	−0.01	−0.02
Pi/ATP during-after visual stimulation (central 31P-MRS)	−0.003	−0.02	.49	−0.01 (−0.05, 0.02) *P* = .49	0.0004	−0.01
Pi/PCr during-before visual stimulation (central 31P-MRS)	−0.005	−0.006	.97	−0.0004 (−0.03, 0.03) *P* = .97	−0.02	−0.02
Pi/PCr during-after visual stimulation (central 31P-MRS)	0.01	−0.005	.52	−0.02 (−0.06, 0.03) *P* = .52	0.01	−0.003

38.8 seconds (25-g cohort). For the mean $\Delta\Psi_m$ the change was 3454 to 3372 (placebo), 2956 to 2948 (5-mg cohort), 3,316 to 3,282 (15-mg cohort) and 3715 to 5279 (25-mg cohort). For the mean percentage of dysfunctional PBMCs the change was from 2.7 to 5.2% (placebo), 3.2 to 3.4% (5-mg cohort), 4.3 to 3.8% (15-mg cohort) and 3.2 to 3.4% (25-mg cohort).

3.4.2 | Part 2

Mitochondrial function

No overall effects were noted on τPCr, $\Delta\Psi_m$ and the percentage of dysfunctional PBMCs after 28 days of treatment. Mean τPCr in the active group did not change from 42.8 seconds (Figure 3A). Mean τPCr in the placebo group also did not significantly change (36.5 to 36.0 s). For the mean $\Delta\Psi_m$ the change was from 3770 to 4124 in the active group and 3125 to 2991 in the placebo group. For the mean percentage of dysfunctional PBMCs the change was from 4.6 to 4.2% in the active group and 2.9 to 4.6% in the placebo group. No overall statistically significant effect of SBT-020 on brain mitochondrial function could be observed compared to placebo (Figure 3B). Furthermore, no effect on $\Delta\Psi_m$ values and half maximal inhibitory concentration values for carvedilol and verapamil could be observed.

Posthoc *analysis on mitochondrial function*

In the low mitochondrial function group (τPCr > 42.4 s) the τPCr decreased with 3.6 s, indicating an improvement in mitochondrial function, while in the high mitochondrial function group (τPCr of < 42.4 s) the τPCr did not decrease.

Patients on active treatment with a low mitochondrial function ($\Delta\Psi_m$ < 3412) had an average increase in mitochondrial membrane potential of 1931 while patients with a high mitochondrial function ($\Delta\Psi_m$ > 3412) had a decrease in mitochondrial membrane potential of 959.

Motor and neurocognitive function

No effect of SBT-020 on cognition could be observed compared to placebo except for the total errors on the visual scanning part of the trail making test (P = .04, 95%CI −0.9 – −0.015). Also, no effect on motor function could be observed.

4 | DISCUSSION

SBT-020 was safe in all dose levels during both parts of the study. Mild injection site reactions were observed frequently throughout the study, but other adverse events were few and equally divided over the active and placebo groups. The exact mechanism of the ISRs is unknown, but plasma histamine levels measured 15 and 30 minutes after administration were not elevated. SBT-020 was rapidly absorbed following subcutaneous dosing, Cmax was observed between 0.5 and 1.0 hours. SBT-020 did not accumulate following repeat dosing, as assessed by comparison of Cmax and AUC(0–τ). Mean terminal half-life values were estimated between 3.13 and 4.14 hours following multiple doses (Day 7). The longer value for the highest dose group on Day 7 may simply reflect more quantifiable data at later time points. All dose levels were safe after single and multiple dosing with ISR being the most common adverse event. SBT-020 did not accumulate following repeat dosing, as judged by comparison of Cmax and AUC (0–τ). Geometric mean terminal half-life values were estimated between 3.47 and 3.74 hours for a single dose (Part 1), and between 3.51 and 5.26 hours following multiple doses (Part 2, Day 7). Geometric mean CLr was estimated between 25.3 and 47.5 mL/min and did not indicate active secretion. There was no evidence that clearance, volume of distribution or bioavailability varied with dose or time. By 24 hours post dose, between 27.5 and 44.9% of the dose was excreted unchanged in urine; the majority was excreted in the first 6 hours postdose.

In our cohort of HD patients, we did not observe an effect of SBT-020 on mitochondrial function in calf muscle or brain. McGhee *et al*. advocate treatment during clinical trials for neurodegenerative disease such as Alzheimer and Parkinson, of at least 6 months, arguing that it is unlikely to observe disease modification before that.[33] This is significantly longer than the 28 days in our study. However, mitochondrial dysfunction plays an important role in the pathophysiology of HD and SBT-020 was previously shown to improve mitochondrial function in preclinical studies in HD. Multiple factors could explain the lack of effect of SBT-020 on mitochondrial function. Most importantly, the mitochondrial function in our patients might not have been impaired sufficiently to be able to improve. Mitochondrial complex defects are present in the striatal cells of late stage HD patients and in late stage disease mouse models, but not in neostriatum and

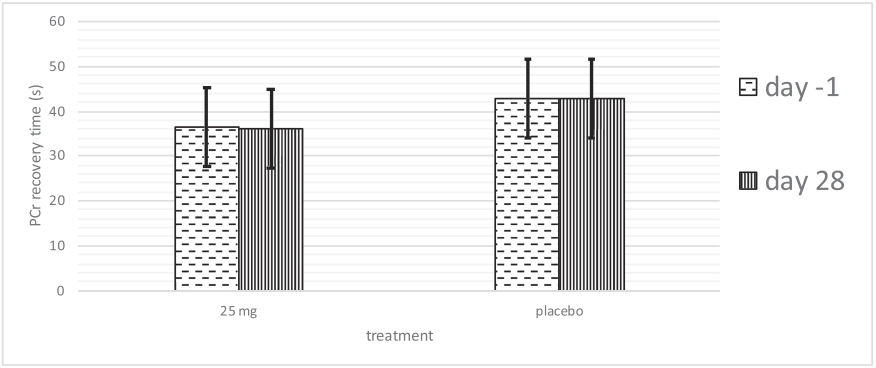

FIGURE 3 Effect of daily administration of 25 mg SBT-020 in Part 2 on (A) peripheral and (B) central mitochondrial function, measured with 31P-spectroscopy. no differences between placebo and SBT-020 were observed. PCr = phosphocreatine

cerebral cortex cells of presymptomatic and mild stage HD patients and transgenic mice in which neuronal loss could not be documented.[34] Mean τPCr (40.2 s) in calf muscle in our patients was longer than previously reported in untrained, healthy volunteers (31.2 s[35]), but shorter than in another cohort of symptomatic HD patients (49.4 s[5]). Mitochondrial function in our cohort was better than expected while both cohorts consisted of similar affected patients: TMS ranged 5–53 (mean 22.7) in our study *vs* TMS ranged 5–55 (mean 25.4) in the study by Saft *et al*. (range 5–55, mean 25.4, standard deviation 14.4), which means that there is poor correlation between UHDRS scores and τPCr.[5] It is important to mention that the aim of the study was primarily to prove the pharmacological principle of SBT-020 in its ability to improve mitochondrial function, whereas improving clinical symptoms was a secondary objective. The observed effects were not clinically meaningful.

SBT-020 does not improve normal functioning mitochondria, so the overall, relatively good, mitochondrial function might have been a relevant factor in the absence of an effect. Nonetheless, PD data in this study indicate that SBT-020 works best when targeting a higher level of mitochondrial dysfunction. When looking at mitochondrial function in PBMCs in Part 2, SBT-020 was most beneficial in the patients with the lowest $\Delta\Psi_m$. Although these effects cannot be viewed as clinically meaningful, patients on active treatment with a $\Delta\Psi_m$ <3412 had an average improvement of 1931 while patients with a $\Delta\Psi_m$ >3412 had a decrease of 959, which indicates a potential pharmacological effect. Also, the patients with the longest τPCr at baseline (> 44 s) in Part 2 showed the highest improvement after 28 days of drug administration (improvement of 3.6 s *vs* a prolonging of 3 s for the patients on active treatment with a baseline τPCr of <44 s). SBT-020 does not improve normal functioning mitochondria, so the overall, relatively good, mitochondrial function might have been a relevant factor in the absence of an effect.

Measuring mitochondrial function *in vivo* inside the brain is challenging. To date, only 31P-MRS has approximated this by measuring the bio-energetics before, during and after visual stimulation.[4] Contrary to the τPCr resulting from exerting skeletal muscle, the bio-energetics in the visual cortex are harder to interpret. In healthy controls, the Pi/PCr ratio increases during visual stimulation, whereas in HD patients the ratio stays the same, which underlines the difference in bio-energetics between the 2 groups.[4] Although no hard conclusion can be drawn from the results, the method is an important tool in assessing mitochondrial function as demonstrated in an earlier clinical trial in HD patients.[36] Another possible cause for a lack of clear effects in this study may be, that the drug did not reach a sufficient concentration at the target site of action in the central nervous system (CNS). In an acute model of CNS neurodegeneration induced by the mitochondrial toxin MPTP, peripheral administration of SBT-20 at 5 mg/kg was sufficient to achieve neuroprotection in the striatum. However, this model may not fully recapitulate the progressive neurodegenerative decline observed in HD, where higher levels of drug exposure over more sustained intervals may be required. Additionally, MPTP itself can be damaging to the blood–brain barrier (BBB),[37] causing leakage through which SBT-020 could have penetrated the BBB in this MPTP-induced model. However, BBB integrity has been observed to be decreased and in patients with neurodegenerative disorders, including HD,[38] which increases the brain delivery of neuropharmaceuticals. Since lumbar punctures to measure SBT-020 concentration in cerebrospinal fluid, a well-known proxy for CNS tissue concentration, was deemed not feasible for this study, this is a possibility that cannot be excluded. A clinical trial with triheptanoin, a C7 fatty acid oil, has previously been conducted in HD patients to improve bioenergetics the visual cortex.[36] The trial reported a normalization of the Pi/PCr ratios between HD patients and healthy controls, although there was no correlation between the normalization and UHDRS score improvement. This proves that it should be possible to pharmacologically influence mitochondrial function in HD patients.

In conclusion, SBT-020 was safe during daily administration for 28 days up to a daily dose of 25 mg in HD patients. PK analysis showed that once daily subcutaneous administration resulted in dose-proportional exposure and no accumulation over a 28-day administration period. No effects were observed on mitochondrial or clinical function and we suspect the mild degree of mitochondrial dysfunction in our patients and short treatment period to be responsible. It is worth mentioning that the results have provided a platform for further studies with SBT-020.

ACKNOWLEDGEMENTS

We would like to thank the HD patients for their participation in the study, The Dutch Huntington's Disease Patient Organization for their help with recruitment and the clinical staff of CHDR and the Leiden University Medical Center for facilitating this trial. This study was funded by Stealth Biotherapeutics Inc. A.B. and L.M. were its employees at the time of the study conduct and S.B. was employed on a consultancy basis.

CONTRIBUTORSS

M.P.J.v.D., E.P.t.H., A.A., L.M., S.C.B.M.M., H.K., J.v.d.G., A.W., R.A.C.R. and G.J.G. were involved in designing the study and writing the study protocol. M.P.J.v.D., I.v.B., S.C.B., E.C. and J.Y.W. performed the clinical part of the study and collected the data. M.P.J.v.D., E.P.t.H., A.A., L.M., I.v.B., S.C.B., P.W.H., M.M., H.K., J.v.d.G., A.W., R.A.C.R. and G.J.G. were involved in the (statistical) analysis in the data. M.P.J.v.D. and G.J.G. wrote the draft manuscript and all authors revised the manuscript to its current form.

COMPETING INTEREST

The authors have no conflicts of interest to declare. The study was approved by the independent ethics committee Stichting BEBO (Assen, the Netherlands) according to the principles of the Helsinki Declaration under registration number NL59198.056.16. Informed consent was obtained from all subjects prior to study enrolment. This study was registered in the EU Clinical Trials Register under EudraCT number 2016–003730-25. S.B., A.A. and L.M. are or were employees of the study's sponsor, Steath BioTherapeutics Inc. H.K. reports grants from the Duchenne Parent Project, NWO and the EU and research

support from Philips Healthcare during the conduct of the study, and consultancy for BioMarin and aTyr Pharma and trial support from ImagingDMD-UF outside the submitted work.

DATA AVAILABILITY STATEMENT

The data that support the findings will be available in EU Clinical Trials Register at https://www.clinicaltrialsregister.eu/ctr-search/search?query=2016-003730-25 following an embargo from the date of publication to allow for commercialization of research findings.

ORCID

Marcus P.J. van Diemen https://orcid.org/0000-0002-6708-1082
Hermien Kan https://orcid.org/0000-0002-5772-7177

REFERENCES

1. The Huntington's Disease Collaborative Research Group. A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*. 1993;72:971-983.
2. Rawlins MD, Wexler NS, Wexler AR, et al. The prevalence of Huntington's disease. *Neuroepidemiology*. 2016;46(2):144-153.
3. Quintanilla RA, Johnson GVW. Role of mitochondrial dysfunction in the pathogenesis of Huntington's disease. *Brain Res Bull*. 2009;80 (4-5):242-247.
4. Mochel F, N'Guyen TM, Deelchand D, et al. Abnormal response to cortical activation in early stages of Huntington disease. *Mov Dis off J Mov Dis Soc*. 2012;27(7):907-910.
5. Saft C, Zange J, Andrich J, et al. Mitochondrial impairment in patients and asymptomatic mutation carriers of Huntington's disease. *Mov Dis off J Movement Dis Soc*. 2005;20(6):674-679.
6. Karaa A, Haas R, Goldstein A, Vockley J, Weaver WD, Cohen BH. Randomized dose-escalation trial of elamipretide in adults with primary mitochondrial myopathy. *Neurology*. 2018;90(14):e1212-e1221.
7. Daubert MA, Yow E, Dunn G, et al. Novel mitochondria-targeting peptide in heart failure treatment: a randomized, placebo-controlled trial of Elamipretide. *Circ Heart Fail*. 2017;10(12). https://doi.org/10.1161/circheartfailure.117.004389
8. Szeto HH, Schiller PW. Novel therapies targeting inner mitochondrial membrane--from discovery to clinical development. *Pharm Res*. 2011;28(11):2669-2679.
9. Kagan VE, Bayir HA, Belikova NA, et al. Cytochrome c/cardiolipin relations in mitochondria: a kiss of death. *Free Radic Biol Med*. 2009;46(11):1439-1453.
10. Kagan VE, Tyurin VA, Jiang J, et al. Cytochrome c acts as a cardiolipin oxygenase required for release of proapoptotic factors. *Nat Chem Biol*. 2005;1(4):223-232.
11. Li K, Li Y, Shelton JM, et al. Cytochrome c deficiency causes embryonic lethality and attenuates stress-induced apoptosis. *Cell*. 2000;101 (4):389-399.
12. Paradies G, Paradies V, De Benedictis V, Ruggiero FM, Petrosillo G. Functional role of cardiolipin in mitochondrial bioenergetics. *Biochim Biophys Acta*. 2014;1837(4):408-417.
13. Yang L, Zhao K, Calingasan NY, Luo G, Szeto HH, Beal MF. Mitochondria targeted peptides protect against 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine neurotoxicity. *Antioxid Redox Signal*. 2009;11(9):2095-2104.
14. Yin X, Manczak M, Reddy PH. Mitochondria-targeted molecules MitoQ and SS31 reduce mutant huntingtin-induced mitochondrial toxicity and synaptic damage in Huntington's disease. *Hum Mol Genet*. 2016;25(9):1739-1753.
15. van den Bogaard SJ, Dumas EM, Teeuwisse WM, et al. Exploratory 7-tesla magnetic resonance spectroscopy in Huntington's disease provides in vivo evidence for impaired energy metabolism. *J Neurol*. 2011;258(12):2230-2239.
16. Zorova LD, Popkov VA, Plotnikov EY, et al. Mitochondrial membrane potential. *Anal Biochem*. 2018;552:50-59.
17. Cossarizza A, Salvioli S. Flow cytometric analysis of mitochondrial membrane potential using JC-1. *Current protocols in cytometry* **Chapter 9**, Unit 9.14 (2001).
18. Finsterer J, Zarrouk Mahjoub S. Mitochondrial toxicity of antiepileptic drugs and their tolerability in mitochondrial disorders. *Expert Opin Drug Metab Toxicol*. 2012;8(1):71-79.
19. Yu Q, Xiao C, Zhang K, et al. The Calcium Channel blocker verapamil inhibits oxidative stress response in Candida albicans. *Mycopathologia*. 2014;177(3-4):167-177.
20. Sgobbo P, Pacelli C, Grattagliano I, Villani G, Cocco T. Carvedilol inhibits mitochondrial complex I and induces resistance to H_2O_2-mediated oxidative insult in H9C2 myocardial cells. *Biochimica et Biophysica Acta - Bioenergetics*. 2007;1767:222-232.
21. Panov AV, Gutekunst CA, Leavitt BR, et al. Early mitochondrial calcium defects in Huntington's disease are a direct effect of polyglutamines. *Nat Neurosci*. 2002;5(8):731-736.
22. Stout JC, Jones R, Labuschagne I, et al. Evaluation of longitudinal 12 and 24 month cognitive outcomes in premanifest and early Huntington's disease. *J Neurol Neurosurg Psychiatry*. 2012;83(7):687-694.
23. Tian JR, Herdman SJ, Zee DS, Folstein SE. Postural control in Huntington's disease (HD). *Acta Otolaryngol Suppl*. 1991;481:333-336.
24. Hart EP, Dumas EM, Schoonderbeek A, Wolthuis SC, van Zwet E, Roos RA. Motor dysfunction influence on executive functioning in manifest and premanifest Huntington's disease. *Mov Dis off J Move Dis Soc*. 2014;29(3):320-326.
25. O'Rourke JJ, Beglinger LJ, Smith MM, et al. The trail making test in prodromal Huntington disease: contributions of disease progression to test performance. *J Clin Exp Neuropsychol*. 2011;33(5):567-579.
26. Lasker AG, Zee DS, Hain TC, Folstein SE, Singer HS. Saccades in Huntington's disease: initiation defects and distractibility. *Neurology*. 1987;37(3):364-370.
27. Hicks SL, Robert MP, Golding CV, Tabrizi SJ, Kennard C. Oculomotor deficits indicate the progression of Huntington's disease. *Prog Brain Res*. 2008;171:555-558.
28. Henderson T, Georgiou-Karistianis N, White O, et al. Inhibitory control during smooth pursuit in Parkinson's disease and Huntington's disease. *Mov Dis off J Mov Dis Soc*. 2011;26(10):1893-1899.
29. Butters N, Wolfe J, Martone M, Granholm E, Cermak LS. Memory disorders associated with huntington's disease: verbal recall, verbal recognition and procedural memory. *Neuropsychologia*. 1985;23(6):729-743.
30. Shoulson I, Fahn S. Huntington disease: clinical care and evaluation. *Neurology*. 1979;29(1):1-3.
31. Huntington Study Group. Unified Huntington's disease rating scale: reliability and consistency. *Mov Dis off J Mov Dis Soc*. 1996;11:136-142.
32. Andreux PA, van Diemen MPJ, Heezen MR, et al. Mitochondrial function is impaired in the skeletal muscle of pre-frail elderly. *Sci Rep*. 2018;8(1):8548. https://doi.org/10.1038/s41598-018-26944-x
33. McGhee DJM, Ritchie CW, Zajicek JP, Counsell CE. A review of clinical trial designs used to detect a disease-modifying effect of drug therapy in Alzheimer's disease and Parkinson's disease. *BMC Neurol*. 2016;16(1):1-13. https://doi.org/10.1186/s12883-016-0606-3
34. Guidetti P, Charles V, Chen EY, et al. Early degenerative changes in transgenic mice expressing mutant huntingtin involve dendritic abnormalities but no impairment of mitochondrial energy production. *Exp Neurol*. 2001;169(2):340-350.
35. van Diemen MPJ, Berends CL, Akram N, et al. Validation of a pharmacological model for mitochondrial dysfunction in healthy

subjects using simvastatin: a randomized placebo-controlled proof-of-pharmacology study. *Eur J Pharmacol*. 2017;815:290-297.
36. Adanyeguh IM, Rinaldi D, Henry PG, et al. Triheptanoin improves brain energy metabolism in patients with Huntington disease. *Neurology*. 2015;84(5):490-495.
37. Zhao C, Ling Z, Newman MB, Bhatia A, Carvey PM. TNF-alpha knockout and minocycline treatment attenuates blood-brain barrier leakage in MPTP-treated mice. *Neurobiol Dis*. 2007;26:36-46.
38. Sweeney MD, Sagare AP, Zlokovic BV. Blood-brain barrier breakdown in Alzheimer disease and other neurodegenerative disorders. *Nat. Rev. Neurol*. 2018;14:133-155.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

How to cite this article: van Diemen MPJ, Hart EP, Abbruscato A, et al. Safety, pharmacokinetics and pharmacodynamics of SBT-020 in patients with early stage Huntington's disease, a 2-part study. *Br J Clin Pharmacol*. 2021;87:2290–2302. https://doi.org/10.1111/bcp.14656