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Research paper

Add-on spironolactone as antagonist of the NRG1-ERBB4 signaling pathway for the treatment of schizophrenia: Study design and methodology of a multicenter randomized, placebo-controlled trial

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ABSTRACT

Background: Preclinical studies recently showed that the mineralocorticoid antagonist spironolactone acts also as an antagonist of the NRG1-ERBB4 signaling pathway and improves schizophrenia-like behaviour in Nrg1 transgenic mouse model. As this signaling pathway is critically linked to the pathophysiology of schizophrenia, especially in the context of working-memory dysfunction, spironolactone may be a novel treatment option for patients with schizophrenia targeting cognitive impairments.

Aims: To evaluate whether spironolactone added to an ongoing antipsychotic treatment improves cognitive functioning in schizophrenia.

Methods: The add-on spironolactone for the treatment of schizophrenia trial (SPIRO-TREAT) is a multicenter randomized, placebo-controlled trial with three arms (spironolactone 100 mg, spironolactone 200 mg and placebo). Schizophrenia patients are treated for three weeks and then followed-up for additional nine weeks. As primary outcome, we investigate changes in working memory before and at the end of the intervention phase. We will randomize 90 patients. Eighty-one patients are intended to reach the primary endpoint measure at the end of the three-week intervention period. Secondary endpoints include other measures of cognition, psychopathology, safety measures and biological measures.

Conclusions: SPIRO-TREAT is the first study evaluating the efficacy of the mineralocorticoid receptor antagonist spironolactone to improve cognitive impairments in schizophrenia patients targeting the NRG1-ERBB4 signaling pathway. With SPIRO-TREAT, we intend to investigate a novel treatment option for cognitive impairments in schizophrenia that goes beyond the established concepts of interfering with dopaminergic neurotransmission as key pathway in schizophrenia treatment.

Clinical trial registration: International Clinical Trials Registry Platform: http://apps.who.int/trialsearch/Trial2.aspx?TrialID=EUCTR2014-001968-35-DE

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1. Introduction

Novel treatment options for schizophrenia that are based in the pathophysiology of the disorder are lacking and pharmaceutical companies are increasingly withdrawing from the field [1]. Especially for cognitive symptoms with their known significant impeding impact on patients' quality of life, effective pharmacological treatment options are yet not available [2,3]. While positive or depressive symptoms are viewed to be effectively treatable with available antipsychotics and antidepressants [4,5], the efficacy of such compounds in the management of cognitive symptoms is not persuasive [6–11].

In that regard, one frequently discussed signaling pathway is the neuregulin (NRG1)-*ERBB4* pathway that is critically linked to the pathophysiology of schizophrenia and especially to the occurrence of working-memory dysfunction [12–15]. Notably, both the membrane-bound ligand *NRG1* and its cognate receptor *ERBB4* were identified as potential risk genes for schizophrenia [16]. A critical involvement of this pathway in the pathophysiology of schizophrenia is further supported by post-mortem findings showing an increased expression of *NRG1* [17,18], a relationship between *ERBB4* splicing and parvalbumin interneuron activity [12], and a *NRG1* induced hyperphosphorylation of *ERBB4* [19]. Mouse models with overexpressed *NRG1*-levels show schizophrenia-like behavior like increased hyperactivity, reduced social interaction and cognitive impairments [15,20–22]. Remarkably, these deficits were reversible when *NRG1*-overexpression was turned off [23]. This effect was accompanied by restoring normal synaptic functioning in glutamatergic axon terminals [23], supporting the idea that such therapeutic interventions in the adult brain may also restore impaired synaptic functioning [24]. Related to this finding, it has been shown that NRG1 is clearly required for normal behavior, that this pathological effect can be reversed, and that overexpressed *NRG1*-levels as a consequence of gain-of-function mutations are risk factors for schizophrenia in mouse models [25].

In light of the evidence that *NRG1-ERBB4* pathway represents a promising target for a new therapeutic concept in schizophrenia [19] and that the repurposing of existing drugs offers a fast track to clinical applications, we recently published a preclinical study. In this preclinical study, we applied a drug repurposing strategy to identify compounds that can improve schizophrenia-relevant behavioral phenotypes in an Nrg1 transgenic mouse model. We screened the NIH-NCC compound library of approved drugs for chemical modulators that cause changes in the activity of *NRG1-ERBB4* signaling, using a cell-based assay [26]. From this screen, the mineralocorticoid antagonist spironolactone was recovered as inhibitor of *ERBB4* activity and reduced phosphorylation levels of *ERBB4* both in vitro in human heterologous T-47D cells and in vivo in Nrg1 transgenic mice [26]. Spironolactone has been introduced for the treatment of heart failure or hyperaldosteronism more than 50 years ago. In our preclinical study, spironolactone caused an increase of enhanced inhibitory neurotransmission in organotypic slice cultures, supporting an *ERBB4* mediated mode-of-action in inhibitory interneurons. To test behavioral improvements, Nrg1 transgenic mice were chronically treated with spironolactone and tested in experimental paradigms reflecting schizophrenia-relevant phenotypes in rodents. Notably, spironolactone-treated Nrg1 transgenic mice displayed improved aspects of positive symptoms as well as improvements in working memory functions [26]. SPIRO-TREAT is the first study evaluating the efficacy and safety of the mineralocorticoid receptor antagonist spironolactone added to an ongoing antipsychotic treatment to improve working memory deficits in patients with schizophrenia.

2. Materials and methods

2.1. Study design

SPIRO-TREAT is a multi-centre trial with three German sites involved. The study is designed as a prospective, randomized, placebo-controlled, double blind, three-arm trial with two arms investigating an active compound (spironolactone 100 mg or 200 mg) and one placebo arm. The clinical trial has been approved by the local ethics committees and the medical regulatory authorities in Germany (Federal Institute for Drugs and Medical Device (Bundesinstitut für Arzneimittel und Medizinprodukte (BfArM)). Prior to the inclusion of the first patient, the study was registered in the EU Clinical Trials Register (https://www.clinicaltrialsregister.eu/ctr-search/search?query=eudract_number:2014-001968-35) and the International Clinical Trials Registry Platform (ICTRP, http://apps.who.int/trialsearch/Trial2.aspx?TrialID=EUCTR2014-001968-35-DE).

2.2. Study sites

The following clinical trial sites are involved: Coordinating site: Department of Psychiatry and Psychotherapy of the Ludwig-Maximilian University Munich (Coordinating Investigators (CIs): P. Falkai; A. Hasan), Department of Psychiatry and Psychotherapy of the Technical University Munich (Principal Investigator (PI): S. Leucht) and Department of Psychiatry and Psychotherapy of the University of Regensburg (PI: B. Langguth). All involved investigators performing patients' ratings are trained for a standardized evaluation of the patients.

2.3. Study population

Inclusion criteria are defined as follows: 1) In- and outpatients (men and women) aged between 18 and 65 with a primary diagnosis of schizophrenia according to ICD-10 confirmed by the Mini-International Neuropsychiatric Interview [27]. 2) Participants are able to sign informed consent, 3) must receive a stable antipsychotic treatment for at least one week, 4) must not be treated with more than two antipsychotics, 5) must have a PANSS total $\leq$75, 6) must have a duration of illness of at least six months. 7) Female participants must have a negative pregnancy test (serum) at baseline and must use a method of contraception that is medically approved by the health authority.

Exclusion criteria are: 1) Incapacity to give informed consent, 2) suicidality or endangerment of others, 3) severe somatic or neurological comorbidities 4) history or assumption of relevant non-compliance that interferes with the ability to participate in a clinical trial, 5) current antipsychotic treatment with clozapine or an antipsychotic with exclusive renal elimination (e.g. amisulpride), 6) planned initiation of a treatment with an antidepressant or mood stabilizer during the intervention period (a prior treatment with an non-renal eliminated antidepressant or a mood-stabilizer other than lithium is permitted), 7) diagnoses of drug dependency other than tobacco or caffeine within the last 6 months prior to inclusion, 8) history of seizures (only relevant for the physiological investigation with transcranial magnetic stimulation (TMS)), 9) documented intolerance to a treatment with spironolactone or placebo capsules, 10) acute kidney failure or anuria, or severe kidney insufficiency (creatinine clearance $<$ 30 ml/min per 1.73 m^2 or serum creatinine $>$ 1.8 mg/dl), 11) clinically relevant hyperkalemia or hyponatremia, 12) clinically relevant hypotension (RR $<$ 100/80 mmHg), simultaneous use of potassium-sparing diuretics, ACE inhibitors, ATII-antagonists, non-steroidal anti-inflammatory drugs (NSAID), thiazide diuretics, carbenoxolone, digoxin or neomycin, 13) coercive treatment 14) treatment-resistant or treatment-naïve schizophrenia, 14) insufficient understanding of German language, 15) pregnancy and 16) absent safe and approved methods of contraception.

2.4. Intervention

Participants are randomized into either one out of two interventional groups (spironolactone 100 mg or 200 mg per day) or one control group (placebo) and are treated for three weeks (see Fig. 1). In contrast to the preclinical study – where a higher dose of spironolactone was administered to mice each day and which corresponds to a calculated average

dose of 400 mg spironolactone for patients [26] – we use lower dosages of 100 mg and 200 mg for this proof-of-concept trial. The reasons for this approach are that the application of 400 mg/day would need a long titration period in humans and that such a dose would significantly increase the risk for life-threatening hyperkalemia and potential cardiac complications (especially in combination with QTc-prolonging antipsychotics). In every group, participants receive two identical capsules per day to maintain the blind. In group I (spironolactone 100 mg), participants receive at the first day 1 capsule with 50 mg spironolactone and 1 capsule with placebo and from day 2 to day 21 two capsules with 50 mg spironolactone in each per day. In group II (spironolactone 200 mg), participants receive at the first day one capsule with 50 mg spironolactone and one capsule with placebo, at day 2 two capsules with 50 mg spironolactone (100 mg in total), at day 3 one capsule with 50 mg spironolactone and one capsule with 100 mg spironolactone (150 mg in total) and from day 4 to day 21 two capsules with 100 mg spironolactone per day (200 mg in total). In group III (placebo), participants receive every day two capsules with placebo. A discontinuation of the allocated intervention, as well as the need for a long-term dose reduction result both in a drop-out. Adherence is assessed by counting the capsules and strategies to improve the adherence include the conduction of at least three study visits per week during the intervention period and the possibility to contact patients via phone in cases of no show-up. Patients who discontinue medication or drop-out for other reasons are offered to perform the V10 visit

Central randomization was performed block wise stratified by study centre at MSZ using nQuery Advisor 7.0. The randomization list was then forwarded to the pharmacy of the University Hospital Munich for labeling and distributing of blinded study medication. Randomization will take place as the patients are assigned the next available patient number in chronological order and receive the corresponding medication kit, which already contains the correct blinded medication. Safety envelopes for emergency unblinding are available at all centres and the integrity of the envelopes is monitored until study end. In order to preserve the allocation concealment, the randomization sequence will not be shared with the study personnel. Please see Table 1 for the synopsis of study visits and assessments.

Unblinding of study medication before database hardlock may only occur on an individual basis if the information can help treat an (S)AE and for safety reasons. The decision to unblinding is at the discretion of the investigator. For this purpose, investigators will have sealed envelopes comprising the information on the type of medication stored used for a given randomization number. All sites will have one sealed envelope for each randomization number. In the case of a medical emergency as described below, the envelope must be opened and the treatment assignment of the respective patient will be unblinded.

Study medication will be applied as an add-on to standard care as defined in the study design. The concurrent medication will be documented in the case report form (CRF). If a second antipsychotic is used, a maximum of 1000 chlorpromazine equivalents should not be exceeded and the target symptom (e.g. sleeping disorder) is to be defined precisely. Lorazepam (max. 3 mg/d), lormetazepam (max. 3 mg/d), diazepam (max. 20 mg/d), zopiclone (max. 7.5 mg/d) and biperiden (max. 4 mg/d) are approved as concurrent medication. The initiation of antidepressants or antiepileptic drugs in the three-week intervention period is not allowed.

The study flow-chart is presented in Fig. 2. The pre-screening and the screening phases can take place in the two weeks prior to randomization. The total study period after randomization is 12 weeks for every patient, including 3 weeks of intervention and 9 weeks of naturalistic follow-up.

2.5. Endpoints and endpoint rationale

The *primary endpoint* is change in working memory performance assessed by the n-back test (2-back level, hit rate) before and after the intervention period. The n-back test is a well-established method for examining working memory [28] and impairments in working-memory are core symptoms of schizophrenia [29]. In SPIRO-TREAT we use a computerized (Presentation Version 16.5, https://www.neurobs.com/) n-back paradigm with three loads (0-back, 1-back, 2-back). Each load is presented in six separate blocks with 14 trials each. On a standard computer screen participants are presented with a number from 1 to 4 every 1800 ms (with numbers being displayed for 400 ms) and asked to press the respective of four response buttons to either the number currently presented (0-back), or the number from the trial before (1-back), or the number from two trials before (2-back). For 0-back there are 14 pre-defined targets are defined per experimental block (with a total of 84 targets), for the 1-back - 13 targets (with a total of 78 targets), and for the 2-back - 12 targets (with a total of 72 targets) [30]. Experimental blocks occur in a pseudorandomized order and prior to each block the instruction for the following task is presented. The sums of hits, errors and missing trials for each load, as well as the respective reaction times are recorded. More details regarding the used n-back are described elsewhere [30].

Secondary endpoints include the change after the intervention period in the remaining n-back test results (hit rates, error rates, and reaction times) and the change after the follow-up period in n-back performance and in other cognitive functions. The verbal declarative memory assessed by the German version of the Rey Auditory Verbal Learning Test (Verbaler Lern-und Merkfähigkeitstest, VLMT) [31], the complex visual scanning, motor speed, the ability to shift strategies assessed by the Trail-Making-Test A (TMT-A) and TMT-B [32] and measures of sustained and selective attention assessed by d2-attention test [33] were used for further cognitive testing. Psychopathological measures include the change in Positive and Negative Syndrome Scale (PANSS) [34] and the frequency of remitters according to the Andreasen criteria [35]. Depressive symptoms are assessed using the Calgary Depression Scale for Schizophrenia (CDSS) [36], disease severity using the Clinical Global Impression scale (CGI) [37] and general functioning using the Global Assessment of Functioning scale (GAF) [38]. Differences between both active study groups in all outcome measures are assessed. Secondary endpoints are assessed directly after the intervention and after the naturalistic follow-up.

Safety measures include physical examinations, electrocardiography (ECG), study laboratory, blood pressure and heart frequency, as well as

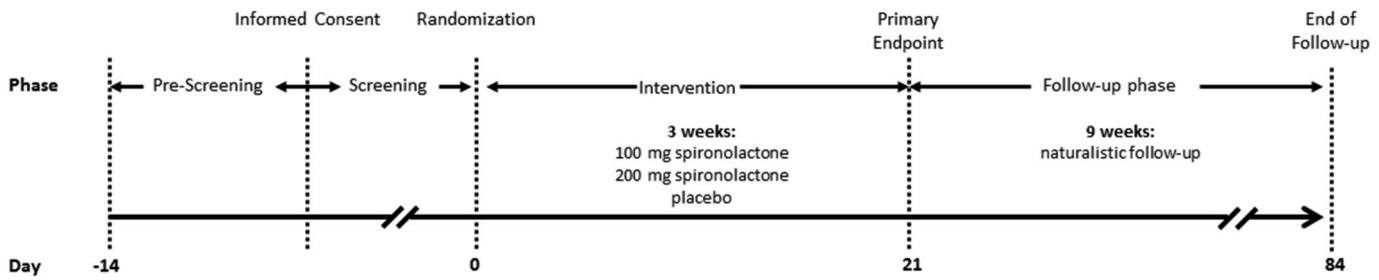

Fig. 1. Sequence of trial milestones per patient.

Table 1
Frequency and scope of study visits.

Study Visite		V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12
Phase	Screening	Baseline	Intervention									Post Intervention	Close-Out
Day	−14 bis −1	0	2	5	7	9	11	14	16	19	21	25	84
Inclusion/Exclusion criteria	X	X											
MINI-Plus interview	X												
Informed consent	X												
Demography		X											
Psychiatric history		X											
Medical history		X											
Randomization		X											
(Serious) adverse events		X	X	X	X	X	X	X	X	X	X	X[a]	X
Co-medication		X			X			X			X		X
n-Back		X									X		X
Neuropsychology (VLMT, TMT, d2)		X									X		X
SiAS		X									X		X
PANSS	X	X	X		X			X			X		X
CDSS		X									X		X
CGI		X	X		X			X			X		X
GAF		X									X		X
ECG		X									X		X
Physical examination		X	X								X		X
Vital signs (BP, HR)	X	X	X		X			X			X	X	X
BMI, waist circumference		X									X		X
Study laboratory		X	X	X	X	X	X	X	X	X	X	X	X
mRNA (optional)		X									X		
TMS (optional)		X									X		
Pregnancy test		X											
Dispense study medication		X			X			X					
Return study medication					X			X			X		

MINI-Plus: MINI-Plus Interview for ICD-10 and DSM-IV diagnosis; PANSS: Positive and Negative Syndrome Scale in Schizophrenia; CDSS: Calgary Depression Rating Scale for Schizophrenia; CGI: Clinical Global Impression; GAF: Global Assessment Scale of Functioning; SiAS: Simpson Angus Scale for EPMS, ECG: electrocardiogram; BP: blood pressure; HR: heart rate; BMI: Body Mass Index, VLMT: Verbaler Lern-und Merkfähigkeitstest (German version of the California Verbal Learning Test); TMT: Trail-Making-Test; d2: d2-attention test, TMS: transcranial magnetic stimulation; (S)AE: (Serious) Adverse Event; mRNA: messenger RNA.
Study laboratory at baseline and V11: sodium, potassium, calcium, creatinine, glomerular filtration rate (GFR), c-reactive protein, aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyltransferase (GGT), blood count, prothrombin time, partial thromboplastin time; study laboratory V2 to V10: sodium, potassium, creatinine, blood count; study laboratory V12: sodium, potassium, calcium, creatinine.
[a] Hospitalization to a psychiatric hospital is not defined as SAE after V11.

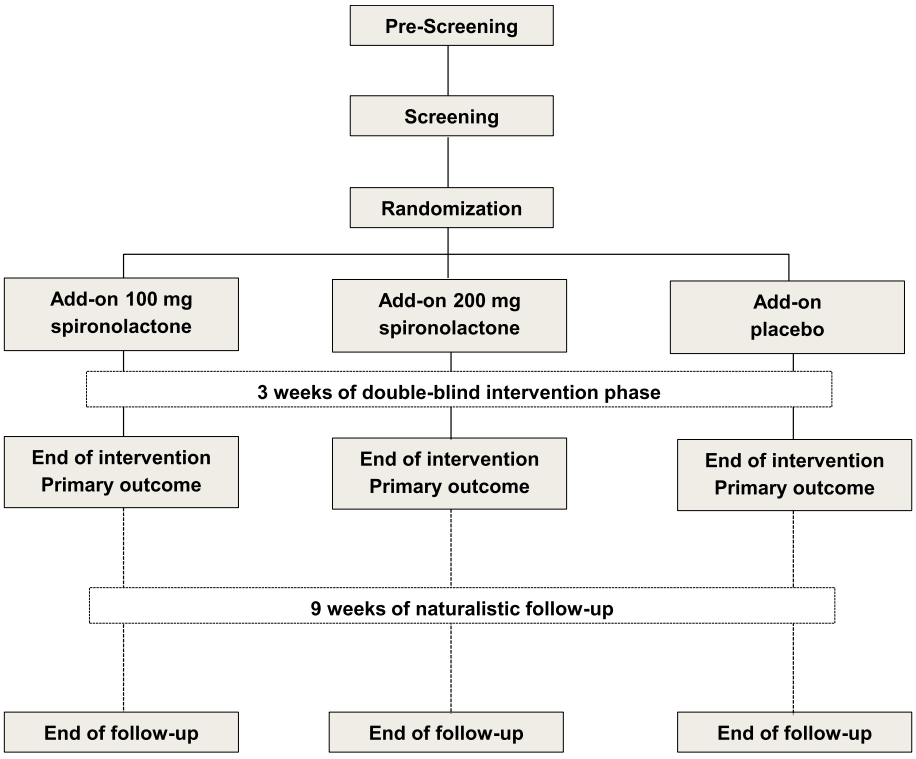

Fig. 2. Trial design.

the assessment of height, body weight and body mass index (BMI). Moreover, the Simpson-Angus scale (SiAS) is used to rate extrapyramidal side effects [39]. Due to the specific side-effect profile of spironolactone, special attention is paid regarding the assessment of potassium and creatinine levels, which are assessed every 2–3 days during the intervention period. Adverse events (AE), severe adverse events (SAE) and suspected unexpected serious adverse reactions (SUSAR) are documented following established definitions and legal requirements. The intensity of AEs is defined according to the common terminology criteria for adverse events (CTCAE Version 4.0, https://www.eortc.be/services/doc/ctc/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf). During the follow-up period (starts after V11), hospitalization to a psychiatric hospital due to the patient's schizophrenia disease was a priori defined not to be a SAE.

Exploratory biological measures include the assessment of mRNA levels in peripheral blood lymphocytes to evaluate whether the intervention modulates mRNA levels of NRG1 type I, II and III, and ERBB4-cyt1 and cyt2 variants. Moreover, cortical excitability and plasticity using TMS and transcranial direct current stimulation are assessed in the model system of the motor cortex as in vivo marker of the excitation/inhibition balance [40]. Both biological measures are optional. For TMS, patients have to sign an additional informed consent.

2.6. Sample size justification and planned data analysis strategy

SPIRO-TREAT is designed as a Phase IIb study. Since spironolactone is applied for the first time for this indication, no previous data is available to estimate the expected effect size. For the primary outcome (change of 2-back performance (sums of hits) before and after intervention), assuming repeated measures ANOVA as analysis method, a type I error probability of $\alpha = 0.05$, a power of $1-\beta = 0.8$, three groups, two measurement time points (V1, V10), and a correlation between the measurements of $r = 0.4$, medium effects of $f = 0.30$ or higher can be detected for the within-subject factor time, for the between-subject factor group, and for interactions between time and group with a total sample size of 81 (27 participants per group) at V10. These calculations were performed with G*power 3.1.7 [41].

To confirm this theoretical framework, multiple analyses on Monte Carlo simulated data for 81 subjects (27 subjects for each of the three groups) at two measurement time points (V1, V10) were performed a) using a linear mixed model and b) using repeated measures ANOVA as analysis methods. From these analyses, the power for both factors and their interactions was sufficiently high for both methods (all $1-\beta \geq 0.8$). Moreover, it resulted that the power for the linear mixed model and for repeated measures ANOVA was identical for complete datasets at V1 and V10.

The intention-to-treat (ITT) population includes all patients as randomized. Assuming a drop-out rate of 10% during the intervention period, 90 patients are planned to be randomized. The per protocol (PP) population will include all participants without major protocol violations. All primary analyses will be performed on the ITT population. For the primary endpoint, a linear mixed model will be performed and in cases of significant 'group x time' interactions, post-hoc comparisons corrected for multiple testing will be performed. Continuous secondary endpoints will be analyses using linear mixed models and subsequent post-hoc tests if the requirements for this strategy are met (tested by Kolmogorov-Smirnov tests and Levene's tests). In cases where the assumptions of normality or variance homogeneity are violated, a monotonic transformation of variables will be performed. If this first step is not successful, corresponding non-parametric tests will be used. Side-effects, AE and SAE will be analyzed with descriptive statistics and likelihood-ratio tests. Demographic information will be shown for each group separately. Dichotomous variables will be analyzed with likelihood-ratio tests and continuous variables will be analyzed using analyses of variance or respective non-parametric tests. An interim analysis is not planned. The statistician will be blinded for group during the phase of data-analyses.

2.7. Organizational framework

Organizational project management, safety management, monitoring and data management is performed by the Münchner Studienzentrum (MSZ), an academic clinical research organization at the Technical University of Munich, school of medicine.

For safety monitoring, an independent safety monitoring board (SMB) is established. The underlying principles for the SMB are ethical and safety aspects for the patients. It is the task of the SMB to examine, whether the conducting of the study is still ethically justifiable, whether safety of the patients is ensured, and whether the process of the study is acceptable. For this, the SMB has to be informed regularly about patient recruitment, and the observed AEs. Occurring serious adverse events (SAEs) will be recorded in a study specific safety form and transferred into a safety database by the safety management (MSZ), which processes further SAE documentation in written form to the SMB, the Coordinating Investigator, the ethics committee and regulatory authority (BfArM). Trial sites must be experienced in clinical trials and must have adequate study infrastructure, e.g. a 'good clinical practice' (GCP)-trained Principle Investigator (PI), PI deputy, study nurses and other trained study personal.

The documentation of the study data in adherence to the GCP-guidelines and the clinical trial protocol is the responsibility of the investigator. Original data (source documents) remain in hospital medical record and information on the case report form must be traceable and consistent with the original data. Source documents are e.g. laboratory results. Original written informed consent signed by the patient is kept by the investigator and a signed copy will be given to the patient. No information in source documents about the identity of the patients will be disclosed.

All study procedures agree with the guidelines of Good Clinical Practice (GCP) of the International Conference on Harmonization of technical requirements for registration of pharmaceuticals for human use (ICH), and the principles of the Declaration of Helsinki. All participating investigators agreed to adhere to the instructions and procedures described in the study protocol and thereby to adhere to the principles of ICH-GCP. The protocol and other required documents were reviewed and approved by the ethics committees and BfArM before study initiation. Any amendments to the protocol other than administrative ones (of which the ethics committee and BfArM will merely be informed), must be reviewed and approved by both. Before inclusion of the first patient, the federal and state authorities will be informed about the study. The safety of the study will be judged by an independent committee of experts on regular basis, at a frequency of at least once a year via personal meeting or telephone conference. The members of this board will have access to the unblinded data, to all SAEs and SUSARs and to the inclusion and drop-out rates. All members of the safety board are independent from the sponsor.

The current protocol version is 4.5 (17.10.2019, amendment 7.0). All protocol versions are available in German language at beate.schossow@mri.tum.de and alkomiet.hasan@med.uni-muenchen.de. At the time of this submission, the clinical trial is open for recruitment, which began in July 2015, and an actual number of 86 participants have been enrolled so far.

3. Discussion

The rational of SPIRO-TREAT (Add-on **spiro**nolactone for the **treat**ment of schizophrenia) is derived from recent preclinical findings showing that the mineralocorticoid antagonist spironolactone can improve schizophrenia-relevant behavioral deficits in a Nrg1-transgenic mouse model by antagonizing increased ERBB4 receptor activity [26]. These findings support the notion that spironolactone may be efficient in an add-on clinical trial in schizophrenia patients. Thus, the

SPIRO-TREAT trial investigates for the first time whether the add-on treatment with the mineralocorticoid antagonist spironolactone is safe and effective to improve cognitive impairments and other symptom domains in patients with schizophrenia. Intriguingly, such an approach follows the idea proposed by the National Institute of Mental Health (NIMH) to identify risk-factors for mental-illness based on genetic and biological research, to repurpose known compounds in preclinical and animal studies and to conduct proof-of-concept trials in patients in academia settings [42,43]. This concept of repurposing of an existing drug or biological rather than the discovery of a new chemical entity has been continuously emphasized by NIMH as a promising potential way regarding the future of drug development for severe mental disorders [42,43].

As outlined above it is unlikely that compounds with novel modes of action for the treatment of schizophrenia, especially for difficult-to-treat domains like cognitive impairments or negative symptoms, will be developed by pharmaceutical companies [1]. Moreover, promising new antipsychotic compounds with alternative modes of actions recently failed before market launch [44,45]. Thus, drug repurposing as proposed by the SPIRO-TREAT trial is one interesting possibility for an alternative pharmacological treatment approach in schizophrenia. In drug repurposing, approved drugs with a known history of their clinical application and potential side effects are tested for new uses, i.e. for new agonistic or antagonistic effects on molecular targets different from the ones they were previously developed for. A particular advantage of the repurposing of approved drugs is that these substances may therefore be immediately tested in a clinical trial, once they qualify for a novel target based on preclinical studies.

The complete concept of SPIRO-TREAT follows an academic conceptual framework of repurposing a drug from bench to bedside. First, members of our neurobiology lab (MCW, MJR) have established a co-culture assay system compatible with high-throughput-screening (HTS) utilizing the split tobacco etch virus (TEV) technology [46,47]. Second, we used this assay to identify an antagonist of ERBB4, namely spironolactone, from a library of approved drugs. Third, we were able to show that spironolactone decreases phosphorylation levels of *ERBB4* in vitro and in vivo and normalizes altered excitation/inhibition of cortical projection neurons [26]. Finally, members of our group showed that applying spironolactone in Nrg1 transgenic mice ameliorates hyperactivity, reverses sensorimotor gating and improves working memory [26]. Thus, SPIRO-TREAT can be viewed as the final step to meet the recommendations of a repurposing process that has been outlined by the NIMH for over a decade [42,43,48].

Overall, the risk-benefit evaluation pretends a short-term use in patients with schizophrenia. Long-term applications of medium to high doses of spironolactone may increase the risk for cardiac arrhythmia via high potassium levels and it could be expected, that this issue might be potentiated when combined for a longer period with antipsychotics. Furthermore, the long-term application may increase the risk for other consequences of hyperkaliemia, for gynecomastia and breast enlargement, for amenorrhea, and for blood count changes – among other relevant long-term side effects. Based on the application in transgenic mice models, which was also limited in time, and considering these outlined potential risks of long termed applications of spironolactone in patients with schizophrenia receiving antipsychotics as standard care, we therefore hypothesize that a targeted, but short-time application of spironolactone can restore altered NRG1-ERBB4 pathway activity. This will potentially lead to an improvement in working memory functions directly after and beyond the intervention phase. Thus, the overarching idea is that a deficient pathway activity can be normalized in the adult brain as it has been proposed in previous animal research [23,24]. Based on this rationale, we decided to apply the intervention for three weeks in our participants corresponding to the treatment duration in the previously published preclinical study [26].

The primary outcome of SPIRO-TREAT is the change in working memory according to the performance in n-back, precisely the change in sum of hits in the 2-back condition, as impairments in working-memory are core deficits in schizophrenia [29,49,50]. However, as schizophrenia is associated with impairments across several cognitive domains [50], SPIRO-TREAT investigates also the impact of the intervention on verbal working memory, complex visual scanning, motor speed, and the ability to shift strategies assessed as well as sustained and selective attention. These explorative assessments will allow for a comprehensive evaluation whether spironolactone is effective in improving core cognitive impairments in schizophrenia or not. Moreover, several measures of psychopathology and functioning are also accessed in SPIRO-TREAT to test whether the assumed cognitive improvement is associated to a clinical improvement.

Another important objective is the safety evaluation of high doses of spironolactone combined with antipsychotics. As most antipsychotics can induce QTc prolongation [51] and as cardiac arrhythmia due to high potassium levels are known side-effects of spironolactone, our trial will also strictly evaluate whether such an approach is safe in this population. Therefore, blood sampling is performed three times per week and ECGs are performed before and after the intervention period to allow for a close monitoring of this potentially severe treatment complication. For that reason, SPIRO-TREAT is also testing two different high doses of spironolactone, namely 100 or 200 mg per day. In the case of a benefit of spironolactone over placebo, but no dose-dependent differences in efficacy, applying a low-dose would increase the safety as severe spironolactone side effects are clearly dose-dependent.

4. Conclusion

In summary, the aim of SPIRO-TREAT is to evaluate whether the application of three weeks of spironolactone add-on to an ongoing antipsychotic treatment can improve working memory deficits in patients with schizophrenia. Upon success, this study will introduce a new therapeutic principle in schizophrenia research based on a strong genetic and biological background that is beyond the established concept of impairments in dopaminergic neurotransmission.

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Authors' contributions

AH, SL, MCW, MJR, SE, BS, SK, TSA and PF designed the study. AH, AR, MCW, IP, EW, SE and PF wrote the first draft of the manuscript. All authors revised the first draft of the manuscript and AH developed the final manuscript version. TSA is responsible for trial statistics. All authors approved the final version of the manuscript.

Declaration of competing interest

AH is co-editor of the German (DGPPN) schizophrenia treatment guideline and first-author of the WFSBP schizophrenia treatment guidelines. He has been on the advisory boards and has received speaker fees from Janssen, Lundbeck and Otsuka. In the last three years **SL** has received honoraria for lectures or as a consultant from LB Pharma, Otsuka, Lundbeck, Boehringer Ingelheim, LTS Lohmann, Janssen, Johnson & Johnson, TEVA, MSD, Sandoz, SanofiAventis, Angelini, Recordati, Sunovion, Geodon Richter. **PF** is a co-editor of the German (DGPPN) schizophrenia treatment guideline and a co-author of the WFSBP schizophrenia treatment guidelines. He is on the advisory boards and receives speaker fees from Janssen, Lundbekc, Otsuka, Servier and Richter. **MCW** und **MJR** are shareholders of Systasy Bioscience GmbH. The company has no financial interest in the study. **WS** has received speaker fees from Mag&More. **AR**, **MH**, **TOS**, **SKK**, **IP**, **LL**, **EW**, **IM**, **IB**,

TSA, **TG**, **PMK, SZ, SK, SE, BS** and **PR** report no conflicts of interest.

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