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Physical Activity in Subacute Stroke (PHYS-Stroke)

Biomarkers And Perfusion – Training-Induced changes after Stroke (BAPTISe)

Final Analysis

Statistical Analysis Plan

Version 1.0 (September 1st, 2017)

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Table of contents

Abbrevations

AE	Adverse Events
ANCOVA	Analysis of covariance
ADL	Activities of daily living
BI	Barthel-Index
BMBF	Bundesministerium für Bildung und Forschung
BMI	Body mass index
CRF	Case Report Form
EVE	Einverständniserklärung (informed consent)
FAC	Functional Ambulation Category
GS	Gait Speed
HR_{max}	Maximum Heart Rate capacity
PHYS	Fitness training Intervention (experimental condition)
PP	Per Protocol
RELAX	Relaxation training intervention (control condition)
SAE	Serious Adverse Events
V1	Visit 1 (Post-Intervention)
V2	Visit 2 (3 months after index stroke)
V3	Visit 3 (6 months after index stroke)

1 Introduction

1.1 Preface

Due to modern therapies an increasing number of stroke patients survive the initial stroke event. From all sequelae of a stroke, the most severe consequences for patients, their families, and the society are restrictions of walking ability and restrictions of activities of daily living such as dressing, self-care, and communication. Therapeutic approaches, such as physiotherapy or occupational therapy are often lengthy and not always successful. Therefore new therapeutic approaches are tested. A promising approach is a fitness-training which is possible even in patients with severe stroke due to body weight-supported treadmill training.

1.2 Purpose of the analyses

These analyses will demonstrate the efficacy and safety of a physical exercise training intervention in stroke patients in the subacute phase.

2 Study Objectives and Endpoints

2.1 Study Objectives

The aim of the PHYS-STROKE[1] trial is to evaluate if a 4-week physical exercise fitness-intervention (PHYS: target intervention) compared to a relaxation intervention (RELAX: control intervention) results in a significantly higher gait speed (in m/s, 10m walk) as well as a better performance of activities of daily living (assessed by the BI) in subacute stroke patients. The underlying physiological changes induced by the interventions will be analyzed in BAPTISe[2], a prospective observational study accompanying PHYS-STROKE. The target and the control intervention are additional interventions to the standard rehabilitation program of each study center.

2.2 Endpoints

Co-Primary endpoints:

- Gait speed (10 m walk) 3 months after stroke.
- BI 3 months after stroke.

Because each primary endpoint can characterize a clinically meaningful benefit of the intervention on its own, we use the “or decision rule”, meaning that the study is regarded as successful in case that

one of the two primary endpoints is significantly better in the intervention group compared to the control group 3 months after stroke.

Secondary endpoints:

1) Gait speed (10m walk) and BI immediately after 4-week Intervention and 6 months after index stroke.

2) The following parameters immediately before and after intervention, as well as 3 months and 6 months after index stroke (or at the time points, where the parameters were assessed, see table 1):

   Functional measures: motor function and mobility (gait endurance: 6-minute walk test (6MWT) including spirometric assessment); Rivermead Mobility Index (RMI), Rivermead Motor Assessment Subdomain Arm; Box and Block-Test (BBT), Medical Research Council Scale (MRC), Resistance to passive movement (REPAS) spasticity scale, Use of walking aids; gait quality (cadence and stride length of walking); modified Rankin scale (mRS); Physical fitness (average number of steps in 24 hours assessed by accelerometer).

   Cognitive measures and questionnaires: cognition [Montreal Cognitive Assessment (MOCA); Trail Making Test A and B; Regensburger Wortflüssigkeitstest (RWT; testing semantic and phonemic word fluency)], quality of life (EQ-5D-5L), sleep (Pittsburgh sleep quality index), mood (Center for Epidemiological Studies Depressions-Scale (CES-D)), Freiburg questionnaire on physical activity (short version).

   Therapeutic time: Total time of therapy (including physiotherapy, occupational therapy, speech and language therapy, neuro-psychological therapy) (Logbook; measured by therapists); Time in rehabilitation center (in days).

   Clinical assessment / vital signs: Systolic/diastolic blood pressure, heart rate, body weight, BMI, waist-to-hip ratio. Assessment of endothelial function (augmentation index (AI) and reactive hyperemia index (RHI), assessed with the EndoPAT2000).

   Laboratory parameters (fasting blood draw): Full blood count, total cholesterols, high density lipoprotein (HDL), low density lipoprotein (LDL), oxidized low density lipoprotein (oxLDL), triglycerides, lipoprotein (a); insulin, glucose, homeostatic model assessment (HOMA) -index, HbA1c, inflammatory markers (e.g. hs-CRP, TNFα, IL-6, IL-11, IL-1b, neopterin), markers of endothelial function (e.g. Asymmetric dimethylarginine (ADMA), vascular endothelial growth factor (VEGF), endoglin, e-selctin, p-selectin, apelin, vascular adhesion molecule-1 (VCAM-1),

intercellular adhesion molecule-1 (ICAM-1), stromal cell-derived factor 1-alpha (SDF-1alpha), endothelial microparticle/microvesicle including proteomics), coagulation parameters (e.g. fibrinogen, INR, PTT), liver enzymes (e.g. AST, ALT, GGT), kidney parameters (e.g. creatinine, eGFR, cystatin c), heart and muscle parameters (e.g. Troponin, CK, CK-MB), hormones (e.g. Prolactin, FSH, LH, IGF-1/Somatomedin, insulin-like growth factor binding protein-3 (IGFBP-3)), steroid-derivates (cortisol, testosterone, estrogen), fibrinogen, neurotrophic facors (brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), granulocyte colony-stimulating factor (GCSF), nerve growth factor (NGF)); astroglial markers (e.g. glial fibrillary acidic protein (GFAP)), as well as other biomarkers: e.g. leptin; uric acid; matrix metalloproteinase-9 (MMP9); Omega-3-index (share of DHA/EPA in Erythrocyte membrane), and hair cortisol concentration.

Magnetic resonance imaging (MRI) parameters: lesion size and infarct growth (DWI-ASPECTS, volumetric analyses), hemorrhagic transformation, recurrent infarction/hemorrhage, blood-brain-barrier disruption, HARM sign, FLAIR hyperintense vessel (FHV) sign, white matter hyperintensities (e.g. Fazekas score, Wahlund score), microbleeds, recanalization rates, cerebral perfusion (CBF, CBV, MTT, TTP) using different modalities (e.g. dynamic susceptibility contrast (DSC), arterial spin labeling (ASL), and T1 book-end approach), microvascular morphology assessed by vessel size index (VSI), vessel size imaging including microvessel densitiy-related quantity (Q), anatomical measurements (e.g. volumetric analyses with voxel based morphometry (VBM) and tractography analyses with diffusion tensor imaging (DTI)), and functional connectivity using resting state analyses.

3) **Safety parameters (severe adverse events)**: monitored continuously throughout the trial, and recorded at baseline, end of intervention , 3 month and 6 months post stroke:

- recurrent fatal or non-fatal cardiovascular or cerebrovascular events
- referral to an acute hospital
- death.

4) **Safety parameters (adverse events)**: self-reported events assessed after each session during intervention time:

- pain

- fatigue
- dizziness
- number and nature of falls
- other.

5) **Intervention (PHYS or RELAX) diaries**: time spent in active phase of intervention each day; heart rate pre and post each intervention; blood pressure (systolic/diastolic) pre and post each intervention; if applicable: walking aid used during intervention; if applicable: treadmill or gait trainer used for intervention; if applicable: amount of body weight support in percent; rating of perceived exertion (visual analog scale)

3 Study Methods

3.1 General Study Design and Plan

PHYS-STROKE is a prospective randomised controlled trial. The target intervention is a physical fitness training. The control intervention is a relaxation programme. BAPTISe is a prospective observational study accompanying PHYS-STROKE.

Time schedule: The total time frame for this study is 4 years and 6 months. The time schedule is as follows:

- month 0-2: presentation of study in study centers, training of raters,
- month 3-39: recruitment of patients (30 months in total)
- month 3-45: baseline- and follow-up visits
- month 45-48: statistical analysis and preparation of scientific publications

3.2 Study Course

Screening and Recruitment

Patient screening and recruitment will be conducted at the rehabilitation departments of the study centers.

Each patient with a stroke diagnosis, who is admitted to a study center during the study period will be registered in a *screening-log* with a screening number keeping the patient identifiers confidential.

Each patient is tested for eligibility by the neurologist in charge. The screening process does not include study-related additional examinations. If a patient is a possible study participant, he or she or his legal representative will be comprehensively informed about the scope of study. After sufficient time for consideration the patient and, if necessary, his/her legal representative will provide informed consent. Patient will simultaneously have the opportunity to additionally participate in the BAPTISe study. If the patient is not eligible for study participation or is not willing to participate, the reason for non-participation will be documented. These patients will not be part of the full analysis set.

Clinical examinations and study related measures

Baseline-Visits

The baseline-visit will be done by the study assessor (a trained and intervention-blinded experienced physiotherapist, occupational therapist, or sport-scientist). During the baseline-visit the following characteristics and parameters will be recorded: socio-demographic information including education, body height and weight, and detailed information on stroke type and location. Additionally the primary and secondary endpoints will be measured. Patients who also participate in the BAPTISe study will receive an MRI (including contrast agent), additional blood draw, and an assessment of endothelial function. For the assessment of hair cortisol, 10 mg of hair segment need to be analyzed. Here, a strand of hair is separated at the back of the head and cut as close to the skull as possible. Hair probes are wrapped in tinfoil. The baseline-visit is study-related.

If exclusion criteria are revealed during the baseline-visit, this will be documented and the study participation of this patient will subsequently be terminated. These patients will not be included in the full analysis set but regarded as *screening failure*.

Intervention:

The intervention will start on the first weekday after the baseline-visit. The time period of the intervention is 4 weeks in total. The intervention will take place on weekdays on a daily basis, i.e., 20 intervention sessions, each lasting 50 minutes. The intervention is study-related.

Study arm 1: Intervention PHYS – training

A PHYS training intervention will be administered.

a) if Functional Ambulation Category (FAC) 0-2: performed in the gait trainer

b) if FAC 3-5: performed as treadmill training, with or without partial bodyweight-support, depending on the needs of the patient.

To transfer a cardiovascular training effect, 50-60% of the maximum heart rate (HR_{max}) should be reached during the training. Therefore, the target heart rate per minute (target-HR; 50-60% of HR_{max}) will be calculated using the formula "180-age" (in years). In case of beta blocker medication intake, the target HR will be reduced by 10 beats per minute. Heart rate during training is controlled via a pulse sensor (Polar FT1 HRM) as well as a screen attached to the treadmill. A graded increase of belt speed, reduction of body weight support and/or inclination will be used to elicit adequate cardiovascular stress to induce an aerobic training effect. During aerobic training, patients will wear a modified parachute harness to prevent falls. The body weight will be either unsupported or supported to a maximum of 15% of body weight according to individual needs. If necessary, one or two therapists will provide help with setting the paretic limb or assisting weight-shifting, and hip and knee extension.

Each session will start with a warm-up phase of 3 minutes, followed by 20 minutes exercise at the target-HR, and a 2 minutes cool-down phase.

Study arm 2: Intervention RELAX – training

Relaxation sessions will avoid any cardiovascular active exercise and aim for <30% of maximum heart rate. Relaxation sessions will involve relaxation and contraction of different muscle groups such as in the face, head, shoulders, arms, legs, chest, back, and abdomen, guided by the therapist. With eyes closed and in a consecutive pattern, the patient is encouraged to concentrate on the sensation of relaxation such as feelings of warmth and heaviness. This progressive training helps the participant achieve physical and mental relaxation and calmness, and does not convey any cardiovascular active training, but the same amount of additional attention by the therapist (to avoid a Hawthorne effect). Cardiovascular response monitoring during the RELAX intervention is identical to that done in the PHYS group.

Further visits directly after intervention (V1), +3 months (V2) and +6 months (V3) post stroke:

- Assessment of primary and secondary endpoints as well as safety parameters (V1 – V3)
- Blood samples (V1-V3)

- Patients who also participate in the BAPTISe study will receive an MRI (including contrast agent), will provide additional blood sample, and obtain an assessment of endothelial function t (V1)

Study participants who also consented to BAPTISe receive a cerebral MRI (including application of contrast agent) with a 3 Tesla MRI scanner (Trio, Siemens) before and after the study intervention. Imaging sequences include standard stroke MRI assessment (FLAIR, DWI, TOF, Perfusion), vessel size imaging for the estimation of microvessel density (Q) and vessel size index (VSI) to assess neo-vascularization of the brain, diffusion tensor imaging (DTI) to determine fractional anisotropy, T1 to assess volume, and a BOLD-signal in resting condition to determine resting state functional connectivity.

3.3 In – and exclusion criteria and general study population

Inclusion criteria for patients

1) Diagnosis of stroke (within 5–45 days after stroke); ischemic or hemorrhagic (cortical, subcortical, brainstem), as determined by initial MRI/CT scan of the brain)
2) Age ≥18 years
3) Able to sit for at least 30 seconds (unsupported or supported - that is, holding onto supports such as the edge of the bed)
4) BI ≤65 at time of inclusion
5) Considered able to perform aerobic exercise, as determined by responsible physician
6) Provision of written informed consent

Additional information for the inclusion criteria:

To reduce the influence of spontaneous recovery a timeframe of 5 – 45 days post stroke onset was chosen for the inclusion time period. To achieve comparability between the patients regarding the severity of functional deficits the BI (as an index for adhering to activities of daily living, range 0 - 100) must be ≤65.

Exclusion criteria for patients

1) Patient considered unable to comply with study requirements
2) Stroke due to intracranial hemorrhage primarily due to bleeding from ruptured aneurysm or arteriovenous malformation
3) Progressive stroke
4) Unable to perform the required exercises due to a) medical, b) musculoskeletal, or c) neurological problems (for details see below, 4a-c)
    a. medical problems: unstable cardiovascular condition, or other serious cardiac conditions (for example, anyone meeting New York Heart Association Class IV criteria, hospitalization for myocardial infarction or heart surgery within 120 days, severe cardiomyopathy or documented serious and unstable cardiac arrhythmias)
    b. musculoskeletal problems: restricted passive range of motion in the major lower limb joints (that is, an extension deficit of >20° for the affected hip or knee joints, or a dorsiflexion deficit of >20° for the affected ankle)
    c. neurological problems: severity of stroke-related deficits
5) Required help of at least 1 person to walk before stroke due to neurological (for example, advanced Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis) or non-neurological (for example, heart failure, orthopedic problems) co-morbidities
6) With life expectancy of less than 1 year as determined by the responsible physician
7) Drug or alcohol addiction within the last 6 months
8) Significant current psychiatric illness defined as psychosis, schizophrenia, acute suicidality, or affective disorder unresponsive to medication including bipolar affective disorder
9) Current participation in another interventional trial

Additional information for the exclusion criteria:

Neurological, medical (particularly cardiovascular), or orthopedic exclusion criteria are necessary to conduct a feasible training intervention without harming the patient.

3.4 Randomization and Blinding

Each participant is assigned to one of two groups, either the PHYS group or the RELAX group by block-randomization with stratification for center, age (≤65 years, >65 years), and severity (FAC 0–3, 4–5). Age and severity might influence functional outcome, and will therefore be included as stratification factors. Moreover, given the pragmatic approach for the application of PHYS training, and given that

different physiotherapists will administer the training in each rehabilitation site, study center will be another stratification factor. The allocation of patients to the two groups (PHYS or RELAX group) will be conducted online by using the web-based randomization tool of the Institute of Medical Informatics, statistics and documentation, Medical University of Graz, Austria; available at http://www.randomizer.at.

Only the patients and the respective therapists who conduct either the PHYS training or the RELAX training have knowledge of the allocation to the intervention.

The study assessors who conduct all baseline and follow-up visits, the MRI study team as well as the study statistician are blinded to the intervention allocation (endpoint-blinded trial).

3.5 Study variables

Table 1: List of all assessments and timing of assessment:

	Screening	Baseline day 0-day 7 (or day 8-day 15*)	Intervention (next working day)	V1 (+1 Mo)	V2 (+3 Mo)	V3 (+6 Mo)
In-/Exclusion criteria	**X**					
Neurological and medical examination	**X**					
Randomisation		**X**				
4 week intervention, daily documentation			**X**			
Documentation of neurological status, grade of disability, medications, type of stroke etc.		**X**				
Gait speed for 10 m walk		**X**		**X**	**X**	**X**
Barthel-Index		**X**		**X**	**X**	**X**
Gait endurance (6-Minute Walk Test)[3]		**X**		**X**	**X**	**X**
Spirometry		**X**		**X**	**X**	**X**

Walking aids	X	X	X	X
Gait parameters (cadence, stride length)	X	X	X	X
Rivermead Mobility Index[4]	X	X	X	X
Rivermead Motor Assessment Subdomain Arm	X	X	X	X
Box and Block Test[5]	X	X	X	X
Medical Research Council Scale	X	X	X	X
REPAS spasticity scale[6]	X	X	X	X
Montreal Cognitive Assessment[7]	X	X	X	X
Trail Making Test A and B	X	X	X	X
Regensburg semantic and phonemic word fluency test	X	X	X	X
modified Rankin Scale (mRS)	X	X	X	X
EQ-5D-5L	X	X	X	X
Pittsburgh Sleep Quality Index	X	X	X	X
Depressions-Scale (CES-D)[8]	X	X	X	X
Freiburg questionnaire on physical activity (short version)				X
Total time of therapy (incl. physiotherapy, occupational therapy, speech and language therapy, neuro-psychological therapy)	X	X	X	X
Laboratory parameters	X	X	X	X
Hair sampling[9]	X			
Spirometry	X	X	X	X
Actimetry for 24 hours	X	X	X	
Actimetry for 7 days				X
Systolic/diastolic blood pressure	X	X	X	X

Heart rate	**X**		**X**	**X**	**X**
Body weight	**X**		**X**	**X**	**X**
Waist-to-hip ratio	**X**		**X**	**X**	**X**
Time in rehabilitation center in days	**X**		**X**	**X**	**X**
MRI incl. vascularization and blood sample (optional; BAPTISe study)	**X**		**X**		
Safety (SAEs)	**X**	**X**	**X**	**X**	**X**
Safety (AEs)		**X**			

* if day 8-15, inclusion and exclusion criteria have to be checked again.

Table 2: Description of study variables:

Gait speed on 10m walkway	Measured in m/s, range: 0-2000, (interval-scaled)
Barthel-Index	Assessment of activities of daily living in 10 domains with each 0, 5, 10 or 15 points, Index comprises summation of all points, range: 0-100 points (ordinal)
Gait endurance over 6 min	Distance in m, range 0 – 1000, (interval-scaled)
Walking aids	Walking aids used during 10 m walk and 6 min walk test, categories in advancing order (assisting person + four point cane, four point cane, cane, walking frame, nothing) (ordinal)
Cadence	Steps per minute during 10m walk, range 0 – 100, (interval-scaled)
Stride length	In meters, Stride length is determined by dividing gait speed by cadence, range 0 – 3, (interval-scaled)
Rivermead Mobility Index	15 items addressing mobility with yes/no rating (1/0) Index comprises number of last successful item, range: 0-15 (ordinal)

Rivermead Motor Assessment Subdomain Arm	15 items addressing mobility of the paretic upper limb with yes/no rating (1/0), Index comprises number of last successful item, range: 0 – 15 (ordinal)
Box and Block Test	Number of correctly moved stones per arm (left and right side) within 60 sec, range: 0 – 100 (ordinal)
Medical Research Council Scale (Force)	Rating of force in the paretic lower limb compared to the non-paretic by the assessor, range: 0 – 5 (ordinal)
REPAS spasticity scale	Rating of resistance against external movement of the upper and lower limbs; 8 items for the upper extremities, 5 items for the lower extremities, 7 sum scores, range: 0 – 4 (items), 0 – 104 (sum scores), (ordinal)
Montreal Cognitive Assessment	Testing of cognitive impairments in 7 domains, Index comprises of summation of all points, range: 0-30
Trail Making Test A and B	Time to complete the test tasks is measured in sec range: 1 – 301
Regensburg semantic and phonemic word fluency test	Number of correctly constructed words in 4 categories, range: 0 – 150 (ordinal)
modified Rankin Scale (mRS)	Index to assess functional impairment, Range: 0 – 6 (ordinal)
Health Questionnaire EQ-5D-5L	Assesses health related quality of life in 5 items, range: 1 – 5 (nominal) as well as a visual analog scale, range: 0 – 100 (ordinal)
Sleep Questionnaire PSQI	Assesses sleep quality in 10 items, which are comprises in 7 components and 1 sum score, range: 0 – 21 (ordinal)
Depression-Scale (CES-D)	20 ordinal Items (range: 0-3), sum score range: 0-60
Freiburg questionnaire on physical activity	8 items addressing overall physical activity, sum score in kcal/day; range: 0 – 10.000 (interval-scaled)

Total time of therapy (incl. physiotherapy, occupational therapy, speech and language therapy, neuro-psychological therapy)	Time in min, range 0 – 10.000 (interval-scaled)
Laboratory	Parameters with respective unit/range (interval-scaled)
Hair cortisol	Cortisol pg/mg, range: 0 – 1000 (interval-scaled)
$V0^2$peak	VO^2 in ml / min, range: 0 – 10.000 (interval-scaled)
Gait economy	VO^2 in ml / min * m, range: 0 – 100 (interval-scaled)
Mean number of steps within 24 h	Steps per day, range : 0 – 50.000 (interval-scaled)
Mean number of steps within 7 days	Steps per day, range : 0 – 50.000 (interval-scaled)
Mean activity per day	Activity counts, range : 0 – 50.000 (interval-scaled)
Systolic / diastolic blood pressure	in mmHg, range: 0 – 500 (interval-scaled)
Pulse	Beats per minute, range: 0 – 400 (interval-scaled)
Weight	Body weight in kg, range: 0 – 400 (interval-scaled)
Waist-to-hip ratio	Ratio of waist to hip circumference in cm, range: 0 – 4 (interval-scaled)
Length of stay in rehabilitation clinic	In days, range: 0 – 400 (interval-scaled)
Volume of brain structures (Lesions, Hippocampus, Thalamus, etc.)	In ml (range: 0 – 2000) or Voxel (range: 0 – 100.000); (interval-scaled)
Fractional anisotropy	Range: 0 – 20 (interval-scaled)
Safety parameter (adverse events)	Number and sort of events, (ordinal)

4 Sample size

A total of 172 patients (86 patients per group) will enter this study. Because of the analysis of two primary outcomes, the significance level was Bonferroni-corrected ($\alpha = 0.05/2 = 0.025$).

First co-primary endpoint: change in BI at 3 months follow-up compared to baseline. We took a German “repetitive locomotor therapy” study (20 minutes on gait trainer + 25 minutes physiotherapy per day five times per week for 4 weeks)[10] as the basis for the power calculation for the outcome

measure BI. A difference of 10 points on the BI is considered a clinically meaningful difference[11]. With a power of 80%, PHYS-STROKE will detect a group difference at a two-sided 0.025 significance level, if the mean difference of improvement on the BI between groups is 10 points if 86 patients in each group will be included. This is based on the assumption that the common standard deviation of the response variable is 21 (at 3 month follow-up).

Second co-primary endpoint: change in gait speed at 3 months follow-up. A difference of 0.1 m/s on short distance walks is considered a clinically meaningful difference[12,13]. A sample size of 86 in each group will have 87% power to detect a difference between groups in means of improvement in gait speed of 0.13 m/s (mean improvement in intervention group of 0.31 m/s and mean improvement in control group 0.18 m/s[10], assuming that the common standard deviation is 0.25 using a two group t-test with a 0.025 two-sided significance level. Drop out was estimated at 25%, resulting in a trial with a total of 215 subjects. This is considered feasible for the six large recruiting centers over a period of 2.5 years recruitment. Group differences for the co-primary endpoints will be analyzed using analysis of covariance (ANCOVA) with baseline measures as covariates with random effects (random intercept) to account for clustering of patients within centers. Here, the t-test was used for sample size estimation in spite of the intended analysis with an ANCOVA model. It can be shown[14] that this is a conservative approach for estimating samples sizes for ANCOVAS, because an ANCOVA with (1 – p2)*n subjects has the same power as a t-test with n subjects where p is the variance deflation factor, calculated by the correlation of baseline and follow-up measures. Assuming the worst case of p = 0 leads to the sample size based on the t-test.

PHYS-STROKE is powered to detect a difference in gait speed of 0.13 m/s, and a difference in BI of 10 points between the two groups.

5 General considerations

5.1 Timing of Analyses

All analyses will be conducted after plausibility testing and after closing of data base by data management. Details of plausibility testing are described in a data validation report (Data validation Report Version 1.0).

5.2 Analysis Populations

Full analysis population: Each patient who received at least one day of intervention treatment will be analyzed irrespective of further adherence within the full analysis set. Only patients who withdraw

informed consent will be excluded from the full analysis set. The main question addressing the efficacy of the trial is analyzed on the basis of this data set. If any reasons for not including a subject arise or the informed consent is withdrawn before first intervention day the patient is considered a screening failure and is not included in the full analysis set.

Per Protocol (PP) Population: The PP analysis comprises all patients who followed the study protocol as intended and received a V2 measurement. Time windows for the data set are described as follows:

Baseline:	within 7 days after Screening visit
V1:	within 4 days after last intervention day
V2:	+/- 14 days after calculated measurement date 90 days (3 months) after stroke onset
V3:	+/- 30 days after calculated measurement date 180 days (6 months) after stroke onset

Affiliation of each patient to one of the above mentioned data sets will be decided before unblinding.

Safety Population: The population for the safety analysis is the same as in the full analysis population.

5.3 Covariates and subgroups

All analyses will be adjusted for center, age and severity of impairment (FAC).

Possible differences in the efficacy of the intervention in pre-specified subgroups will be tested with analyses of interaction. Further subgroup analyses are conducted in an explorative manner.

Potential differences in the efficacy (secondary/exploratory efficacy analyses) of the intervention for the end points gait speed and ADL (at time points V1, V2 and V3) are analyzed for the following subgroups (subgroups defined by using baseline information):

- based on dichotomization with regard to the severity of impairment (FAC 0 – 2 & FAC 3 – 5)
- based on dichotomization with regard to mobility impairment (Rivermead Mobility Index; median split)
- based on dichotomization with regard to the time point of study inclusion (5 – 15 days vs. 16 – 45 days)
- based on dichotomization with regard to the Baseline MoCA sum score (median split)
- based on a categorization of baseline lesion volume of the BAPTISe study sub-population

- based on lesion location (cortical vs. subcortical)
- based on defined age categories (dichotomized: <70 and ≥70 years; three groups: ≤65, 66-75, and ≥75 years)
- based on categorized levels of blood-derived biomarkers (i.e., markers of inflammation, immunoreactivity, metabolism, endothelial function, hormones, and neurotrophic factors; listed above)
- based on categorized levels of MRI-based biomarkers (i.e., cerebral perfusion changes, vessel size imaging with vessel size index and microvessel density (Q), DTI, volume, and resting state analyses)

5.4 Missing data

Missing data due to missing assessments or due to attrition will be imputed by using all relevant information. Therefore multiple imputation based on 10 imputed data sets generated by the R package `mice`[15] will be used. This procedure will only be implemented if MAR (missing at random) can be assumed for the patient with missing data. To ensure this assumption, reasons for missing values will be categorized. If MAR cannot be assumed for patients, no imputation will be performed and these patients will not be analyzed at the specific time points.

Reasons for drop out will be reported, summarised and discussed with regard to possible relation to treatment.

5.5 Interim Analysis (only for BAPTISe)

Within the BAPTISe Study, a blinded interim analysis (blinded for treatment allocation) was done after recruitment of the first 24 patients. The aim of the interim analysis of the first 24 recruited patients was to identify the most promising biomarkers and to exclude less promising markers from further analysis that are not associated with the treatment defined by effect sizes for differential changes between groups of less than 0.3. Interim analyses were done using ANCOVA with baseline marker as covariate for each marker and each time point testing differences between treatment groups. In case of skewed distributed data, measures were transformed before analysis. Group differences with a partial eta square of less than 0.022 (corresponding to Cohen's d of 0.3) were considered as less promising. If at least at one time point (V1, V2, or V3) partial eta square or Cohen's d was higher than 0.022 or 0.3, respectively, for group differences, the biomarker was suggested to keep as promising. However, the final decision of which biomarker to keep in the study was based not only on statistical

arguments, but also on financial costs, feasibility and clinical relevance (e.g. leukocytes). This resulted in a final set of 50 biomarkers (leukocytes, erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, red blood cell distribution width (RDW), glucose, HbA1c, fibrinogen, aspartate transaminase (AST), alanine transaminase (ALT), Gamma-glutamyltransferase (GGT), high density lipoprotein (HDL), low density lipoprotein (LDL), oxidized low density lipoprotein (oxLDL), triglycerides, lipoprotein (a), creatinine, estimated glomerular filtration rate (eGFR), cortisol, glial fibrillary acidic protein (GFAP), insulin, homeostatic model assessment (HOMA)-index, thyroid-stimulating hormone (TSH), luteinizing hormone (LH), follicle-stimulating hormone (FSH), estradiol, tumor necrosis factor-alpha (TNF-alpha), interleukin-6 (IL-6), high-sensitive C-reactive protein (hs-CRP), brain-derived neurotrophic factor (BDNF), interleukin-1-beta (IL-1b), apelin, insulin-like growth factor binding protein-3 (IGFBP-3), creatine kinase (CK-MB), cystatin, uric acid, matrix metalloproteinase-9 (MMP9), neopterin, intercellular adhesion molecule-1 (ICAM-1), vascular adhesion molecule-1 (VCAM-1), Asymmetric dimethylarginine (ADMA), e-selectin, p-selectin, vascular endothelial growth factor (VEGF), granulocyte colony-stimulating factor (GCSF), troponin-T.

The interim analysis was exploratory and did not affect type I error.

5.6 Multicenter trial

Seven recruiting centers participated in this mulitcenter trial (Kliniken Beelitz-Heilstätten, Medical Park Berlin Humboldt Mühle, Brandenburg Klinik, Median Klinik Grünheide, Ev. Geriatriezentrum Berlin, Charité- Universitätsmedizin Berlin (CBF), Neurologische Frührehabilitation, Vivantes Klinikum Neukölln, Neurologische Frührehabilitation). To account for the clustering of patients in centers all analyses will be done by using mixed models (random intercept models) while using the first center, where the patient was treated as cluster variable.

5.7 Multiple Testing

For the analyses of the two primary endpoints, the significance level will be adjusted for multiple testing by using Bonferroni correction ($\alpha1=\alpha2=0.05/2=0.025$, two sided). For all other secondary and exploratory analyses, no adjustment for multiple testing will be applied.

6 Summary of study data

6.1 Subject disposition

Definitions

Screening Failure: A patient who withdraws or is withdrawn from the study for any reason before receiving first day of intervention will be considered a screening failure. For reporting, screening failures are divided between 'being randomized' and 'not yet randomized'. Screening Failures are neither included in the full analysis set, nor in the PP set.

Withdrawal from treatment: A patient who received less than 75% of the intended intervention treatments for any reasons or missed intervention on more than five consecutive days (see 6.4) will be considered a 'withdrawal from treatment'. If possible V2 measurements will be acquired. Those subjects will be included in the full analysis set.

Lost-to-follow-up: Patients who did not receive a V2 assessment for any reason will be considered 'Lost-to-follow-up'. Those subjects will be included in the full analysis set.

6.2 Protocol deviations

Inclusion – and exclusion criteria: Patients not meeting the exclusion criteria will be considered a screening failure and will therefore be excluded from analyses. Patients will still be included if minor violations of inclusion criteria are found such as:

- no imaging is available confirming the stroke but obvious signs of stroke are present
- subjects included earlier than 5 days post stroke, if the intervention did not start before the fifth day post stroke
- subjects included after 45 days post stroke, if the intervention started within 55 days post stroke

Patients with minor violations will be analyzed according to the allocated analysis set (full analysis set vs. PP set). To ensure that the pre-defined minor protocol violations do not influence the main findings, the PP set will be additionally analyzed without the protocol violators (sensitivity analysis).

6.3 Demographic and Baseline Variables and Medical Conditions

Sociodemographic variables: Age, gender, smoking habit (past, former, present and pack years), occupation, years of education, height.

Comorbidities: Migraine, amyotrophic lateral sclerosis, multiple sclerosis, Idiopathic Parkinson's Disease, Arterial hypertension, cardiomyopathy, myocardial infarction >120 days, peripheral artery disease, previous stroke or previous transient ischemic attack, atrial fibriallation, hypercholesteremia, thyroid disease, diabetes mellitus, coagulation dysfunction, sleep apnoe, cancer.

Medical conditions in regard to the index stroke: Date of stroke, date of latest imaging, type of stroke, affected hemisphere, affected vessel circulation, TOAST classification, thrombolytic therapy if applicable, NIHSS.

Initial symptoms after index stroke: sensory hemiparesis and affected side, motor hemiparesis and affected side, aphasia, neglect, other.

Medication: before stroke onset (self-reported), after discharge from stroke unit, during trial participation

Plus all study variables (see chapter 2.2 as well as table 2)

6.4 Treatment Compliance

Intervention: Patients were required to receive at least 75 % days of intervention. If a patient is not able to receive the intervention on a specific day, the intervention day is added after the last intervention day if possible. Additionally patients are allowed to miss training only up to five consecutive days.

7 Efficacy-Analyses

7.1 Primary Efficacy Analysis

Group differences for the co-primary endpoints will be analysed using two separate analyses of covariance for each of the outcomes (ANCOVA) with baseline measures as covariates and an additional random effect (random intercept model) to account for clustering of the patients in centres. The

outcomes at follow-up (3 months after stroke: Gait speed, BI) are the dependent variables in these analyses, and baseline scores and group are independent variables. Additionally, the analyses will be adjusted for age and severity (as assessed by the FAC) of stroke. The study is seen as successful if at least one of the two primary endpoints can be demonstrated to be significantly better in the intervention group than in the control group ("or decision rule") with a two-sided significance level of 0.025 for each of the analyses.

7.2 Secondary/exploratory Analyses

Secondary analyses will test differences between intervention and control group with regard to additional time points and end points. Differential results for specific subgroups will also be explored. All secondary endpoints will be analyzed in separate mixed models with the endpoint measures at the specific time points after intervention as dependent variables at level 1, the individuals as level 2 clusters (random intercept models), the centers as level three clusters. Independent variables will be the specific baseline measures (e.g. for analyzing gait speed as dependent variable, baseline gait speed as independent variable), time as continuous measure in days after stroke, age and severity of stroke (as assessed by FAC). The association between time and dependent measure will be checked and appropriately modelled (e.g. curvilinear associations). If necessary models will be calculated as random intercept and random slope models with an additional variance estimation for the slope of time.

Intervention immanent measures will be analyzed exploratory to evaluate how the interventions were implemented in detail.

Additionally in BAPTISe we will analyze how changes in MRI measures and blood markers are related to each other and to differences in functional outcome, in particular gait speed, BI (co-primary endpoints of PHYS-STROKE) and mRS, after adjustment for treatment exposure using mixed regression models.

8 Safety analyses

For each assessment (baseline, end of intervention, 3 and 6 months post-stroke), the following parameters will be reported separately as incidences (n, %) in total and by intervention group: recurrent fatal or non-fatal cardiovascular or cerebrovascular events; referral to an acute hospital; death. Additionally for each intervention, reports of number of patients with the presence of self-reported pain, fatigue, dizziness, falls, and note other adverse events will be given in total and by intervention group. The safety analysis will be done in the full analysis population. When calculating

the incidence of adverse events, or any sub-classification thereof by treatment, time period, etc., each subject will only be counted once and any repetitions will be ignored; the denominator will be the total population size. Group differences will be tested at different time points using Fisher's exact test. Additionally intervention documentation will be analyzed for heart rate and blood pressure response, fatigue, and self-reported exhaustion (via a visual analog scale).

9 Other analyses

For this large multicenter trial additional exploratory analyses will be conducted, some of which will not be pre-specified here in detail.

9.1 Spirometry

The analysis of the mobile spirometry data (k4b2, Cosmed) is done according to the SOP: ‚K4b2 Spirometry: Datenauswertung bei 6min Geh-Test'. Spirometric data assessed during 6min walk test is used to compute gait economy (VO^2 in ml / min * m)[16,17]. It is hypothesized that the PHYS intervention will reduce energy expenditure during walking and thus improve gait economy between baseline and V1 more than the RELAX intervention. Gait economy will be a better explanatory variable for changes in gait speed than cardiorespiratory functioning (as assessed via VO^2peak).

9.2 Accelerometry

The analysis of the accelerometer data (GT3x, Actigraphcorp) is done according to the SOP: , Akzelerometrie GT3x Analyse'. Functional data analysis as described by Goldsmith et al.[18] is used to examine the influence of the intervention on circadian activity and sleep at time point V3. The hypothesis is that participants of the PHYS intervention will be spending more time in moderate to high exhausting activities (as defined by time spent in moderate to vigorous activity) and better sleep quality (as defined by reduced time awake after sleep onset, and longer total sleep time) at time point V3 than patients of the RELAX group.

9.3 MRI analysis

The following analyses will be conducted including standard pre-processing:

Lesion analysis will be performed following standard manual by clusterize toolbox[19] with lesion volume and location at baseline and clinical outcome at timepoints V1, V2 and V3 as endpoints. It is hypothesized that lesion location but not volume of lesion will affect rehabilitation of clinical outcome.

Influence of intervention on resting state connectivity between time points Baseline and V1 will be performed using pre-processing pathways such as described in Prehn[20] et al and subsequently analyzed with CONN toolbox[21] with cognition as covariate. It is hypothesized that both interventions will influence functional connectivity but with regard to different brain networks.

Influence of intervention on fractional anisotropy between time points Baseline and V1 will be analyzed using Diffusion Tensor Imaging and ExploreDTI[22] software. Mean diffusivity and partial anisotropy will be computed in the corticospinal tract and correlated to motor recovery.

Volumetric measurement of areas indirectly affected by the lesions will be conducted using co-registration to a standardized atlas[23,24] and correlated to functional recovery. It is hypothesized that PHYS-Training will improve re-organization of remaining cortical and subcortical areas, and consequently modulate volume of those areas.

9.4 Audio data analysis

Cluster analysis[25] of verbal fluency (audio data of the Regensburger Wortflüssigkeitstest) will be conducted . Exploratory analysis on recovery of word production as expressed by time between switching of semantic clusters and its correlation to motor recovery will be performed.

10 Figures and tables

To adhere to the guidelines of reporting scientific findings data will be presented at least with the following figures and tables

10.1 Tables

Baseline Characteristics:

	All **n =**	**PHYS Training** **n =**	**RELAX Training** **n =**
Demographics			
Age (years), mean (SD), median (IQR)			
Sex (n, % female)			
Education (years), mean (SD)			
Occupation (n, % in categories)			
Previous stroke (n, %)			
Stroke type (n, % in categories)			
Area of stroke (n, % in categories)			
Thrombolysis (n, %)			
TOAST (n, % in categories)			
NIHSS, median (IQR)			
Medical history			
Neurological comorbidities (n, % in categories)			
Medical comorbidities (n, % in categories)			
Other comorbidities (n, % in categories)			

Smoking, (n, % in categories, pack years)	
Physical, cognitive exam	
Body Mass Index, mean (SD)	
Blood pressure, mmHg, mean (SD)	
modified Rankin Scale, median (IQR)	
FAC, median (IQR)	
BI, median (IQR)	
Gait speed (m/s), median (IQR)	
Need for walking aid (n, % in categories)	
EQ-5D-5L sum score, median (IQR)	
CES-D sum score, median (IQR)	
Laboratory data, mean (SD) per category	
Medication (n, % in categories)	
Intervals after index event	
Days to baseline, median (IQR)	

Days to start intervention, median (IQR)	
Initial symptoms	
Initial aphasia (n, %)	
Initial neglect (n, %)	

Clinical baseline values of both groups. Values in mean (SD, standard deviation), median and interquartile range (IQR)

Safety parameters:

Event	**Fitness Training N=**	**Relaxation Training N=**	***P*-Wert**
Serious Adverse Events			
Recurrent cerebrovascular event			
Recurrent cardiovascular event			
Referral to acute hospital			
Death			
Adverse Events			
Pain			
Fatigue			
Dizziness			
Falls (n, % in categories)			
Other			

Values in absolute numbers and (%); all AEs and SAEs will be reported for baseline and each time point (baseline, V1, V2, V3), if AEs or SAEs occur more than once in a patient at the particular time point, it is reported as one event.

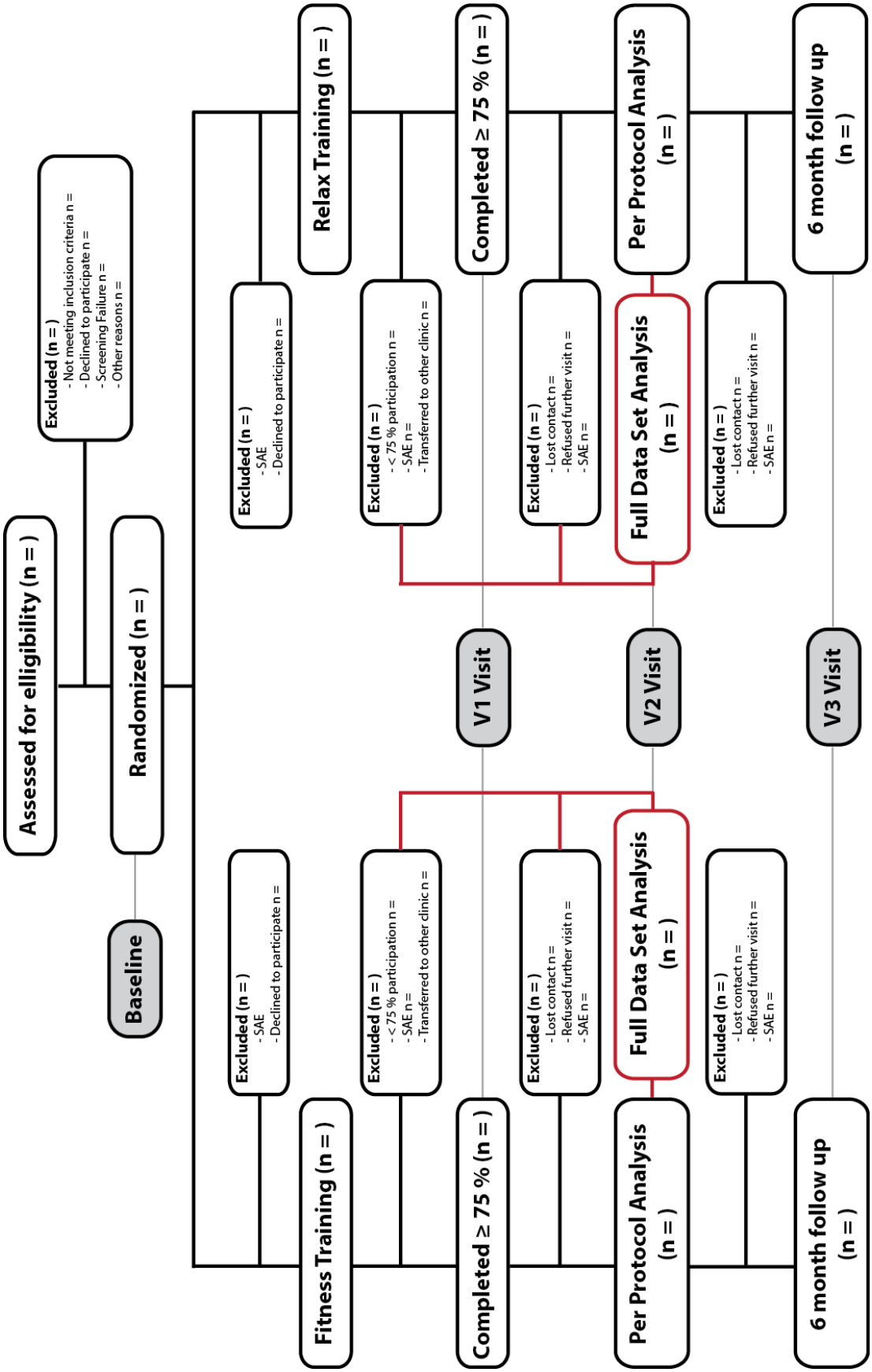
Main results:

Table title	number	population	endpoints	Time points	Covariates or subgroups	Summary statistics	Formal analysis
Summary of baseline characteristics	1	Full analysis	NA	baseline	Treatment	N (%), mean (SD), median (IQR)	NA
Summary of primary endpoint analysis	2	Full analysis /per Protocol	Gait speed, Barthel Index	Baseline, 3 months	Treatment	Mean (SD)	Linear mixed model adjusted for centre heterogeneity, random intercept, adjustment for age and severity
Summary of secondary endpoint analysis	3	Full analysis / Per Protocol	See list of secondary endpoints	Baseline, after intervention, 3 months, 6 months	Treatment, subgroups see list of subgroups above	N (%), mean (SD), median (IQR)	Mixed model (linear or other link function depending on the distribution

			of the endpoint, 3 levels: 1. Measure, 2. Individual, 3. Centre)

10.2 Figures

Flow-Chart according to CONSORT statement



For primary and secondary endpoints, boxplot of endpoints by time point and group, and additionally spaghetti plots for individual changes over time by group will be generated.

11 Summary of Changes to the protocol

In the course of the study four amendments (02.09.2013, 23.04.2015, 15.11.2016 & 21.08.2017) were approved by the ethics committee of the Charite Universitätsmedizin Berlin. The following data was added:

7 day accelerometry	Amendment 2 (Votum 23.04.2015)
Freiburg questionnaire on physical activity (short version)	Amendment 2 (Votum 23.04.2015)
Audio recording of the Regensburg semantic and phonemic word fluency test	Amendment 3 (Votum 15.11.2016)
Small administrative changes (no additional assessments)	Amendment 4 (Votum n.a. yet)

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