STUDY PROTOCOL

Study Title: A randomized controlled trial with Rituximab for Psychotic disorder in adults

Protocol Number: RCT-Rits	**EU CT Number:** 2024-512408-20-00
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SPONSOR AND NATIONAL COORDINATING INVESTIGATOR

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LISTED USED ACRONYMS AND ABBREVIATIONS

AAR-R = Any Adverse Reactions-Revised
AE = Adverse event
ANCOVA = Analysis of covariance
ASD = Autism Spectrum Disorder
CGI-I = Clinical Global Impression-Improvement scale
CGI-S = Clinical Global Impression-Severity scale
CNS = Central Nervous System
DMC = Data Monitoring Committee
DSM-5 = The Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition
DSUR = Development Safety Update Report
IVIG = intravenous immunoglobulin
MAP-SR = The Motivation and Pleasure - Self Report
M.I.N.I. = Mini International Neuropsychiatric Interview
MRI = Magnetic Resonance Imaging
MS = Multiple Sclerosis
NMDA = N-methyl-D-aspartate
OCD = Obsessive-Compulsive Disorder
PANDAS = Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infection
PANS = Pediatric Acute-onset Neuropsychiatric Syndrome
PANSS = Positive and Negative Syndrome Scale
PGE = Patient's Global Evaluation of severity
PI = Principal investigator
PML = Progressive Multifocal Leukoencephalopathy
PSP = Personal and Social Performance Scale
RA = Rheumatoid arthritis
RCT = Randomized controlled trial
SAE = Serious adverse event
SLE = Systemic Lupus Erythematosus
SNS = Self-assessment of Negative Symptoms
SD = Standard Deviation
SSD = Schizophrenia Spectrum Disorder
SUSAR = Serious Adverse Event Suspected Unexpected Serious Adverse Reaction
USM = Urgent Safety Measures
VAS = Visual Analogue Scale
TNF = Tumor Necrosis Factor
Y-BOCS = Yale-Brown Obsessive Compulsive Scale

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SIGNATURE PAGE

Sponsor

I am responsible for ensuring that this protocol includes all essential information to be able to conduct this trial. I will submit the protocol and all other important trial-related information to the responsible investigator(s) so that they can conduct the trial correctly. I am aware that it is my responsibility to hold the staff members who work with this trial informed and trained.

Sponsor's signature Date

Printed name

Coordinating Investigator

I have read this protocol and agree that it includes all essential information to be able to conduct the trial. By signing my name below, I agree to conduct the trial in compliance with this clinical trial protocol, the EU Regulation on clinical trials of medicinal products for human use (EU 536/2014), the Declaration of Helsinki, ICH-GCP (Good Clinical Practice) guidelines and the current national regulations governing the conduct of this clinical trial.

I will submit this protocol and all other important trial-related information to the principal investigators who participate in this trial, so that they can conduct the trial correctly. I am aware of my responsibility to continuously keep the principal investigators who work with this trial informed and trained.

Coordinating Investigator's signature Date

Printed name

Principal Investigator

I have read this protocol and agree that it includes all essential information to be able to conduct the trial. By signing my name below, I agree to conduct the trial in compliance with this clinical trial protocol, the EU Regulation on clinical trials of medicinal products for human use (EU 536/2014), the Declaration of Helsinki, ICH-GCP (Good Clinical Practice) guidelines and the current national regulations governing the conduct of this clinical trial.

I will submit this protocol and all other important trial-related information to the staff members and investigators who participate in this trial, so that they can conduct the trial correctly. I am aware of my responsibility to continuously keep the staff members and investigators who work with this trial informed and trained.

I am aware that quality control of this trial will be performed in the form of monitoring and eventual audit and inspection.

Principal Investigator's signature Date

Printed name

1 SYNOPSIS

TITLE	A randomized controlled trial with Rituximab for Psychotic disorder in adults
STUDY CENTER	Psychiatry Unit, Örebro University Hospital
AIM	To investigate whether a single dose of rituximab added to treatment as usual (TAU) in patients with schizophrenia spectrum disorder (SSD) will lead to clinically relevant overall improvement compared to NaCl (placebo) at endpoint week 12 and week 24.
OBJECTIVES	Primary objective: 1. To investigate whether psychiatric patients with schizophrenia spectrum disorder (SSD) are significantly improved after treatment with the immunomodulatory drug rituximab (anti-CD20 antibodies) compared to placebo.
OUTCOME	Primary outcome 1. Proportion of responders, defined as much, or very much improved since baseline (CGI-I) up to week 12, endpoint data could be collected within another 4 weeks. Secondary outcomes: The secondary outcome measures are 2. Symptom change, measured as change in Positive and Negative Syndrome Scale (PANSS) score from baseline up to week 12. If a patient is unavailable at week 12, endpoint data could be collected within another 4 weeks. 3. Proportion of responders, defined as much, or very much improved since baseline (CGI-I) up to week 24. 4. Improvement (CGI-I) up to week 12 and 24 compared to baseline. 5. Improvement at week 12 and 24, corresponding to CGI-I of 1 or 2 among participants defined as having treatment resistant SSD 6. Change in Personal and Social Performance Scale (PSP), measuring overall disability from baseline up to week 12 and 24. 7. Severity according to CGI-S at weeks 12 and 24 compared to baseline. 8. Improvement in PANSS up to week 24 compared to baseline 9. Differences in patient self-rated health (VAS-health) and PGE since baseline at week 12 and 24.

10. Baseline levels of inflammatory markers in relation to treatment response.
11. Safety and tolerability of rituximab during treatment for SSD.
12. Change in brain morphology and/or activity in fMRI.
13. Mental health symptom domains (Cross-cutting symptom measure of global symptom severity, CCSM) in relationship to response.
14. Improvement in the three PANSS subscales and in the PANSS Marder negative factor at week 12 and 24.

If a patient is unavailable at week 12 or 24, data could be collected within another 4 weeks.

RESPONSE CRITERIA

Treatment response is defined as a clinician rated CGI-I score of 1 or 2, corresponding to very much improved or much improved at week 12.

POPULATION

120 psychiatric patients (108 completers endpoint I)

INCLUSION CRITERIA

1. patient ages 18 to 55 years
2. duration of illness exceeding 1 year
3. diagnosed with Schizophrenia spectrum disorder (SSD) according to DSM-5
4. if female and with any risk for pregnancy, willing to use contraceptives or abstinence if normal and preferred lifestyle.
5. subjects should be judged by the investigator to be lucid and oriented to person, place, time, and situation when giving the informed consent.
6. insufficiently recovered from previous antipsychotic treatments.
7. a minimum score of 4 in CGI-S at baseline.

EXCLUSION CRITERIA

1. pregnancy or breast-feeding
2. weight below 40 kg
3. clinically relevant ongoing infection at the discretion of the physician
4. chronic infections
5. positive test for hepatitis B, hepatitis C, HIV, or TB prior to treatment
6. malignancy currently or within 2 years prior to inclusion
7. current severe heart failure (NYHA grade IV) or any other severe heart disease (e.g. or history of cardiac arrhythmia or myocardial infarction)
8. any change of antipsychotic medication within the previous 4 weeks

9. unable to make an informed decision to consent to the trial
10. ongoing clozapine treatment
11. ongoing immunomodulatory treatment
12. treatments with monoclonal antibodies within 1 year before the inclusion

STUDY DESIGN

Phase II, Proof-of-concept study.
Randomized, placebo-controlled, double-blinded intervention study.
Participants will be treated with rituximab or placebo on one single occasion and followed for 12 and 24 weeks.
If a patient is unavailable at week 12 or week 24, endpoint data could be collected within another 4 weeks.
An interim analysis will be made when 32 patients have reached endpoint I (week 12 24) and again after 64 patients.

STATISTICAL ANALYSIS

Determination of Sample Size
In this study, a sample size of 120 participants is assumed to be large enough to determine differences between groups with a power of 80% and an alpha level of 5%.

We anticipate a PANSS baseline score of 85, a mean reduction of 4 points in the PANSS score in the placebo group and a reduction of 14 in the rituximab treatment group, resulting in endpoint scores of 81 (SD=17) and 71 (SD=17), respectively. We anticipate a drop- out rate of 10 % Then, a sample size of 51 in each group will be sufficient to reach a power of 0.8. For subgroup analyses additional patients are needed.

Categorical outcome: We expect 33 % in the rituximab treated group to be much or very much improved (i.e., score 1 or 2 on CGI-I), compared to an expected 10 % in the placebo group. To reach a power of 0.8 and a significance level of 0.05 (two-sided) we need to include 47 patients in each group. To allow for a 10 % drop-out we need 52 participants per group. We aim to include a total of 120 participants to account for loss of degrees of freedom when statistically adjusting for center and prognostic factors in the model.

Primary objective: Effect of rituximab on schizophrenia symptoms measured with CGI-Improvement.
The primary outcome measure is proportion of responders according to CGI-Improvement.

In order to examine whether baseline characteristics can predict response in the rituximab treated arm, baseline values of all measurements will be compared between the two groups "responders" and "non- responders" after completion of the study using t-tests or Mann-Whitney tests, as applicable. In addition, for each measurement, a generalized linear model (ANCOVA) will be built.

Analyses of other clinical data related to clinical response:
For results on the scales (PSP, CGI-I, CGI-S, VAS-health, PANSS subscales and Marder negative factor) and response in the treatment resistant participants the week 12 scores and the Δ- values (difference between scores at week 12 and baseline) will be compared between the treatment groups using t-tests, Mann-Whitney tests and chi-square test, as applicable. They will also be included in an analysis of covariance (ANCOVA) model, controlling for baseline measures and other possible confounders, as demographic variables.

STUDY TERMINATION

LPLV – Last Patient Last Visit

2 BACKGROUND AND RATIONALE

Purpose and aims
Recent insights into the field show that aberrant immune activities may contribute to schizophrenia spectrum disorder (SSD) of different severity. Our previous studies indicate that patients can be successfully treated with potent immunomodulatory drugs. This application aims to enable an RCT with rituximab and placebo in patients with SSD.
We aim to investigate whether psychiatric symptoms of adult psychiatric patients, diagnosed with SSD will be significantly more improved after treatment with the immunomodulatory drug rituximab compared to placebo when added to their ongoing psychotropic treatment. The primary outcome measure is the Clinical Global Impressions Scale -Improvement (CGI-I).

Research questions
I: Does addition of rituximab to standard psychiatric treatment improve psychotic symptoms (treatment resistant or not)?
II: Does overall disability improve with the addition of rituximab?
III: Are clinical or biological markers related to treatment response?
IV: Is rituximab safe and tolerable for patients with SSD?

Theory and Background
1. Schizophrenia – impact and unmet needs
Schizophrenia spectrum disorder (SSD) is a mental illness with a lifetime prevalence of 1.8 % of the population (Chang et al. 2017) and often has its onset at a young age, between 18-25 years. SSD is disabling to the patient and most often, it has a lifelong negative socioeconomic impact on not only the patient but also his/her family.

The current treatment of schizophrenia is far from satisfactory. Pharmacotherapy based on antipsychotic drugs, all of which block the dopamine-2 (D_2) receptors, forms the main specific part of treatment programs. It is established that D_2 receptors mediate some key symptoms in the pathogenesis of schizophrenia. However, the most burdensome, so-called negative symptoms, do not respond well to D_2-blocking treatment. D_2 blockers are also linked to several troublesome adverse effects, e.g. extrapyramidal symptoms, sexual dysfunction and emotional numbness. Other significant side-effects that affect adherence to treatment are weight gain, diabetes and hyperlipidemia. Because of the lack of adherence, a substantial proportion is treated in compulsory care with long-acting antipsychotic injectables. There is a selective negative attitude among users to treatment with the present antipsychotic drugs. Thus, there is a need for investigating drugs with other mechanisms than D_2-blockers that may be more accepted by patients with SSD.
Treatment resistance is another important issue. Approximately 30 % of the patients will not improve from anti-psychotic drugs. Accordingly, several lines of research try to delineate the pathophysiology of schizophrenia to find other targets, thereby providing novel, more acceptable treatments (Kaar et al. 2020). During the last decade, inflammatory processes and immune dysregulation have become the main focus of aetiological research, hopefully informing the development of novel treatment paradigms (Khandaker et al. 2015, Müller 2018). And even if no anti-inflammatory treatment yet has shown clinically useful efficacy, considering the intricacies of the immune system, alternative targets such as the monoclonal antibody rituximab are worth pursuing.

1. Inflammation in psychiatric disorders

Presently, inflammation and immunological mechanisms are a major area of general psychiatric research, like in other fields of medicine. Sickness behaviour, i.e. depression-like symptoms in response to an immunological challenge, provides a link to evolutionary explanations of a neuro-immune axis (Lasselin et al. 2020). As regards major depression, an immunometabolic subtype of the disorder has recently been suggested (Milaneschi et al. 2020). Low-grade peripheral inflammation and microglia in the brain are here believed to interact with mesolimbic and corticostriatal circuits in forming mental and behavioural symptoms.

2. Inflammation and immunological factors in schizophrenia

Multiple evidence indicates a role of inflammation and immunological mechanisms in schizophrenia (Khandaker et al. 2015, Khandaker & Dantzer 2016, Müller 2018, Miller & Goldsmith 2020, Thylur & Goldsmith 2022). The association between inflammatory cytokines and schizophrenia is well established in the literature, as confirmed by systematic reviews (Goldsmith et al. 2016, Dawidowski et al. 2021). However, delineating the onset, underlying causes and immunological mechanisms leading to this inflammatory phenotype constitutes a demanding challenge, which the research community still have not mastered. Nevertheless, many intriguing findings provide clues to ways of tackling the dysregulated immunology of schizophrenia.

3.1. Genetics of schizophrenia linked to immunology

There is a strong genetic association between the major histocompatibility complex (MHC) locus and schizophrenia, established by genome-wide association studies (GWAS, Ripke et al. 2014). The expression of the genetic loci linked to schizophrenia was enriched by B-lymphocytes involved in acquired immunity (CD19 and CD20 lines). Out of multiple cell lines in the body, this B-cell enrichment was equalled only by cells from cortical brain regions, providing strong genetic support for immune dysregulation, specifically implicating B-lymphocytes as a pathogenetic mechanism in schizophrenia. Further research has shown that these associations to a great extent are driven by the complement system (Sekar et al. 2016, Cooper et al. 2017, Ji et al. 2022). This system has a crucial role in synaptic pruning during development. Excessive pruning may disable important synaptic connections in the brain, resulting in a vulnerability to neuropsychiatric disorders, including SSD (Woo et al. 2020).

Links to autoimmune, inflammatory disorders are suggested by epidemiological research (Benros et al. 2011). Intriguingly, genetic links from schizophrenia to systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA) have been localised to the MHC region, despite divergent clinical manifestations (Kamitaki et al. 2020, Zamanpoor et al. 2020).

Interestingly, two cases of bone marrow transplantation further highlight the link between immune genetics and schizophrenia: One case was reported with complete remission from treatment-resistant schizophrenia after a bone marrow transplantation (Miyaoka et al. 2017). Conversely, another reports the onset of treatment-resistant psychosis after receiving bone marrow from a donor with schizophrenia (Sommer et al. 2015).

Accordingly, immunogenetics strongly supports the immune system’s involvement in schizophrenia pathogenesis and suggests connections with chronic rheumatological diseases.

3.2. Maternal immune activation (MIA), prenatal infections and the neurodevelopmental theory

MIA, induced through prenatal exposure to one of several infectious diseases, has been implicated as a risk factor for schizophrenia (Saatci et al. 2021, Cheslack-Postava et al. 2021). Maternal infection in combination with irregular foetal immune responses to e.g. *Toxoplasma gondii* or cytomegalovirus may contribute to subsequent risk for psychosis (Blomström et al. 2015). The

role of CNS-active autoantibodies generated by influenza infection has also been suggested (Kępińska et al. 2020). Further support for MIA in schizophrenia is the increased rate of minor physical anomalies observed among these patients (Sreeraj et al. 2021).

MIA and related findings form the basis of a neurodevelopmental theory of schizophrenia, where genetic vulnerability interacts with early environmental adversities to shape the phenotype of schizophrenia, including immune dysregulation.

3.3. Cytokines in schizophrenia

Increased plasma protein levels of cytokines and cytokine mRNA expression are widely reported in schizophrenia (Goldsmith et al. 2016, Dawidowski et al. 2021).
Of the general proinflammatory cytokines, interleukin-6 (IL-6) is most consistently elevated and has been suggested as a trait marker of schizophrenia, while it also seems to correlate with symptom severity. Other proinflammatory cytokines, such as IL-1β, TNF-α and IFN-γ are also elevated in most studies. The anti-inflammatory cytokines IL-10 and TGF-β are also mostly elevated, indicating a dysregulated state of the adaptive immune system. Other cytokines reported as elevated or deviating in schizophrenia are IL-1 receptor antagonist (IL-1RA), the soluble receptor of IL-2 (sIL-2R), IL-4, IL-8 and IL-17.
Some of the cytokines, e.g. IFN-γ seem to decrease with antipsychotic drug treatment and others, e.g. IL-8, are associated with negative symptoms and treatment resistance. Associations with brain morphology, e.g. localised cortex thinning or abnormal folding, have been reported for mRNA expression of *IL6*, *TNFR1*, *TNFR2* and *IL1B* cytokine related genes. Recently, such changes in brain structure were genetically associated with IL-6 by Mendelian randomisation (Williams et al. 2022), indicating that IL-6 is of central aetiological relevance.
Our research group has found similar associations (Hylén et al. 2020). We compared a mixed sample of markedly ill psychiatric patients (N = 40) with healthy subjects regarding inflammatory-related proteins, using electrochemiluminescence ELISA and real-time qPCR technology. Fourteen of these patients suffered from schizophrenia spectrum disorder (SSD), and, in this small group, IL-1RA ($p < 0.001$), IL-18 ($p = 0.006$), IL-6 ($p < 0.002$) and TNF-α ($p = 0.005$) protein levels were significantly higher compared to matched controls. Also, the mRNA gene expression of *CASP1* (caspase-1, part of the inflammasome complex) was elevated ($p = 0.008$). Together, our data indicate that individuals with schizophrenia have increased inflammatory activity.

3.4. Autoantibodies in psychoses

Autoimmune inflammatory diseases are often characterised by antibodies directed against “self” components, so-called autoantibodies. These autoantibodies, however, are not always the central pathogenetic factor; instead, T-lymphocytes may be responsible for the main pathological processes, which is believed to be the case in multiple sclerosis (MS). Yet, in 2007, a new diagnostic entity was described, anti-NMDA-receptor encephalitis, where specific autoantibodies may diffuse across a disrupted blood-brain barrier and cause loss of function of glutamate receptors in the brain (Dalmau et al. 2011). Although patients affected by anti-NMDA-receptor encephalitis present a clinical picture resembling SSD and run a risk of being misdiagnosed (Ariño et al. 2021), research has shown that only a small minority of people with SSD have detectable antibodies against the NMDA-receptor or other CNS structures.
Accordingly, research on autoantibody mechanisms has not explained the immune dysregulation in SSD, and other mechanisms should be explored.

3.5. Recent developments in immune-schizophrenia research

A notable finding in recent research is that inflammation has a closer association with the negative

symptoms, specifically reward processing and motivational deficits, than with positive symptoms (Goldsmith and Rapaport 2020; Thylur & Goldsmith 2022). Also, the possible contributing role of latent infection with *Toxoplasma gondii* has been further studied in clinical samples (Fond et al. 2018) and linked to the kynurenine pathway and the complement system (Pearce et al. 2020, Severance et al. 2021). It has been shown that the subependymal zone of the lateral ventricles is another entry point of peripheral immune cells (North et al. 2021). The roles of astrocytes and regulatory T-cells (Tregs) have also been emphasised (Dietz et al. 2020, Corsi-Zuelli et al. 2021). Dysfunctional Tregs have been suggested as a pathophysiological mechanism that links schizophrenia to autoimmune disorders, such as MS and Rheumatoid arthritis (RA). Flow cytometry of immune cells from 40 patients with schizophrenia (Sahbaz et al. 2020) indicated dysfunctional Tregs and an increased percentage of B-cells (CD19 and CD20) compared to controls. In addition, the percentage of B-cells correlated positively with the severity of psychosis.

4. Immunomodulatory treatments

Antipsychotic drugs blocking the D_2 receptors seem to have subtle anti-inflammatory effects (Baumeister et al. 2016) although the clinical relevance of this is disputed. However, based on the inflammatory aspect of SSD, several trials with anti-inflammatory agents for SSD have been published (Rappard & Mueller 2004, Deakin et al. 2018, Sommer et al. 2021, Weiser et al. 2021, Nasib et al. 2021). The outcome with these anti-inflammatory augmentations (celecoxib, minocycline, simvastatin, aspirin and prednisolone) has been unconvincing, but according to a meta-analysis (Jeppesen et al. 2020) anti-inflammatory add-on treatment to antipsychotics did show some, albeit clinically insignificant, improvement in psychotic disorders. We suggest that these disappointing results with anti-inflammatory augmentation may be related to the emphasis on suppressing the microglial activity, rather than enhancing the Tregs.

A small number of monoclonal antibodies are presently undergoing studies for SSD (Chaves & Vieira-Coelho 2020). However, tocilizumab (IL-6-antagonist), the first monoclonal antibody to be tested for schizophrenia, was not effective (Girgis et al. 2018). Hitherto, rituximab has not been tested in SSD, apart from our open yet unpublished pilot study.

Intriguingly, clozapine the most effective antipsychotic drug when compared to the others in both treatment-resistant and non-resistant schizophrenia (Mizuno et al. 2020), has several anti-inflammatory properties (Leykin et al. 1997; Giridharan et al. 2020). It has been suggested that clozapine's immunomodulatory effects are related to its superior efficacy.

5. Effects of Rituximab on the brain

Rituximab is a monoclonal antibody that binds to a membrane protein, CD20, which is located on the surface of pre-B cells and mature B lymphocytes. The binding of the antibody to a B cell leads to cytolysis (cell death). The action of rituximab in CNS disorders is thought to be primarily associated with depletion of B cells in the periphery which subsequently leads to decreased B cell entry into the CNS (from the bloodstream), i.e. not depending on cytolytic action against B cells within the CNS.

The B cell fills multiple functions in the immune system, including as antigen presenter (activating T cells and other cells), secretion of immunomodulating cytokines, and production of antibodies. In rituximab treatment, the antigen-presenting and immunomodulating functions of the B cells are thought to be largely the property that gives rise to the treatment effect, i.e. not the elimination of the antibodies. CD20 is not expressed on hematopoietic stem cells, pro B cells (precursor to pre-B cells and B lymphocytes), plasma cells (challenged B lymphocytes producing antibodies) or in normal tissue, which means: 1) the effect of rituximab is reversible and upon completion of treatment, the patient eventually recovers its B cells, which thus regain their function in the immune system, 2) already engineered plasma cells will continue to produce

antibodies, and 3) tissues in the body that do not express CD20 are not affected. As rituximab is used on a long-term basis by thousands of people, its side effects in the short and long term are well-known.
Several findings support that there is a B and T cell involvement in SSD. In addition, communication between B and T cells clarifies how an anti-B cell therapy affects more immune system processes than just the production of antibodies. It is hypothesized that the role of the immune system in mental illness is mediated by the interaction between B and T cells, glia cells and cytokines. A possible effect of rituximab treatment may be that decreased activation via B cells contributes to the normalization of T-cell-driven inflammation in the brain, possibly through enhancement of T regulatory cells (Tregs) which in turn leads to a normalization of glial activity and eventually a decrease in symptoms. In summary, we consider that we have presented a scientifically based hypothesis that CD-20 antibodies may decrease the symptoms and improve the function of persons with SSD.

6. Why do we want to study rituximab rather than other immunomodulatory drugs?
Rituximab is a standard treatment for primary B cell type lymphoma, including CNS lymphoma. It is also a standard therapy for RA and difficult-to-treat MS. The effect of rituximab on neuroinflammation has clearly been shown by its effect in the treatment of MS, a disorder of the brain.
In 2019 we reported the case of a difficult-to-treat patient with obsessions and psychotic symptoms who fulfilled the diagnostic criteria for Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections (PANDAS). When she fell ill with an autoimmune disorder (neuromyelitis optica) she received rituximab and recovered from all psychiatric symptoms (Bejerot et al. 2019).

Between 2020 and 2022, we have run two open trials with a single intravenous dose of rituximab 1000 mg without placebo control. One study included 9 patients diagnosed with treatment-resistant SSD and the other 10 treatment-resistant patients with obsessive-compulsive disorder (OCD). Preliminary results show that seven out of nine SSD patients improved significantly at one or several time-points (12, 20 or 40 weeks), but only two of the OCD patients were much improved. One patient deteriorated, but she refused all antipsychotic drugs which presumably caused the deterioration. Rather few side-effects, apart from symptoms of running nose, a well-known side effect from rituximab, were reported by the participants. Symptoms such as anxiety and depression, also acknowledged side-effects from rituximab, were rarely reported (unpublished data). However, one of the SSD patients developed abdominal pain which was reported as a SUSAR. After examination by several surgeons, the patient was deemed as having so-called functional symptoms, i.e. no physical disorder could explain the symptoms and they remitted fully. One OCD patient deteriorated compared to her status at inclusion at the one year follow up, presumably due to long-term COVID-19 (which also a close family member of the patient developed, which suggests a genetic susceptibility). This patient had contracted COVID-19 prior to inclusion in the study and was fully recovered but relapsed nevertheless after rituximab treatment.

Rituximab is included in the treatment guidelines for anti-NMDA receptor encephalitis (Wandinger et al. 2011) and is generally well-tolerated (Edwards et al. 2004). Moreover, Rituximab targets B-lymphocytes of CD19-CD20 lineage, exactly the cells that were found to enrich the schizophrenia genome in the above-mentioned GWAS study and were associated with psychosis severity (Sahbaz et al. 2020). While rituximab selectively targets B-cells, studies have suggested that the treatment effect is largely antibody-independent and that other B-cell functions

such as antigen presentation and cytokine production contribute to the improvement of autoimmune diseases after B-cell depleting therapies (Hauser, 2015). Recent research shows that rituximab indirectly may enhance Treg function (Eggenhuizen et al. 2021) which leads to improved control of pathologically active T cells.

In the present study, we want to test if rituximab can ameliorate symptoms in patients with SSD in a placebo-controlled setting. Although the experience with immunomodulatory drugs such as corticosteroids and intravenous immunoglobulin (IVIG) is longer as compared to rituximab, they have drawbacks: corticosteroids increase the risk for mania and IVIG is a blood-derived product,

which makes its availability limited and recurrent treatments costly and complicated. Other monoclonal antibodies that are presently tested for psychosis, but yet unpublished (i.e. canakinumab (targeting IL-1β), infliximab (targeting TNF-α) and siltuximab (targeting IL-6)), are presumably not overall safer than rituximab. Finally, rituximab is regarded as sufficiently safe in large populations suffering from autoimmune disorders and likely equally safe in psychiatric patients. Accordingly, we expect the presumed benefits of rituximab to overrun the potential harm caused by this drug in patients with SSD.

Clinical significance and Originality
At present, difficult-to-treat persons with SSD are often treated with high doses of antipsychotic drugs. In spite of this many continue to suffer from their symptoms in addition to suffering from significant side effects. The inflammatory hypothesis of schizophrenia and our own experience make us predict that rituximab treatment could improve the condition.

If a controlled study can confirm the positive results from our open trial on patients with SSD, it would be nothing less than ground-breaking. We can expect effects on positive, negative and general symptoms in SSD, which otherwise is only obtained with clozapine. Clozapine, however, requires a daily intake in addition to frequent blood tests, while rituximab presumably can be administered twice yearly. Although rituximab is not without side effects, the severe weight gain and metabolic syndrome associated with clozapine treatment are not present in patients treated with rituximab.

Our experience of the treatment and the patient group
Already in 2013, we started our research on children and young adults assessed for Pediatric Autoimmune Neuropsychiatric Disorder Associated with Streptococcal Bacteria (PANDAS) and Pediatric Acute Neuropsychiatric Syndrome (PANS) (EPN in Stockholm 2014/551-31/2 and 2015 / 964-31). We interviewed 53 patients and their families in their homes across Sweden. These children and young adults had obsessive-compulsive symptoms, but approximately 25 % also suffered from psychotic symptoms and some were diagnosed with schizophrenia (Hesselmark & Bejerot 2019). Our findings are consistent with other researchers in the field of PANS suggesting psychosis to be present in 28 % of the cases (Thienemann et al. 2021). As was mentioned previously we published a case report on one of the PANDAS patients in 2019 who received rituximab and recovered.

More recently we have investigated gene expression of inflammatory markers in a pooled sample of 39 markedly ill psychiatric patients (children and adults) with diagnoses such as SSD, OCD, non-suicidal self-injury disorder and autism spectrum disorder, and compared the markers with those of healthy, age and sex-matched controls from the same area (EPN in Uppsala, 2016/091). Our data (as described above) show large differences in gene expressions between the patients and

the controls (Hylén et al., 2020) as well as elevated plasma levels of cytokines which support the notion of inflammatory activation in psychiatric patients. Our results strongly suggest that comorbid, severely ill psychiatric patients have an up-regulated immune system with an inflammatory activity that does not correspond to detectable inflammation in the peripheral body. Hence, neuroinflammatory activity is a likely explanation.

Between 2019 and 2022, we have run two open trials with a single intravenous dose of rituximab 1,000 mg without placebo control. One included 9 patients diagnosed with treatment-resistant schizophrenia spectrum disorder and the other 10 treatment-resistant patients with OCD (EPN 2019-00260 and 2019-00256), both were registered at CLINICAL TRIALS: NCT03983031 and NCT03983018). All participants were followed for one year. Primary outcome was measured at week 20. Results showed vast improvement of SSD, but less so for OCD. The mean baseline PANSS score in the SSD group was 99 (SD 32) and severity according to CGI-S was 5.8 corresponding to severely ill. At 20 weeks PANSS score had dropped to a mean of 73 (n=8, p=0.03, t=2.7, Cohen's *d* =0.94) and CGI-S had improved to 4.4, corresponding to being in between moderately and markedly ill. 4 out of 8 patients with SSD were deemed as much or very much improved according to CGI-I (one drop out) at week 20. Only one patient who refused all antipsychotic drugs, deteriorated. Intriguingly, even better response was seen in week 12 (Bejerot et al., 2023).

There is always a risk for placebo response in an open uncontrolled study, however, among the patients with OCD, only 2/10 was much improved according to the Y-BOCS. The mean reduction in Y-BOCS was only 2 points (from 27 to 25, n.s.) and the patients' overall function was likewise marginally improved. Our data suggest that the placebo response is negligible among markedly ill chronic psychiatric patients, at least those with OCD. We have no reason to believe that the placebo response differs in markedly ill patients with SSD.

Overall, our present research group consists of clinical researchers with extensive experience with patients with severe psychiatric illnesses. The PI has worked decades as a senior consultant and since 2015 assessed patients with SSD, OCD and Autism spectrum disorder at the Psychiatric Clinic in Örebro on weekly basis. Also, a PhD and collaborator in this study, has extensive clinical experience with severe treatment-resistant psychiatric patients. We will also collaborate with an immunologist and a senior consultant at the Rheumatology clinic with extensive experience with rituximab treatments. For statistical analyses and analyses of the MRIs experts are consulted. Also, we have collaborations with researchers and clinicians at other sites and universities in this study.

3. BENEFIT-RISK ASSESSMENT

Patients will share personal information, which could be perceived as intrusive. However, all collected data will be pseudonymized and data will only be presented at a group level. Blood sampling can cause pain, but only for a brief period. Lumbar puncture may cause low-back pain and headache, but it is a clinical routine, usually well tolerated. The pain is seldom long-lasting and local anaesthetics are applied prior to the lumbar puncture for all patients.

The patients' expectations of the study and its intervention may be high, which can generate disappointment among those who do not experience a positive effect from the study drug.

Side effects of rituximab treatment may occur, some of those can be serious, but rituximab is usually well tolerated. Among patients with rheumatoid arthritis, even mild side effects are reported to be rare. According to the Summary of Products Characteristics (SmPC), between 1/100 and 1/1000 patients report depression and/or increased nervousness as a side effect from rituximab. Rituximab is a standard treatment for multiple sclerosis and rheumatoid arthritis in Sweden. Approximately 5,000 patients with rheumatoid arthritis are treated with rituximab (48/100 000) and as rituximab is prescribed off label for MS another 5 to15,000 patients receive this treatment yearly for this indication (227/100,000 are diagnosed with MS, Danish data). In Örebro, rituximab is administered to the rheumatoid arthritis patients on a day-ward at Örebro University hospital, run by specially trained nurses.

The side effects of rituximab include infusion reactions, severe skin and mouth reactions, hepatitis B virus reactivation and in extremely rare cases (2.56 per 100,000 patients) (Berger et al. 2018), progressive multifocal leukoencephalopathy (PML), a serious brain infection caused by the JC virus. We will collaborate with a senior rheumatologist who is experienced with rituximab. Since the treatment may activate latent infections, all patients will be screened for hepatitis, tuberculosis, and HIV prior to the study. The patients will be pre-medicated to reduce the risk of infusion-related side effects, and they are carefully monitored during the course of the study.

Treatment with rituximab may induce an increased risk for falling ill with COVID-19. Notably, increased death rate from COVID-19 has been associated with rituximab treatment (Andersen et al. 2022; Patel et al. 2022). Therefor all patients are offered vaccination for COVID-19, at least 4 weeks prior to the rituximab treatment.
Neutrophiles count, protein fractioning, and C-reactive protein (CRP), Hb and trombocytes will be checked at baseline and both endpoints (at 12 and 24 weeks) to ensure that patients´ blood count recover after treatment. Also, if there are signs of infection (other than mild symptoms such as a running nose) neutrophiles will be checked on additional occasions.

Early onset of severe psychiatric disability is associated with a particularly poor long-term prognosis. This is often the case for schizophrenia. Delayed treatment may cause persistent brain damage if associated with inflammation in the brain. We believe that earlier treatments may have better effects on the symptoms than treatment that are first provided after many years with the disease. Also, rituximab is frequently used for treating children and adolescents with MS (Salzer et al. 2016). Thus, it is not warranted to exclude 18-year-olds from participation in a trial with a medication that may prove helpful.

The patients will remain on their standard psychiatric treatment throughout the course of the study and are allowed to adapt their medication dose accordingly. Therefore, we do not put the patient’s mental health at risk. The side effects of rituximab do not coincide with those that can be expected from psychiatric medication, which implies low risk for add-on side effects.

To conclude, SSD may cause severe suffering in the patient and his/her family members. It is also associated with poor quality of life and increased suicide risks. Accordingly, the possible benefits from treatment with rituximab must be balanced against the risk of taking the drug. Moreover, even if the treatment proves ineffective for treating psychiatric symptoms, the trial would nevertheless be valuable; we will publish the results and other research groups can take part of our findings.

4. TRIAL OBJECTIVES

Objective

To investigate whether psychiatric patients diagnosed with schizophrenia spectrum disorder (SSD) are improved after an intravenous treatment with the immunomodulatory drug rituximab (anti-CD20 antibodies) in comparison with patients receiving NaCl (placebo).
Treatment response in this study is defined as a clinician rated CGI-I score of 1 or 2, corresponding to very much improved or much improved at week 12.

4.1 Primary objective

1. Proportion of responders to treatment, i.e. rated as much or very much improved since baseline according to CGI-I up to week 12.

4.2 Secondary objectives

1. Change in symptoms measured as change in Positive and Negative Syndrome Scale (PANSS) score from baseline up to week 12.
2. Proportion of responders, defined as much, or very much improved since baseline (CGI-I) up to week 24.
3. Improvement since baseline (CGI-I) up to week 12 and 24.
4. Improvement at week 12 and 24, corresponding to CGI-I of 1 or 2, among participants defined as having treatment resistant SSD.
5. Change in Personal and Social Performance Scale (PSP) measuring overall disability from baseline up to week 12 and 24.
6. Severity according to CGI-S compared to baseline at week 12 and week 24.
7. Improvement in PANSS up to week 24 compared to baseline.
8. Differences in patient self-rated health (VAS-health) and PGE since baseline at week 12 and 24.
9. Baseline levels of inflammatory markers in relation to treatment response.
10. Safety and tolerability of rituximab during treatment for SSD
11. Change in brain morphology and/or activity in fMRI.
12. Mental health symptom domains (Cross-cutting symptom measure of global symptom severity, CCSM) in relationship to response.
13. Improvement in the three PANSS subscales and in the PANSS Marder negative factor at week 12 and 24.

If a patient is unreachable at week 2 or 7, or the clinician is unavailable we will accept extension of another 2 weeks, i.e. data can be collected no later than week 4 and week 9 respectively.

If a patient is unreachable at endpoint I or II, we will accept extension of the 12 or 24 weeks with additional 4 weeks.
Assessments of primary and secondary outcomes will be performed continuously during the trial on all participants.

4.3 Primary endpoint (variable)

1) Clinical Global Impression-Improvement scale (CGI-I) measures change of symptoms on a 7-

point Likert scale. A score of 1 is very much improved, 2 is much improved, 3 is minimally improved and 4 corresponds to no change, whereas scores of 5, 6 and 7 correspond to deterioration. The time span considered is the week before the rating. CGI-I will also be administered to the next-of-kin (Guy, 1976). In both the clinical and next-of-kin assessment, clinical response in this study is regarded as a score of 1 or 2 corresponding to very much improved or much improved. Investigators may score CGI-I into half points, but those will be transformed into whole numbers in the analyses of the data, i.e 2.5 will be transformed into a 2, 1.5 into a 1, etc.

4.4. Secondary endpoint (variables)

2) Positive and Negative Syndrome Scale (PANSS) is a measure for severity in patients with schizophrenia (Kay et al. 1987). It is widely used in the study of antipsychotic therapy and is known as the "gold standard" for the assessment of schizophrenia. To assess a patient using PANSS, an approximately 45-minute clinical interview is conducted. The patient is rated from 1 to 7 on 30 different symptoms based on the interview as well as reports from family members or caretaking healthcare workers. The time span considered is the week before the rating.
Seven items measure "positive" symptoms, 7 items "negative" symptoms, and 16 items general psychopathology. Each item is graded between 1 and 7 resulting in a total score between 30 which correspond to no symptoms and a maximum of 210 points. We will evaluate the item A6 for depression separately at baseline and endpoints in order to assess depressive symptoms.

   Improvements will be calculated by reducing the baseline scores with 30 points (which equals no symptoms) to reach a PANSS 0 score.

   The researchers who will assess the patients with PANSS will receive rater training prior to the study, to obtain satisfactory interrater reliability (Müller et al. 1998).

3) Clinical Global Impression-Improvement scale (CGI-I) week 12 and 24.

4) Clinical Global Impression-Improvement at week 12 and 24, corresponding to CGI-I of 1 or 2, among participants defined as having treatment resistant SSD. Definition of treatment response is "not sufficiently improved
   (remaining having at least moderate severity and at least moderate functional impairment) despite having tried at least two different antipsychotic drugs in the recommended dose, each for at least 6 weeks, one of which is from the second-generation antipsychotics" (Howes et al 2017).

5) Personal and Social Performance Scale (PSP) (Morosini et al., 2000) is a 100-point single-item rating scale, subdivided into 10 equal intervals. The ratings are based on the assessment of patient's functioning in four main areas: 1) socially useful activities, 2) personal and social relationships, 3) self-care, and 4) disturbing and aggressive behaviors. In responders, we expect a 25 % reduction or more in the PSP score.

6) Clinical Global Impression-Severity scale (CGI-S) is a clinician rated measure of overall clinical severity in the context of the diagnostic group. It is rated on a scale between 1 and 7. A person with no clinical complaints or problems (which is most people in a population) will get a score of

1. The score 7, which indicates the highest level of severity is phrased as “Among the most extremely ill patients”. A score of 2 is borderline ill, 3 is mildly ill, 4 is moderately ill, 5 markedly ill and 6 severely ill (Guy, 1976). In the present study we expect patients to be moderately ill (or worse) and will improve by at least 1 point at week 12 with rituximab treatment.

7) VAS-health is a patient self-evaluated health measure using a horizontal visual analogue scale ranging from 0 (= worst imaginable health) to 100 (= best imaginable health) the day of the visit. A 25 % reduction from baseline will be regarded as a self-reported treatment response measure.

Patient’s Global Evaluation (PGE) (Zohar & Judge, 1996) provides a self-administered global measure of improvement on a 7-point Likert scale identical to the CGI-I scoring system. In this study minimal improvement, i.e. a score of 3 (or below) is regarded as treatment response as individuals with SSD frequently tend to lack ability to observe improvements.

8) Baseline levels of inflammatory markers in relation to treatment response will be examined at both endpoints. Change in inflammatory markers in relation to response will also be investigated. Severity and frequencies of adverse events according to Any Adverse Reactions-Revised (AAR-R).

9) Severity and frequencies of adverse events according to Any Adverse Reactions-Revised (AAR-R).

10) Change in brain activity (e.g. blood flow and Default mode network) and morphology before and after treatment and in relation to response.

11) Cross-cutting symptom measure of global symptom severity, CCSM (APA, 2013) is a patient rated measure included in the DSM-5, which assesses mental health domains that are important across psychiatric diagnoses. It includes 13 domains and each of the items are rated 0-4 on a Likert scale. We will investigate if each endorsed domain is associated with response.

12) Improvement in the three PANSS subscales and in the PANSS Marder negative factor score (Marder et al., 1997) at week 12 and 24. The PANSS subscales include the Negative, Positive and General psychopathology subscales. The Marder negative factor includes the following PANSS items: Blunted affect, Emotional withdrawal, Poor rapport, Passive social withdrawal, Lack of spontaneity, Motor retardation, and Active social avoidance (i.e. items N1, N2, N3, N4, N6, G7, G16).

4.5 Methods for measurement of endpoints for clinical safety

Adverse events will be checked with the following questions at each visit:

- Have you noticed any new symptoms or problems since your last visit? If yes, which?
- Do you gather these symptoms can be related to a possible rituximab treatment?
- Have you noticed if any of your previous side effects have attenuated or increased? If yes, which ones?
- Have you changed your medication since last visit, if yes which ones and what are the

current doses?

Any Adverse Reactions-Revised (AAR-R) is a 26 item questionnaire, based on a report of adverse events published in a report, Relapsed or Refractory, Low-Grade or Follicular NHL, Receiving Single-agent Rituxan which includes 40 symptoms https://www.rxlist.com/rituxan-drug.htm#side_effects) and developed by the research group to identify side effects known to be related to rituximab treatment. Common side effects of rituximab include headache, fever, chills, stomach pain, nausea, diarrhea, heartburn, flushing, night sweats, weakness, muscle or joint pain, back pain, or dizziness. These items, and others, are included in the AAR-R questionnaire and for each item severity and frequency is described.

5. TRIAL DESIGN AND PROCEDURES

5.1 Overall trial design

A multi-center randomized double-blinded placebo-controlled proof-of-concept trial.

Recruitment and Procedures

Patients with SSD will be recruited from psychiatric departments at several sites in Sweden. The central site is Örebro with recruitment within Region Örebro county. We will also inform patient interest associations for psychiatric disorders, outpatient clinics for psychosis and housing for psychiatric patients with chronic SSD about the study.
Patients who meet the inclusion criteria will be asked to participate in this study. They will receive written and verbal information about the study and receive a signed copy. Participants will furthermore be requested to give consent that the researchers collect information from an informant (personnel or next-of-kin) on the patient´s symptoms.

An extensive psychiatric interview (The PANSS and the psychosis section in the M.I.N.I.) will be performed with each patient and, if accepted by the patient his/her next of kin, at baseline. We may also review medical records in order to document previous symptomatology and treatments. All participants will be encouraged to undergo a physical examination. However only the interview is a prerequisite for participation. If clinical routine is online assessments, this is accepted in the current study.

Blood tap prior to treatment to rule out conditions that may be harmful if treatment with rituximab is initiated is obligatory, whereas all other blood tests and spinal tap are optional.

Patients are requested to remain on their standard psychiatric treatment until endpoint I at 12 weeks (obligatory), and treatment should be stable for the last 1 month prior to inclusion. Patients who change their antipsychotic medication are however not withdrawn from the study but this information will be included in the final analyses.

Patients will be allocated to treatment with rituximab or placebo by a blinded block procedure. Five assessments will be performed at pre-decided time points **(see table 1**) with a clinician (or for

intermediate assessment possibly a research nurse) in addition to the visit at the day of rituximab/placebo treatment (week 0). Screening prior to inclusion can be divided into more than one visit if deemed necessary. Within 4 weeks after inclusion in the study, the patient should be

administered the treatment with rituximab/placebo. Other sites than Örebro may administer the rituximab infusion, although other sites can send their patients to Örebro for this procedure.

If a participant is unavailable at endpoint I (week 12) or endpoint II (week 24), another 4 weeks extension for the final outcome assessments are accepted, i.e. endpoint I assessment can be made no later than week 16, and endpoint II no later than week 28.

Side effects will be monitored at each visit. Patients will continue their regular visits with the psychiatric staff. After the termination of the study and when the results are published, patients will be informed whether they were treated with rituximab or placebo.

5.2 Procedures and flow chart

Patients with SSD who consent to participate in the trial will be assessed by a clinician (preferably by his/her local psychiatrist) and interviewed with a background questionnaire that includes questions on psychiatric and somatic comorbidities, and symptom onset. The psychosis diagnosis is validated with items drawn from the psychosis section of the M.I.N.I. vs 7, in addition to the PANSS interview (where the depression item is also assessed separately). Additional measures are the PSP scale, the CGI-Severity rating and the patient-rated health on a VAS scale and self-assessed psychiatric symptoms (Cross-cutting symptom measure of global symptom severity, CCSM) and self-rated negative symptoms with the 15 item Motivation and pleasure- self report (MAP-SR) questionnaire. A short physical assessment should also be done. Blood samples will be collected for security measures and for research purposes (optional). Lumbar punctures and fMRI scans at baseline and endpoint I are also optional. Lumbar puncture and fMRI may not be offered at all sites due limited resources.

If the patient fulfils the inclusion criteria and none of the exclusion criteria, the patient will be offered to receive the treatment (single dose of placebo or rituximab infusion) within 4 weeks. Treatments are offered at the Rheumatology department, Örebro university hospital but other clinics with experience of rituximab treatments may be involved to minimize traveling (i.e. hospital units with access to trained personnel). Patients will take premedication two hours before the intravenous infusion which takes approximately 4 hours. Long-distance patients will be offered transportation to and from Örebro, and an overnight stay at a local hotel together with a support person (e.g. staff, family member). After receiving the treatment, the patient can return home the same day.

Patients are followed up, preferably by his/her investigator at 5 time points including endpoint I and II. One month prior to inclusion and during the study up to week 12 (endpoint I), the patient is requested not to change his/her standard psychotropic medication. Any changes in the prescription of antipsychotic drugs should be noted in the journal.

Data collection, management, and analysis

Demographic background data and treatment history will be collected from interview, rating scales and medical records at baseline and on each visit, in addition to data from physical assessments and laboratory assessments (see below).
Assessments will be made with established and well-validated rating scales and questionnaires (see list of all rating scales and questionnaires below). Sampling of biological materials will be done by a standard operating procedure (SOP) used for sampling for other current cohorts. All data collection forms will be stored at a safe place within Örebro hospital, Region Örebro County.

Collected data will be registered in an electronic data capture (EDC) platform (*SMART trial, renamed into "Greenlight Guru Clinical version" but we use the term SMART trial in this proposal)*).

Blood and CSF will be stored at local biobanks. In Örebro that is biobank 454.

Demographics, comorbidities, treatment history and vaccine history

- Background data collection form

Diagnostic measure

- Mini International Neuropsychiatric Interview (M.I.N.I. version 7) (Sheehan et al., 1998) is a structured clinical interview with a section for psychotic disorders, which is the only section that will be administered.

Physical examination

- General condition; blood pressure; heart rate; height and weight (can be performed by research nurse)
- Basic neurological examination (can be performed online if this is the clinical routine)

Brain imaging

- fMRI for assessment of altered activity and extra cellular water, which may be a sign of inflammation.

Patients will undergo an fMRI-based assessment at baseline and endpoint I and are optional. Lumbar puncture and fMRI may not be offered at all sites due limited resources. Prior to scanning patients will be asked to complete a MRI checklist. Patients will also be asked if they have taken benzodiazepines and/or hypnotics within 24 hours of initiating MRI; those who have will be asked to come back for a later session (>24 hours later). During scanning, patients will undergo the following protocol:

1. Diffusion weighted imaging: 90 gradient orientations distributed in two shells of b=1000 and b=2000, plus 2 b0 images; 2 mm resolution isotropic—20-minute scan time. We will estimate neural inflammation using a measure of regional extra-cellular free water derived from the diffusion weighted imaging data. We will apply to these data a free-water estimation algorithm that calculates the degree of cellularly constrained versus extra-cellular water. This method has been used reliably to determine levels of regional neuro-inflammation in psychiatric conditions.
2. Brain-blood perfusion imaging: arterial spin labeling scan, 40 labeled and 40 unlabeled scans, 30 axial 5-mm thick axial slices, 4x4 mm in-plane resolution—6-minute scan time.
3. Resting functional MRI (fMRI) scanning: blood-oxygen level dependent scan, SMS factor = 3, GRAPA = OFF, 32 4-mm thick axial slices with 3x3 mm in-plane resolution—12-minute scan time.
4. High-resolution anatomical MRI scans:
    a. Whole-head, T1-weighted MRI scan, 1-mm isotropic voxels—6-minute scan time.

b. Whole-head, T2-weighted MRI scan, 1-mm isotropic voxels—6-minute scan time.

Table 1 Study plan

Week		
-4 – 0	Screening prior to treatment with rituximab Decision on inclusion / exclusion **Baseline**	• Consent form (signed up to 8 weeks prior to treatment) • Evaluation of suitability • Information card about drug study participation • Information to not use attenuated vaccines, what risks are involved and signs of immunosuppression • Check anti-hypertensive medication* • Demographics questionnaire (including vaccination i.e. COVID-19, varicella check-up) • Treatment history, and Five-to Fifteen brief with next-of kin (optional) • Infection history • Assessments (MINI (section psychosis), PANSS, PSP, CGI-S, Patient evaluated VAS-health), MAP-SR, AAR-R, Global symptom severity • Physical examination including weight • Urine: Pregnancy test (women), drug screen • Blood sample for biomarkers (optional) • Lumbar puncture for biomarkers (optional) • fMRI scan (optional)
-2	Security laboratory test	• Test of hepatitis, HIV, TB, immunoglobulins, SR, CRP, Hb, Leukocytes, Trombocytes, Krea, 5-part, ALAT, ASAT, ALP, protein fractioning
0	*Rituximab dose 1000mg*/ placebo & auxiliary drugs	• Progress notes
2 (+2)		• Adverse Event assessment: open check-up questions • Patient evaluated improvement (PGE) • CGI-I next of kin (if doable) • Progress notes
7 (+2)		• Adverse Event assessment: open check-up questions • Patient evaluated improvement (PGE) • CGI-I next of kin (if doable) • Progress notes
12 (+4)	**Endpoint I**	• PANSS • PSP • CGI-S • CGI-I** • Patient evaluated improvement (PGE) • Patient evaluated VAS-health, MAP-SR, Global symptom severity • Adverse Event assessment (including AAR-R) • Blood test security: CRP, neutrophiles, protein fractioning, thrombocytes, Hb • Progress notes • Blood sample for biomarkers (optional) • Lumbar puncture for biomarkers and/or fMRI (optional) • Weight • Qualitative interview with informant and Qualitative interview and SNS-with patient (optional)

24 (+4)	**Endpoint II**	• PANSS • PSP • CGI-S • CGI-I** • Patient evaluated improvement (PGE) • Patient evaluated VAS-health, MAP-SR, Global symptom severity • Adverse Event assessment (including AAR-R) • Blood test security: neutrophiles, CRP, Hb, protein fractioning, thrombocytes • Progress notes

*If the patient is treated with antihypertensive medication these should be withdrawn 12 h prior to the rituximab treatment; **Evaluated by clinician and if doable next-of-kin.

Table 2

Week		**-4 – 0**	**0**	2 (+2)	7 (+2)	12 (+4)	24 (+4)
Visit		**1**	**2**	3	4	5	6
Activity/ ***Assessment number***	**Time to complete**	**Baseline**	**Treatment**	**Check-up**	**Check-up**	**Endpoint I**	**Endpoint II**
ENROLEMENT Eligibility screen, Consent form, Demographics, Treatment history, Infection history, Physical examination, Routine blood tests, Test of hepatitis, HIV, and TB. Pregnancy test in urine, Drug screen in urine	40 min	x					
INTERVENTION Rituximab/placebo infusion and auxiliary drugs	4.5h		x				
ASSESSMENTS MINI interview, psychosis section	10 min	x					
PANSS, PSP	40 min	x				x	x
CGI-S (clinician)	5 min	x				x	x
CGI-I (next of kin)	10 min			x	x	x	x
CGI-I (clinician)	5 min					x	x
PGE (patient evaluated improvement)	5 min			x	x	x	x
The Five to Fifteen – Brief rating scale (parent)	5 min	x					
Patient evaluated health (VAS-health), DSM-5 and MAP-SR	10 min	x				x	x
Adverse Event assessment: open check-up questions	5 min			x	x	x	x
Adverse Event assessment: AAR-R	10 min	x				x	x
Blood test security: neutrophiles, CRP, protein fractioning, Hb, trombocytes	10 min	x				x	x
Blood sample for analyses of B-cell depletion, proteins and gene expression are optional	15 min	x				x	
Cerebrospinal fluid tap and fMRI are optional, weight	40 / 60 min	x				x	
Qualitative interview with a parent and/or spouse and qualitative interview and SNS with patient (optional)	30-60 min x 2					x	
Progress notes (in patient's medical journal)	10 min	x	x	x	x	x	x

CGI-S = Clinical Global Impression-Severity scale; CGI-I = Clinical Global Impression-Improvement scale; fMRI = functional Magnetic Resonance Imaging; PGE = Patient's Global Evaluation of improvement; PSP = Personal and Social Performance Scale; AAR-R = Any Adverse Reactions -Revised, VAS-health = visual analogue scale assessing patient´s evaluation of health; DSM-5 (Cross-cutting symptom measure of global symptom severity (CCSM)), MAP-SR= Motivation and pleasure- self report; SNS = Self-assessment of Negative Symptoms
.

5.3 Biological Sampling Procedures

Blood will be drawn at baseline (before intervention) for security purposes. Analyses will include routine safety biochemistry, recommended for rituximab in clinical use:

- Serology for hepatitis B and C, HIV and TB
- Protein fractioning (including immunoglobulines)
- Pregnancy test (urine)
- Illicit drug test (urine)

Also, all subjects will be asked if they have received standard vaccinations and if they have had chicken pox, i.e. immunity to Varicella zoster (positive antibodies if unknown). If not, then vaccination is encouraged to precede the treatment with rituximab and Covid-19 vaccination is offered to all participants. Patients will be informed that they are immunocompromised – a consequence of the rituximab treatment –which results in an insufficiently strong immune response for infections.

1- 14 days prior to treatment (after inclusion):

- SR, CRP, Hb, Leukocytes, Trombocytes, Kreatine, 5-part, ALAT, ASAT, ALP, protein fractioning

Week 12 and 24:

- On week 12 and 24 CRP, neutrophiles, protein fractioning, trombocytes, and Hb will be checked for security.

Inflammatory related blood tests for research purposes (optional):

Aliquots of plasma will be collected by drawing whole blood in EDTA-tubes (7 mL) and stored in a local biobank, at baseline, and week 12 (+4) (optional). This will be used for investigating changes in circulating, disease-associated markers, examples being immunological mediators, metabolites, markers of tissue damage etc. The remaining sample will also be stored in a local biobank for genetic analyses including common, functional polymorphisms and methylation patterns in disease-associated genes.

RNA will be stored in the local biobank, by collection of whole blood in PAXgene tubes (2.5 mL), at baseline, and week 12 (+4). This will be used for investigating changes in gene expression of disease-associated genes, examples being immune genes, metabolic enzymes, transcriptional regulators, etc.

Circulating mononuclear cells will be isolated and cryopreserved by drawing whole blood (8 mL) in Vacutainer® CPT-tubes, using standard procedures, at baseline and week 12 (+4). Cryopreserved mononuclear cells will be stored in a local biobank and used for later analysis of immune cell populations and potential functional studies.

***Cerebrospinal fluid (CSF)* for research purposes** (optional):
CSF will be collected for investigating changes in circulating, disease-associated markers, examples being immunological mediators, metabolites, markers of tissue damage, etc. at baseline

and week 12 (+4).

To evaluate CSF levels of inflammatory cytokines, a lumbar puncture (LP) will be performed as described. LPs will be done in the L3/L4 or L4/L5 interspace following administration of local anesthetic.

Tubes will be gently mixed to avoid gradient effects. CSF samples will be centrifuged at 2000g, 8°C, for 10 minutes to remove cells (for gene expression analyses) and other insoluble material, aliquoted into 1 ml tubes to eliminate the need for repeated freezing and thawing and stored at -80^{o}C. From the CSF samples, we will obtain biomarkers of inflammatory signaling using ELISA and Mesoscale methods. Mesoscale allows for complex multiplexing of up to 17 analytes in one sample and will be the method of choice. The lumbar puncture is optional.

5.3.1. Handling, storage and destruction of biological samples

Handling, storage and destruction of biological samples will follow SOP (standard operation procedure) guidelines of the biobank. Only accredited labs will be used for chosen analysis. Destruction of biological samples will be done at the latest 10 years after study termination.

5.3.2 Total Volume of Blood and CSF per Subject

The total volume of blood taken from each subject during the trial is a maximum of 300mL.
A volume of up to 20 ml of CSF will be collected in silicone-coated tubes for research for each subject.

5.3.3. Biobank

All samples taken in this trial are registered in a biobank at the local hospital and handled according to the current biobank laws and regulations. For patients that are treated at the Örebro university hospital the biobank no 454 (in Örebro) will store the samples. The samples are pseudonymized to protect the subject´s identity. All samples and the identification/code list are stored securely and separately to prevent access by unauthorized persons.

5.4 End of Trial

Patients may withdraw from the study at any time, of their own choice or by investigator decision, due to safety reasons or in case the patient is not able to comply with the protocol. Data collected up to the end of follow-up will be used in the final analysis of the study. If a patient wants to discontinue the study participation, data collected until that time point will be analysed in the study.

Early termination of the study may occur due to decision of regulatory authority or ethics committee, interim analyses, safety issues or sponsor decision. The study is completed when the last patient has done their last study visit.

6. SUBJECT SELECTION

6.1 Inclusion Criteria

To be eligible for inclusion into this study, each patient must fulfil the following criteria:

1) Age: 18 to 55 years

2) Duration of psychiatric illness: exceeding 1 year

3) Diagnosed with Schizophrenia spectrum disorder (SSD) according to Diagnostic and Statistical Manual for Mental Disorders, 5th edition (DSM-5)

4) If female and with any risk for pregnancy: willing to use contraceptives* or abstinence if normal and preferred lifestyle

5) Subjects should be judged by the investigator to be lucid and oriented to person, place, time, and situation when giving the informed consent

6) Insufficiently recovered from previous antipsychotic treatments

7) A minimum score of 4 in CGI-Severity at baseline

*Fertile women will only be included in the study if they are using/agree to use adequate contraception during treatment with the study drug and for a period of 12 months after the last treatment. Adequate contraception is defined as at least 1 of the following: 1) abstinence, 2) oral contraceptive (combined or progesterone alone), 3) implants of levonorgestrel, 4) estrogenic vaginal ring, 5) percutaneous contraceptive patches, 6) injectable progestogen, 7) intrauterine device (IUD) or 8) male partner sterilization. Non-fertile women are defined as any female that is surgically sterile (documented hysterectomy and/or bilateral oophorectomy or tubal ligation).

Participants with impaired communication skills may not be able to respond to all questions. However, as such patients have a guardian or next-to-kin we will obtain independent information whether the patient is improved or not according to the rating scales and clinical interviews. Thus, our primary outcome measure can be used at all times regardless of the state of the patient.

6.2 Exclusion Criteria

Patients are to be excluded from the trial if ***any*** of the following criteria is fulfilled:

1) pregnancy or breast-feeding
2) weight below 40 kg
3) clinically relevant ongoing infection at the discretion of the physician
4) chronic infections
5) positive test for hepatitis B, hepatitis C, HIV, or TB prior to treatment
6) malignancy currently or within 2 years prior to inclusion
7) current severe heart failure (NYHA grade IV) or any other severe heart disease (e.g. or history of cardiac arrhythmia or myocardial infarction)
8) any change of antipsychotic medication within the previous 4 weeks
9) unable to make an informed decision to consent to the trial
10) ongoing clozapine treatment
11) ongoing immunomodulatory treatment
12) treatments with monoclonal antibodies within 1 year prior to the inclusion

6.3 Screening

Subject eligibility (that subjects fulfill all inclusion criteria and do not meet any exclusion criteria) is established before inclusion. A brief structured interview for screening purposes (in Swedish) is included in the study.

6.4 Withdrawal criteria

The patients can at any time choose to stop participating in the study without giving any reason for this. If possible, the reason for the patient leaving the study will be recorded. Data collected up to the subject's leaving the study will be used in the analysis of the study.
The investigator can remove a patient from the study due to safety reasons or in case the patient is not able to comply with the protocol.

7. TRIAL TREATMENTS

7.1 Description of investigational medicinal product

Active substance: Rituximab (Mabthera, Rixathon, Ruxience or Truxima)
1,000 mg concentrate for solution for infusion,100 mL.
ATC-code: L01XC02
Rituximab is a sterile, clear, colourless, preservative-free liquid concentrate designed for intravenous administration. The product is supplied at a concentration of 10 mg/mL in 500-mg (50-mL) single-use vials. Rituximab is formulated for intravenous administration in sodium chloride 9 mg/mL, sodium citrate dihydrate 7.35 mg/mL, polysorbate-80 0.7 mg/mL, and sterile water for infusion. The pH is adjusted to 6.5.

Placebo: Physiological saline 500mL (0.9 %, any brand provided by the pharmacy) will be administered intravenously as placebo.

7.2 Dose and administration

Rituximab (or placebo) will be administered on one single occasion.
A rituximab dose of 1,000 mg (100mL) will be given as intra-venous infusion in 400 mL physiological saline (0.9 %, any brand provided by the pharmacy) or 500 mL physiological saline. Before administration of rituximab (or placebo) all patients will be given pre-medication to prevent or reduce possible side effects from rituximab, presented below.

The rituximab treatment is administrated at the Rheumatology clinic at Örebro University Hospital and Neurology clinic at Karolinska hospital. Additional treatment sites may be added if they are trained for providing rituximab treatment.

Each bag for infusion will be prepared just in time for each visit. A blinded study nurse, trained in the administration of rituximab, will administer the treatment to the patients.

7.3 Storage and preparation of study medication

A list for the randomizing will be constructed. An unblinded nurse who at each site is informed about the randomisation and will prepare the saline bag (500mL) for each patient infusion. If the

patient is allocated to the rituximab treatment arm the nurse will add 100mL rituximab (and draw 100mL saline from the bag) or, if the patient is allocated to placebo, prepare the 500mL saline bag. Rituximab or placebo will be administered according to the randomization number provided. All bags will be labelled with a study specific label. Each bag for infusion will be prepared just in time for each visit and delivered to the clinical facility for administration. A blinded study nurse will administer the treatment to the patient.

Study drug, rituximab and saline for dilution and for placebo, will be stored at the department of Rheumatology at Örebro University hospital for treatments in Örebro and at local clinical units for rituximab treatment for patients receiving the treatment on other sites.

7.4 Drug accountability and treatment compliance

A Drug Dispensing Log will be used during the study for traceability of all dispensed study drug or placebo. The Drug Dispensing Log is unblinded and can only be used by unblinded study nurse/s who prepares the study drug/placebo. The log will be kept in a secure place to ensure restricted access by others. The study participant's medical records will be updated with information about the administered study infusion. No information will be stated in medical record whether the participant received Rituximab or Placebo.
The study drug is kept in the normal drug storage room at the clinic where the infusion will be prepared. All handling of study drug/placebo as well as activities for stock maintenance will be performed according to the clinic's normal routine.

The administration of premedication prior to study drug/placebo infusion (Paracetamol, Desloratadin and Betapred) will be recorded in the study participant's medical records.

Compliance with the study drug is assured as the patient receives study drug/placebo at the hospital as an infusion.

7.5 Randomization

The first 32 patients will be randomized 1:1 to either rituximab or placebo, using block-randomization (blocks of e.g. 2, 4, 6, 8 or 10). A second block randomization 1:1 for another 32 patients will be made after 32 patients have reached endpoint I. After 64 patients have reached endpoint I a third block randomization will be made for the remaining patients and if there is no need to terminate the study prematurely.

Randomization codes will not be available to the study staff. Emergency unblinding is only allowed in case of serious concerns about patient safety. All study staff and patients will be blinded to treatment allocation.

7.6 Blinding

Un-blinded study staff at treatment site will prepare the saline bag for each patient infusion, adding rituximab. For subjects randomized to placebo, nothing will be added to the saline. All bags will be labelled with a study specific label (Appendix 3, Labelling of study medication).

7.7 Code breaking

Emergency Unblinding means that you can break the blindness for a specific patient. A list of each participant's code and information on whether the subject had received placebo or rituximab will be stored at the emergency psychiatric unit located at Örebro university hospital and thus available at all times. Thus, the on-duty clinician at Örebro university hospital will enable code breaking if he/she is informed about a serious adverse event in a study participant that requires emergency code breaking. The study participant's safety will always be the first consideration when a decision is made to break the code or not. The PI should be contacted before code breaking, if possible. The reason for breaking the code and what treatment the study subject was on will be recorded in the medical record. Study participation will be discontinued for a study subject for whom the code has been broken.

7.8 Auxiliary medical products

Premedication is administered once, 1-2 hours prior to rituximab/placebo and consists of T. Paracetamol 1000 mg (p.o), T Desloratadin 10 mg (p.o) and twelve T. Betapred (Betamethasone) 0.5 mg p.o (dissolved in a glass of water) in order to reduce the incidence of infusion-related reactions. All auxiliary products have marketing authorization and will be used according to the terms in its approval. All administered premedications will be recorded in the study participant's medical record.

Desloratadin is an antihistamine, used to relieve allergic symptoms and Betapred is a corticoid steroid used with the same aim in this study. Paracetamol is administrated for pain relief. Risks involved with taking these drugs on one single occasion are viewed as low, but side-effects may appear.

7.9 Concomitant use of other medicinal products and treatments

Patients should remain on their stabilized treatments with anti-psychotic drugs during the (12 weeks) study (up to endpoint I) and ideally until endpoint 2 (24 weeks). Other medications considered necessary for the safety and well-being of the subject may be provided at the discretion of the investigators, unless otherwise specified in the exclusion criteria. Concomitant medication should be recorded in the Case Report Form (CRF).

7.10 Destruction

The saline bags must be administered on the day of preparation and cannot be stored for use at a later visit. If, for any reason, a prepared bag is not used on the day of preparation it should be discarded.

7.11 Treatment after trial end

This is decided by the treating physician and not by the research group.

8. METHODS FOR MEASUREMENT OF ENDPOINTS FOR CLINICAL EFFICACY AND SAFETY

8.1.1 Primary Endpoint

The primary endpoint (endpoint I) is performed at week 12. Clinical efficacy is measured using CGI-I, see Table 1 and 2. Response is defined as < 3 in CGI-I, i.e. much or very much improved.

8.1.2 Secondary Endpoints

At endpoint II on week 24, patients are assessed with identical measures as at endpoint I. Questions on the presence of adverse events are asked at each visit after treatment. If a patient is unavailable at week 12 or week 24, endpoint data could be collected within another 4 weeks.

9. HANDLING OF ADVERSE EVENTS

9.1 Definitions

An Adverse Event (AE) is any untoward medical occurrence in a patient/healthy volunteer administered a pharmaceutical product and which does not necessarily have a causal relationship with that treatment or usage. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical (investigational) product, whether or not related to the product.

9.1.1 Adverse Events, AE

The coordinating investigator, principal investigator or any investigator or study nurse delegated to work in the study will record any AEs after the study drug has been administered. Only the coordinating investigator, principal investigators or investigator is allowed to determine the seriousness and severity (mild, moderate, severe) and whether the AE is related to the study medication. All AEs in the study will be reported to the sponsor via SMART-trial.

9.1.2 Adverse reaction

All AEs that are observed from the time of the administration of study medication until the patient leaves the study will be registered, analysed and summarized at the end of the study. Only serious adverse events will be recorded in the medical record.

9.1.3. Serious Adverse Event, SAE

Each adverse event is to be classified by the investigator as serious or non-serious. An adverse event that meets one or more of the following criteria/outcomes is classified as serious:

- Results in death
- Is life-threatening, i.e., the patient was, in the opinion of the Investigator, at immediate risk of death from the event as it occurred (It does not include an event that, had it occurred in a more severe form, might have caused death.)
- Results in persistent or significant disability/incapacity
- Requires in-patient hospitalization or prolongs hospitalization assumed to be related to rituximab treatment (i.e. not solely due to the mental disorder)

- Is a congenital anomaly/birth defect
- Is another medically significant event that, based upon appropriate medical judgment, may jeopardize the volunteer and may require medical or surgical intervention to prevent one of the outcomes listed above (e.g., allergic bronchospasm requiring intensive treatment in an emergency room or home, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse)

SAEs are observed from the time of the administration of study medication. Any SAE that occurs during the study will be documented and reported to sponsor within 24 hours after the investigator becomes aware of the SAE. SAEs will be reported electronically in SMART trial. For instances where SMART trial cannot be used of some reason, SAE will be reported on a SAE form and emailed to sponsor. SAEs will be documented, analysed and summarized at the end of the trial on a paper form and in SMART trial. SAEs will be followed until the participation in the trial is ended or they are resolved.

9.1.4 Suspected Unexpected Serious Adverse Reaction, SUSAR

The sponsor reports all SUSARs that arise during the trial to The Medicinal Product Agency and the Swedish Ethical Review Authority (Etikprövningsmyndigheten). Reports will be done by sending the CIOMS form to the MPA since sponsor does not have the resources to report electronically to the EudraVigilance database.

A SUSAR that results in death or is life-threatening must be reported promptly and no later than 7 days after it occurred and was known by sponsor. Relevant follow-up information should be sent within another 8 days. Any other SUSARs should be reported as soon as possible but not later than 15 days after being brought to sponsors attention.

SUSARs should, if possible, be reported unblinded, that is, should state to which trial medicinal product the subject had a reaction. The investigator should only unblind the treatment allocation for a subject if unblinding is relevant to the subject's safety. Unblinded data should only be available to persons performing safety evaluations during the clinical trial. Placebo should only be reported if it is suspected that any component of the placebo treatment has caused the reaction.

9.2 Assessment of Adverse events

Patients will be asked if they have experienced any AE by means of the following questions, administered at each visit:

- Have you noticed any new symptoms or problems since your last visit? If yes, which?
- Do you gather these symptoms can be related to a rituximab treatment?
- Have you noticed if any of your previous side effects have attenuated or increased? If yes, which ones?

In addition, the questionnaire Any Adverse Reactions-Revised (AAR-R) targeting possible rituximab side-effects will be used at endpoint.

9.2.1 Assessment of causal relationship

Causal relationship is more likely if the symptoms are listed in the AAR-R, but it is up to the investigator to draw a conclusion.

9.2.2 Assessment of intensity

Intensity of adverse events is assessed with the AAR-R with the alternatives “mild”, “moderate” or “severe” and lasting either “occasionally”, “daily basis” or “ongoing”. Assessment of intensity is done by the investigator.

9.2.3 Assessment of seriousness

Seriousness of adverse events is assessed with the AAR-R by the investigator.

9.3 Reporting and registration of Adverse events

At each trial visit, adverse events (AE) are registered, starting after start of treatment with the investigational medicinal product, up to patients’ last visit. AE that occurs during the trial and which are observed by the investigator/trial nurse or reported by the subject will be registered in the CRF regardless of whether they are related to the investigational medicinal product or not. Data will be reported on AE log paper and then inserted in SMART trial.

Assessment of causal relationship, severity, and whether the AE is considered to be an SAE will be made by the investigator. At minimum for each AE/SAE, a description of the event is recorded (diagnosis/symptom if diagnosis is missing), if the symptoms are continuing, causal relationship, severity, if the AE is considered to be an SAE, measures and outcome.

Adverse events are reported by open questions and the AAR-R and will be registered in SMART trial. In our pilot trial we noticed that most individuals with SSD cannot recollect when adverse events start or remit, therefor the AAR-R only include present symptoms. If other events have occurred prior to the visit and the patient can remember them, they will be identified with the open questions and documented in the AE log.

9.3.1 Reporting of Adverse events (AE)

Adverse events are documented in the AAR-R and in all events will be documented in SMART trial.

9.3.2 Reporting of Serious Adverse events (SAE)

SAEs will be reported in SMART trial. Serious Adverse Event (SAE) Report Form will be used in case reporting in SMART trial is not possible.
All SAEs are reported to the sponsor within 24 hours of the investigator being informed of the SAE.
Follow-up information describing the outcome and handling of the SAE is reported as soon as this information is available. The original should be kept in the Investigator Site File.

9.3.3 Reporting of Suspected Unexpected serious Adverse Reactions (SUSAR)

Those SAE which are assessed by sponsor to be SUSAR are reported via a CIOMS form to the Swedish Medical Products Agency /EudraVigilance database according to the specified time frames.
SUSAR that are fatal or life-threatening are reported as soon as possible and no later than 7 days after the serious adverse event has become known to the sponsor. Relevant follow-up information is sent thereafter within an additional 8 days. Other SUSAR are reported as soon as possible and no later than 15 days after they have come to the sponsor's knowledge.
Information about SUSAR occurring during the trial is compiled by the sponsor and sent to the principal investigators at all participating sites. In order to preserve the integrity of the trial, it is recommended that reporting of SUSAR to investigators in a blinded study is made without unblinding, that is, without specifying which investigational medicinal product the subject received.

9.4 Follow -up of Adverse events

AE are followed up on endpoint II, which correspond to 24 weeks post treatment with the same methods as on endpoint I according to clinical routines.

9.5 Independent Data monitoring Committee

A research group associated with Örebro University will constitute a data monitoring committee (DMC) independent of the investigators and PIs. If any patient develops severe side effect, such as progressive multifocal leukoencephalopathy (PML), the study shall be stopped. The DMC will also decide on whether the study should be terminated in advance when data on the interim analysis is available (after 32 and 64 participants have reached endpoint I). The DMC will be unblinded to the data for the interim analyses. The DMC will keep all information confidential except to state if the study should be terminated or proceed.

9.6 Annual Safety report

The sponsor will submit an annual report on the safety of each investigational medicinal product used in a clinical trial. The safety report should be written according to the format Development Safety Update Report, (DSUR).
The safety report defines for which time period the report applies and a list of all SAE that have occurred, as well as possible SUSAR. A summary assessment of the safety situation for the subjects and a benefit/risk evaluation will be included.

The annual report will not contain any personal information of study participants.

9.7 Procedures in case of emergencies, overdose or pregnancy

If an unforeseen event is likely to have a serious impact on the benefit/risk relationship, the sponsor and investigator will take appropriate Urgent Safety Measures (USM) necessary to protect the subjects. Examples of such measures are to temporarily suspend the clinical trial or to introduce supplementary monitoring measures.
If a subject who participates in a clinical trial becomes pregnant, this person will be followed up until the birth has taken place. If the fetus/child has any congenital malformation, it will be reported as a serious adverse event (SAE).

9.8 Reference safety information

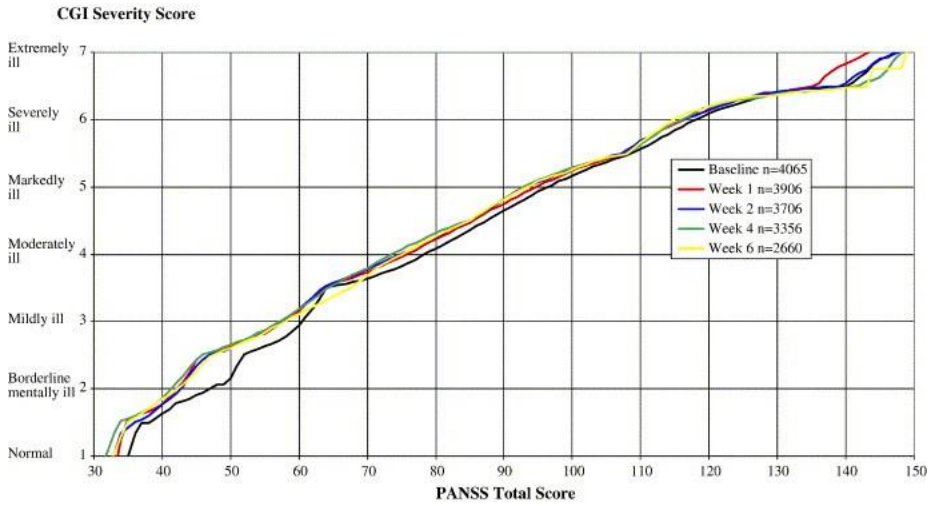
The approved and current SPC for rituximab and NaCl (placebo) will be used as reference safety information.

10. STATISTICS

10.1 Analysis population

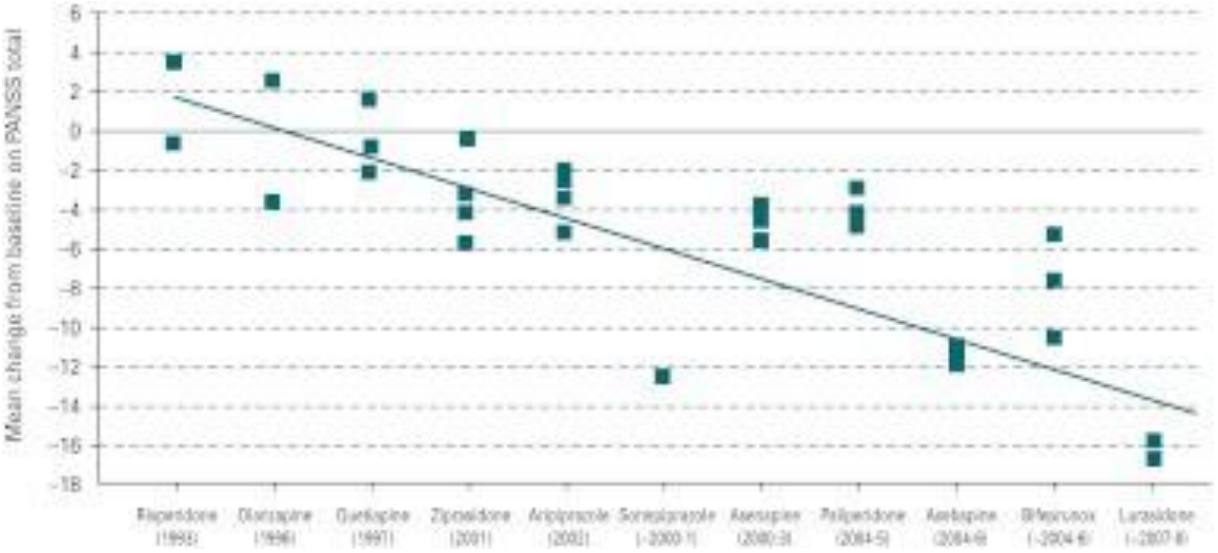
Based on a pooled database with more than 4 000 patients with an acute exacerbation of SSD, the mean PANSS score at inclusion was 94 ± 19 and the mean CGI-Severity score was 4.8 ± 0.9 corresponding to in between moderately (CGI=4) and markedly ill (CGI=5) (Leucht et al. 2005, figure 1. below). Chronic schizophrenia patients´ PANSS score is expected to be somewhat lower than patients´ in acute phases.

Fig. 1 Clinical severity in relation to PANSS score



Different studies report vast variations in placebo response in randomized placebo-controlled trials on schizophrenia, which make sample size calculation difficult to estimate. The placebo response has increased considerably in more recent conducted studies (Alphs et al. 2012, see figure 2). Up to 2008 the median placebo response score in PANSS on RCTs with antipsychotic drugs was 4 points (Alphs et al. 2012). In a more recent RCT with the muscarinic receptor agonist xanomeline for patients with acute exacerbation or relapse, the placebo group had a 5.9 points reduction compared to the treatment group who had a 17.4 points reduction (Brannan et al. 2021). A meaningful difference in PANSS score between placebo and the active drug was suggested to be 5 points (Scott et al. 2020).

Fig. 2 Mean change in PANSS total score in the placebo treated group in RCTs with antipsychotic drugs published between 1993 and 2008. Later conducted trials compared to earlier conducted trials, show an increased placebo response. 12 of the placebo-treated study groups had a mean PANSS reduction of ≤ 4 while 11 of the placebo treated study groups had PANSS scores > 4 (Alphs et al. 2012).



An important factor that may increase the placebo response is the selection of patients. If inclusion criteria demand patients to be in relapse or in an acute exacerbation, the natural course of the disease could lead to regression to the mean, which comes out as a placebo response (Alphs et al. 2012). Detailed evaluation of all participants in acute trials compared to those who completed these trials suggests that the differences in placebo effect may also be driven by those subjects who completed the study and not by those who dropped out early. It was also observed that the placebo effect was most obvious in subjects originating from the USA. Other reasons for high dropout rates are difficult assessments that may exhaust the patient and lack of incentives.

We will mostly include chronic stable patients in Sweden, use very few assessments, only administer one single dose of the research drug and offer a reimbursement at the endpoints, which should reduce drop-out rates and placebo responses.

10.2 Statistical analyses

10.2.1 Statistical methods

Analyses of clinical data related to clinical response: For results on the scales (PANSS, PSP, CGI-S, CGI-I, VAS-health, PANSS subscales and Marder negative factor) and response in the treatment resistant participants the week 12 and 24 scores and the Δ-values (difference between scores at week 12 and baseline and scores at week 24 and baseline) will be compared between the treatment groups by means of t-tests or Mann-Whitney tests, as applicable. This data will

also be included in an analysis of covariance model, controlling for baseline measures and other possible confounders, as demographic variables. Missing data will be handled as Last Observation Carried Forward, in addition to completer analysis.

To examine whether baseline characteristics can predict response in the rituximab treated arm, baseline values of all measurements will be compared between the two groups “responders” and “non-responders” after completion of the study by means of t-tests or Mann-Whitney tests, as applicable.

To examine whether changes in inflammatory markers and other measurements correlate with clinical response, the difference between baseline values and week-12 and 24 values (Δ-values) of all relevant measurements will be compared between the two groups “responders” and “non-responders” after completion of the study by means of t-tests or Mann-Whitney tests. In addition, for each of these measurements, a generalized linear model (ANCOVA) will be built and a PCA analysis will be performed.

10.2.2 Dropouts

We expect a 10 % drop out rate. Since the treatment is given as an infusion only once we expect treatment adherence to be excellent, compared to if medication was dosed daily. However, there is a considerable risk of dropouts regarding the subsequent data collection including the primary outcome, which is a rating of clinical change at week 12. Therefore, a reimbursement will be offered to the participants at endpoint I and II.

10.3. Adjustment of significance and confidence interval

No adjustments will be made.

10.4 Sample size calculation

A total sample of 120 is estimated (108 completers at endpoint I).

Placebo response: A high placebo response is expected in studies which include patients with acute exacerbation of the SSD due to the regression to the mean. As most of our participants are anticipated to be stable, not in an acute phase, the placebo response could be expected to be low. We anticipate the placebo response (i.e. much or very much improved according to CGI-I) to be 10 %.

The pilot study: In our pilot study, the 9 treatment resistant SSD patients had a mean baseline PANSS score of 99 (SD 32) and CGI-S of 5.8 (SD 0.7). At 12 weeks their mean PANSS score was 62.2 (SD 27), i.e. a 37 points reduction (SD 20.5) and 78 % reached a ≥ 30% reduction in PANSS. CGI-S dropped to 4.1 (SD 1.4). Six out of 9 (67 %) were considered much improved at week 12 (i.e. CGI-I < 3). No patient dropped out prior to week 12.

Anticipated baseline score: In the present study we will include chronic patients expected to be moderately or markedly ill, corresponding to a CGI-S score of 4 to 5. Our sample size calculation in the present study is based on an estimated mean PANSS baseline score of 85 (SD 17) and a mean CGI-S score of 4.5 (SD 0.7).

In the placebo group we anticipate a 4 points reduction in PANSS, based on a number of

published studies (Leucht et al. 2005). In the rituximab treatment group, we anticipate a 14 points reduction in PANSS. According to Leucht (2022), a 10 points difference in PANSS score between placebo and treatment group is the most common outcome across RCT studies with antipsychotic drugs.

Sample size calculation: A mean baseline PANSS score of approximately 85 (SD =17) is anticipated.

Primary (categorical) outcome: We expect 33 % in the rituximab treated group to be much or very much improved (i.e., score 1 or 2 on CGI-I), compared to an expected 10 % in the placebo group. To reach a power of 80 % with a significance level of 5 % (two-sided), we need to include 47 patients in each group. To allow for a 10 % drop-out, we need (47/0.9 = 52 participants per group. The effect size, Cohen's *d* is 0.58.

Secondary outcome: We expect a mean reduction of 4 points in the PANSS score in the placebo group and a reduction of 14 in the rituximab treatment group, resulting in endpoint scores of 81 and 71, respectively. With a SD of 17 in both groups and 10 point differences between groups, an effect size Cohen's *d* of 0.59 is expected. With an estimated dropout of 10 %, a sample size of 51 in each group is needed. The calculations are based on two sided tests with power (1 – risk of making a type II error) of 80 % and a significance level (risk of type I error) of 5 %.

Subgroup analyses:
We aim to include a total of 120 participants to account for loss of degrees of freedom when statistically adjusting for center and prognostic factors in the model.

Response criteria: much or very much improved according to CGI-I.

Sample size calculation was made by a statistician using R version 4.1.1.

10.5 Interim analysis

When 32 participants have reached endpoint I, an interim analysis will be performed. Criteria for trial termination is lack of response, i.e., less than 25 % of the rituximab-treated participants have responded < 3 on CGI-I). Otherwise, the study will proceed. A second interim analyses will be made with the same criteria as described above, when 64 patients have reached endpoint I.

11. QUALITY CONTROL AND QUALITY ASSURANCE

11.1 Quality Assurance and Sponsor oversight

11.2 Monitoring

The study will be monitored before start, during the study and after the study is completed. The monitoring will be performed to assure that the rights and wellbeing of the patients are protected,

that the study is conducted according to the protocol, that all essential documents are available, and that data are collected, documented, and reported according to ICH-GCP (Good Clinical Practice) and applicable ethical and regulatory rules and directives.

The aim of the monitoring is also to secure that collected data are correct, complete, legible, and verifiable from source documents if applicable.

Monitoring is planned to be done by monitors from the Clinical Trials Unit (Enheten för kliniska studier) at Örebro University Hospital (EKS). Monitoring may also be done by monitors from other local Clinical Research Units/Organisations.

11.3 Source data

The investigator must keep source documents for each subject in the trial. A document describing what has been classified as source data in the trial (source data reference document) should be included in the Investigator Site File (ISF). The investigator must ensure that all source documents are accessible for monitoring and other quality control activities.

Source data is defined before trial start at each individual site and can, in cases where source data is not registered in another document, consist of the CRF. This should be decided in consultation with the monitor and clearly stated in the source data reference document.

Access to trial-related documentation, such as subjects' medical records, CRFs, other source data and other trial documentation will be provided for monitoring and auditing purposes.

11.4 Deviations, serious breaches and other reporting obligations

The responsible investigator shall, without delay, report to the sponsor any serious breaches and deviations from the trial protocol, ICH-GCP and other regulations that significantly and directly affect, or with high likelihood could affect, the subjects’ safety and integrity or the reliability and robustness of the data generated in the trial. The sponsor should assess the suspected serious breach and the consequences of deviations that have occurred, and, without delay but no later than 7 days (from knowledge) report these to the Swedish Medical Products Agency.

Other unforeseen events that may affect the benefit/risk relationship must be reported without undue delay, but no later than 15 days after the sponsor becomes aware of the event.

11.5 Audits and inspections

Authorized representatives for the sponsor and Competent Authorities (CA) may carry out audits or inspections at the trial site, including source data verification. The investigator must ensure that all source documents are available for audits and inspections. The purpose of an audit or inspection is to systematically and independently review all trial-related activities and documents, so as to determine whether these activities were performed, registered, analyzed and reported correctly according to protocol, ICH- GCP and applicable regulations.

12. ETHICS

12.1 Compliance to the protocol, ICH-GCP and regulations

The trial will be conducted in compliance with the protocol, in accordance with the latest adopted version of the Helsinki declaration, ICH-GCP and the applicable regulatory requirements.

12.2 Ethical review of the trial

The final protocol for clinical trials on medicinal products must be approved, as a part of the application for a permit for clinical trials, by both the Swedish Ethical Review Authority and the Swedish Medical Products Agency before the trial can be conducted. The final version of the informed consent form and other information provided to subjects, must be approved, or given a written positive opinion by the Swedish Ethical Review Authority. The authority must be informed of any changes in the trial protocol in accordance with current requirements.

12.3 Procedures for obtaining Informed Consent

Patients who may be suitable to participate in the study will be informed about the study during one of their regular visits at the clinic/digitally and through personnel at inpatient clinics. Patients will be given written information about the study, time to read it and the opportunity to ask the investigator any questions they may have. All patients will be asked to sign the written consent. This could be done up to 8 weeks prior to the study treatment in order to give time for vaccination procedures if needed. Individuals who do not want to participate are not included in the study. The patients are informed that their participation is voluntary and that they may choose to withdraw from the study at any time, without repercussions. Only patients able to make an informed decision about the study will be included.

If the patient agrees to participate in the study they will sign the informed consent form together with the investigator, who will then sign the consent form as well. A copy of the signed informed consent will be given to the patient.

Clinical routine visits for this patient group are commonly performed digitally at several participating study sites. If deemed to be the best option, digital visits will be allowed in this study, both for the Informed consent process and study visits. Many patients are used to digital meetings as their standard contact with their doctor, and will therefore most likely, also feel most comfortable with this method. The Informed consent process can be done online if the following procedure is followed: Patient and clinician can both hear and see one another, and patient is able to ask questions in real time. The clinician and patient then have a copy each of the informed consent. Control of patient’s identity is needed if any uncertainty regarding identity exists. The signed consent form is shown on the screen by both parts, signed simultaneously and checked that they agree. The two signature pages will be merged to one complete document and kept in the patients file or investigator file. One copy will be given to the patient.
If an approved system for electronic signatures will be implemented in the future, or may already be implemented at some clinics, this will also be an accepted method for signing the consent form.

Two versions with information on the study are offered to the patient, of which one is brief and easy to read whereas the other one is detailed and comprehensive. Thereby we can offer

information that fits patients of diverse cognitive levels, commonly seen in individuals with SSD. Next-to-kin are always invited to participate on each visit if this is accepted by the patient. If patient has given his/her consent, next-of-kin will be asked to participate in a qualitative interview concerning the patient's behavior post treatment (after having passed endpoint I). A parent or someone who knew the patient in childhood will be asked to fill out the Five to Fifteen – Brief, a 24 item rating scale for assessing neurodevelopmental symptoms (Lugnegård & Bejerot, 2019). The patient will be interviewed with similar questions and is asked to fill out the 20 item questionnaire Self-evaluation of Negative Symptoms (SNS) (Dollfus et al. 2016) that is regarded to be less sensitive to change after treatment.

12.4 Data protection

Data management and data handling will be completed in accordance with applicable regulations. Only study personnel will have access to and handle study data. The study participants will be pseudonymized. Personal identification numbers in the data processing file will be replaced by serial numbers and information regarding the relationship between personal identification numbers and serial numbers are stored in a separate, code protected file.

12.5 Insurances

The volunteers are covered by The Swedish Patient's insurance and The Swedish Pharmaceutical Insurance during the study.

13 SUBSTANTIAL CHANGES TO THE TRIAL

Amendments, protocol additions and essential changes of the protocol may only be implemented after approval of a written application to The Ethics Committee and/or The Medicinal Products Agency.

14. COLLECTION, HANDLING AND ARCHIVING OF DATA

14.1 Case Report Form, CRF

A study database with all patients included in the study will be generated. The patients' identity will be pseudonymized, names and social security numbers will not be entered in the CRF. The code list, identifying the patients, will be kept locked up, only accessible to study personnel.

14.2 Notification of trial completion, reporting, and publication

The study will end when the last patient has completed the last visit. Data collected during the study will be archived for 25 years after the study has been completed.

Study results will be summarized and submitted in a report to the Medicinal Products Agency and the Ethics Committee within 12 months after completion of the trial.

The results will be submitted for publishing in suitable scientific journals and may be presented at meetings and conferences.

16. REFERENCES

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17 APPENDIX 1, DECLARATION OF HELSINKI

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI
Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly, Helsinki, Finland, June 1964 and amended by the:
29th WMA General Assembly, Tokyo, Japan, October 1975
35th WMA General Assembly, Venice, Italy, October 1983
41st WMA General Assembly, Hong Kong, September 1989
48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996
52nd WMA General Assembly, Edinburgh, Scotland, October 2000
53rd WMA General Assembly, Washington DC, USA, October 2002 (Note of Clarification added)
55th WMA General Assembly, Tokyo, Japan, October 2004 (Note of Clarification added)
59th WMA General Assembly, Seoul, Republic of Korea, October 2008
64th WMA General Assembly, Fortaleza, Brazil, October 2013

PREAMBLE

1. The World Medical Association (WMA) has developed the Declaration of Helsinki as a statement of ethical principles for medical research involving human subjects, including research on identifiable human material and data.
The Declaration is intended to be read as a whole and each of its constituent paragraphs should be applied with consideration of all other relevant paragraphs.
2 Consistent with the mandate of the WMA, the Declaration is addressed primarily to physicians. The WMA encourages others who are involved in medical research involving human subjects to adopt these principles.

GENERAL PRINCIPLES

3. The Declaration of Geneva of the WMA binds the physician with the words, “The health of my patient will be my first consideration,” and the International Code of Medical Ethics declares that, “A physician shall act in the patient's best interest when providing medical care.”

4. It is the duty of the physician to promote and safeguard the health, well-being and rights of patients, including those who are involved in medical research. The physician's knowledge and conscience are dedicated to the fulfilment of this duty.

5. Medical progress is based on research that ultimately must include studies involving human subjects.
6. The primary purpose of medical research involving human subjects is to understand the causes, development and effects of diseases and improve preventive, diagnostic and therapeutic interventions (methods, procedures and treatments). Even the best proven interventions must be evaluated continually through research for their safety, effectiveness, efficiency, accessibility and quality.
7. Medical research is subject to ethical standards that promote and ensure respect for all human subjects and protect their health and rights.
8. While the primary purpose of medical research is to generate new knowledge, this goal can never take precedence over the rights and interests of individual research subjects.
9. It is the duty of physicians who are involved in medical research to protect the life, health, dignity, integrity, right to self-determination, privacy, and confidentiality of personal information of research subjects. The responsibility for the protection of research subjects must always rest with the physician or other health care professionals and never with the research subjects, even though they have given consent.
10. Physicians must consider the ethical, legal and regulatory norms and standards for research involving human subjects in their own countries as well as applicable international norms and standards. No national or international ethical, legal or regulatory requirement should reduce or eliminate any of the protections for research subjects set forth in this Declaration.

11. Medical research should be conducted in a manner that minimises possible harm to the environment.
12. Medical research involving human subjects must be conducted only by individuals with the appropriate ethics and scientific education, training and qualifications. Research on patients or healthy volunteers requires the supervision of a competent and appropriately qualified physician or other health care professional.
13. Groups that are underrepresented in medical research should be provided appropriate access to participation in research.
14. Physicians who combine medical research with medical care should involve their patients in research only to the

extent that this is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

15. Appropriate compensation and treatment for subjects who are harmed as a result of participating in research must be ensured.

RISKS, BURDENS AND BENEFITS

16. In medical practice and in medical research, most interventions involve risks and burdens.
Medical research involving human subjects may only be conducted if the importance of the objective outweighs the risks and burdens to the research subjects.

17. All medical research involving human subjects must be preceded by careful assessment of predictable risks and burdens to the individuals and groups involved in the research in comparison with foreseeable benefits to them and to other individuals or groups affected by the condition under investigation.
Measures to minimise the risks must be implemented. The risks must be continuously monitored, assessed and documented by the researcher.

18. Physicians may not be involved in a research study involving human subjects unless they are confident that the risks have been adequately assessed and can be satisfactorily managed.
When the risks are found to outweigh the potential benefits or when there is conclusive proof of definitive outcomes, physicians must assess whether to continue, modify or immediately stop the study.

VULNERABLE GROUPS AND INDIVIDUALS

19. Some groups and individuals are particularly vulnerable and may have an increased likelihood of being wronged or of incurring additional harm.
All vulnerable groups and individuals should receive specifically considered protection.

20. Medical research with a vulnerable group is only justified if the research is responsive to the health needs or priorities of this group and the research cannot be carried out in a non-vulnerable group. In addition, this group should stand to benefit from the knowledge, practices or interventions that result from the research.

SCIENTIFIC REQUIREMENTS AND RESEARCH PROTOCOLS

21. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and adequate laboratory and, as appropriate, animal experimentation. The welfare of animals used for research must be respected.

22. The design and performance of each research study involving human subjects must be clearly described and justified in a research protocol.
The protocol should contain a statement of the ethical considerations involved and should indicate how the principles in this Declaration have been addressed. The protocol should include information regarding funding, sponsors, institutional affiliations, potential conflicts of interest, incentives for subjects and information regarding provisions for treating and/or compensating subjects who are harmed as a consequence of participation in the research study.
In clinical trials, the protocol must also describe appropriate arrangements for post-trial provisions.

RESEARCH ETHICS COMMITTEES

23. The research protocol must be submitted for consideration, comment, guidance and approval to the concerned research ethics committee before the study begins. This committee must be transparent in its functioning, must be independent of the researcher, the sponsor and any other undue influence and must be duly qualified. It must take into consideration the laws and regulations of the country or countries in which the research is to be performed as well as applicable international norms and standards but these must not be allowed to reduce or eliminate any of the protections for research subjects set forth in this Declaration.
The committee must have the right to monitor ongoing studies. The researcher must provide monitoring information to the committee, especially information about any serious adverse events. No amendment to the protocol may be made without consideration and approval by the committee. After the end of the study, the researchers must submit a final report to the committee containing a summary of the study’s findings and conclusions.

PRIVACY AND CONFIDENTIALITY

24. Every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information.

INFORMED CONSENT

25. Participation by individuals capable of giving informed consent as subjects in medical research must be voluntary. Although it may be appropriate to consult family members or community leaders, no individual capable of giving informed consent may be enrolled in a research study unless he or she freely agrees.

26. In medical research involving human subjects capable of giving informed consent, each potential subject must

be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail, post-study provisions and any other relevant aspects of the study. The potential subject must be informed of the right to refuse to participate in the study or to withdraw consent to participate at any time without reprisal.

Special attention should be given to the specific information needs of individual potential subjects as well as to the methods used to deliver the information.
After ensuring that the potential subject has understood the information, the physician or another appropriately qualified individual must then seek the potential subject's freely-given informed consent, preferably in writing. If the consent cannot be expressed in writing, the non-written consent must be formally documented and witnessed.
All medical research subjects should be given the option of being informed about the general outcome and results of the study.
27. When seeking informed consent for participation in a research study the physician must be particularly cautious if the potential subject is in a dependent relationship with the physician or may consent under duress. In such situations the informed consent must be sought by an appropriately qualified individual who is completely independent of this relationship.
28. For a potential research subject who is incapable of giving informed consent, the physician must seek informed consent from the legally authorised representative. These individuals must not be included in a research study that has no likelihood of benefit for them unless it is intended to promote the health of the group represented by the potential subject, the research cannot instead be performed with persons capable of providing informed consent, and the research entails only minimal risk and minimal burden.
29. When a potential research subject who is deemed incapable of giving informed consent is able to give assent to decisions about participation in research, the physician must seek that assent in addition to the consent of the legally authorised representative. The potential subject's dissent should be respected.
30. Research involving subjects who are physically or mentally incapable of giving consent, for example, unconscious patients, may be done only if the physical or mental condition that prevents giving informed consent is a necessary characteristic of the research group. In such circumstances the physician must seek informed consent from the legally authorised representative. If no such representative is available and if the research cannot be delayed, the study may proceed without informed consent provided that the specific reasons for involving subjects with a condition that renders them unable to give informed consent have been stated in the research protocol and the study has been approved by a research ethics committee. Consent to remain in the research must be obtained as soon as possible from the subject or a legally authorised representative.
31. The physician must fully inform the patient which aspects of their care are related to the research. The refusal of a patient to participate in a study or the patient's decision to withdraw from the study must never adversely affect the patient-physician relationship.
32. For medical research using identifiable human material or data, such as research on material or data contained in biobanks or similar repositories, physicians must seek informed consent for its collection, storage and/or reuse. There may be exceptional situations where consent would be impossible or impracticable to obtain for such research. In such situations the research may be done only after consideration and approval of a research ethics committee.

USE OF PLACEBO

33. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best proven intervention(s), except in the following circumstances:
Where no proven intervention exists, the use of placebo, or no intervention, is acceptable; or
Where for compelling and scientifically sound methodological reasons the use of any intervention less effective than the best proven one, the use of placebo, or no intervention is necessary to determine the efficacy or safety of an intervention and the patients who receive any intervention less effective than the best proven one, placebo, or no intervention will not be subject to additional risks of serious or irreversible harm as a result of not receiving the best proven intervention.
Extreme care must be taken to avoid abuse of this option.

POST-TRIAL PROVISIONS

34. In advance of a clinical trial, sponsors, researchers and host country governments should make provisions for post-trial access for all participants who still need an intervention identified as beneficial in the trial. This information must also be disclosed to participants during the informed consent process.

RESEARCH REGISTRATION AND PUBLICATION AND DISSEMINATION OF RESULTS

35. Every research study involving human subjects must be registered in a publicly accessible database before recruitment of the first subject.
36. Researchers, authors, sponsors, editors and publishers all have ethical obligations with regard to the publication

and dissemination of the results of research. Researchers have a duty to make publicly available the results of their research on human subjects and are accountable for the completeness and accuracy of their reports. All parties should adhere to accepted guidelines for ethical reporting. Negative and inconclusive as well as positive results must be published or otherwise made publicly available. Sources of funding, institutional affiliations and conflicts of interest must be declared in the publication. Reports of research not in accordance with the principles of this Declaration should not be accepted for publication.

UNPROVEN INTERVENTIONS IN CLINICAL PRACTICE

37. In the treatment of an individual patient, where proven interventions do not exist or other known interventions have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorised representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. This intervention should subsequently be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information must be recorded and, where appropriate, made publicly available.

ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

31. The physician may combine medical research with medical care only to the extent that the research is justified by its potential preventive, diagnostic or therapeutic value and if the physician has good reason to believe that participation in the research study will not adversely affect the health of the patients who serve as research subjects.

32. The benefits, risks, burdens and effectiveness of a new intervention must be tested against those of the best current proven intervention, except in the following circumstances:

• The use of placebo, or no treatment, is acceptable in studies where no current proven intervention exists; or

• Where for compelling and scientifically sound methodological reasons the use of placebo is necessary to determine the efficacy or safety of an intervention and the patients who receive placebo or no treatment will not be subject to any risk of serious or irreversible harm. Extreme care must be taken to avoid abuse of this option.

33. At the conclusion of the study, patients entered into the study are entitled to be informed about the outcome of the study and to share any benefits that result from it, for example, access to interventions identified as beneficial in the study or to other appropriate care or benefits.

34. The physician must fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study or the patient’s decision to withdraw from the study must never interfere with the patient-physician relationship.

35. In the treatment of a patient, where proven interventions do not exist or have been ineffective, the physician, after seeking expert advice, with informed consent from the patient or a legally authorized representative, may use an unproven intervention if in the physician's judgement it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, this intervention should be made the object of research, designed to evaluate its safety and efficacy. In all cases, new information should be recorded and, where appropriate, made publicly available.