Clinical pharmacological aspects of mitochondrial function in muscle

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CHAPTER V

SAFETY, PHARMACOKINETICS AND PHARMACODYNAMICS OF SBT-020 IN PATIENTS WITH EARLY STAGE HUNTINGTON'S DISEASE, A TWO-PART STUDY

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INTRODUCTION 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 two-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 (PBMCS).

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, PBMCS or visual cortex was not changed overall due to treatment with SBT-020. In a post hoc analysis, patients with a higher degree of mitochondrial dysfunction (below the median ($\Delta\Psi m < 3412$ and $\tau PCr > 42.5$ s)) showed more improvement than patients with a relatively lower level of mitochondrial dysfunction.

DISCUSSION 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.

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 within 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 (ETC), thereby improving the electron flow between complexes. SBT-020 (aka SS-20, H-Phe-D-Arg-Phe-Lys-NH_2) is one of the Szeto-Schiller (SS) proteins, a novel class of small tetra-peptides 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 (MPTP), 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 nigrastriatal neurons (STHDHQ111/Q111) by normalizing mitochondrial structure and function.[14]

The primary objectives in the current study were assessment of safety, tolerability and pharmacokinetics 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 UHDRS and a battery of neurocognitive tests.

MATERIAL AND METHODS

Trial design

This phase II study was conducted at the Centre for Human Drug Research (CHDR, Leiden, The Netherlands) as a single-center, 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 one of three dose cohorts (5mg, 15mg or 25mg) 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.

Dosing rationale

The dose of SBT-020 was chosen based on pre-clinical and clinical studies. In a pharmacodynamic, pre-clinical study on the neuroprotective effects of SBT-020 in 1-Methyl-4-Phenyl-1,2,3,6-Tetrahydropyridine (MPTP) treated mice, a single dose of 4 mg/kg of SBT-020 attenuated 40% of the MPTP-induced dopamine depletion.[13] In a pre-clinical study on ischemia/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 (HED) of 0.76 mg/kg. For a 60kg human this corresponds to a starting dose of 4.5mg.

5, 10, 20 and 30mg doses 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, 25mg 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 25mg single and multiple dose part was performed prior to the 28-day multiple dose part.

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 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 (LUMC, Leiden, NL). 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 pharmacokinetic (PK) and pharmacodynamic (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.

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, gamma 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 MRI scans performed; significant cardiac abnormalities on the resting ECG (QTCF >450 or <300 msec, 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 (non-active hay fever was acceptable).

Concomitant medications

Paracetamol (up to 4 g/day) and ibuprofen (1 g/day) 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.

PK 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 (Supplementary Table 5.9) 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 day 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.

PK concentration measurement

Concentrations of SBT-020 were measured by a validated LC/MS 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.

Pharmacodynamics

During Part 1 and 2, measurements for PD were performed at various time points to assess effects on mitochondrial and clinical functioning (Supplementary Table 5.10). 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.

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 was acquired before, during and after exercise using a pulse-acquire sequence with a time resolution of 2 seconds (flip angle 45 degrees, 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 below 6.8[16] and rescanning after a 10-minute break was allowed to reach an end-exercise pH of >6.8. Outlying data (up to 10% of total), deviating more than 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.

Visual cortex

31P-MRS of the brain was performed on a 3 Tesla MRI scanner (Philips, Best, The Netherlands). A custom-made 6cm 31P transmit/receive surface coil was used to detect signals from the visual cortex while limiting muscle contamination. A small sphere (Æ 10mm) 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 (FIDS) 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 analyzed 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]

Peripheral blood mononuclear cells

The ΔΨm can be used as a general outcome for mitochondrial health, because most mitochondrial inhibition or damage results in a decrease of ΔΨm.[17] The ΔΨm of live PBMCS was assessed using the JC-1 dye and flowcytometry (method described elsewhere).[18] Healthy mitochondria emit different fluorescent (FL-2) than dysfunctional mitochondria (FL-1). Treating a small fraction of cells with the uncoupling agent CCCP carbonyl cyanide m-chlorophenyl hydrazine (CCCP) to act as positive control, the ΔΨm was calculated:

$$[(\Delta\Psi)]\_M = ((FL2/FL1) / ([FL2]\_CCCP / [FL1]\_CCCP)) \times 100$$

In Part 2 of the study, the 'stressability' of the ΔΨm was additionally assessed by *ex vivo* titration of mitotoxic medications verapamil and carvedilol.[19] Verapamil decreases the calcium fluctuation under stress by increasing sensitivity to H_2O_2, and enhances oxidative stress by increasing ROS levels.[20] Carvedilol has an adverse effect on mitochondrial complex I, resulting in a decreasing activity of this complex and, therefore, an increase in ROS production.[21] When challenged with cyanide (an inhibitor of complex IV), the ΔΨm of HD patients collapsed to a much greater extent than in heathy controls.[22] The same was found in a study using Ca^2+ as a stressor.[23] Freshly isolated PBMCS were incubated with a concentration range (0mM, 0.125mM, 0.25mM, 0.5mM, 1mM and 2mM) of verapamil and carvedilol, at pre-dose baseline and after 27 days of SBT-020 administration. With the titration curve, we calculated the half maximal inhibitory concentration (IC50) values per timepoint, per mitotoxic compound.

Motor and neurocognitive assessments

To assess the neurocognitive and motor functioning, we used a comprehensive set of tests (Supplementary Table 5.10). The assessments were selected as they have been proven sensitive to detect cognitive and motor deterioration in HD.[24-31] The Single Digit Modalities Task (SDMT), Stroop, and Trail Making Test (TMT) were paper-and-pencil tasks, the Sustained Attention to Response Task (SART), Adaptive Tracking, and Visual Verbal Learning Test (VVLT) were computerized and were administered using the CHDR's NeuroCart®.

Unified Huntington's Disease Rating Scale (UHDRS)

The UHDRS is a clinical rating scale, which is used to assess the total motor score (TMS, range 0-124) and the total functional capacity (TFC, range 0-13) (described in detail elsewhere).[32,33] The UHDRS was performed by certified physicians. A higher TMS indicates increased motor symptoms and a lower TFC indicates increased functional disability.

Sample size calculation

Sample size was calculated based on τPCR data in the literature. The effect size (9.8s) was set on the difference between the means of asymptomatic HD patients (43.0s)[5] and heathy controls (33.2s), because the goal of the treatment was to normalize mitochondrial function in HD patients. The variability was set on the standard deviation (8.2s) of pre-frail sedentary elderly.[34] This meant that a sample size of 12 in each group would have a power of 0.80 using a two-sample t-test with a 0.05 two-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 32.4s instead of the earlier reported mean value of 43s, which led to exclusions of most of the screened patients. However, the standard deviation was considerably lower in our measurements (4.0s instead of the reported 8.2s).[5] Therefore, we amended the study protocol to set the inclusion threshold on at least 32.4s, 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 5.0s, using a two-sample t-test with a 0.05 two-sided significance level. It was subsequently decided to not change the sample size.

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 analyzed 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 analyzed 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.

Post hoc analysis

A post hoc 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 versus relatively high mitochondrial function. To divide the active cohort (n = 11), the median values of the τPCR (42.4s) and ΔΨm (3412) prior to drug administration in Part 2 were

used as cutoff values. Patients with a low mitochondrial function were defined as a τPCR > 42.4s and ΔΨm < 3412 and patients with a high mitochondrial function as a τPCR < 42.4s and ΔΨm > 3412.

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.

Pharmacokinetic analysis

PK analysis was performed, using SAS v9.4, by a study-independent CHDR statistician. Plasma PK parameters were derived by non-compartmental analysis of the plasma concentration data. Data below the limit of quantification (BLOQ) before T_{max} were replaced with zero, data after T_{max} were excluded from the analysis. No outlying data were removed.

RESULTS

Demographics

Supplementary Figure 5.1 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 TMS of 18.9 (range 6-47), mean TFC of 9.9 (range 7-13) and mean τPCR of 40.2s (range 33.3-57.5). Demographics and baseline values are listed in Table 5.1. All 24 patients successfully passed rescreening for Part 2, but 1 patient dropped out due to SAEs before drug administration and 1 patient withdrew consent after inclusion due to the perceived study burden. Hence 22 patients completed Part 2.

SAFETY

Adverse events

The frequency of treatment-emergent adverse events (TEAEs) per treatment is listed in Table 2 (with a detailed overview in Supplementary Table 5.5). 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 25mg dose cohort.

There were two SAEs in one 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.

Pharmacokinetics

A non-compartmental PK analysis (Supplementary Table 6 for plasma and Supplementary Table 7 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 T_{max} (around 1h post-dose) was observed in all subjects, independent of dose. Plasma concentration vs time profiles were consistent with extravascular dosing and the variability was less than 26% for both C_{max} and $AUC_{0\text{-}last}$. At day 7, the median percentage of extrapolated AUC was less than 8% (max 12.5%) in the 5mg cohort and less than 2% in the 15mg and 25mg cohorts. Exposures between Day 1 and Day 7 were approximately 10% higher in the 25mg dose cohort, but within reasonable variability. The apparent elimination half-life at day 7 appeared to be fairly independent of dose (3.13h in the 5mg cohort, 3.94 in the 15mg cohort and 4.14h in the 25mg 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.

Pharmacodynamics

A summary of the statistical analysis (results of the ANCOVA analysis and the least square means (LSM) change from baseline) of the mitochondrial function tests has been listed in Table 5.3 for Part 1 and in Table 5.4 for Part 2. The results of the neurocognitive and motor function tests can be found in Supplementary Table 5.8.

Part 1

No overall or dose-related effects were noted on τPCR, ΔΨm and the percentage of dysfunctional PBMCS after 7 days of treatment. The mean τPCR changed from 38.8s to 33.6s (placebo) 41.9s to 42.5s (5mg cohort), 40.0s to 43.1s (15mg cohort) and 39.2s to 38.8s (25mg cohort). For the mean ΔΨm the change was 3454 to 3372 (placebo), 2956 to 2948 (5mg cohort), 3316 to 3282 (15mg cohort) and 3715 to 5279 (25mg cohort). For the mean percentage of dysfunctional PBMCS the change was from 2.7% to 5.2% (placebo), 3.2% to 3.4% (5mg cohort), 4.3% to 3.8% (15 mg cohort) and 3.2% to 3.4% (25mg cohort).

Part 2

Mitochondrial function

No overall effects were noted on τPCR, ΔΨm and the percentage of dysfunctional PBMCS after 28 days of treatment. Mean τPCR in the active group did not change from 42.8 sec (Figure 5.3A). Mean τPCR in the placebo group also did not significantly change (36.5s to 36.0s). For the mean ΔΨm 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 5.3B). Furthermore, no effect on ΔΨm values and IC50 values for carvedilol and verapamil could be observed.

Post hoc analysis on mitochondrial function

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

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

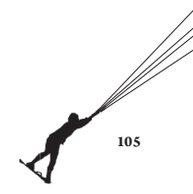
Motor and neurocognitive function

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 TMT (CI95% -0.9–-0.015, p = 0.04). Also, no effect on motor function could be observed.

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 AEs 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 SC dosing, T_{max} was observed between 0.5 h and 1.0 h. SBT-020 did not accumulate following repeat dosing, as assessed by comparison of C_{max} and $AUC_{0\text{-}tau}$. Mean terminal T½ values were estimated between 3.13 h and 4.14 h 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 injection site reactions (ISR) being the most common adverse event. SBT-020 did not accumulate following repeat dosing, as judged by comparison of C_{max} and $AUC_{0\text{-}tau}$. Geometric mean terminal T½ values were estimated between 3.47 h and 3.74 h for a single dose (Part 1), and between 3.51 h and 5.26 h following multiple doses (Part 2, Day 7). Geometric mean CLr was estimated between 25.3 mL/min 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 h post dose, between 27.5% and 44.9% of the dose was excreted unchanged in urine; the majority was excreted in the first 6 h post dose.

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's and Parkinson's disease, of at least 6 months, arguing that it is unlikely to observe



disease modification before that.[35] 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 pre-clinical 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 cerebral cortex cells of pre-symptomatic and mild stage HD patients and transgenic mice in which neuronal loss could not be documented.[36] Mean τPCR (40.2s) in calf muscle in our patients was longer than previously reported in untrained, healthy volunteers (31.2s),[37] but shorter than in another cohort of symptomatic HD patients (49.4s).[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 versus TMS ranged 5-55 (mean 25.4) in the study by Saft *et al.* (range 5-55, mean 25.4, SD 14.4), which means that there is a 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 indicates 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 versus 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 bioenergetics between the two 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.[38] 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 CNS. In an acute model of CNS neurodegeneration induced by the mitochondrial toxin MPTP, peripheral administration of SBT-20 at 5mg/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),[39] causing leakage through which SBT-020 could have penetrated the BBB in this MTPT induced model. However, BBB integrity has been observed to be decreased and in patients with neurodegenerative disorders , including HD,[40] which increases the brain delivery of neuropharmaceuticals. Since lumbar punctures to measure SBT-020 concentration in cerebrospinal fluid (CSF), 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, C7 fatty acid oil, has previously been conducted in HD patients to improve bioenergetics the visual cortex.[38] 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 25mg in HD patients. PK analysis showed that once daily SC 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.

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TABLE 1 Demographics. Demographics and baseline values for the UHDRS sub-scores and the PCr recovery time of 31P-MRS of the calf muscle.

	Mean	SD	Min	Max
Number of patients (n)	24			
Age (years)	47.5	9.3	20	64
Sex (% female)	50%			
BMI (kg/m²)	25.9 (4.8)	24.7	18.6	39.7
CAG repeat (number)	44.3	4.4	39	60
Age of disease onset (years)	40.6	9.7	19	59
Time since HD-related complaints (years)	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

UHDRS = Unified Huntington's Disease Rating Scale, TMS = Total Motor Score, TFC = Total Functional Capacity, τPCr = PCr recovery time, SD = standard deviation.

TABLE 2 Adverse events. 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)

TABLE 3 Pharmacodynamics Part 1. Summary of PD results in Part 1.

	LS Means				Contrasts (CI95%)			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	0.3104	6.50472 (-3.4601, 16.4696) p=0.1844	8.80711 (-1.0294, 18.6436) p=0.0757	5.47741 (-5.5072, 16.4620) p=0.3047	-5.40847	1.09625	3.39864	0.06894
PCr/ATP resting phase 31P-MRS scan	3.8055	3.7838	3.8400	3.8510	0.9045	-.02172 (-.22404, 0.18060) p=0.8235	0.03449 (-.17248, 0.24146) p=0.7295	0.04551 (-.17953, 0.27054) p=0.6750	0.04631	0.02459	0.08080	0.09181
PCr/Pi resting phase 31P-MRS scan	10.4685	9.9289	11.5607	11.2289	0.2035	-.53955 (-2.2149, 1.13580) p=0.5060	1.09226 (-.68069, 2.86521) p=0.2110	0.76042 (-1.0773, 2.59818) p=0.3948	-0.30014	-0.83969	0.79212w	0.46028
Percentage of dysfunctional PMBCs (%)	5.80	3.82	2.80	3.75	0.0337	-1.980 (-3.819, -0.141) p=0.0363	-3.002 (-4.980, -1.025) p=0.0051	-2.052 (-3.894, -0.210) p=0.0310	2.410	0.430	-0.593	0.358
Mitochondrial membrane potential (Delta Psi)	2898.74	3162.27	2882.36	4411.95	0.0919	263.528 (-1151.3, 1678.31) p=0.7001	-16.375 (-1411.2, 1378.48) p=0.9806	1513.21 (114.067, 2912.36) p=0.0356	-457.692	-194.164	-474.067	1055.521

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.

TABLE 4 Pharmacodynamics Part 2. Summary of mitochondrial function PD results of Part 2.

	LS Means			Contrasts (CI95%)	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	0.63	2.8 (-9.3, 15.0) p=0.63	-1.7	1.2
PCR/ATP resting phase 31P-MRS scan	3.8	3.7	0.54	-.07 (-0.3, 0.2) p=0.54	0.03	-0.04
PCR/PI resting phase 31P-MRS scan	10.1	10.2	0.9	0.1 (-2.0, 2.1) p=0.94	0.2	0.3
Percentage of dysfunctional PMBCs (%)	4.83	4.08	0.62	-0.75 (-3.93, 2.42) p=0.62	1.062	0.308
Mitochondrial membrane potential (Delta Psi)	3025.07	4090.47	0.17	1065.4 (-495.2, 2626.0) p=0.17	-422.405	642.997
PCR/ATP after visual stimulation (central 31P-MRS)	0.9	0.9	0.57	-0.02 (-0.1, 0.05) p=0.57	-0.003	-0.02
PCR/ATP before visual stimulation (central 31P-MRS)	0.8	0.8	0.71	-0.01 (-0.09, 0.06) p=0.71	-0.04	-0.05
PCR/ATP during visual stimulation (central 31P-MRS)	0.8	0.8	0.41	-0.03 (-0.1, 0.04) p=0.41	-0.03	-0.06
PI/ATP after visual stimulation (central 31P-MRS)	0.2	0.2	0.66	0.0073 (-0.0270, 0.0415) p=0.6613	-0.01	0.0001
PI/ATP before visual stimulation (central 31P-MRS)	0.2	0.2	0.80	-0.002 (-0.02, 0.02) p=0.80	0.008	0.005
PI/ATP during visual stimulation (central 31P-MRS)	0.2	0.2	0.57	-0.004 (-0.02, 0.01) p=0.57	-0.007	-0.01
PI/PCR after visual stimulation (central 31P-MRS)	0.2	0.2	0.32	0.02 (-0.02, 0.06) p=0.32	-0.01	0.01
PI/PCR before visual stimulation (central 31P-MRS)	0.2	0.2	0.94	0.0007 (-0.02, 0.02) p=0.93	0.02	0.02
PI/PCR during visual stimulation (central 31P-MRS)	0.2	0.2	0.81	0.004 (-0.03, 0.03) p=0.81	0.003	0.006
PCR/ATP during-before visual stimulation (central 31P-MRS)	0.02	-0.03	0.22	-0.04 (-0.11, 0.03) p=0.23	0.02	-0.02
PCR/ATP during-after visual stimulation (central 31P-MRS)	-0.05	-0.05	0.92	-0.004 (-0.07, 0.06) p=0.92	-0.03	-0.03
PI/ATP during-before visual stimulation (central 31P-MRS)	-0.002	-0.01	0.42	-0.009 (-0.03, 0.01) p=0.42	-0.01	-0.02
PI/ATP during-after visual stimulation (central 31P-MRS)	-0.003	-0.02	0.49	-0.01 (-0.05, 0.02) p=0.49	0.0004	-0.01
PI/PCR during-before visual stimulation (central 31P-MRS)	-0.005	-0.006	0.97	-0.0004 (-0.03, 0.03) p=0.97	-0.02	-0.02
PI/PCR during-after visual stimulation (central 31P-MRS)	0.01	-0.005	0.52	-0.02 (-0.06, 0.03) p=0.52	0.01	-0.003

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, tPCr = PCr recovery time.

TABLE 5 Adverse events detailed. Overview of treatment-emergent adverse events in Part 1&2.

Treatment	Organ system	Patients affected	TEAE	Frequency
		PART 1		
5 mg	General disorders	6/6	· Injection site erythema	23
			· Injection site swelling	9
			· Fatigue	1
			· Feeling cold	1
	Nervous system disorders	3/6	· Headache	3
			· Somnolence	1
	Eye disorder	1/6	· Ocular hyperaemia	1
	Musculoskeletal and connective tissue disorders	1/6	· Muscle strain	1
	Skin and subcutaneous tissue disorders	1/6	· Contact dermatitis	1
15 mg	General disorders	6/6	· Injection site erythema	38
			· Injection site swelling	11
			· Injection site pruritus	6
			· Injection site pain	5
			· Injection site haematoma	1
	Nervous system disorders	2/6	· Headache	1
			· Dizziness	1
	Gastrointestinal disorders	1/6	· Toothache	1
25 mg	General disorders	6/6	· Injection site erythema	37
			· Injection site swelling	22
			· Injection site pruritus	18
			· Injection site pain	12
			· Injection site warmth	1
			· Increased energy	1
	Nervous system disorders	2/6	· Paraesthesia	1
			· Somnolence	1
	Gastrointestinal disorders	1/6	· Diarrhea	1
	Psychiatric disorders	1/6	· Flat affect	1
		PART 2		
25 mg	General disorders	11/11	· Injection site erythema	77
			· Injection site swelling	72
			· Injection site pain	3
			· Injection site paraesthesia	2
			· Injection site haematoma	
			· Injection site irritation	2
			· Malaise	
			· Chest pain	1
				1
				1
	Infections and infestations	5/11	· Nasopharyngitis	3
			· Root canal infection	1
			· Lice infestation	1
	Nervous system disorders	3/11	· Headache	3

TABLE 6 Plasma PK analysis SBT-02. Results from non-compartmental PK analysis of SBT-020 in plasma.

Parameter	n	Median	SD	Min	Max
			DAY 1		
			5 MG COHORT		
C_{max} (ng/ml)	6	199.00	56.23	174.00	311.00
T_{max} (h)	6	0.62	0.25	0.50	1.00
T_{lag} (h)	6	0.00	0.00	0.00	0.00
$AUC_{0\text{-}last}$ (ng*h/ml)	6	861.47	221.21	601.29	1203.44
			15 MG COHORT		
C_{max} (ng/ml)	6	886.50	169.89	603.00	1020.00
T_{max} (h)	6	0.75	0.21	0.50	1.00
T_{lag} (h)	6	0.00	0.00	0.00	0.00
$AUC_{0\text{-}last}$ (ng*h/ml)	6	3374.82	942.05	2770.29	5260.16
			25 MG COHORT		
C_{max} (ng/ml)	6	973.00	165.33	858.00	1310.00
T_{max} (h)	6	0.75	0.23	0.50	1.03
tlag (h)	6	0.00	0.00	0.00	0.00
$AUC_{0\text{-}last}$ (ng*h/ml)	6	4618.57	489.18	4241.35	5475.74
			DAY 7		
			5 MG COHORT		
C_{max} (ng/ml)	6	211.50	55.14	132.00	276.00
$AUC_{0\text{-}last}$ (ng*h/ml)	6	817.12	279.30	597.96	1325.97
$AUC_{0\text{-}imf}$ (ng*h/ml)	6	882.24	281.19	653.23	1342.77
Terminal T½ (h)	6	2.99	0.55	2.70	4.17
V_F (L)	6	23.98	5.02	19.65	32.05
			15 MG COHORT		
C_{max} (ng/ml)	6	726.00	165.63	494.00	956.00
$AUC_{0\text{-}last}$ (ng*h/ml)	6	3301.97	733.75	2660.93	4510.37
$AUC_{0\text{-}imf}$ (ng*h/ml)	6	3332.43	743.28	2704.67	4583.36
Terminal T½ (h)	6	3.90	0.28	3.64	4.31
V–F (L)	6	24.40	5.82	17.78	34.45
			25 MG COHORT		
C_{max} (ng/ml)	6	1135.00	240.00	897.00	1610.00
$AUC_{0\text{-}last}$ (ng*h/ml)	6	5220.46	396.11	4805.33	5754.24
$AUC_{0\text{-}imf}$ (ng*h/ml)	6	5267.38	396.52	4871.52	5801.97
Terminal T½ (h)	6	4.00	0.39	3.80	4.68
V–F (L)	6	27.77	3.69	23.64	34.08

TABLE 7 Urine PK analysis SBT-02. SBT-020 urine PK analysis of day 1 and day 7 of all active cohorts in Part 1.

Time (h)	n	Median_Ae (ng)	SD_Ae (ng)	Mean_Ae (ng)	Median_Fe (%)	SD_Fe (%)	Mean_Fe (%)
5 MG COHORT							
5.833	6	1018333	400502	1084116	20.370	8.011	21.683
11.833	6	273721	105752	301853	5.475	2.115	6.035
23.833	5	91615	64678	110647	1.830	1.291	2.212
TotalSum	6	1351659	356971	1478175	27.030	7.146	29.562
15 MG COHORT							
5.833	6	5249174	1094235	5050779	34.995	7.293	33.673
11.833	6	1200864	370868	1221642	8.005	2.473	8.142
23.833	5	352929	124618	333208	2.350	0.833	2.220
TotalSum	6	6581210	1111212	6550094	43.870	7.409	43.665
25 MG COHORT							
5.833	6	8369072	2423278	7747313	33.475	9.693	30.988
11.833	6	1427505	606069	1458579	5.710	2.423	5.833
23.833	6	401561	274109	445017	1.605	1.097	1.780
TotalSum	6	9898159	2875633	9650909	39.590	11.502	38.602

Ae = amount excreted, Fe = fraction excreted.

TABLE 8 Neurocognitive and motor function. Summary of neurocognitive and motor function PD results of Part 2.

Parameter	Placebo	25 mg SBT-020	Treatment P-value	25 mg SBT-020 Placebo	placebo	25 mg SBT-020
Total score of SDMT paper task	40	38	0.1915	-1.9 (-5.0, 1.1) p=0.1915	2.2	0.2
Stroop: Number incorrect answers card 3	3	3	0.9195	00.1 (-1.4, 1.5) p=0.9195	-0.3	-0.2
Stroop: Time completing card 1 (sec)	68	65	0.4605	-2.8 (-10.6, 5.0) p=0.4605	1.9	-0.9
Stroop: Time completing card 2 (sec)	87	83	0.1960	-4.6 (-11.9, 2.6) p=0.1960	0.6	-4.1
Stroop: Time completing card 3 (sec)	135	133	0.8797	-1.6 (-24.0, 20.7) p=0.8797	-5.8	-7.4
Stroop: Difference in time card (3-2) (sec)	49	48	0.9149	-1.3 (-26.3, 23.7) p=0.9149	-4.2	-5.5
Total errors TMT visual Scanning	1	0	0.0435	-0.5 (-0.9,-0.0) p=0.0435	0.5	0.0
Time TMT visual Scanning (sec)	27	24	0.2316	-2.7 (-7.2, 1.8) p=0.2316	-0.8	-3.4
Total errors TMT Digit Sequencing	0	0	0.3789	-0.2 (-0.6, 0.2) p=0.3789	0.0	-0.2
Time TMT Digit Sequencing (sec)	42	42	0.9119	0.6 (-9.8, 10.9) p=0.9119	-8.0	-7.4
Total errors TMT Letter Sequencing	0	0	0.1707	-0.4 (-0.9, 0.2) p=0.1707	0.1	-0.3
Time TMT Letter Sequencing (sec)	46	46	0.9478	-0.4 (-11.5, 10.8) p=0.9478	-6.1	-6.5
Total errors TMT Letter-Digit Sequencing	1	1	0.6886	0.2 (-0.7, 1.1) p=0.6886	0.0	0.2
Time TMT Letter-Digit Sequencing (sec)	119	125	0.6695	5.2 (-19.7, 30.1) p=0.6695	-1.6	3.5
Time TMT Motor Speed (sec)	34	34	0.9329	-0.4 (-9.0, 8.3) p=0.9329	-4.0	-4.4
VVLT: Word recall correct 1	5.2	5.8	0.5005	0.61 (-1.24, 2.45) p=0.5005	-1.03	-0.42
VVLT: Word recall correct 2	6.9	8.3	0.1438	1.32 (-0.49, 3.14) p=0.1438	-0.48	0.84
VVLT: Word recall correct 3	8.8	10.1	0.2732	1.29 (-1.10, 3.69) p=0.2732	0.04	1.33
VVLT: Delayed word recall correct	4.0	6.2	0.1969	2.12 (-1.20, 5.44) p=0.1969	-0.33	1.79
VVLT: Delayed word recognition correct	20.1	21.7	0.4398	1.52 (-2.52, 5.56) p=0.4398	-0.40	1.13

TABLE 8 (*Continuation of previous page*)

Parameter	Placebo	25 mg SBT-020	Treatment P-value	25 mg SBT-020 Placebo	placebo	25 mg SBT-020
VVLT: Delayed word recognition RT correct (msec)	993.9	1066.2	0.3601	72.27 (-89.02, 233.55) p=0.3601	-2.36	69.91
SART total commission errors	10.2	9.0	0.4220	-1.13 (-4.02, 1.76) p=0.4220	1.34	0.21
SART mean RT correct	453.6	440.2	0.5321	-13.36 (-57.29, 30.57) p=0.5321	12.77	-0.59
SART total omission errors	13.0	12.3	0.8535	-0.71 (-8.67, 7.24) p=0.8535	-1.96	-2.67
SART post error slowing	0.3005	0.2844	0.8436	-.01609 (-.18442, 0.15225) p=0.8436	0.03848	0.02240
SART RT variability	0.3488	0.3205	0.4351	-.02830 (-.10257, 0.04598) p=0.4351	0.02201	-0.00629
SART total error score	23.1	21.4	0.6517	-1.72 (-9.59, 6.14) p=0.6517	-0.68	-2.41
Adaptive tracking (%)	16.20	16.51	0.8286	0.307 (-2.626, 3.239) p=0.8286	-1.394	-1.088
UHDRS: Total Motor Score	23	22	0.7335	-1.0 (-6.7, 4.8) p=0.7335	0.4	-0.6
UHDRS: Total Functional Capacity	9	9	0.9832	0.0 (-1.4, 1.5) p=0.9832	0.6	0.6
Tapping: Mean of 5 trials (taps/10 sec)	48.35	47.56	0.7411	-0.782 (-5.664, 4.100) p=0.7411	-2.509	-3.291
Saccadic eye movements: Inaccuracy (%)	8.4	7.5	0.4248	-0.93 (-3.33, 1.48) p=0.4248	0.36	-0.57
Saccadic eye movements: Peak Velocity (deg/s)	417.9	409.4	0.6510	-8.48 (-47.47, 30.51) p=0.6510	-27.62	-36.10
Saccadic eye movements: Reaction Time (sec)	0.255	0.274	0.0674	0.0192 (-0.0015, 0.0400) p=0.0674	0.0022	0.0214
Smooth Pursuit (%)	36.5	37.6	0.6208	1.08 (-3.41, 5.57) p=0.6208	-1.19	-0.11
Body sway (mm)	723.4	700.5	0.7987	-3.2% (-25.4%, 25.7%) p=0.7987	0.6%	-2.6%
IC50 of PMBCS in CAR concentration (mM)	0.445	0.485	0.4681	0.0401 (-0.0740, 0.1542) p=0.4681	0.0533	0.0935
IC50 of PMBCS in VER concentration (mM)	0.632	0.667	0.6327	0.0353 (-0.1173, 0.1879) p=0.6327	0.0534	0.0887
IC50 of ΔΨm in CAR concentration (mM)	0.348	0.329	0.6748	-0.0183 (-0.1082, 0.0716) p=0.6748	0.0765	0.0582
IC50 of ΔΨm in VER concentration (mM)	0.573	0.589	0.8434	0.0161 (-0.1522, 0.1845) p=0.8434	0.0298	0.0459

TABLE 9 **PK timepoints.** Timepoints of plasma samples for PK.

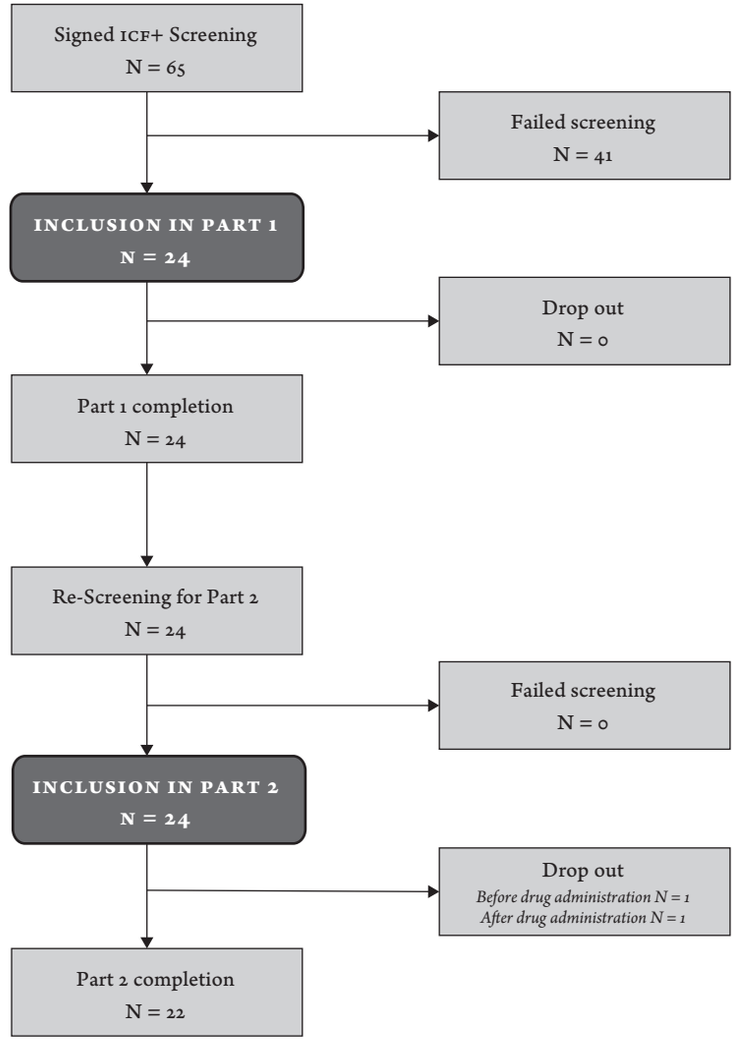
PART 1	
Day	**Time relative to drug administration**
Day 1	-15 min +30 min +45 min +1 h +2 h +4 h +6 h +8 h +10 h +24 h
Day 7	- 15 min +30 min +45 min +1.25 h +2.5 h +4 h +6 h +8 h +10 h +24 h

PART 2	
Day	**Time relative to drug administration**
Day 1	-1 h
Day 7	-15 min
Day 14	-15 min
Day 21	-15 min
Day 27	-5 min +1 h
Day 28	+1 h

TABLE 10 **Neurocognitive and motor test battery.** List of neurocognitive and motor tests and outcome parameters.

Test	Function evaluated	Method	Outcome parameter	Ref
NEUROCOGNITIVE ASSESSMENTS				
Symbol Digit Modalities Test (SDMT)	Speed of processing	Pairing symbols to numbers according to a preset key. A higher score indicates a better performance.	Total number of correct responses in 90 seconds.	26
Stroop test	Information processing and executive functioning, especially cognitive control and inhibitory processes	Color, word and interference tasks were used to determine. Higher scores indicate a better performance.	Total number of correct responses in 45 seconds per trial	41
Trail Making Test (TMT)	Attention and cognitive flexibility: perceptual processing, visual scanning, attention, executive functioning (response inhibition, set-SHIFTing), processing speed, and working memory	Connecting numbers and/or letters in ascending order.	Completion time in seconds and number of errors for each trial	42
Visual Verbal Learning Test (VVLT)	Various components of learning (including acquisition, consolidation, storage, and retrieval of memories).	Recall of words	Total number correct	31
Sustained Attention to Response Task (SART)	Attentional control.	Patients have to press a button when the number 3 appears on the screen, but withhold a response if 0-2 or 4-9 is shown 43 .	The total number of (commission and omission) errors and the mean reaction time of all correct response trials	16 17
Adaptive tracking	Pursuit tracking, in which the neocortex, basal nuclei, brain stem and cerebellum are involved.	Tracking a moving dot on a screen, using a joystick.	average performance (%)	44
MOTOR ASSESSMENTS				
Finger tapping task	Motor activation and fluency	Computerized finger tapping task (adapted from the Halstead Reitan Test Battery)	Mean tapping rate and standard deviation	45
Saccadic eye movements	Motor activation and fluency	Capturing eye movement following a horizontally moving light (jumping side-to-side) on a computer screen.	Saccadic reaction time (seconds), saccadic peak velocity (degrees/second), saccadic inaccuracy (%)	28 29
Smooth pursuit	Motor activation and fluency	Capturing eye movement following a continuously moving light on a computer screen.	Percentage of time the eyes are in smooth pursuit of the target (%)	28 29
Body sway	Postural stability	Standing still with eyes closed, measuring sway with the Celesco® string potentiometer	Antero-posterior sway (in mm)	25

FIGURE 1 **Subject allocation.** Flow chart patient disposition Parts 1 and 2.



ICF = informed consent form.

FIGURE 2 A-B **Plasma SBT concentrations.** 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.

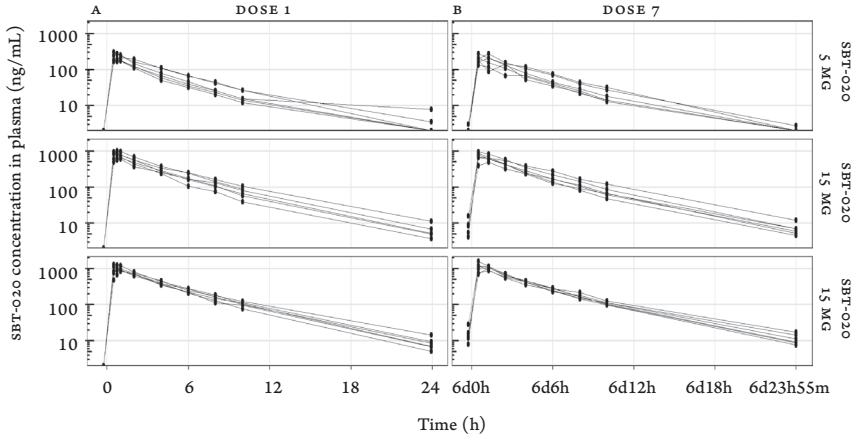
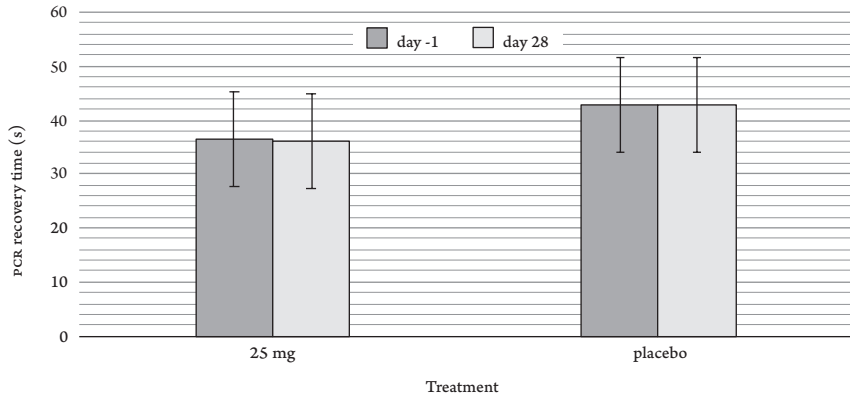


FIGURE 3 **Peripheral mitochondrial function.** Effect of daily administration of 25mg SBT-020 in Part 2 on peripheral mitochondrial function, measured with 31P-MRS. No differences between placebo and SBT-020 were observed.



PCr = phosphocreatine, Pi = inorganic phosphate, 31P-MRS.