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BMJ Open

Protocol for the Psychosis Immune Mechanism Stratified Medicine (PIMS) trial: A randomised double-blind placebo-controlled trial of single dose tocilizumab in patients with psychosis

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3 1 **Protocol for the Psychosis Immune Mechanism Stratified Medicine (PIMS) trial: A**
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5 2 **randomised double-blind placebo-controlled trial of single dose tocilizumab in patients**
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8 3 **with psychosis**
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35 ABSTRACT

36 **Introduction:** Evidence suggests a potentially causal role of interleukin 6 (IL-6), a
37 pleiotropic cytokine that generally promotes inflammation, in the pathogenesis of psychosis,
38 but no interventional studies elucidating potential mechanisms in patients with psychosis,
39 stratified using inflammatory markers, have been conducted. Tocilizumab is a humanised
40 monoclonal antibody targeting the IL-6 receptor to inhibit IL-6 signalling licensed in the UK
41 for treatment of rheumatoid arthritis. The primary objective of this study is to test whether IL-
42 6 contributes to the pathogenesis of psychosis, and to examine potential mechanisms by
43 which IL-6 affects psychotic symptoms. A secondary objective is to examine characteristics
44 of inflammation-associated psychosis.

45 **Methods and analysis:** A proof-of-concept study employing a randomised, parallel-group,
46 double-blind, placebo-controlled design testing the effect of IL-6 inhibition on anhedonia in
47 patients with psychosis. Approximately 60 participants with diagnosis of schizophrenia and
48 related psychotic disorders (ICD-10 codes F20, F22, F25, F28, F29) with evidence of low-
49 grade inflammation (IL-6 ≥ 0.7 pg/ml) will receive either one intravenous infusion of
50 tocilizumab (4.0mg/kg; max 800mg) or normal saline. Psychiatric measures and blood
51 samples will be collected at baseline, and 7-, 14-, and 28-days post-infusion. Cognitive and
52 neuroimaging data will be collected at baseline and 14 days post-infusion. In addition,
53 approximately 30 patients with psychosis without evidence of inflammation (IL-6 < 0.7 pg/ml)
54 and 30 matched healthy controls will be recruited to complete identical baseline assessments
55 to allow for comparison of the characteristic features of inflammation-associated psychosis.

56 **Ethics and dissemination:** The study is sponsored by the University of Bristol and has been
57 approved by the Cambridge East Research Ethics Committee (reference: 22/EE/0010; IRAS
58 project ID: 301682). Study findings will be published in peer-review journals. Findings will
59 be also disseminated by scientific presentation and by other means.

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60 **Trial registration number:** ISRCTN 23256704

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62 **KEYWORDS:** Psychotic Disorders; Negative Symptoms; Interleukin 6; Immunotherapy;
63 Tocilizumab; Clinical Trial.

For peer review only

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3 64 ARTICLE SUMMARY
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6 65 **Strengths and limitations of this study**
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- 8 66 • Adopting a randomised controlled trial (RCT) design and patient selection based on
9
10 67 elevated level of IL-6 (in addition to other criteria) will help examine the causal role
11
12 68 of IL-6, and the therapeutic potential of targeting IL-6 pathway, in psychosis.
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15 69 • The use of target specific intervention (anti-IL6R monoclonal antibody tocilizumab)
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17 70 will help assess the clinical relevance of IL-6 and related up- and downstream
18
19 71 inflammatory cytokines in psychosis.
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22 72 • The use of neuroimaging, cognitive tests, and extensive peripheral blood biomarker
23
24 73 exploration before and after tocilizumab treatment to assess potential mechanisms of
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26 74 effect.
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29 75 • One dose of tocilizumab is unlikely to be sufficient to test the efficacy of this drug as
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31 76 potential treatment for psychosis.
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34 77 • Tocilizumab inhibits both anti-inflammatory (classic) and pro-inflammatory (trans)
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36 78 pathways of IL-6 that may have complementary or differential effects relevant to
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38 79 potential therapeutic effects.
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3 81 **Word count:** 3,956
4

5 82 INTRODUCTION
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8 83 **Scientific background and study rationale**
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10 84 The neuroimmune hypothesis of schizophrenia proposes that mild peripheral immune
11
12 85 activation gives rise to an inflammatory response in the brain and neurobiological changes
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14 86 associated with psychotic illness [1–4]. Meta-analytic evidence is clear that circulating
15
16 87 concentrations of interleukin 6 (IL-6) and other inflammatory proteins, such as C-reactive
17
18 88 protein (CRP), are increased in patients with psychosis, including treatment naïve first
19
20 89 episode psychosis (FEP) [5], compared with controls [6–9]. Prospective cohort studies show
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22 90 that these indices of mild immune activation precede the onset of symptoms [10,11].
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26 91 Furthermore, genetic variants known to increase IL-6 concentrations are associated with
27
28 92 genetic risk of schizophrenia [12,13]. These Mendelian randomization studies eliminate the
29
30 93 possibility that raised IL-6 concentrations are a consequence of environmental exposures
31
32 94 associated with schizophrenia, such as obesity and smoking and instead suggest that IL-6 has
33
34 95 a causal role in psychosis. Extending this approach using the UK Biobank population, we
35
36 96 found that genetically-predicted levels of IL-6 were associated with reduced grey matter
37
38 97 primarily in the middle temporal gyrus, a region whose gene expression profile is enriched
39
40 98 for IL-6 pathway proteins and for neuropsychiatric disorder ontologies [14]. Moreover,
41
42 99 clinical studies report correlations between IL-6 levels and structural brain changes in
43
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45 100 individuals with schizophrenia [15], with reduced grey matter volume being exaggerated in
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47 101 patients with psychosis and elevated inflammatory cytokines [16]. Though this causal
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50 102 evidence strongly implicates IL-6, only an intervention study in patients can test the causal
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53 103 hypothesis.
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56 104 The neuroimmune hypothesis generally assumes that microglia, the brain's resident
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58 105 immune cells, are activated and pathogenic in schizophrenia. This is supported by traditional
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3 106 neuropathological studies and initial in-vivo PET imaging studies [17–19], possibly reflecting
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5 107 impaired cellular control of inflammation or oxidative defence. Inflammatory damage may
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8 108 also account for evidence of oxidative stress from MRS glutathione studies [20]. However,
9
10 109 whether microglia are the direct target of IL-6 is unclear and it is not certain that IL-6 can
11
12 110 cross the blood-brain barrier and/or increase its permeability to circulating inflammatory
13
14 111 cells, cytokines, and chemokines [2,21]. Additionally, it is increasingly uncertain whether
15
16 112 microglial inflammation, as traditionally understood, occurs in schizophrenia [22,23]. Recent
17
18 113 meta-analyses of PET radioligand binding studies report decreased rather than increased
19
20 114 radioligand binding to activated microglia [24,25]. This may account for the unexpected lack
21
22 115 of therapeutic benefit of the anti-microglial antibiotic, minocycline, in recent large clinical
23
24 116 trials [26,27]. Furthermore, large transcriptomic studies in post-mortem brains report no
25
26 117 change or reduction in microglial gene expression but increases in astrocytic expression
27
28 118 [23,28–32]. It is increasingly understood that both peripheral immune responses and brain
29
30 119 glial function are regulated by specialised T cells (Tregs), a subset of which reside in brain
31
32 120 parenchyma [33,34]. A novel proposal is that Treg hypofunction accounts for mild peripheral
33
34 121 immune disinhibition and dysregulated astroglial-microglial interaction, such that microglia
35
36 122 are driven into a developmental, synapse-pruning phenotype while astroglia disrupt
37
38 123 neurotransmitter function [33,34]. Importantly, there are bidirectional interactions between
39
40 124 IL-6 and Treg function [34]. Crucially, we will measure IL-6 in addition to cellular and
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42 125 molecular markers of immune function and investigate how they correlate with central
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44 126 markers and clinical state.
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51 127 Previous attempts testing the inflammatory hypothesis in therapeutic clinical trials
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53 128 have been attempted. However, little evidence of overall efficacy has been found [35]. These
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55 129 trials have generally tested broad spectrum agents, such as non-steroidal anti-inflammatory
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57 130 drugs, with no attempt to stratify patients according to evidence of inflammation. A trial
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3 131 using tocilizumab, a humanised monoclonal antibody (mAb) against the IL-6 receptor
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5 132 currently licensed in the UK for treatment of rheumatoid arthritis (RA) and severe
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7 133 coronavirus disease, reported no improvements in any clinical measure in a small sample of
8
9 134 36 patients with established schizophrenia [36]. However, as mentioned previously, no
10
11 135 stratification by inflammatory markers or any mechanistic immune measures was applied.
12
13 136 Low-grade inflammation is associated with poor response to antipsychotic drugs [37], but
14
15 137 immunotherapy is unlikely to be relevant for all patients with psychosis. Meta-analysis
16
17 138 suggests that evidence of immune activation, defined by elevated CRP levels, is present in a
18
19 139 quarter to one third of patients with schizophrenia [38]. A randomised controlled trial of
20
21 140 infliximab, an anti-tumour necrosis factor alpha (TNF- α) mAb, reported that antidepressant
22
23 141 response was associated with higher CRP levels at baseline [39], suggesting that patients with
24
25 142 evidence of immune activation may be better candidates for immunotherapy trials. As far as
26
27 143 we are aware, no previous clinical trial has selected patients with schizophrenia based on
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29 144 evidence of immune activation.
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35 145 Selection of patients with particular symptom profiles and/or stage of illness may also
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37 146 be a useful strategy that needs to be employed in immunotherapy trials for schizophrenia. A
38
39 147 wide variety of symptoms occur in schizophrenia such as hallucinations, delusions,
40
41 148 anhedonia, cognitive dysfunction, and affective symptoms and presentation of these
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43 149 symptoms differ from one individual to another. Some symptoms may be more related to
44
45 150 inflammation than others. For instance, a recent study from the ALSPAC birth cohort
46
47 151 reported that out of 20 positive and negative symptoms, CRP is particularly associated with
48
49 152 anhedonia and auditory hallucinations [40]. Anhedonia and amotivation are strongly
50
51 153 associated with poor functional outcomes in depression and schizophrenia, and present a
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53 154 formidable barrier to returning to work or building relationships [41,42]. Patients with
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55 155 psychotic disorders also present with cognitive deficits in a range of domains [43]. Available
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3 156 antipsychotic medications have a limited effects on poor cognitive functioning in psychosis
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5 157 [44]. Illness stage may also be of relevance. Meta-analytic data has revealed no differences in
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7 158 IL-6 levels between stable, medicated patients with schizophrenia and controls, although
8
9 159 compared with controls, IL-6 levels were similarly elevated in patients with FEP and those
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11 160 with acute relapse [7]. A separate meta-analysis found evidence of elevated blood cytokine
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13 161 levels in acutely and chronically ill patients with schizophrenia [6]. Focusing on particular
14
15 162 inflammation-related symptoms and/or illness stage may increase the chance of success for
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17 163 immunotherapy trials.
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24 165 **Proposed study**

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26 166 The proposed two-year study is a UK multi-site (Bristol, Birmingham, and Cambridge)
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28 167 proof-of-concept, randomised, parallel-group, double-blind, placebo-controlled trial.
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33 169 *Study aims and hypotheses*

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35 170 The primary aim of this trial is to examine potential mechanisms by which IL-6 affects
36
37 171 anhedonia, psychotic symptoms, and cognition. Our primary hypothesis is that inhibition of
38
39 172 IL-6 signalling with a single intravenous infusion of anti-IL6R monoclonal antibody,
40
41 173 tocilizumab, in individuals with psychosis and elevated IL-6 at baseline will attenuate
42
43 174 symptoms of anhedonia and amotivation in patients with psychosis, relative to placebo. This
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45 175 will provide further evidence for a potential causal role of inflammation in psychosis. Our
46
47 176 secondary hypothesis is that reduction in peripheral inflammation after tocilizumab infusion
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49 177 in patients with psychosis and evidence of inflammation will be associated with central
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51 178 measures of oxidative stress and relevant resting state brain function.
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56 179 We will also conduct deep immunophenotyping of peripheral blood mononuclear cell
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58 180 subsets (CD4⁺, CD8⁺, Tregs, natural killer and natural killer-T cells, monocytes, and B cells)
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3 181 to characterise their absolute number, frequency, and function. Our primary mechanistic
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5 182 outcome is the level of IL-6/STAT3 signalling inhibition within both innate and adaptive
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8 183 immune cells using multi-colour flow cytometry with an established optimised pSTAT3
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10 184 phosflow assay. This will help identify the potential cellular impact of peripheral
11
12 185 inflammation in psychosis, which is largely unknown. Functional assessment of IL-6/STAT3
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14 186 signalling in immune cell subsets and their response to exogenous IL-6 stimulation will
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16 187 inform abnormal immune response in psychosis and allow measurement of response to
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18 188 tocilizumab at the cellular level.

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21 189 A secondary objective is to carry out an observational study to examine clinical and
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23 190 biomarker differences and similarities between patients with psychotic disorder with and
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25 191 without evidence of inflammation and healthy controls (HCs). We hypothesise that
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27 192 individuals with psychotic disorder and evidence of inflammation, compared to those without
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29 193 evidence of inflammation and HCs, will have increased symptoms of anhedonia and
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31 194 amotivation, poorer cognitive functioning, and cellular and brain-based measures of immune
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33 195 dysfunction.

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38 39 40 41 197 METHODS

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43 198 This protocol has been prepared in accordance with the Standard Protocol Items:
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45 199 Recommendations for Interventional Trials (SPIRIT) 2013 statement [45]. Please see
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47 200 supplementary eTable 1 for the SPIRIT checklist.

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51 52 202 **Patient and public involvement**

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54 203 The study protocol was prepared in collaboration with individuals with lived experience of
55
56 204 mental illness who contributed to the development of participant information sheet, consent
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58 205 forms, and data collection procedures.

206 **Study design and sample**

207 See Figure 1 for an overview of study design. Individuals residing in Bristol, Birmingham, or
208 Cambridge in the United Kingdom will be recruited. Approximately 60 participants with
209 psychosis and evidence of inflammation (i.e., IL-6 ≥ 0.7 pg/ml) will be randomised to receive
210 either one intravenous infusion of tocilizumab (drug) or normal saline (placebo). For the
211 secondary, observational study, we will compare baseline characteristics of the intervention
212 cohort with approximately 30 participants with psychosis without evidence of inflammation
213 (i.e., IL-6 < 0.7 pg/ml), and approximately 30 HCs. Participants without evidence of
214 inflammation and controls will not be randomised as they will not receive any intervention.
215 Neuroimaging will only be undertaken by those without MRI contraindications who have
216 given specific informed consent for MRI. Participants not eligible or not consenting for MRI
217 will take part in all other aspects of the study.

219 **Intervention**

220 Single intravenous infusion of tocilizumab (4.0mg/kg; max 800mg in total) or normal saline
221 given to participants with psychosis and evidence of inflammation. Tocilizumab blocks both
222 IL-6 classic and trans-signalling – the latter being responsible for most of the inflammatory
223 effects of IL-6 – providing broad inhibition of IL-6 signalling and a strong test of a casual
224 role for IL-6 in psychosis [46]. Tocilizumab is the first-in-class, humanized monoclonal
225 antibody against the IL-6R, commercially available and licensed in the UK for treatment of
226 RA. Approved dosage of tocilizumab for treatment of RA is 2, 4, or 8mg/kg; max 800mg in
227 total. In RA, a single tocilizumab infusion has shown to improve clinical and laboratory
228 measures within 48 hours, with most noticeable results in one-to-two weeks [47,48]. The
229 follow-up schedule for our study is in keeping with this observation.

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3 **230 Eligibility criteria**
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5 231 We will recruit participants aged 18-40 years. Patient participants must meet International
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7 232 Classification of Diseases 10th Revision (ICD-10) criteria for a diagnosis of schizophrenia
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9 233 and related psychoses (ICD-10 code F20, F22, F25, F28, F29) at the time of eligibility
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11 234 assessment, be within three years of first diagnosis of psychotic disorder, be on a stable
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13 235 treatment regime with no recent (within two weeks) initiation, cessation, or change in class of
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15 236 antipsychotic medication, and have a Positive and Negative Syndrome Scale (PANSS) item
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17 237 score ≥ 3 on P1 (delusions), P2 (conceptual disorganisation), P3 (hallucinatory behaviour), or
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19 238 P6 (suspiciousness/persecution). Additionally, patients recruited to the interventional arm
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21 239 will be required to have serum IL-6 levels ≥ 0.7 pg/ml and a Temporal Experience of Pleasure
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23 240 Scale (TEPS) anticipatory pleasure score ≤ 41 (based on item numbers 1, 3, 7, 11, 12, 14, 15,
24
25 241 16, 17, and 8) and consummatory pleasure score ≤ 36 (based on item numbers 2, 4, 5, 6, 8, 9,
26
27 242 10, and 13). Finally, COVID-19 anti-body titre test will be used to determine adequate levels
28
29 243 of immune response via the following cut-offs (for poor response): 400IU Roche/700IU
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31 244 Abbot assay.
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37 245 HCs will have no current or lifetime history of psychiatric diagnosis, as determined by
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39 246 the Mini-International Neuropsychiatric Interview (MINI). See Table 1 for complete
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41 247 inclusion and exclusion criteria. HCs will be matched to patient participants at the group level
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43 248 by age and sex.
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251 **Table 1. PIMS Trial inclusion and exclusion criteria.**

| Group | Inclusion criteria | Exclusion criteria |
|------------------|---|---|
| All participants | <ul style="list-style-type: none"> - Provide informed consent. - Understand written and spoken English. - Able and willing to consent to blood sampling. - Willing to abstain from strenuous exercise for 72 hours prior to assessment. | <ul style="list-style-type: none"> - Pregnancy (confirmed by urine pregnancy test) or breast feeding. - Body mass index >35. - Current or lifetime diagnosis of antisocial personality disorder, autism or other neurodevelopmental disorder, major traumatic brain injury. - Currently active diagnosed eating disorder likely to compromise ability to take part. - History of alcohol or substance use disorder (abuse/dependence) within six months prior to eligibility assessment (nicotine and caffeine dependence are not exclusionary). - Current use of medication likely to compromise interpretation of immunological data. - Known active current or history of recurrent bacterial, viral, fungal, mycobacterial, or other opportunistic infections. - Current infection with VZV, TB, Hepatitis B, Hepatitis C, or HIV confirmed by blood test. Chest X-ray will also be performed to assess for TB. - Any major episode of infection requiring hospitalisation or treatment with IV antibiotics within 4 weeks of eligibility assessment. - Unstable cardiac, pulmonary, renal, hepatic, endocrine, hematologic, or active infectious disease, including current or prior malignancy. - Diverticulitis, inflammatory bowel disease, or uncontrolled gastric/duodenal ulcer. - Concomitant auto-immune or auto-inflammatory rheumatological disease. - Concomitant treatment with any biologic drugs. - Current and active ischemic heart disease. - Uncontrolled hypertension defined as systolic blood pressure > 170 or diastolic blood pressure > 110. - History of severe allergic or anaphylactic reactions to human, humanized or murine monoclonal antibodies. - No history of chicken pox infection or no history of varicella zoster immunity. |

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|---|---|-----------------------------|
| Additional criteria for neuroimaging (optional) | - Able and willing to consent to MRI scanning | - Contraindications to MRI. |
| Additional criterion for healthy controls | - No current or lifetime psychiatric diagnosis. | |
| Additional criteria for all individuals with psychosis | <ul style="list-style-type: none"> - Meet ICD-10 criteria for a diagnosis of schizophrenia and related psychoses (code F20, F22, F25, F28, F29) at the time of eligibility assessment, as determined by the Mini-International Neuropsychiatric Interview. - Be within three years of first diagnosis of psychotic disorder. - On stable treatment regime with no recent (within 2 weeks) initiation, cessation, or change in class of antipsychotic medication. - No indication or other reason for preclusion into research (e.g., significant risk of suicidal behaviour or risk to others) as determined by their clinical team. - Positive and Negative Syndrome Scale item score ≥ 3 on P1 (delusions), P2 (conceptual disorganisation), P3 (hallucinatory behaviour), OR P6 (suspiciousness/persecution). | |
| Additional criteria for intervention group | <ul style="list-style-type: none"> - Serum IL-6 level ≥ 0.7pg/ml at eligibility and baseline assessment. - Temporal Experience of Pleasure Scale anticipatory pleasure score ≤ 41 (based upon item numbers 1, 3, 7, 11, 12, 14, 15, 16, 17, and 8) and consummatory pleasure score ≤ 36 (based upon item numbers 2, 4, 5, 6, 8, 9, 10, and 13). - Evidence of COVID-19 immunity required prior to infusion, confirmed before randomisation using evidence of vaccination and antibody titre test. | |
| Additional criterion for patients with psychosis without inflammation | - Serum IL-6 level < 0.7 pg/ml at eligibility and baseline assessment. | |

254 **Study outcomes**

255 The primary outcome is anhedonia, defined as anticipatory and consummatory pleasure
 256 scores, assessed by the TEPS [49] at approximately day 14 post-infusion. The primary
 257 mechanistic outcome is the level of IL-6/STAT3 signalling inhibition post-tocilizumab
 258 infusion in both innate and adaptive immune cells using multi-colour flow cytometry and an
 259 established optimised pSTAT3 phosflow assay. We will also collect data on several
 260 tertiary/exploratory measures including positive and negative symptoms of psychosis,
 261 depressive symptoms, fatigue, quality of life and subjective wellbeing, cognitive function,
 262 peripheral blood inflammatory markers, cortisol, cell expression, including DNA and RNA
 263 sequencing, functionality, and neuroimaging measures, including functional resting state MRI
 264 and MRS outcomes (Table 2).

265
 266 **Table 2. PIMS trial study measures**

| Domain | Tool | Source | Validated Tool | Time of assessment |
|----------------------------|---|------------------------------|----------------|-----------------------------------|
| Sociodemographic/lifestyle | Screening questionnaire | Self-report | | Screening |
| | Medical History Questionnaire | Self-report/General practice | | Eligibility |
| | Substance Use Questionnaire | Self-report | | Eligibility |
| | Physical Measurements Form | Self-report | | Baseline |
| | Sociodemographic Questionnaire | Self-report | | Baseline |
| Psychiatric | The Temporal Experience of Pleasure Scale | Self-report | ✓ | Eligibility, baseline, follow-ups |
| | The Positive and Negative Syndrome Scale | Interviewer assessed | ✓ | Eligibility, baseline, follow-ups |
| | The Mini-International Neuropsychiatric Interview | Interviewer assessed | ✓ | Eligibility |
| | Psychiatric History Questionnaire | Self-report | | Baseline |

| | | | | |
|--------------|---|----------------------|---|-----------------------|
| | The Scale for the Assessment of Negative Symptoms | Self-report | ✓ | Baseline, follow-ups |
| | The Calgary Depression Scale for Schizophrenia | Interviewer assessed | ✓ | Baseline, follow-ups |
| | Multi-dimensional Fatigue Inventory | Self-report | ✓ | Baseline, follow-ups |
| | European Quality of Life-5 Dimensions Three-Level Version | Self-report | ✓ | Baseline, follow-ups |
| | Visual Analogue Scale for Subjective Wellbeing | Self-report | ✓ | Baseline, follow-ups |
| Cognitive | National Adult Reading Test for estimated premorbid IQ | Interviewer assessed | ✓ | Baseline, follow-up 2 |
| | CANTAB Reaction Time test | Computer task | ✓ | Baseline, follow-up 2 |
| | Symbol Coding Test | Paper task | ✓ | Baseline, follow-up 2 |
| | CANTAB Rapid Visual Information Processing test | Computer task | ✓ | Baseline, follow-up 2 |
| | CANTAB Paired Associates Learning test | Computer task | ✓ | Baseline, follow-up 2 |
| | CANTAB One Touch Stockings of Cambridge test | Computer task | ✓ | Baseline, follow-up 2 |
| Biologic | Inflammatory markers, cardiometabolic markers,IDO activation, white cell phenotyping | Laboratory tests | | Baseline, follow-ups |
| Genetic | Gene expression/genotyping | Blood (RNA, DNA) | | Baseline, follow-ups |
| Neuroimaging | MRI Screening Questionnaire | | | Baseline, follow-up 2 |
| | Structural MRI, 1H-MRS measure of glutathione in the prefrontal cortex area, resting state fMRI | | | Baseline, follow-up 2 |

268 **Sample size and statistical power**

269 We will recruit approximately 60 patients with psychosis. However, currently there are no
270 trials of immunotherapies for anhedonia in schizophrenia making accurate power calculation
271 difficult. This study is a proof-of-concept experiment designed to test whether inhibition of
272 IL-6 signalling leads to changes in psychotic symptoms. It could also inform likely statistical
273 power for future trials testing efficacy of the drug as a treatment of schizophrenia, which is
274 not the intention of this study

275

276 **Randomisation and blinding**

277 An external agency independent of the study team will arrange random allocation to
278 tocilizumab or normal saline group 1:1, ensuring two groups are comparable regarding
279 anhedonia severity and sex. Randomisation will be stratified by site. Randomising agency
280 will provide the randomisation code to the relevant hospital pharmacy who will dispense
281 tocilizumab or normal saline according to the randomisation schedule. Dispensing
282 pharmacies will keep a log of products dispensed. Infusions will be prepared and
283 administered at clinical research facilities (CRFs). Infusion packs will be prepared by trained
284 staff not part of the core study team, ensuring blinding of treatment allocation. Infusion packs
285 containing drug or placebo will be visually indistinguishable from each other, ensuring that
286 both participants and study team remain blind regarding treatment allocation.

287

288 **Statistical analysis**

289 For randomised participants, an intention-to-treat approach will be taken for data analysis by
290 including all randomised participants in statistical analyses, regardless of the treatment they
291 received (if any). We will compare outcome measures between treatment and placebo groups
292 controlling for baseline scores. This mechanistic experiment will focus on overall pattern of

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3 293 results and their effect sizes rather than *P*-values for individual tests of statistical significance.
4

5 294 The secondary mechanistic and observational analysis will compare psychotic symptoms,
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7
8 295 cognitive function, blood, neuroimaging, and other biomarkers between and across study
9

10 296 groups using appropriate statistical tests.
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15 16 299 STUDY PROCEDURE

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18 300 An overview of study procedures is presented in Figure 1 and all study measures are detailed
19

20 301 in Table 2. Recruitment will take place in Bristol, Birmingham, and Cambridge and
21

22
23 302 assessments at University and NHS research facilities.
24

25 303
26

27 304 **Participant identification**

28
29 305 Potential participants with psychosis will be identified by NHS Psychosis Early Intervention
30

31 306 (EI) teams. HCs will be recruited through advertisement methods in Birmingham and
32

33 307 Cambridge. Potential participants will complete a screening questionnaire to confirm their
34

35 308 eligibility to participate. If deemed eligible, participants will be invited to an appointment to
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37 309 complete a full eligibility assessment.
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43 311 **Eligibility assessment**

44
45 312 Assessments will be carried out to establish eligibility and to obtain informed consent.
46

47
48 313 Patients will complete the MINI to confirm ICD-10 diagnosis of schizophrenia and related
49

50 314 psychoses, the PANSS to confirm the presence of positive symptoms of psychosis, and the
51

52 315 TEPS to confirm eligibility based on anticipatory and consummatory pleasure sum scores. A
53

54 316 blood sample will be collected from patients for serum IL-6 measurement. An MRI screening
55

56 317 questionnaire will be administered to those willing to give informed consent for
57

58 318 neuroimaging.
59
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3 319 **Baseline assessment**
4

5 320 All participants (60 inflamed psychosis, 30 non-inflamed psychosis, and 30 HCs) will attend
6
7 321 a baseline assessment comprising psychiatric measures, cognitive tasks, blood sampling, and
8
9 322 neuroimaging (optional). This will be the final study contact for patients without evidence of
10
11 323 inflammation and HCs. Patients with evidence of inflammation will undergo further tests to
12
13 324 establish safety/eligibility to receive tocilizumab, including a chest X-ray and blood tests to
14
15 325 exclude pregnancy and certain infections, such as TB, HIV, and COVID-19. Eligible
16
17 326 participants will be randomised and invited for infusion.
18
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25 328 **Intervention**

26 329 Intravenous infusion of tocilizumab or normal saline will be given continuously over one
27
28 330 hour at CRFs in Bristol, Birmingham, and Cambridge by trained clinical staff under the
29
30 331 supervision of a designated study doctor. Participants will remain under clinical observation
31
32 332 for a further 1-hour period after the end of infusion.
33
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37 334 **Follow-up assessments**

38 335 Follow-up assessments will take place approximately 7-, 14-, and 28-days post-infusion, and
39
40 336 will collect similar data to the baseline assessment. Cognitive tasks and neuroimaging
41
42 337 (optional) will be administered only on day 14. Around 42 days post-infusion, participants
43
44 338 will be contacted by phone to provide a final debrief; at which point they will exit the study.
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51 340 **RISK MANAGEMENT**

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53 341 **Psychosis-related risks**

54 342 All patients will be under the care of a specialist NHS psychosis EI service. Participation will
55
56 343 not involve any treatment modifications or significant delays in receiving treatment. If a
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3 344 patient becomes distressed during an assessment, or does not wish to continue for any reason,
4
5 345 the researcher will stop the assessment. Participants may withdraw at any time without giving
6
7 346 a reason. If there is any concern for the participant's safety, the research team will liaise with
8
9
10 347 participant's GP and/or mental health team as needed.
11

12 348

14 349 **Procedure-related risks**

16 350 *Venepuncture*

17
18
19 351 Blood taking is associated with mild discomfort and other side effects are rare. Efforts will be
20
21 352 made to minimise discomfort. Blood taking will be performed by a nurse, doctor, or research
22
23 353 team member trained in venepuncture.
24

25 354

26 355 *Chest X-ray*

27
28
29 356 This study will use a typical effective radiation dose of 0.014 mSv; equivalent to 2.5 days of
30
31 357 average natural background radiation in the UK. The risk of developing cancer as a
32
33 358 consequence of participating in this study is 0.0001%. Only non-pregnant, adult participants
34
35 359 will be included.
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38 360

39 361 *Neuroimaging*

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42 362 Discomfort during MRI will be minimised by using mirrors to allow participants to view
43
44 363 outside of the machine, providing ear plugs and a panic button, and allowing participants to
45
46 364 communicate with the researcher and scan operator throughout. Mild transient vertigo may be
47
48 365 experienced when being moved into the MRI machine. Risk of dislodgement or malfunction
49
50 366 of medical implants or metallic foreign objects will be minimised by screening participants to
51
52 367 ensure no metal is present on or within the body.
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3 369 *IL-6 levels*
4

5 370 Some participants will show evidence of inflammation in the blood (IL-6 ≥ 0.7 pg/ml). This is
6
7 371 not necessarily a cause for concern. In people with FEP, ~50% have serum IL-6 levels
8
9 372 > 0.7 pg/ml. Reasons for this in the absence of an acute infection or chronic inflammatory
10
11 373 illness could include obesity, smoking, alcohol use, and lack of exercise, so knowledge of
12
13 374 'inflammation status' may prompt participants to adopt a healthier lifestyle. If serum IL-6
14
15 375 level is high (i.e., IL-6 ≥ 0.7 pg/ml) along with elevated CRP (> 20 mg/L) without any apparent
16
17 376 explanation, such as infection or chronic inflammatory illness, we will inform the
18
19 377 participant's GP and the participant will be excluded from the study.
20
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26 379 *Risk to research staff*
27

28 380 Staff will follow local safety procedures when lone working. No other risks are anticipated.
29
30

31 381

32
33 382 **Safety considerations for infusion and monitoring of adverse reaction**
34

35 383 *Before infusion*
36

37 384 Participants will be selected based on strict inclusion and exclusion criteria. Additionally, we
38
39 385 will carry out tests for TB, HIV, VZV antibody, and Hepatitis B and C because, though
40
41 386 unlikely after a single dose, tocilizumab could make these infections worse. Female
42
43 387 participants of childbearing age will be given a pregnancy test, which must be negative.
44
45 388 Participants who are sexually active will be asked to use at least one form of effective
46
47 389 contraception for six weeks post-infusion. Male participants will also be asked not to donate
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49 390 sperm samples for six weeks post-infusion.
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3 394 *During infusion*
4

5 395 Infusions will be given under supervision of a designated study doctor. Participants will be
6
7 396 monitored for possible side effects, which will be managed in line with use of tocilizumab for
8
9 397 treating patients with RA.
10

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12 398
13

14 399 *After infusion*
15

16 400 Participants will remain under observation for one-hour post-infusion. Participants will be
17
18 401 advised to seek help if they feel unwell after leaving the assessment centre and will be given
19
20 402 an information sheet containing a telephone number their health professionals can call. If
21
22 403 necessary, we will unblind the participant and inform their health professional whether they
23
24 404 received tocilizumab or normal saline. Adverse reactions will be recorded at each follow-up
25
26 405 visit. Additional, safety blood tests will be done at second follow-up (e.g., WBC count, liver
27
28 406 function, lipids).
29
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35 408 ETHICS AND DISSEMINATION
36

37 409 The study will be conducted in accordance with the REC, Health Research Authority (HRA),
38
39 410 and local Research and Development (R&D) department approvals and guidelines (REC
40
41 411 reference: 22/EE/0010). The study team will prepare protocol amendments as required and
42
43 412 ethics approval will be sought before implementing any changes to the approved protocol.
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45

46 413 The ISRCTN Trial Registry and the Research Governance Office will be informed of any
47
48 414 amendments to the protocol.
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52

53 416 **Consent**
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55 417 Informed consent will be obtained prior to eligibility assessments for participation in the
56
57 418 study. This will include consent to randomise, for contact with their GP to inform them about
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3 419 participation, access GP/psychiatric records to verify medical history to establish eligibility,
4
5 420 and to inform the participant's GP any results/outcomes as necessary. Consent for additional
6
7 421 tests to establish safety for tocilizumab infusion and for storing biological samples will also
8
9 422 be obtained.
10
11

12 423

14 424 **Study management**

16
17 425 The study is sponsored by the University of Bristol. The sponsor, the Chief Investigator
18
19 426 (GMK), and the co-Lead (RU) will have overall responsibility for the study. A named
20
21 427 principal investigator will take clinical responsibility for study activities at each site. The
22
23 428 study does not require the formal arrangement of a steering committee because, according to
24
25 429 the HRA, it is not a Clinical Trial of an Investigational Medicinal Product. However, to
26
27 430 enhance monitoring of the study, a study management group will be established, comprising
28
29 431 academic and clinical experts in psychiatry, rheumatology, neuroscience, and immunology.
30
31

32 432

35 433 **Data management and retention of samples**

37 434 All potential participants will be assigned a unique study-specific participant ID number. All
38
39 435 data will be subject to good practice as laid down in the Data Protection Act. Each study
40
41 436 stage is tracked so that participant's (de-identified) status within the study is known, and
42
43 437 assessment and other appointment dates are forecasted. This information is held on a secure,
44
45 438 password-protected database. Anonymised data from assessments will be uploaded to a
46
47 439 secure, password-protected database using secure web-based data entry systems. Minimal
48
49 440 personal data (age, sex) will be indexed by each participant's unique ID number. Blood
50
51 441 samples collected in this study may be stored for up to 10 years after the completion for
52
53 442 additional research. Stored samples will be coded throughout the sample storage and analysis
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3 443 process and will not be labelled with personal identifiers. Participants may withdraw their
4
5 444 consent for their samples to be stored for future research.
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9
10 446 **Dissemination plan**

11
12 447 Study results will be published in peer-review journals and will conform to the guidelines of
13
14 448 the International Committee of Medical Journal Editors. Findings will be disseminated at
15
16 449 conferences, departmental talks, and via social and traditional media.
17
18
19 450

20
21 451 **AUTHORS CONTRIBUTIONS**

22
23 452 ÉMF wrote first draft of the PIMS trial protocol and of this manuscript. SLG, MK, GKM,
24
25 453 BD, DJ, JS, and NMB contributed to study design and protocol development and revised
26
27 454 manuscript drafts. RU contributed to study design and study protocol, and revised manuscript
28
29 455 drafts. GMK devised study design and trial protocol, and revised drafts. ÉMF and SLG
30
31 456 developed study materials and liaised with REC and HRA regarding approvals. AM, JR,
32
33 457 FCZ, and HH contributed to the revision of the manuscript and validation of operating
34
35 458 procedures and mechanistic protocols. RU and GMK co-lead the MRC grant that funds the
36
37 459 PIMS trial and provide overall supervision and oversight for the project.
38
39
40 460

41
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2
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18
19
20
21
22 476

23 477 COMPETING INTERESTS STATEMENT

24 478 ÉMF, SLG, AM, JR, FCZ, HH, MK, GKM, BD, DJ, JS, RU, and GMK have no conflicts of
25
26 479 interest to report. NMB holds shares and is a Director of Celentyx Ltd.
27
28
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31 480

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3 645 FIGURE LEGEND
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5 646 Figure 1. Overview of study design
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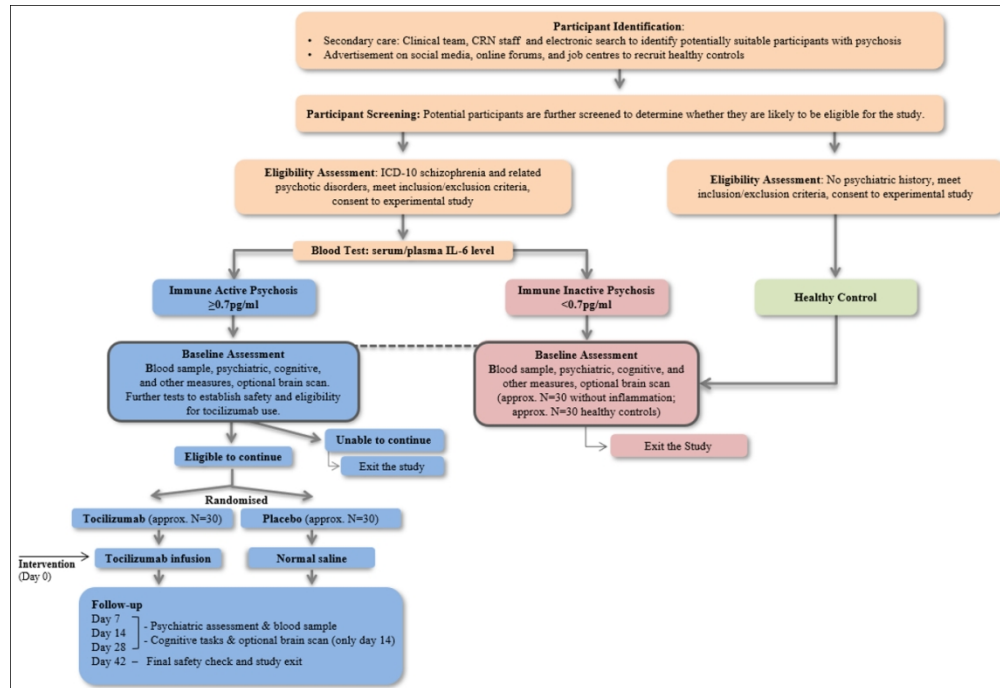


Figure 1. Overview of study design

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Foley et al. Protocol for the Psychosis Immune Mechanism Stratified Medicine (PIMS) trial: A randomised double-blind placebo-controlled trial of single dose tocilizumab in patients with psychosis.

eTable1: SPIRIT 2013 Checklist – Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description | Addressed on page number |
|-----------------------------------|---------|--|---------------------------|
| Administrative information | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | 4 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | 4 (ISRCTN 23256704) |
| Protocol version | 3 | Date and version identifier | - |
| Funding | 4 | Sources and types of financial, material, and other support | 24 - 25 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | 1, 24 |
| | 5b | Name and contact information for the trial sponsor | 23 |
| | 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 23-25 |

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| | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | 23 - 25 |
| Introduction | | | |
| Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 6-9 |
| | 6b | Explanation for choice of comparators | 6-9 |
| Objectives | 7 | Specific objectives or hypotheses | 9-10 |
| Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | 9 |
| Methods: Participants, interventions, and outcomes | | | |
| Study setting | 9 | Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 9, 11 |
| Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | 12, Table 1 |
| Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 11, 19, 21-22, Figure 1 |
| | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) | 22 |
| | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | - |
| | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | 12, Table 1 |

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| 1 | Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 9-10, Figure 1, Table 2 |
| 2 | | | | |
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| 6 | Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 18-19, Figure 1 |
| 7 | | | | |
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| 9 | Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 17 |
| 10 | | | | |
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| 13 | Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | 18 |
| 14 | | | | |
| 15 | Methods: Assignment of interventions (for controlled trials) | | | |
| 16 | Allocation: | | | |
| 17 | | | | |
| 18 | Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 17 |
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| 25 | Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 17 |
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| 29 | Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 17 |
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| 33 | Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 17 |
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| 36 | | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | 22 |
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46**Methods: Data collection, management, and analysis**

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|----------------------------|-----|--|-----------------------|
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 10-15, 18-19, Table 2 |
| | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | 17-18 |
| Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | 23-24 |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 17-18 |
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 17-18 |
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | - |
| Methods: Monitoring | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | 23 |
| | 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | - |
| Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 20-22 |

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| 1 | Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | - |
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| 4 | Ethics and dissemination | | | |
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| 6 | Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | 22 |
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| 8 | | | | |
| 9 | Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | 22 |
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| 14 | Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 22-23 |
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| 17 | | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | 22-23 |
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| 20 | Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 23-24 |
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| 24 | Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 25 |
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| 27 | Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 23 |
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| 30 | Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | 22 |
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| 33 | Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 24 |
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| 38 | | 31b | Authorship eligibility guidelines and any intended use of professional writers | 24 |
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| 40 | | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | - |
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| Appendices | | | |
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | Available in full protocol |
| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | 23-24 |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons "[Attribution-NonCommercial-NoDerivs 3.0 Unported](#)" license.

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BMJ Open

Protocol for the Psychosis Immune Mechanism Stratified Medicine (PIMS) trial: A randomised double-blind placebo-controlled trial of single dose tocilizumab in patients with psychosis

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|---------------------------------|---|
| Journal: | <i>BMJ Open</i> |
| Manuscript ID | bmjopen-2022-067944.R1 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 28-Nov-2022 |
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| Primary Subject Heading: | Mental health |

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| Secondary Subject Heading: | Immunology (including allergy) |
| Keywords: | Schizophrenia & psychotic disorders < PSYCHIATRY, IMMUNOLOGY, Clinical trials < THERAPEUTICS, Magnetic resonance imaging < RADIOLOGY & IMAGING |
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Manuscripts

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3 1 **Protocol for the Psychosis Immune Mechanism Stratified Medicine (PIMS) trial: A**
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5 2 **randomised double-blind placebo-controlled trial of single dose tocilizumab in patients**
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12 5 Éimear M. Foley^{a,b}; Sian Lowri Griffiths^c; Alexander Murray^c; Jack Rogers^c; Fabiana Corsi-
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14 6 Zuelli^{c,d,e}; Hannah Hickinbotham^f; Ella Warwick^c; Martin Wilson^c; Muzaffer Kaser^{f,g};
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16 7 Graham K. Murray^{f,g}; Bill Deakin^h; Deepak Jadonⁱ; John Suckling^{f,g}; Nicholas M. Barnes^e;
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18 8 Rachel Upthegrove^{c,j,†}; and Golam M. Khandaker^{a,b,k,l,†,*}; for the PIMS Collaboration
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35 ABSTRACT

36 **Introduction:** Evidence suggests a potentially causal role of interleukin 6 (IL-6), a
37 pleiotropic cytokine that generally promotes inflammation, in the pathogenesis of psychosis,
38 but no interventional studies elucidating potential mechanisms in patients with psychosis,
39 stratified using inflammatory markers, have been conducted. Tocilizumab is a humanised
40 monoclonal antibody targeting the IL-6 receptor to inhibit IL-6 signalling licensed in the UK
41 for treatment of rheumatoid arthritis. The primary objective of this study is to test whether IL-
42 6 contributes to the pathogenesis of psychosis, and to examine potential mechanisms by
43 which IL-6 affects psychotic symptoms. A secondary objective is to examine characteristics
44 of inflammation-associated psychosis.

45 **Methods and analysis:** A proof-of-concept study employing a randomised, parallel-group,
46 double-blind, placebo-controlled design testing the effect of IL-6 inhibition on anhedonia in
47 patients with psychosis. Approximately 60 participants with diagnosis of schizophrenia and
48 related psychotic disorders (ICD-10 codes F20, F22, F25, F28, F29) with evidence of low-
49 grade inflammation (IL-6 ≥ 0.7 pg/ml) will receive either one intravenous infusion of
50 tocilizumab (4.0mg/kg; max 800mg) or normal saline. Psychiatric measures and blood
51 samples will be collected at baseline, and 7-, 14-, and 28-days post-infusion. Cognitive and
52 neuroimaging data will be collected at baseline and 14 days post-infusion. In addition,
53 approximately 30 patients with psychosis without evidence of inflammation (IL-6 < 0.7 pg/ml)
54 and 30 matched healthy controls will be recruited to complete identical baseline assessments
55 to allow for comparison of the characteristic features of inflammation-associated psychosis.

56 **Ethics and dissemination:** The study is sponsored by the University of Bristol and has been
57 approved by the Cambridge East Research Ethics Committee (reference: 22/EE/0010; IRAS
58 project ID: 301682). Study findings will be published in peer-review journals. Findings will
59 be also disseminated by scientific presentation and by other means.

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60 **Trial registration number:** ISRCTN 23256704

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62 **KEYWORDS:** Psychotic Disorders; Negative Symptoms; Interleukin 6; Immunotherapy;
63 Tocilizumab; Clinical Trial.

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3 64 ARTICLE SUMMARY
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6 65 **Strengths and limitations of this study**
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- 8 66 • Adopting a randomised controlled trial (RCT) design and patient selection based on
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10 67 elevated level of IL-6 (in addition to other criteria) will help examine the causal role
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12 68 of IL-6, and the therapeutic potential of targeting IL-6 pathway, in psychosis.
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15 69 • The use of target specific intervention (anti-IL6R monoclonal antibody tocilizumab)
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17 70 will help assess the clinical relevance of IL-6 and related up- and downstream
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19 71 inflammatory cytokines in psychosis.
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22 72 • The use of neuroimaging, cognitive tests, and extensive peripheral blood biomarker
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24 73 exploration before and after tocilizumab treatment to assess potential mechanisms of
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26 74 effect.
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29 75 • One dose of tocilizumab is unlikely to be sufficient to test the efficacy of this drug as
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31 76 potential treatment for psychosis.
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34 77 • Tocilizumab inhibits both anti-inflammatory (classic) and pro-inflammatory (trans)
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36 78 pathways of IL-6 that may have complementary or differential effects relevant to
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38 79 potential therapeutic effects.
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3 81 **Word count:** 4,245
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5 82 INTRODUCTION
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8 83 **Scientific background and study rationale**
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10 84 The neuroimmune hypothesis of schizophrenia proposes that mild peripheral immune
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12 85 activation gives rise to an inflammatory response in the brain and neurobiological changes
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14 86 associated with psychotic illness [1–4]. Meta-analytic evidence is clear that circulating
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16 87 concentrations of interleukin 6 (IL-6) and other inflammatory proteins, such as C-reactive
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18 88 protein (CRP), are increased in patients with psychosis, including treatment naïve first
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20 89 episode psychosis (FEP) [5], compared with controls [6–9]. Prospective cohort studies show
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22 90 that these indices of mild immune activation precede the onset of symptoms [10,11].
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26 91 Furthermore, genetic variants known to increase IL-6 concentrations are associated with
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28 92 genetic risk of schizophrenia [12,13]. These Mendelian randomization studies eliminate the
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30 93 possibility that raised IL-6 concentrations are a consequence of environmental exposures
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32 94 associated with schizophrenia, such as obesity and smoking and instead suggest that IL-6 has
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34 95 a causal role in psychosis. Extending this approach using the UK Biobank population, we
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36 96 found that genetically-predicted levels of IL-6 were associated with reduced grey matter
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38 97 primarily in the middle temporal gyrus, a region whose gene expression profile is enriched
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40 98 for IL-6 pathway proteins and for neuropsychiatric disorder ontologies [14]. Moreover,
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42 99 clinical studies report correlations between IL-6 levels and structural brain changes in
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45 100 individuals with schizophrenia [15], with reduced grey matter volume being exaggerated in
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47 101 patients with psychosis and elevated inflammatory cytokines [16]. Though this causal
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49
50 102 evidence strongly implicates IL-6, only an intervention study in patients can test the causal
51
52
53 103 hypothesis.
54

55
56 104 The neuroimmune hypothesis generally assumes that microglia, the brain's resident
57
58 105 immune cells, are activated and pathogenic in schizophrenia. This is supported by traditional
59
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1
2
3 106 neuropathological studies and initial in-vivo PET imaging studies [17–19], possibly reflecting
4
5 107 impaired cellular control of inflammation or oxidative defence. Inflammatory damage may
6
7
8 108 also account for evidence of oxidative stress from MRS glutathione studies [20]. However,
9
10 109 whether microglia are the direct target of IL-6 is unclear and it is not certain that IL-6 can
11
12 110 cross the blood-brain barrier and/or increase its permeability to circulating inflammatory
13
14 111 cells, cytokines, and chemokines [2,21]. Additionally, it is increasingly uncertain whether
15
16 112 microglial inflammation, as traditionally understood, occurs in schizophrenia [22,23]. Recent
17
18 113 meta-analyses of PET radioligand binding studies report decreased rather than increased
19
20 114 radioligand binding to activated microglia [24,25]. This may account for the unexpected lack
21
22 115 of therapeutic benefit of the anti-microglial antibiotic, minocycline, in recent large clinical
23
24 116 trials [26,27]. Furthermore, large transcriptomic studies in post-mortem brains report no
25
26 117 change or reduction in microglial gene expression but increases in astrocytic expression
27
28 118 [23,28–32]. It is increasingly understood that both peripheral immune responses and brain
29
30 119 glial function are regulated by specialised T cells (Tregs), a subset of which reside in brain
31
32 120 parenchyma [33,34]. A novel proposal is that Treg hypofunction accounts for mild peripheral
33
34 121 immune disinhibition and dysregulated astroglial-microglial interaction, such that microglia
35
36 122 are driven into a developmental, synapse-pruning phenotype while astroglia disrupt
37
38 123 neurotransmitter function [33,34]. Importantly, there are bidirectional interactions between
39
40 124 IL-6 and Treg function [34]. Crucially, we will measure IL-6 in addition to cellular and
41
42 125 molecular markers of immune function and investigate how they correlate with central
43
44 126 markers and clinical state.
45
46
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50
51 127 Previous attempts testing the inflammatory hypothesis in therapeutic clinical trials
52
53 128 have been attempted. However, little evidence of overall efficacy has been found [35]. These
54
55 129 trials have generally tested broad spectrum agents, such as non-steroidal anti-inflammatory
56
57 130 drugs, with no attempt to stratify patients according to evidence of inflammation. A trial
58
59
60

1
2
3 131 using tocilizumab, a humanised monoclonal antibody (mAb) against the IL-6 receptor
4
5 132 currently licensed in the UK for treatment of rheumatoid arthritis (RA) and severe
6
7 133 coronavirus disease, reported no improvements in any clinical measure in a small sample of
8
9
10 134 36 patients with established schizophrenia [36]. However, as mentioned previously, no
11
12 135 stratification by inflammatory markers or any mechanistic immune measures was applied.
13
14 136 Low-grade inflammation is associated with poor response to antipsychotic drugs [37], but
15
16 137 immunotherapy is unlikely to be relevant for all patients with psychosis. Meta-analysis
17
18 138 suggests that evidence of immune activation, defined by elevated CRP levels, is present in a
19
20 139 quarter to one third of patients with schizophrenia [38]. A randomised controlled trial of
21
22 140 infliximab, an anti-tumour necrosis factor alpha (TNF- α) mAb, reported that antidepressant
23
24 141 response was associated with higher CRP levels at baseline [39], suggesting that patients with
25
26 142 evidence of immune activation may be better candidates for immunotherapy trials. As far as
27
28 143 we are aware, no previous clinical trial has selected patients with schizophrenia based on
29
30 144 evidence of immune activation.

31
32
33
34
35 145 Selection of patients with particular symptom profiles and/or stage of illness may also
36
37 146 be a useful strategy that needs to be employed in immunotherapy trials for schizophrenia. A
38
39 147 wide variety of symptoms occur in schizophrenia such as hallucinations, delusions,
40
41 148 anhedonia, cognitive dysfunction, and affective symptoms and presentation of these
42
43 149 symptoms differ from one individual to another. Some symptoms may be more related to
44
45 150 inflammation than others. For instance, meta-analytic data suggests that elevated
46
47 151 proinflammatory cytokines are associated with negative psychotic symptomatology [40].
48
49 152 Moreover, a recent study from the ALSPAC birth cohort reported that out of 20 positive and
50
51 153 negative symptoms, CRP is particularly associated with anhedonia and auditory
52
53 154 hallucinations [41]. Lastly, results from work we have completed to date as part of the MRC-
54
55 155 funded larger PIMS collaboration (MR/S037675/1), suggest that anhedonia may be a
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1
2
3 156 promising target in early phases of established psychotic disorder. Anhedonia and
4
5 157 amotivation are strongly associated with poor functional outcomes in depression and
6
7 158 schizophrenia, and present a formidable barrier to returning to work or building relationships
8
9
10 159 [42,43]. Patients with psychotic disorders also present with cognitive deficits in a range of
11
12 160 domains [44]. Available antipsychotic medications have a limited effects on poor cognitive
13
14 161 functioning in psychosis [45]. Illness stage may also be of relevance. Meta-analytic data has
15
16 162 revealed no differences in IL-6 levels between stable, medicated patients with schizophrenia
17
18 163 and controls, although compared with controls, IL-6 levels were similarly elevated in patients
19
20 164 with FEP and those with acute relapse [7]. A separate meta-analysis found evidence of
21
22 165 elevated blood cytokine levels in acutely and chronically ill patients with schizophrenia [6].
23
24 166 Focusing on particular inflammation-related symptoms, such as anhedonia, and/or illness
25
26 167 stage may increase the chance of success for immunotherapy trials.
27
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32

33 169 **Proposed study**

34
35 170 The proposed two-year study is a UK multi-site (Bristol, Birmingham, and Cambridge)
36
37 171 proof-of-concept, randomised, parallel-group, double-blind, placebo-controlled trial.
38
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40
41

42 173 *Study aims and hypotheses*

43
44 174 The primary aim of this trial is to examine potential mechanisms by which IL-6 affects
45
46 175 anhedonia, psychotic symptoms, and cognition. Our primary hypothesis is that inhibition of
47
48 176 IL-6 signalling with a single intravenous infusion of anti-IL6R monoclonal antibody,
49
50 177 tocilizumab, in individuals with psychosis and elevated IL-6 at baseline will attenuate
51
52 178 symptoms of anhedonia and amotivation in patients with psychosis, relative to placebo. This
53
54 179 will provide further evidence for a potential causal role of inflammation in psychosis. Our
55
56 180 secondary hypothesis is that reduction in peripheral inflammation after tocilizumab infusion
57
58
59
60

1
2
3 181 in patients with psychosis and evidence of inflammation will be associated with central
4
5 182 measures of oxidative stress and relevant resting state brain function.
6
7

8 183 We will also conduct deep immunophenotyping of peripheral blood mononuclear cell
9
10 184 subsets (CD4⁺, CD8⁺, Tregs, natural killer and natural killer-T cells, monocytes, and B cells)
11
12 185 to characterise their absolute number, frequency, and function. Our primary mechanistic
13
14 186 outcome is the level of IL-6/STAT3 signalling inhibition within both innate and adaptive
15
16 187 immune cells using multi-colour flow cytometry with an established optimised pSTAT3
17
18 188 phosflow assay. This will help identify the potential cellular impact of peripheral
19
20 189 inflammation in psychosis, which is largely unknown. Functional assessment of IL-6/STAT3
21
22 190 signalling in immune cell subsets and their response to exogenous IL-6 stimulation will
23
24 191 inform abnormal immune response in psychosis and allow measurement of response to
25
26 192 tocilizumab at the cellular level.
27
28
29

30 193 A secondary objective is to carry out an observational study to examine clinical and
31
32 194 biomarker differences and similarities between patients with psychotic disorder with and
33
34 195 without evidence of inflammation and healthy controls (HCs). We hypothesise that
35
36 196 individuals with psychotic disorder and evidence of inflammation, compared to those without
37
38 197 evidence of inflammation and HCs, will have increased symptoms of anhedonia and
39
40 198 amotivation, poorer cognitive functioning, and cellular and brain-based measures of immune
41
42 199 dysfunction.
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47 200

48 49 50 201 METHODS

51
52 202 This protocol has been prepared in accordance with the Standard Protocol Items:
53
54 203 Recommendations for Interventional Trials (SPIRIT) 2013 statement [46]. Please see
55
56 204 supplementary eTable 1 for the SPIRIT checklist. The planned start date for the PIMS trial
57
58 205 was 1st November 2021, however, this was delayed due to the COVID-19 pandemic. We
59
60

1
2
3 206 began recruiting at our site in Birmingham in November 2022, and we soon expect
4
5 207 recruitment to begin at our Bristol and Cambridge sites. The planned end date is 31st May
6
7
8 208 2024.
9

10 209

11 210 **Patient and public involvement**

12
13
14
15 211 The study protocol was prepared in collaboration with individuals with lived experience of
16
17 212 mental illness who contributed to the development of participant information sheet, consent
18
19 213 forms (Appendix I, II, and III), and data collection procedures.
20
21

22 214

23 215 **Study design and sample**

24
25
26 216 See Figure 1 for an overview of study design. Individuals residing in Bristol, Birmingham, or
27
28
29 217 Cambridge in the United Kingdom will be recruited. Approximately 60 participants with
30
31 218 psychosis and evidence of inflammation (i.e., IL-6 ≥ 0.7 pg/ml) will be randomised to receive
32
33 219 either one intravenous infusion of tocilizumab (drug) or normal saline (placebo). For the
34
35
36 220 secondary, observational study, we will compare baseline characteristics of the intervention
37
38 221 cohort with approximately 30 participants with psychosis without evidence of inflammation
39
40 222 (i.e., IL-6 < 0.7 pg/ml), and approximately 30 HCs. Participants without evidence of
41
42
43 223 inflammation and controls will not be randomised as they will not receive any intervention.
44
45 224 Neuroimaging will only be undertaken by those without MRI contraindications who have
46
47 225 given specific informed consent for MRI. Participants not eligible or not consenting for MRI
48
49 226 will take part in all other aspects of the study.
50

51 227

52 228 **Intervention**

53
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55
56 229 Single intravenous infusion of tocilizumab (4.0mg/kg; max 800mg in total) or normal saline
57
58
59 230 given to participants with psychosis and evidence of inflammation. Tocilizumab blocks both
60

1
2
3 231 IL-6 classic and trans-signalling – the latter being responsible for most of the inflammatory
4
5 232 effects of IL-6 – providing broad inhibition of IL-6 signalling and a strong test of a casual
6
7 233 role for IL-6 in psychosis [47]. Tocilizumab is the first-in-class, humanized monoclonal
8
9 234 antibody against the IL-6R, commercially available and licensed in the UK for treatment of
10
11 235 RA. Approved dosage of tocilizumab for treatment of RA is 2, 4, or 8mg/kg; max 800mg in
12
13 236 total. In RA, a single tocilizumab infusion has shown to improve clinical and laboratory
14
15 237 measures within 48 hours, with most noticeable results in one-to-two weeks [48,49]. The
16
17 238 follow-up schedule for our study is in keeping with this observation.
18
19
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23

24 240 **Eligibility criteria**

25
26 241 We will recruit participants aged 18-40 years. Patient participants must meet International
27
28 242 Classification of Diseases 10th Revision (ICD-10) criteria for a diagnosis of schizophrenia
29
30 243 and related psychoses (ICD-10 code F20, F22, F25, F28, F29) at the time of eligibility
31
32 244 assessment, be within three years of first diagnosis of psychotic disorder, be on a stable
33
34 245 treatment regime with no recent (within two weeks) initiation, cessation, or change in class of
35
36 246 antipsychotic medication, and have a Positive and Negative Syndrome Scale (PANSS) item
37
38 247 score ≥ 3 on P1 (delusions), P2 (conceptual disorganisation), P3 (hallucinatory behaviour), or
39
40 248 P6 (suspiciousness/persecution). Additionally, patients recruited to the interventional arm
41
42 249 will be required to have serum IL-6 levels ≥ 0.7 pg/ml and a Temporal Experience of Pleasure
43
44 250 Scale (TEPS) anticipatory pleasure score ≤ 41 (based on item numbers 1, 3, 7, 11, 12, 14, 15,
45
46 251 16, 17, and 8) and consummatory pleasure score ≤ 36 (based on item numbers 2, 4, 5, 6, 8, 9,
47
48 252 10, and 13). The threshold of serum IL-6 ≥ 0.7 pg/mL as evidence of inflammation for this
49
50 253 particular trial was chosen based on observations from the Personalised Prognostic Tools for
51
52 254 Early Psychosis Management (PRONIA) cohort [<https://www.pronia.eu>]. In 192 first-episode
53
54 255 psychosis patients included in the PRONIA study, the median value of serum IL-6 was
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56
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256 0.49pg/mL (25th percentile 0.22pg/mL; 75th percentile 1.11pg/mL), and the mean was
 257 0.79pg/mL (SD \pm 0.84). Based on these observations, we chose the cut-off of 0.7pg/mL for
 258 patient selection in current trial. Finally, COVID-19 anti-body titre test will be used to
 259 determine adequate levels of immune response via the following cut-offs (for poor response):
 260 400IU Roche/700IU Abbot assay.

261 HCs will have no current or lifetime history of psychiatric diagnosis, as determined by
 262 the Mini-International Neuropsychiatric Interview (MINI). See Table 1 for complete
 263 inclusion and exclusion criteria. HCs will be matched to patient participants at the group level
 264 by age and sex.

265
 266 **Table 1. PIMS Trial inclusion and exclusion criteria.**

| Group | Inclusion criteria | Exclusion criteria |
|------------------|---|---|
| All participants | <ul style="list-style-type: none"> - Provide informed consent. - Understand written and spoken English. - Able and willing to consent to blood sampling. - Willing to abstain from strenuous exercise for 72 hours prior to assessment. | <ul style="list-style-type: none"> - Pregnancy (confirmed by urine pregnancy test) or breast feeding. - Body mass index >35. - Current or lifetime diagnosis of antisocial personality disorder, autism or other neurodevelopmental disorder, major traumatic brain injury. - Currently active diagnosed eating disorder likely to compromise ability to take part. - History of alcohol or substance use disorder (abuse/dependence) within six months prior to eligibility assessment (nicotine and caffeine dependence are not exclusionary). - Current use of medication likely to compromise interpretation of immunological data. - Known active current or history of recurrent bacterial, viral, fungal, mycobacterial, or other opportunistic infections. - Current infection with VZV, TB, Hepatitis B, Hepatitis C, or HIV confirmed by blood test. Chest X-ray will also be performed to assess for TB. - Any major episode of infection requiring hospitalisation or treatment with IV antibiotics within 4 weeks of eligibility assessment. |

| | | |
|--|---|--|
| | | <ul style="list-style-type: none"> - Unstable cardiac, pulmonary, renal, hepatic, endocrine, hematologic, or active infectious disease, including current or prior malignancy. - Diverticulitis, inflammatory bowel disease, or uncontrolled gastric/duodenal ulcer. - Concomitant auto-immune or auto-inflammatory rheumatological disease. - Concomitant treatment with any biologic drugs. - Current and active ischemic heart disease. - Uncontrolled hypertension defined as systolic blood pressure > 170 or diastolic blood pressure > 110. - History of severe allergic or anaphylactic reactions to human, humanized or murine monoclonal antibodies. - No history of chicken pox infection or no history of varicella zoster immunity. |
| Additional criteria for neuroimaging (optional) | - Able and willing to consent to MRI scanning | - Contraindications to MRI. |
| Additional criterion for healthy controls | - No current or lifetime psychiatric diagnosis. | |
| Additional criteria for all individuals with psychosis | <ul style="list-style-type: none"> - Meet ICD-10 criteria for a diagnosis of schizophrenia and related psychoses (code F20, F22, F25, F28, F29) at the time of eligibility assessment, as determined by the Mini-International Neuropsychiatric Interview. - Be within three years of first diagnosis of psychotic disorder. - On stable treatment regime with no recent (within 2 weeks) initiation, cessation, or change in class of antipsychotic medication. - No indication or other reason for preclusion into research (e.g., significant risk of suicidal behaviour or risk to others) as determined by their clinical team. - Positive and Negative Syndrome Scale item score ≥ 3 on P1 (delusions), P2 (conceptual disorganisation), P3 (hallucinatory behaviour), OR P6 (suspiciousness/persecution). | |

| | | |
|---|--|--|
| Additional criteria for intervention group | <ul style="list-style-type: none"> - Serum IL-6 level ≥ 0.7pg/ml at eligibility and baseline assessment. - Temporal Experience of Pleasure Scale anticipatory pleasure score ≤ 41 (based upon item numbers 1, 3, 7, 11, 12, 14, 15, 16, 17, and 8) and consummatory pleasure score ≤ 36 (based upon item numbers 2, 4, 5, 6, 8, 9, 10, and 13). - Evidence of COVID-19 immunity required prior to infusion, confirmed before randomisation using evidence of vaccination and antibody titre test. | |
| Additional criterion for patients with psychosis without inflammation | <ul style="list-style-type: none"> - Serum IL-6 level < 0.7pg/ml at eligibility and baseline assessment. | |

269

270 Study outcomes

271 The primary outcome is anhedonia, defined as anticipatory and consummatory pleasure
 272 scores, assessed by the TEPS [50] at approximately day 14 post-infusion. The primary
 273 mechanistic outcome is the level of IL-6/STAT3 signalling inhibition post-tocilizumab
 274 infusion in both innate and adaptive immune cells using multi-colour flow cytometry and an
 275 established optimised pSTAT3 phosflow assay. We will also collect data on several
 276 tertiary/exploratory measures including positive and negative symptoms of psychosis,
 277 depressive symptoms, fatigue, quality of life and subjective wellbeing, cognitive function
 278 (psychomotor speed, attention and memory, and executive function), peripheral blood
 279 inflammatory markers, cortisol, cell expression, including DNA and RNA sequencing,
 280 functionality, and neuroimaging measures, including functional resting state MRI and MRS
 281 outcomes (Table 2). Where possible, blood samples will be collected during working hours

282 and time of sampling will be recorded. However, a specified time window will not be given
 283 to ease burden on patients and to maximise participation.

284
 285 **Table 2. PIMS trial study measures**

| Domain | Tool | Source | Validated Tool | Time of assessment |
|----------------------------|---|------------------------------|----------------|-----------------------------------|
| Sociodemographic/lifestyle | Screening questionnaire | Self-report | | Screening |
| | Medical History Questionnaire | Self-report/General practice | | Eligibility |
| | Substance Use Questionnaire | Self-report | | Eligibility |
| | Physical Measurements Form | Self-report | | Baseline |
| | Sociodemographic Questionnaire | Self-report | | Baseline |
| Psychiatric | The Temporal Experience of Pleasure Scale | Self-report | ✓ | Eligibility, baseline, follow-ups |
| | The Positive and Negative Syndrome Scale | Interviewer assessed | ✓ | Eligibility, baseline, follow-ups |
| | The Mini-International Neuropsychiatric Interview | Interviewer assessed | ✓ | Eligibility |
| | Psychiatric History Questionnaire | Self-report | | Baseline |
| | The Scale for the Assessment of Negative Symptoms | Self-report | ✓ | Baseline, follow-ups |
| | The Calgary Depression Scale for Schizophrenia | Interviewer assessed | ✓ | Baseline, follow-ups |
| | Multi-dimensional Fatigue Inventory | Self-report | ✓ | Baseline, follow-ups |
| | European Quality of Life-5 Dimensions Three-Level Version | Self-report | ✓ | Baseline, follow-ups |
| Cognitive | Visual Analogue Scale for Subjective Wellbeing | Self-report | ✓ | Baseline, follow-ups |
| | National Adult Reading Test for estimated premorbid IQ | Interviewer assessed | ✓ | Baseline, follow-up 2 |

| | | | | |
|--------------|---|------------------|---|-----------------------|
| | CANTAB Reaction Time test | Computer task | ✓ | Baseline, follow-up 2 |
| | Symbol Coding Test | Paper task | ✓ | Baseline, follow-up 2 |
| | CANTAB Rapid Visual Information Processing test | Computer task | ✓ | Baseline, follow-up 2 |
| | CANTAB Paired Associates Learning test | Computer task | ✓ | Baseline, follow-up 2 |
| | CANTAB One Touch Stockings of Cambridge test | Computer task | ✓ | Baseline, follow-up 2 |
| Biologic | Inflammatory markers, cardiometabolic markers,IDO activation, white cell phenotyping | Laboratory tests | | Baseline, follow-ups |
| Genetic | Gene expression/genotyping | Blood (RNA, DNA) | | Baseline, follow-ups |
| Neuroimaging | MRI Screening Questionnaire | | | Baseline, follow-up 2 |
| | Structural MRI, 1H-MRS measure of glutathione in the prefrontal cortex area, resting state fMRI | | | Baseline, follow-up 2 |

287

288 **Sample size and statistical power**

289 We will recruit approximately 60 patients with psychosis. However, currently there are no
 290 trials of immunotherapies for anhedonia in schizophrenia making accurate power calculation
 291 difficult. This study is a proof-of-concept experiment designed to test whether inhibition of
 292 IL-6 signalling leads to changes in psychotic symptoms. It could also inform likely statistical
 293 power for future trials testing efficacy of the drug as a treatment of schizophrenia, which is
 294 not the intention of this study. The exact statistical tests and techniques that will be applied to
 295 the data will depend on the objective of specific analysis and data characteristics (e.g.,

1
2
3 296 variable type, distribution). These details will be specified in analysis plans and registered
4
5 297 online before participants are unblinded and any data analysis is performed.
6
7
8 298

9
10 299 **Randomisation and blinding**

11
12 300 An external agency independent of the study team will arrange random allocation to
13
14 301 tocilizumab or normal saline group 1:1, ensuring two groups are comparable regarding
15
16 302 anhedonia severity and sex. Randomisation will be stratified by site. Randomising agency
17
18 303 will provide the randomisation code to the relevant hospital pharmacy who will dispense
19
20 304 tocilizumab or normal saline according to the randomisation schedule. Dispensing
21
22 305 pharmacies will keep a log of products dispensed. Infusions will be prepared and
23
24 306 administered at clinical research facilities (CRFs). Infusion packs will be prepared by trained
25
26 307 staff not part of the core study team, ensuring blinding of treatment allocation. Infusion packs
27
28 308 containing drug or placebo will be visually indistinguishable from each other, ensuring that
29
30 309 both participants and study team remain blind regarding treatment allocation.
31
32
33
34
35

36 310

37
38 311 **Statistical analysis**

39
40 312 For randomised participants, an intention-to-treat approach will be taken for data analysis by
41
42 313 including all randomised participants in statistical analyses, regardless of the treatment they
43
44 314 received (if any). We will compare outcome measures between treatment and placebo groups
45
46 315 controlling for baseline scores. This mechanistic experiment will focus on overall pattern of
47
48 316 results and their effect sizes rather than *P*-values for individual tests of statistical significance.
49
50 317 The secondary mechanistic and observational analysis will compare psychotic symptoms,
51
52 318 cognitive function, blood, neuroimaging, and other biomarkers between and across study
53
54 319 groups using appropriate statistical tests.
55
56
57
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59 320
60

322 STUDY PROCEDURE

323 An overview of study procedures is presented in Figure 1 and all study measures are detailed
324 in Table 2. Recruitment will take place in Bristol, Birmingham, and Cambridge and
325 assessments at University and NHS research facilities.

326

327 **Participant identification**

328 Potential participants with psychosis will be identified by NHS Psychosis Early Intervention
329 (EI) teams. HCs will be recruited through advertisement methods in Birmingham and
330 Cambridge. Potential participants will complete a screening questionnaire to confirm their
331 eligibility to participate. If deemed eligible, participants will be invited to an appointment to
332 complete a full eligibility assessment.

333

334 **Eligibility assessment**

335 Assessments will be carried out to establish eligibility and to obtain informed consent.
336 Patients will complete the MINI to confirm ICD-10 diagnosis of schizophrenia and related
337 psychoses, the PANSS to confirm the presence of positive symptoms of psychosis, and the
338 TEPS to confirm eligibility based on anticipatory and consummatory pleasure sum scores. A
339 blood sample will be collected from patients for serum IL-6 measurement. An MRI screening
340 questionnaire will be administered to those willing to give informed consent for
341 neuroimaging.

342

343 **Baseline assessment**

344 All participants (60 inflamed psychosis, 30 non-inflamed psychosis, and 30 HCs) will attend
345 a baseline assessment comprising psychiatric measures, cognitive tasks, blood sampling, and
346 neuroimaging (optional). This will be the final study contact for patients without evidence of

1
2
3 347 inflammation and HCs. Patients with evidence of inflammation will undergo further tests to
4
5 348 establish safety/eligibility to receive tocilizumab, including a chest X-ray and blood tests to
6
7 349 exclude pregnancy and certain infections, such as TB, HIV, and COVID-19. Eligible
8
9
10 350 participants will be randomised and invited for infusion.
11

12 351

14 352 **Intervention**

16 353 Intravenous infusion of tocilizumab or normal saline will be given continuously over one
17
18 354 hour at CRFs in Bristol, Birmingham, and Cambridge by trained clinical staff under the
19
20
21 355 supervision of a designated study doctor. Participants will remain under clinical observation
22
23
24 356 for a further 1-hour period after the end of infusion.
25

26 357

28 358 **Follow-up assessments**

30 359 Follow-up assessments will take place approximately 7-, 14-, and 28-days post-infusion, and
31
32
33 360 will collect similar data to the baseline assessment. Cognitive tasks and neuroimaging
34
35 361 (optional) will be administered only on day 14. Around 42 days post-infusion, participants
36
37 362 will be contacted by phone to provide a final debrief; at which point they will exit the study.
38

39 363

42 364 **RISK MANAGEMENT**

44 365 **Psychosis-related risks**

46 366 All patients will be under the care of a specialist NHS psychosis EI service. Participation will
47
48 367 not involve any treatment modifications or significant delays in receiving treatment. If a
49
50
51 368 patient becomes distressed during an assessment, or does not wish to continue for any reason,
52
53 369 the researcher will stop the assessment. Participants may withdraw at any time without giving
54
55
56 370 a reason. If there is any concern for the participant's safety, the research team will liaise with
57
58 371 participant's GP and/or mental health team as needed.
59
60

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3 3724
5 373 **Procedure-related risks**6
7 374 *Venepuncture*8
9 375 Blood taking is associated with mild discomfort and other side effects are rare. Efforts will be
10
11 376 made to minimise discomfort. Blood taking will be performed by a nurse, doctor, or research
12
13 377 team member trained in venepuncture.
1415
16 37817
18 379 *Chest X-ray*19
20 380 This study will use a typical effective radiation dose of 0.014 mSv; equivalent to 2.5 days of
21
22 381 average natural background radiation in the UK. The risk of developing cancer as a
23
24 382 consequence of participating in this study is 0.0001%. Only non-pregnant, adult participants
25
26 383 will be included.
27
28 38429
30 385 *Neuroimaging*31
32 386 Discomfort during MRI will be minimised by using mirrors to allow participants to view
33
34 387 outside of the machine, providing ear plugs and a panic button, and allowing participants to
35
36 388 communicate with the researcher and scan operator throughout. Mild transient vertigo may be
37
38 389 experienced when being moved into the MRI machine. Risk of dislodgement or malfunction
39
40 390 of medical implants or metallic foreign objects will be minimised by screening participants to
41
42 391 ensure no metal is present on or within the body.
43
44 39245
46 393 *IL-6 levels*47
48 394 Some participants will show evidence of inflammation in the blood ($IL-6 \geq 0.7\text{pg/ml}$). This is
49
50 395 not necessarily a cause for concern. In people with FEP, ~50% have serum IL-6 levels
51
52 396 $>0.7\text{pg/ml}$. Reasons for this in the absence of an acute infection or chronic inflammatory
53
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56
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1
2
3 397 illness could include obesity, smoking, alcohol use, and lack of exercise, so knowledge of
4
5 398 'inflammation status' may prompt participants to adopt a healthier lifestyle. If serum IL-6
6
7 399 level is high (i.e., IL-6 ≥ 0.7 pg/ml) along with elevated CRP (>20 mg/L) without any apparent
8
9 400 explanation, such as infection or chronic inflammatory illness, we will inform the
10
11 401 participant's GP and the participant will be excluded from the study.
12
13
14

15 402

16
17 403 *Risk to research staff*18
19 404 Staff will follow local safety procedures when lone working. No other risks are anticipated.
20
21

22 405

23
24 406 **Safety considerations for infusion and monitoring of adverse reaction**25
26 407 *Before infusion*27
28 408 Participants will be selected based on strict inclusion and exclusion criteria. Additionally, we
29
30 409 will carry out tests for TB, HIV, VZV antibody, and Hepatitis B and C because, though
31
32 410 unlikely after a single dose, tocilizumab could make these infections worse. Female
33
34 411 participants of childbearing age will be given a pregnancy test, which must be negative.
35
3637 412 Participants who are sexually active will be asked to use at least one form of effective
38
39 413 contraception for six weeks post-infusion. Male participants will also be asked not to donate
40
41 414 sperm samples for six weeks post-infusion.
42
43

44 415

45
46 416 *During infusion*47
48 417 Infusions will be given under supervision of a designated study doctor. Participants will be
49
50 418 monitored for possible side effects, which will be managed in line with use of tocilizumab for
51
52 419 treating patients with RA.
53
54

55 420

56
57 421 *After infusion*
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3 422 Participants will remain under observation for one-hour post-infusion. Participants will be
4
5 423 advised to seek help if they feel unwell after leaving the assessment centre and will be given
6
7 424 an information sheet containing a telephone number their health professionals can call. If
8
9 425 necessary, we will unblind the participant and inform their health professional whether they
10
11 426 received tocilizumab or normal saline. Adverse reactions will be recorded at each follow-up
12
13 427 visit. Additional, safety blood tests will be done at second follow-up (e.g., WBC count, liver
14
15 428 function, lipids).
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430 ETHICS AND DISSEMINATION

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22
23 431 The study will be conducted in accordance with the REC, Health Research Authority (HRA),
24
25 432 and local Research and Development (R&D) department approvals and guidelines (REC
26
27 433 reference: 22/EE/0010). The study team will prepare protocol amendments as required and
28
29 434 ethics approval will be sought before implementing any changes to the approved protocol.
30
31 435 The ISRCTN Trial Registry and the Research Governance Office will be informed of any
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33 436 amendments to the protocol.
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438 **Consent**

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41 439 Informed consent will be obtained prior to eligibility assessments for participation in the
42
43 440 study (Appendix I, II, and II). This will include consent to randomise, for contact with their
44
45 441 GP to inform them about participation, access GP/psychiatric records to verify medical
46
47 442 history to establish eligibility, and to inform the participant's GP any results/outcomes as
48
49 443 necessary. Consent for additional tests to establish safety for tocilizumab infusion and for
50
51 444 storing biological samples will also be obtained.
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446 **Study management**

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3 447 The study is sponsored by the University of Bristol. The sponsor, the Chief Investigator
4
5 448 (GMK), and the co-Lead (RU) will have overall responsibility for the study. A named
6
7 449 principal investigator will take clinical responsibility for study activities at each site. The
8
9 450 study does not require the formal arrangement of a steering committee because, according to
10
11 451 the HRA, it is not a Clinical Trial of an Investigational Medicinal Product. However, to
12
13 452 enhance monitoring of the study, a study management group will be established, comprising
14
15 453 academic and clinical experts in psychiatry, rheumatology, neuroscience, and immunology.
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21 455 **Data management and retention of samples**

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23 456 All potential participants will be assigned a unique study-specific participant ID number. All
24
25 457 data will be subject to good practice as laid down in the Data Protection Act. Each study
26
27 458 stage is tracked so that participant's (de-identified) status within the study is known, and
28
29 459 assessment and other appointment dates are forecasted. This information is held on a secure,
30
31 460 password-protected database. Anonymised data from assessments will be uploaded to a
32
33 461 secure, password-protected database using secure web-based data entry systems. Minimal
34
35 462 personal data (age, sex) will be indexed by each participant's unique ID number. Blood
36
37 463 samples collected in this study may be stored for up to 10 years after the completion for
38
39 464 additional research. Stored samples will be coded throughout the sample storage and analysis
40
41 465 process and will not be labelled with personal identifiers. Participants may withdraw their
42
43 466 consent for their samples to be stored for future research.
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49 50 51 468 **Dissemination plan**

52
53 469 Study results will be published in peer-review journals and will conform to the guidelines of
54
55 470 the International Committee of Medical Journal Editors. Findings will be disseminated at
56
57 471 conferences, departmental talks, and via social and traditional media.
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5 473 AUTHORS CONTRIBUTIONS

6
7 474 ÉMF wrote first draft of the PIMS trial protocol and of this manuscript. SLG, MK, GKM,
8
9 475 BD, DJ, JS, and NMB contributed to study design and protocol development and revised
10
11 476 manuscript drafts. RU contributed to study design and study protocol, and revised manuscript
12
13 477 drafts. GMK devised study design and trial protocol, and revised drafts. ÉMF and SLG
14
15 478 developed study materials and liaised with REC and HRA regarding approvals. AM, JR,
16
17 479 FCZ, HH, EW, and MW contributed to the revision of the manuscript and validation of
18
19 480 operating procedures and mechanistic protocols. RU and GMK co-lead the MRC grant that
20
21 481 funds the PIMS trial and provide overall supervision and oversight for the project.
22
23
24
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26 482

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29
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31
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50 494 Technology Assessment, European Commission - Research: The Seventh Framework
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52 495 Programme, and personal fees from Sunovion, outside the submitted work. GMK
53
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2
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4
5 497 funders had no role in the design of this study.
6
7
8 498

9
10 499 COMPETING INTERESTS STATEMENT

11
12 500 ÉMF, SLG, AM, JR, FCZ, HH, EW, MW, MK, GKM, BD, DJ, JS, RU, and GMK have no
13
14 501 conflicts of interest to report. NMB holds shares and is a Director of Celentyx Ltd.
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671 FIGURE LEGEND

672 Figure 1. Overview of study design

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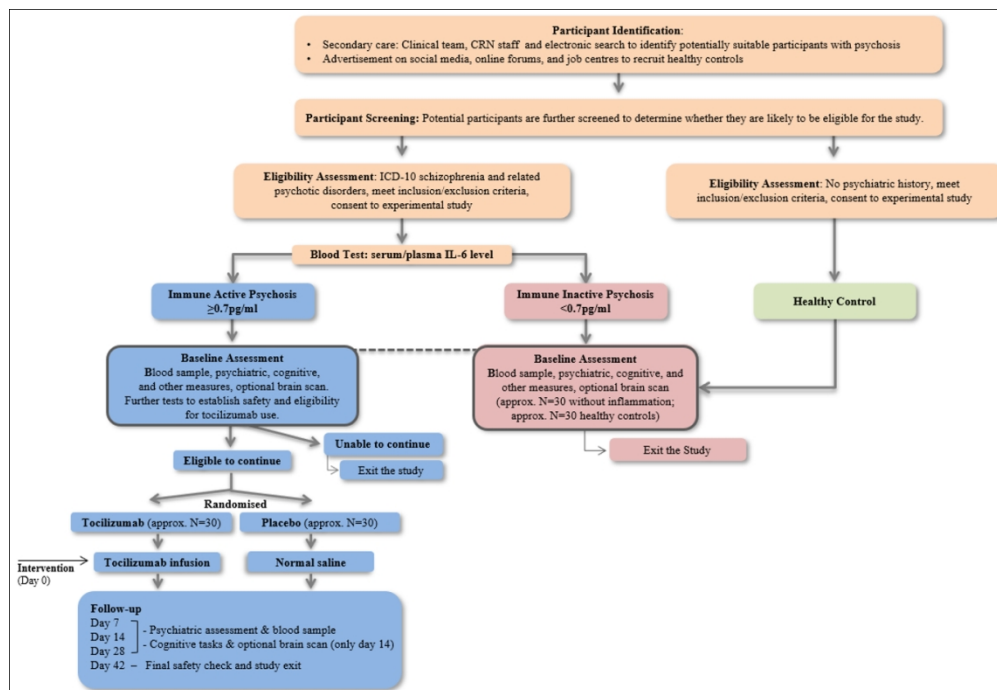


Figure 1. Overview of study design

240x165mm (144 x 144 DPI)

Supplementary Material

Foley et al. Protocol for the Psychosis Immune Mechanism Stratified Medicine (PIMS) trial: A randomised double-blind placebo-controlled trial of single dose tocilizumab in patients with psychosis.

Table of Contents

eTable1: SPIRIT 2013 Checklist..... 2

Appendix I – Consent Form for Screening: All Participants..... 8

Appendix II – Informed Consent Form for Study Participation: Patients 10

Appendix III – Informed Consent Form for Study Participation: Healthy Controls..... 13

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eTable1: SPIRIT 2013 Checklist – Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description | Addressed on page number |
|-----------------------------------|---------|--|---------------------------|
| Administrative information | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | 4 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | 4 (ISRCTN 23256704) |
| Protocol version | 3 | Date and version identifier | - |
| Funding | 4 | Sources and types of financial, material, and other support | 24 - 25 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | 1, 24 |
| | 5b | Name and contact information for the trial sponsor | 23 |
| | 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 23-25 |

| | | | | |
|----|---|-----|--|-------------------------|
| 1 | | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | 23 - 25 |
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| 9 | Introduction | | | |
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| 11 | Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 6-9 |
| 12 | | | | |
| 13 | | 6b | Explanation for choice of comparators | 6-9 |
| 14 | | | | |
| 15 | Objectives | 7 | Specific objectives or hypotheses | 9-10 |
| 16 | | | | |
| 17 | Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | 9 |
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| 21 | | | | |
| 22 | Methods: Participants, interventions, and outcomes | | | |
| 23 | | | | |
| 24 | Study setting | 9 | Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 9, 11 |
| 25 | | | | |
| 26 | Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | 12, Table 1 |
| 27 | | | | |
| 28 | Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 11, 19, 21-22, Figure 1 |
| 29 | | | | |
| 30 | | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) | 22 |
| 31 | | | | |
| 32 | | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | - |
| 33 | | | | |
| 34 | | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | 12, Table 1 |
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| 1 | Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 9-10, Figure 1, Table 2 |
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| 6 | Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 18-19, Figure 1 |
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| 8 | | | | |
| 9 | Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 17 |
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| 13 | Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | 18 |
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| 15 | Methods: Assignment of interventions (for controlled trials) | | | |
| 16 | Allocation: | | | |
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| 18 | Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 17 |
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| 25 | Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 17 |
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| 29 | Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 17 |
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| 33 | Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 17 |
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| 36 | | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | 22 |
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46**Methods: Data collection, management, and analysis**

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| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 10-15, 18-19, Table 2 |
| | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | 17-18 |
| Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | 23-24 |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 17-18 |
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 17-18 |
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | - |
| Methods: Monitoring | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | 23 |
| | 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | - |
| Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 20-22 |

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|----|---------------------------------|-----|---|-------|
| 1 | Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | - |
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| 4 | Ethics and dissemination | | | |
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| 6 | Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | 22 |
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| 9 | Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | 22 |
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| 14 | Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 22-23 |
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| 16 | | | | |
| 17 | | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | 22-23 |
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| 20 | Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 23-24 |
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| 24 | Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 25 |
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| 27 | Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 23 |
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| 30 | Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | 22 |
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| 33 | Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 24 |
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| 38 | | 31b | Authorship eligibility guidelines and any intended use of professional writers | 24 |
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| 40 | | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | - |
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|----------------------------|----|--|------------------------|
| Appendices | | | |
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | Appendix I, II and III |
| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | 23-24 |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons [“Attribution-NonCommercial-NoDerivs 3.0 Unported”](https://creativecommons.org/licenses/by-nc-nd/3.0/) license.

Appendix I – Consent Form for Screening: All Participants

Informed Consent Form for Screening

REC No. 22/EE/0010, Date: 08.02.2022, Version: 1.2

Psychosis Immune Mechanism Stratified Medicine Trial: The PIMS Trial

PARTICIPANT ID: _____

Thank you for considering taking part in the PIMS Trial. The research team must explain the eligibility assessment of the study to you before you agree to take part. If you have any questions arising from the Participant Information Sheet or from the explanation already given to you, please ask a member of the research team before you decide to participate. You will be given a copy of this Informed Consent Form to keep for future reference.

Please read the statements below and insert your initial in the box next to each statement if you agree with them:

| Statement | Initial Here | |
|--|--------------|----|
| 1. I have read and understood the information sheet version XX, DD.MM.YYYY and have had the opportunity to ask questions. | | |
| 2. I understand that my participation is voluntary and I am free to withdraw at any time without giving a reason, without my current or future medical care or legal rights being affected. | | |
| 3. I agree to provide blood samples for eligibility screening. I understand my blood samples will be analysed to test for evidence of immune activation. The purposes and possible risks of donating these samples have been explained to me. I understand that donated samples will be considered a gift but I will have the right to withdraw permission for analysis. | | |
| 4. I agree that my GP can be told that I am participating in the eligibility assessment of the PIMS Trial, and can be informed if any unexpected results are found pertaining specifically to my health. | | |
| 5. I consent for my GP/Psychiatrist to share information from my medical record in order to confirm my eligibility to take part in this study. The study team may access my GP/Psychiatrist records if necessary. | | |
| <i>Optional (Not agreeing to these will not exclude you from this study). Please tick Yes / No (as appropriate)</i> | Yes | No |
| 6. I agree that the samples can be stored after completion of the screening analysis, for use in future, ethically approved, non-genetic studies, even if I am deemed non-eligible to partake in the PIMS study. | | |
| 7. I agree that data and samples can be stored after completion of the PIMS Trial for use in future, ethically approved, genetic studies. This includes the main stocks of any genetic material collected, such as DNA and RNA. | | |

If you want to participate in the screening session of the PIMS Trial, please sign your name below:

Participant Signature _____

Participant Full Name _____

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11 **The researcher who has explained this study to you also needs to sign this form:**
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15 Staff Signature _____
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18 Staff Full Name _____
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21 Date ____ / ____ / ____
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24 **Thank you for your help.**
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26 By completing and returning this form, you are giving us your consent that the personal
27 information you provide will be treated as strictly confidential and handled in accordance with
28 the provisions of the UK Data Protection Act 2018.
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30 **When completed: 1 for participant; and 1 for researcher site file.*
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Appendix II – Informed Consent Form for Study Participation: Patients

Informed Consent Form for Study Participation

REC No. 22/EE/0010, Date: 24.08.2022, Version: 1.5

Psychosis Immune Mechanism Stratified Medicine Trial: The PIMS Trial

PARTICIPANT ID: _____

Thank you for taking part in the PIMS Trial eligibility assessment. Based on this assessment, you are eligible to take part in the PIMS Trial. Before you agree to take part, the research team must explain the study to you. If you have any questions arising from the Participant Information Sheet or from the explanation already given to you, please ask a member of the research team before you decide to participate. You will be given a copy of this Informed Consent Form to keep for future reference.

Please read the statements below and insert your initial in the box next to each statement if you agree with them:

| Statement | Initial Here |
|---|--------------|
| 1. I have read and understood the information sheet version XX dated DD.MM.YYYY and have had the opportunity to ask questions. | |
| 2. I agree to take part in the PIMS Trial. I understand that my participation is voluntary and I am free to withdraw at any time without giving a reason, without my current or future medical care or legal rights being affected. | |
| 3. I understand that confidentiality and anonymity will be maintained and it will not be possible to identify me in any publications. | |
| 4. I agree to partake in interviews, complete questionnaires, and cognitive tests as part of this study. I understand what will happen during the study assessments. | |
| 5. I agree to provide blood samples. The purposes and possible risks of donating these samples have been explained to me. I understand that donated samples will be considered a gift but I will have the right to withdraw permission for analysis. | |
| 6. I understand that blood samples collected from me will be used to measure non-genetic factors such as biochemical changes in the blood. | |
| 7. I agree that the samples and information I provide can be stored, used and shared between PIMS Trial sites and with collaborators/contractors for the purpose of the study. | |
| 8. I understand that blood samples collected will be stored at PIMS Trial centres. | |
| 9. I understand that any of my samples (labelled with an anonymous ID only), or any information obtained from them, including the sequence of my genetic material, may be sent to specialist research laboratories in the UK and abroad for analyses and the results returned to PIMS Trial centres. Researchers at these laboratories have no access to personal information about study participants. | |
| 10. I agree, if necessary, to provide blood/urine samples to test for pregnancy, COVID-19 immunity, Hepatitis B, Hepatitis C, HIV, VZV and Tuberculosis, and to undergo a chest X ray. | |
| 11. I agree to being randomised into the tocilizumab or placebo group if deemed eligible to take part. | |

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| 12. I understand that taking part will involve the administration of a single intravenous infusion of the anti-inflammatory drug tocilizumab or normal saline. | | |
| 13. I agree that my GP can be told that I am participating in this study, and about any findings that require further attention. | | |
| 14. I understand that information related to my participation in this study may be accessed by responsible individuals from the sponsors of this study for quality control purposes. I give permission for these individuals to have access to this data. | | |
| 15. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from research team, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. | | |
| <i>Optional (Not agreeing to these will not exclude you from this study). Please tick Yes / No (as appropriate)</i> | Yes | No |
| 16. I agree to undergo brain scans as part of the PIMS Trial. | | |
| 17. I agree to be contacted in future by researchers to participate in follow up studies to this project, or in future studies of a similar nature. | | |
| 18. I understand that researchers may use the blood samples for genetic analysis. | | |
| 19. I agree that the samples can be stored after completion of the PIMS Trial for use in future, ethically approved, non-genetic studies. | | |
| 20. I agree that the information I give can be stored after completion of the PIMS Trial for use in future, ethically approved, non-genetic studies. | | |
| 21. I agree that data and samples can be stored after completion of the PIMS Trial for use in future, ethically approved, genetic studies. This includes the main stocks of any genetic material collected, such as DNA and RNA. | | |

If you want to participate in the PIMS Trial, please sign your name below:

Participant Signature _____

Participant Full Name _____

Date ____/____/____

Research staff who has explained this study to you also needs to sign this form:

Staff Signature _____

Staff Full Name _____

Date ____/____/____

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5 **Thank you for your help.**
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7 By completing and returning this form, you are giving us your consent that the personal
8 information you provide will be treated as strictly confidential and handled in accordance with
9 the provisions of the UK Data Protection Act 2018.
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11 **When completed: 1 for participant; and 1 for researcher site file.*
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For peer review only

Appendix III – Informed Consent Form for Study Participation: Healthy Controls

Healthy Controls Informed Consent Form for Study Participation

REC No. 22/EE/0010, Date: 17.02.2022, Version: 1

Psychosis Immune Mechanism Stratified Medicine Trial: The PIMS Trial

PARTICIPANT ID: _____

Thank you for taking part in the PIMS Trial eligibility assessment. Based on this assessment, you are eligible to take part in the PIMS Trial. Before you agree to take part, the research team must explain the study to you. If you have any questions arising from the Participant Information Sheet or from the explanation already given to you, please ask a member of the research team before you decide to participate. You will be given a copy of this Informed Consent Form to keep for future reference.

Please read the statements below and insert your initial in the box next to each statement if you agree with them:

| Statement | Initial Here | |
|---|--------------|----|
| 1. I have read and understood the information sheet version XX dated DD.MM.YYYY and have had the opportunity to ask questions. | | |
| 2. I agree to take part in the PIMS Trial. I understand that my participation is voluntary, and I am free to withdraw at any time without giving a reason, without my current or future medical care or legal rights being affected. | | |
| 3. I understand that confidentiality and anonymity will be maintained, and it will not be possible to identify me in any publications. | | |
| 4. I agree to partake in interviews, complete questionnaires, and cognitive tests as part of this study. I understand what will happen during the study assessments. | | |
| 5. I agree to provide blood samples. The purposes and possible risks of donating these samples have been explained to me. I understand that donated samples will be considered a gift, but I will have the right to withdraw permission for analysis. | | |
| 6. I understand that my blood samples collected will be stored at PIMS Trial centres. | | |
| 7. I understand that blood samples collected from me will be used to measure non-genetic factors such as biochemical changes in the blood. | | |
| 8. I understand that any of my samples (labelled with an anonymous ID only), or any information obtained from them, including the sequence of my genetic material, may be sent to specialist research laboratories in the UK and abroad for analyses and the results returned to PIMS Trial centres. Researchers at these laboratories have no access to personal information about study participants. | | |
| 9. I agree that the samples and information I provide can be stored, used and shared between PIMS Trial sites and with collaborators/contractors for the purpose of the study. | | |
| 10. I understand that information related to my participation in this study may be accessed by responsible individuals from the sponsors of this study for quality control purposes. I give permission for these individuals to have access to this data. | | |
| <i>Optional (Not agreeing to these will not exclude you from this study). Please tick Yes / No (as appropriate)</i> | Yes | No |

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| 11. I agree to undergo brain scans as part of the PIMS Trial. | | |
| 12. I agree to be contacted in future by researchers to participate in follow up studies to this project, or in future studies of a similar nature. | | |
| 13. I understand that researchers may use the blood samples for genetic analysis. | | |
| 14. I agree that the samples can be stored after completion of the PIMS Trial for use in future, ethically approved, non-genetic studies. | | |
| 15. I agree that the information I give can be stored after completion of the PIMS Trial for use in future, ethically approved, non-genetic studies. | | |
| 16. I agree that data and samples can be stored after completion of the PIMS Trial for use in future, ethically approved, genetic studies. This includes the main stocks of any genetic material collected, such as DNA and RNA. | | |

If you want to participate in the PIMS Trial, please sign your name below:

Participant Signature _____

Participant Full Name _____

Date ____ / ____ / _____

Research staff who has explained this study to you also needs to sign this form:

Staff Signature _____

Staff Full Name _____

Date ____ / ____ / _____

Thank you for your help.

By completing and returning this form, you are giving us your consent that the personal information you provide will be treated as strictly confidential and handled in accordance with the provisions of the UK Data Protection Act 2018.

**When completed: 1 for participant; and 1 for researcher site file.*

BMJ Open

Protocol for the Psychosis Immune Mechanism Stratified Medicine (PIMS) trial: A randomised double-blind placebo-controlled trial of single dose tocilizumab in patients with psychosis

| | |
|---------------------------------|--|
| Journal: | <i>BMJ Open</i> |
| Manuscript ID | bmjopen-2022-067944.R2 |
| Article Type: | Protocol |
| Date Submitted by the Author: | 25-Jan-2023 |
| Complete List of Authors: | <p>Foley, Éimear; University of Bristol, MRC Integrative Epidemiology Unit, Population Health Sciences; Bristol Medical School, Centre for Academic Mental Health, Population Health Sciences</p> <p>Griffiths, Sian Lowri; University of Birmingham, Institute for Mental Health and Centre for Human Brain Health</p> <p>Murray, Alexander; University of Birmingham, Institute for Mental Health and Centre for Human Brain Health</p> <p>Rogers, Jack; University of Birmingham, Institute for Mental Health and Centre for Human Brain Health</p> <p>Corsi-Zuelli, Fabiana; University of Birmingham, Institute for Mental Health and Centre for Human Brain Health; University of Sao Paulo, Department of Neuroscience and Behaviour</p> <p>Hickinbotham, Hannah; University of Cambridge, Department of Psychiatry</p> <p>Warwick, Ella; University of Birmingham, Institute for Mental Health and Centre for Human Brain Health</p> <p>Wilson, Martin; University of Birmingham, Institute for Mental Health and Centre for Human Brain Health</p> <p>Kaser, Muzaffer; University of Cambridge, Psychiatry; Cambridgeshire and Peterborough NHS Foundation Trust</p> <p>Murray, Graham K.; University of Cambridge, Psychiatry; Cambridgeshire and Peterborough NHS Foundation Trust</p> <p>Deakin, Bill; The University of Manchester, Faculty of Medicine, Biology and Health</p> <p>Jadon, Deepak; University of Cambridge, Medicine</p> <p>Suckling, John; University of Cambridge, Psychiatry; Cambridgeshire and Peterborough NHS Foundation Trust</p> <p>Barnes, Nicholas ; University of Birmingham, Institute of Clinical Sciences</p> <p>Uptegrove, Rachel; University of Birmingham, Institute for Mental Health and Centre for Human Brain Health; Early Intervention Service, Birmingham Women's and Children's NHS Foundation Trust</p> <p>Khandaker, Golam M.; University of Bristol, MRC Integrative Epidemiology Unit, Population Health Sciences; NIHR Bristol Biomedical Research Centre</p> |
| Primary Subject Heading: | Mental health |

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|----------------------------|--|
| Secondary Subject Heading: | Immunology (including allergy) |
| Keywords: | Schizophrenia & psychotic disorders < PSYCHIATRY, IMMUNOLOGY, Clinical trials < THERAPEUTICS, Magnetic resonance imaging < RADIOLOGY & IMAGING |
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SCHOLARONE™
Manuscripts

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3 1 **Protocol for the Psychosis Immune Mechanism Stratified Medicine (PIMS) trial: A**
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5 2 **randomised double-blind placebo-controlled trial of single dose tocilizumab in patients**
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12 5 Éimear M. Foley^{a,b}; Sian Lowri Griffiths^c; Alexander Murray^c; Jack Rogers^c; Fabiana Corsi-
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14 6 Zuelli^{c,d,e}; Hannah Hickinbotham^f; Ella Warwick^c; Martin Wilson^c; Muzaffer Kaser^{f,g};
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16 7 Graham K. Murray^{f,g}; Bill Deakin^h; Deepak Jadonⁱ; John Suckling^{f,g}; Nicholas M. Barnes^e;
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18 8 Rachel Upthegrove^{c,j,†}; and Golam M. Khandaker^{a,b,k,l,†,*}; for the PIMS Collaboration
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8 28 ^l Avon and Wiltshire Mental Health Partnership NHS Trust, Bristol, United Kingdom
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12 30 [†] Joint senior authors
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17 32 * **Corresponding author and address:** Prof Golam M. Khandaker, MRC Integrative
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19 33 Epidemiology Unit, Oakfield House, Oakfield Grove, Clifton, Bristol, BS8 2BN, UK
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35 ABSTRACT

36 **Introduction:** Evidence suggests a potentially causal role of interleukin 6 (IL-6), a
37 pleiotropic cytokine that generally promotes inflammation, in the pathogenesis of psychosis.
38 However, no interventional studies in patients with psychosis, stratified using inflammatory
39 markers, have been conducted to assess the therapeutic potential of targeting IL-6 in
40 psychosis and to elucidate potential mechanism of effect. Tocilizumab is a humanised
41 monoclonal antibody targeting the IL-6 receptor to inhibit IL-6 signalling licensed in the UK
42 for treatment of rheumatoid arthritis. The primary objective of this study is to test whether IL-
43 6 contributes to the pathogenesis of first episode psychosis, and to examine potential
44 mechanisms by which IL-6 affects psychotic symptoms. A secondary objective is to examine
45 characteristics of inflammation-associated psychosis.

46 **Methods and analysis:** A proof-of-concept study employing a randomised, parallel-group,
47 double-blind, placebo-controlled design testing the effect of IL-6 inhibition on anhedonia in
48 patients with psychosis. Approximately 60 participants with diagnosis of schizophrenia and
49 related psychotic disorders (ICD-10 codes F20, F22, F25, F28, F29) with evidence of low-
50 grade inflammation (IL-6 ≥ 0.7 pg/ml) will receive either one intravenous infusion of
51 tocilizumab (4.0mg/kg; max 800mg) or normal saline. Psychiatric measures and blood
52 samples will be collected at baseline, and 7-, 14-, and 28-days post-infusion. Cognitive and
53 neuroimaging data will be collected at baseline and 14 days post-infusion. In addition,
54 approximately 30 patients with psychosis without evidence of inflammation (IL-6 < 0.7 pg/ml)
55 and 30 matched healthy controls will be recruited to complete identical baseline assessments
56 to allow for comparison of the characteristic features of inflammation-associated psychosis.

57 **Ethics and dissemination:** The study is sponsored by the University of Bristol and has been
58 approved by the Cambridge East Research Ethics Committee (reference: 22/EE/0010; IRAS

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59 project ID: 301682). Study findings will be published in peer-review journals. Findings will
60 also be disseminated by scientific presentation and other means.

61 **Trial registration number:** ISRCTN 23256704

62
63 **KEYWORDS:** Psychotic Disorders; Negative Symptoms; Interleukin 6; Immunotherapy;
64 Tocilizumab; Clinical Trial.

For peer review only

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3 65 ARTICLE SUMMARY
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6 66 **Strengths and limitations of this study**
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- 8 67 • Adopting a randomised controlled trial (RCT) design and patient selection based on
9
10 68 elevated level of IL-6 (in addition to other criteria) will help examine the causal role
11
12 69 of IL-6, and the therapeutic potential of targeting IL-6 pathway, in psychosis.
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15 70 • The use of target specific intervention (anti-IL6R monoclonal antibody tocilizumab)
16
17 71 will help assess the clinical relevance of IL-6 and related up and downstream
18
19 72 inflammatory cytokines in psychosis.
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22 73 • The use of neuroimaging, cognitive tests, and extensive peripheral blood biomarker
23
24 74 exploration before and after tocilizumab treatment to assess potential mechanisms of
25
26 75 effect.
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29 76 • One dose of tocilizumab is unlikely to be sufficient to test the efficacy of this drug as
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31 77 potential treatment for psychosis, which is not the intention of this study.
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34 78 • Tocilizumab inhibits both IL-6 classical and trans signalling, and consequently the
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36 79 trial will not be able to distinguish between the effects of modulating these two
37
38 80 signalling pathways specifically in psychosis.
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3 82 **Word count:** 4,336
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5 83 INTRODUCTION
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8 84 **Scientific background and study rationale**
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10 85 The neuroimmune hypothesis of schizophrenia proposes that mild peripheral immune
11
12 86 activation gives rise to an inflammatory response in the brain and neurobiological changes
13
14 87 associated with psychotic illness [1–4]. Meta-analytic evidence is clear that circulating
15
16 88 concentrations of interleukin 6 (IL-6) and other inflammatory proteins, such as C-reactive
17
18 89 protein (CRP), are increased in patients with psychosis, including treatment naïve first
19
20 90 episode psychosis (FEP) [5], compared with controls [6–9]. Prospective cohort studies show
21
22 91 that these indices of mild immune activation precede the onset of symptoms [10,11].
23
24 92 Furthermore, genetic variants known to increase IL-6 concentrations are associated with
25
26 93 genetic risk of schizophrenia [12,13]. These Mendelian randomization studies eliminate the
27
28 94 possibility that raised IL-6 concentrations are a consequence of environmental exposures
29
30 95 associated with schizophrenia, such as obesity and smoking and instead suggest that IL-6 has
31
32 96 a causal role in psychosis. Extending this approach using the UK Biobank population, we
33
34 97 found that genetically-predicted levels of IL-6 were associated with reduced grey matter
35
36 98 primarily in the middle temporal gyrus, a region whose gene expression profile is enriched
37
38 99 for IL-6 pathway proteins and for neuropsychiatric disorder ontologies [14]. Moreover,
39
40 100 clinical studies report correlations between IL-6 levels and structural brain changes in
41
42 101 individuals with schizophrenia [15], with reduced grey matter volume being exaggerated in
43
44 102 patients with psychosis and elevated inflammatory cytokines [16]. Though this causal
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46 103 evidence strongly implicates IL-6, only an intervention study in patients can test the causal
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48 104 hypothesis.
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56 105 The neuroimmune hypothesis generally assumes that microglia, the brain's resident
57
58 106 immune cells, are activated and pathogenic in schizophrenia. This is supported by traditional
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3 107 neuropathological studies and initial in-vivo PET imaging studies [17–19], possibly reflecting
4
5 108 impaired cellular control of inflammation or oxidative defence. Inflammatory damage may
6
7
8 109 also account for evidence of oxidative stress from MRS glutathione studies [20]. However,
9
10 110 whether microglia are the direct target of IL-6 is unclear and it is not certain that IL-6 can
11
12 111 cross the blood-brain barrier and/or increase its permeability to circulating inflammatory
13
14 112 cells, cytokines, and chemokines [2,21]. Additionally, it is increasingly uncertain whether
15
16 113 microglial inflammation, as traditionally understood, occurs in schizophrenia [22,23]. Recent
17
18 114 meta-analyses of PET radioligand binding studies report decreased rather than increased
19
20 115 radioligand binding to activated microglia [24,25]. This may account for the unexpected lack
21
22 116 of therapeutic benefit of the anti-microglial antibiotic, minocycline, in recent large clinical
23
24 117 trials [26,27]. Furthermore, large transcriptomic studies in post-mortem brains report no
25
26 118 change or reduction in microglial gene expression but increases in astrocytic expression
27
28 119 [23,28–32]. It is increasingly understood that both peripheral immune responses and brain
29
30 120 glial function are regulated by specialised T cells (Tregs), a subset of which reside in brain
31
32 121 parenchyma [33,34]. A novel proposal is that Treg hypofunction accounts for mild peripheral
33
34 122 immune disinhibition and dysregulated astroglial-microglial interaction, such that microglia
35
36 123 are driven into a developmental, synapse-pruning phenotype while astroglia disrupt
37
38 124 neurotransmitter function [33,34]. Importantly, there are bidirectional interactions between
39
40 125 IL-6 and Treg function [34]. Crucially, we will measure IL-6 in addition to cellular and
41
42 126 molecular markers of immune function and investigate how they correlate with central
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44 127 markers and clinical state.
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51 128 Previous attempts testing the inflammatory hypothesis in therapeutic clinical trials
52
53 129 have been attempted. However, little evidence of overall efficacy has been found [35]. These
54
55 130 trials have generally tested broad spectrum agents, such as non-steroidal anti-inflammatory
56
57 131 drugs, with no attempt to stratify patients according to evidence of inflammation. A trial
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3 132 using tocilizumab, a humanised monoclonal antibody (mAb) against the IL-6 receptor
4
5 133 currently licensed in the UK for treatment of rheumatoid arthritis (RA) and severe
6
7 134 coronavirus disease, reported no improvements in any clinical measure in a small sample of
8
9 135 36 patients with established schizophrenia [36]. However, as mentioned previously, no
10
11 136 stratification by inflammatory markers or any mechanistic immune measures was applied.
12
13 137 Low-grade inflammation is associated with poor response to antipsychotic drugs [37], but
14
15 138 immunotherapy is unlikely to be relevant for all patients with psychosis. Meta-analysis
16
17 139 suggests that evidence of immune activation, defined by elevated CRP levels, is present in a
18
19 140 quarter to one third of patients with schizophrenia [38]. A randomised controlled trial of
20
21 141 infliximab, an anti-tumour necrosis factor alpha (TNF- α) mAb, reported that antidepressant
22
23 142 response was associated with higher CRP levels at baseline [39], suggesting that patients with
24
25 143 evidence of immune activation may be better candidates for immunotherapy trials. As far as
26
27 144 we are aware, no previous clinical trial has selected patients with schizophrenia based on
28
29 145 evidence of immune activation.
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35 146 Selection of patients with particular symptom profiles and/or stage of illness may also
36
37 147 be a useful strategy that needs to be employed in immunotherapy trials for schizophrenia. A
38
39 148 wide variety of symptoms occur in schizophrenia such as hallucinations, delusions,
40
41 149 anhedonia, cognitive dysfunction, and affective symptoms and presentation of these
42
43 150 symptoms differ from one individual to another. Some symptoms may be more related to
44
45 151 inflammation than others. For instance, meta-analytic data suggests that elevated
46
47 152 proinflammatory cytokines are associated with negative psychotic symptomatology [40].
48
49 153 Moreover, a recent study from the ALSPAC birth cohort reported that out of 20 positive and
50
51 154 negative symptoms, CRP is particularly associated with anhedonia and auditory
52
53 155 hallucinations [41]. Lastly, results from work we have completed to date as part of the MRC-
54
55 156 funded larger PIMS collaboration (MR/S037675/1), suggest that anhedonia may be a
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3 157 promising target in early phases of established psychotic disorder. Anhedonia and
4
5 158 amotivation are strongly associated with poor functional outcomes in depression and
6
7 159 schizophrenia, and present a formidable barrier to returning to work or building relationships
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9
10 160 [42,43]. Patients with psychotic disorders also present with cognitive deficits in a range of
11
12 161 domains [44]. Available antipsychotic medications have a limited effects on poor cognitive
13
14 162 functioning in psychosis [45]. Illness stage may also be of relevance. Meta-analytic data has
15
16 163 revealed no differences in IL-6 levels between stable, medicated patients with schizophrenia
17
18 164 and controls, although compared with controls, IL-6 levels were similarly elevated in patients
19
20 165 with FEP and those with acute relapse [7]. A separate meta-analysis found evidence of
21
22 166 elevated blood cytokine levels in acutely and chronically ill patients with schizophrenia [6].
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24 167 Focusing on particular inflammation-related symptoms, such as anhedonia, and/or illness
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26 168 stage may increase the chance of success for immunotherapy trials.
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33 170 **Proposed study**

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35 171 The proposed study is a UK multi-site (Birmingham, Bristol, and Cambridge) proof-of-
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37 172 concept, randomised, parallel-group, double-blind, placebo-controlled trial.
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42 174 *Study aims and hypotheses*

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44 175 The primary aim of this trial is to examine potential mechanisms by which IL-6 affects
45
46 176 anhedonia, psychotic symptoms, and cognition. Our primary hypothesis is that inhibition of
47
48 177 IL-6 signalling with a single intravenous infusion of anti-IL6R monoclonal antibody,
49
50 178 tocilizumab, in individuals with psychosis and elevated IL-6 at baseline will attenuate
51
52 179 symptoms of anhedonia and amotivation in patients with psychosis, relative to placebo. This
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54 180 will provide further evidence for a potential causal role of inflammation in psychosis. Our
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56 181 secondary hypothesis is that reduction in peripheral inflammation after tocilizumab infusion
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3 182 in patients with psychosis and evidence of inflammation will be associated with central
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5 183 measures of oxidative stress and relevant resting state brain function.
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8 184 We will also conduct deep immunophenotyping of peripheral blood mononuclear cell
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10 185 subsets (CD4⁺, CD8⁺, Tregs, natural killer and natural killer-T cells, monocytes, and B cells)
11
12 186 to characterise their absolute number, frequency, and function. The level of IL-6/STAT3
13
14 187 signalling inhibition within both innate and adaptive immune cells will also be examined
15
16 188 using multi-colour flow cytometry with an established optimised pSTAT3 phosflow assay.
17
18 189 This will help identify the potential cellular impact of peripheral inflammation in psychosis,
19
20 190 which is largely unknown. Functional assessment of IL-6/STAT3 signalling in immune cell
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22 191 subsets and their response to exogenous IL-6 stimulation will inform abnormal immune
23
24 192 response in psychosis and allow measurement of response to tocilizumab at the cellular level.
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28 193 A secondary objective is to carry out an observational study to examine clinical and
29
30 194 biomarker differences and similarities between patients with psychotic disorder with and
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32 195 without evidence of inflammation and healthy controls (HCs). We hypothesise that
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34 196 individuals with psychotic disorder and evidence of inflammation, compared to those without
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36 197 evidence of inflammation and HCs, will have increased symptoms of anhedonia and
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38 198 amotivation, poorer cognitive functioning, and cellular and brain-based measures of immune
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40 199 dysfunction.
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47 201 METHODS

48 202 This protocol has been prepared in accordance with the Standard Protocol Items:
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50 203 Recommendations for Interventional Trials (SPIRIT) 2013 statement [46]. Please see
51
52 204 supplementary eTable 1 for the SPIRIT checklist. The planned start date for the PIMS trial
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54 205 was 1st November 2021, however, this was delayed due to the COVID-19 pandemic. We
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56 206 began recruiting at our site in Birmingham in November 2022, and we soon expect
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3 207 recruitment to begin at our Bristol and Cambridge sites. The planned end date is 31st May
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5 208 2024.
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10 210 **Patient and public involvement**

11
12 211 The study protocol was prepared in collaboration with individuals with lived experience of
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14 212 mental illness who contributed to the development of participant information sheet, consent
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16 213 forms (Appendix I, II, and III), and data collection procedures.
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22 215 **Study design and sample**

23
24 216 See Figure 1 for an overview of study design. Individuals residing in Birmingham, Bristol, or
25
26 217 Cambridge in the United Kingdom will be recruited. Approximately 60 participants with first
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28 218 episode psychosis and evidence of inflammation (i.e., IL-6 ≥ 0.7 pg/ml) will be randomised to
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30 219 receive either one intravenous infusion of tocilizumab (drug) or normal saline (placebo). For
31
32 220 the secondary, observational study, we will compare baseline characteristics of the
33
34 221 intervention cohort with approximately 30 participants with psychosis without evidence of
35
36 222 inflammation (i.e., IL-6 < 0.7 pg/ml), and approximately 30 HCs across Birmingham and
37
38 223 Cambridge. Participants without evidence of inflammation and controls will not be
39
40 224 randomised as they will not receive any intervention. Neuroimaging will only be undertaken
41
42 225 by those without MRI contraindications who have given specific informed consent for MRI.
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44 226 Participants not eligible or not consenting for MRI will take part in all other aspects of the
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46 227 study.
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54 229 **Intervention**

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56 230 Single intravenous infusion of tocilizumab (4.0mg/kg; max 800mg in total) or normal saline
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58 231 given to participants with psychosis and evidence of inflammation. Tocilizumab blocks both
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3 232 IL-6 classic and trans-signalling – the latter being responsible for most of the inflammatory
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5 233 effects of IL-6 – providing broad inhibition of IL-6 signalling and a strong test of a casual
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7 234 role for IL-6 in psychosis [47]. Tocilizumab is the first-in-class, humanized monoclonal
8
9 235 antibody against the IL-6R, commercially available and licensed in the UK for treatment of
10
11 236 RA. Approved dosage of tocilizumab for treatment of RA is 2, 4, or 8mg/kg; max 800mg in
12
13 237 total. In RA, a single tocilizumab infusion has shown to improve clinical and laboratory
14
15 238 measures within 48 hours, with most noticeable results in one-to-two weeks [48,49]. The
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17 239 follow-up schedule for our study is in keeping with this observation.
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240

241 **Eligibility criteria**

242 We will recruit participants aged 18-40 years. Patient participants must meet International
243 Classification of Diseases 10th Revision (ICD-10) criteria for a diagnosis of schizophrenia
244 and related psychoses (ICD-10 code F20, F22, F25, F28, F29) at the time of eligibility
245 assessment, be within three years of first diagnosis of psychotic disorder, be on a stable
246 treatment regime with no recent (within two weeks) initiation, cessation, or change in class of
247 antipsychotic medication, and have a Positive and Negative Syndrome Scale (PANSS) item
248 score ≥ 3 on P1 (delusions), P2 (conceptual disorganisation), P3 (hallucinatory behaviour), or
249 P6 (suspiciousness/persecution). Additionally, patients recruited to the interventional arm
250 will be required to have serum IL-6 levels ≥ 0.7 pg/ml and a Temporal Experience of Pleasure
251 Scale (TEPS) anticipatory pleasure score ≤ 41 (based on item numbers 1, 3, 7, 11, 12, 14, 15,
252 16, 17, and 8) and consummatory pleasure score ≤ 36 (based on item numbers 2, 4, 5, 6, 8, 9,
253 10, and 13). The threshold of serum IL-6 ≥ 0.7 pg/mL as evidence of inflammation for this
254 particular trial was chosen based on observations from the Personalised Prognostic Tools for
255 Early Psychosis Management (PRONIA) cohort [<https://www.pronia.eu>]. In 192 first-episode
256 psychosis patients included in the PRONIA study, the median value of serum IL-6 was

257 0.49pg/mL (25th percentile 0.22pg/mL; 75th percentile 1.11pg/mL), and the mean was
 258 0.79pg/mL (SD \pm 0.84). Based on these observations, we chose the cut-off of 0.7pg/mL for
 259 patient selection in current trial. Finally, COVID-19 anti-body titre test will be used to
 260 determine adequate levels of immune response via the following cut-offs (for poor response):
 261 400IU Roche/700IU Abbot assay.

262 HCs will have no current or lifetime history of psychiatric diagnosis, as determined by
 263 the Mini-International Neuropsychiatric Interview (MINI). See Table 1 for complete
 264 inclusion and exclusion criteria. HCs will be matched to patient participants at the group level
 265 by age and sex.

266
 267 **Table 1. PIMS Trial inclusion and exclusion criteria.**

| Group | Inclusion criteria | Exclusion criteria |
|------------------|---|---|
| All participants | <ul style="list-style-type: none"> - Provide informed consent. - Understand written and spoken English. - Able and willing to consent to blood sampling. - Willing to abstain from strenuous exercise for 72 hours prior to assessment. | <ul style="list-style-type: none"> - Pregnancy (confirmed by urine pregnancy test) or breast feeding. - Body mass index >35. - Current or lifetime diagnosis of antisocial personality disorder, autism or other neurodevelopmental disorder, major traumatic brain injury. - Currently active diagnosed eating disorder likely to compromise ability to take part. - History of alcohol or substance use disorder (abuse/dependence) within six months prior to eligibility assessment (nicotine and caffeine dependence are not exclusionary). - Current use of medication likely to compromise interpretation of immunological data. - Known active current or history of recurrent bacterial, viral, fungal, mycobacterial, or other opportunistic infections. - Current infection with VZV, TB, Hepatitis B, Hepatitis C, or HIV confirmed by blood test. Chest X-ray will also be performed to assess for TB. - Any major episode of infection requiring hospitalisation or treatment with IV antibiotics within 4 weeks of eligibility assessment. |

| | | |
|--|---|--|
| | | <ul style="list-style-type: none"> - Unstable cardiac, pulmonary, renal, hepatic, endocrine, hematologic, or active infectious disease, including current or prior malignancy. - Diverticulitis, inflammatory bowel disease, or uncontrolled gastric/duodenal ulcer. - Concomitant auto-immune or auto-inflammatory rheumatological disease. - Concomitant treatment with any biologic drugs. - Current and active ischemic heart disease. - Uncontrolled hypertension defined as systolic blood pressure > 170 or diastolic blood pressure > 110. - History of severe allergic or anaphylactic reactions to human, humanized or murine monoclonal antibodies. - No history of chicken pox infection or no history of varicella zoster immunity. |
| Additional criteria for neuroimaging (optional) | - Able and willing to consent to MRI scanning | - Contraindications to MRI. |
| Additional criterion for healthy controls | - No current or lifetime psychiatric diagnosis. | |
| Additional criteria for all individuals with psychosis | <ul style="list-style-type: none"> - Meet ICD-10 criteria for a diagnosis of schizophrenia and related psychoses (code F20, F22, F25, F28, F29) at the time of eligibility assessment, as determined by the Mini-International Neuropsychiatric Interview. - Be within three years of first diagnosis of psychotic disorder. - On stable treatment regime with no recent (within 2 weeks) initiation, cessation, or change in class of antipsychotic medication. - No indication or other reason for preclusion into research (e.g., significant risk of suicidal behaviour or risk to others) as determined by their clinical team. - Positive and Negative Syndrome Scale item score ≥ 3 on P1 (delusions), P2 (conceptual disorganisation), P3 (hallucinatory behaviour), OR P6 (suspiciousness/persecution). | |

| | | |
|---|--|--|
| Additional criteria for intervention group | <ul style="list-style-type: none"> - Serum IL-6 level ≥ 0.7pg/ml at eligibility and baseline assessment. - Temporal Experience of Pleasure Scale anticipatory pleasure score ≤ 41 (based upon item numbers 1, 3, 7, 11, 12, 14, 15, 16, 17, and 8) and consummatory pleasure score ≤ 36 (based upon item numbers 2, 4, 5, 6, 8, 9, 10, and 13). - Evidence of COVID-19 immunity required prior to infusion, confirmed before randomisation using evidence of vaccination and antibody titre test. | |
| Additional criterion for patients with psychosis without inflammation | <ul style="list-style-type: none"> - Serum IL-6 level < 0.7pg/ml at eligibility and baseline assessment. | |

270

271 **Study outcomes**

272 The primary outcome is anhedonia, defined as anticipatory and consummatory pleasure
 273 scores, assessed by the TEPS [50] at approximately day 14 post-infusion. We will also collect
 274 data on several secondary/exploratory measures including 1) clinical outcomes, namely
 275 positive and negative symptoms of psychosis, depressive symptoms, fatigue, general quality
 276 of life and subjective wellbeing, 2) cognitive function (psychomotor speed, attention,
 277 memory, and executive function), 3) neuroimaging outcomes based on comparisons of brain
 278 structure, function, and oxidative stress levels using MRI and MRS outcomes, 4) blood
 279 biomarker outcomes, namely peripheral blood inflammatory markers, biochemical assays,
 280 including cortisol and cardiometabolic markers, and peripheral blood cellular
 281 immunophenotyping, and 5) genetic outcomes including DNA and RNA sequencing and
 282 epigenetic mechanism assessment with methylation assays (see Table 2 for more
 283 details). Where possible, blood samples will be collected during working hours and time of

284 sampling will be recorded. However, a specified time window will not be given to ease
 285 burden on patients and to maximise participation.

286
 287 **Table 2. PIMS trial study measures**

| Domain | Tool | Source | Validated Tool | Time of assessment |
|----------------------------|---|------------------------------|----------------|-----------------------------------|
| Sociodemographic/lifestyle | Screening questionnaire | Self-report | | Screening |
| | Medical History Questionnaire | Self-report/General practice | | Eligibility |
| | Substance Use Questionnaire | Self-report | | Eligibility |
| | Physical Measurements Form | Self-report | | Baseline |
| | Sociodemographic Questionnaire | Self-report | | Baseline |
| Psychiatric | The Temporal Experience of Pleasure Scale | Self-report | ✓ | Eligibility, baseline, follow-ups |
| | The Positive and Negative Syndrome Scale | Interviewer assessed | ✓ | Eligibility, baseline, follow-ups |
| | The Mini-International Neuropsychiatric Interview | Interviewer assessed | ✓ | Eligibility |
| | Psychiatric History Questionnaire | Self-report | | Baseline |
| | The Scale for the Assessment of Negative Symptoms | Self-report | ✓ | Baseline, follow-ups |
| | The Calgary Depression Scale for Schizophrenia | Interviewer assessed | ✓ | Baseline, follow-ups |
| | Multi-dimensional Fatigue Inventory | Self-report | ✓ | Baseline, follow-ups |
| | European Quality of Life-5 Dimensions Three-Level Version | Self-report | ✓ | Baseline, follow-ups |
| Cognitive | Visual Analogue Scale for Subjective Wellbeing | Self-report | ✓ | Baseline, follow-ups |
| | National Adult Reading Test for | Interviewer assessed | ✓ | Baseline, follow-up 2 |

| | | | | |
|--------------|---|------------------|---|-----------------------|
| | estimated premorbid IQ | | | |
| | CANTAB Reaction Time test | Computer task | ✓ | Baseline, follow-up 2 |
| | Symbol Coding Test | Paper task | ✓ | Baseline, follow-up 2 |
| | CANTAB Rapid Visual Information Processing test | Computer task | ✓ | Baseline, follow-up 2 |
| | CANTAB Paired Associates Learning test | Computer task | ✓ | Baseline, follow-up 2 |
| | CANTAB One Touch Stockings of Cambridge test | Computer task | ✓ | Baseline, follow-up 2 |
| Biologic | Inflammatory markers, cardiometabolic markers,IDO activation, white cell phenotyping | Laboratory tests | | Baseline, follow-ups |
| Genetic | RNA and DNA sequencing, methylation assay | Blood (RNA, DNA) | | Baseline, follow-ups |
| Neuroimaging | MRI Screening Questionnaire | | | Baseline, follow-up 2 |
| | Structural MRI, 1H-MRS measure of glutathione in the prefrontal cortex area, resting state fMRI | | | Baseline, follow-up 2 |

289

290 **Sample size and statistical power**

291 We will recruit approximately 60 patients with psychosis. However, currently there are no

292 trials of immunotherapies for anhedonia in schizophrenia making accurate power calculation

293 difficult. This study is a proof-of-concept experiment designed to test whether inhibition of

294 IL-6 signalling leads to changes in psychotic symptoms. It could also inform likely statistical

295 power for future trials testing efficacy of the drug as a treatment of schizophrenia, which is

296 not the intention of this study. The exact statistical tests and techniques that will be applied to

1
2
3 297 the data will depend on the objective of specific analysis and data characteristics (e.g.,
4
5 298 variable type, distribution). These details will be specified in analysis plans and registered
6
7
8 299 online before participants are unblinded and any data analysis is performed.
9

10 300

11 301 **Randomisation and blinding**

12
13
14 302 An external agency independent of the study team will arrange random allocation to
15
16
17 303 tocilizumab or normal saline group 1:1, ensuring two groups are comparable regarding
18
19 304 anhedonia severity and sex. Randomisation will be stratified by site. Randomising agency
20
21
22 305 will provide the randomisation code to the relevant hospital pharmacy who will dispense
23
24 306 tocilizumab or normal saline according to the randomisation schedule. Dispensing
25
26 307 pharmacies will keep a log of products dispensed. Infusions will be prepared and
27
28 308 administered at clinical research facilities (CRFs). Infusion packs will be prepared by trained
29
30 309 staff not part of the core study team, ensuring blinding of treatment allocation. Infusion packs
31
32 310 containing drug or placebo will be visually indistinguishable from each other, ensuring that
33
34
35 311 both participants and study team remain blind regarding treatment allocation.
36

37 312

38 313 **Statistical analysis**

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42 314 For randomised participants, an intention-to-treat approach will be taken for data analysis by
43
44 315 including all randomised participants in statistical analyses, regardless of the treatment they
45
46 316 received (if any). We will compare outcome measures between treatment and placebo groups
47
48 317 controlling for baseline scores. This mechanistic experiment will focus on overall pattern of
49
50 318 results and their effect sizes rather than *P*-values for individual tests of statistical significance.
51
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53 319 The secondary mechanistic and observational analysis will compare psychotic symptoms,
54
55 320 cognitive function, blood, neuroimaging, and other biomarkers between and across study
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57
58 321 groups using appropriate statistical tests.
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67 324 **STUDY PROCEDURE**

8
9 325 An overview of study procedures is presented in Figure 1 and all study measures are detailed
10
11 326 in Table 2. Recruitment will take place in Birmingham, Bristol, and Cambridge and
12
13 327 assessments at University and NHS research facilities.
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16 328

17
18 329 **Participant identification**

19
20 330 Potential participants with psychosis will be identified by NHS Psychosis Early Intervention
21
22 331 (EI) teams. HCs will be recruited through advertisement methods in Birmingham and
23
24 332 Cambridge. Potential participants will complete a screening questionnaire to confirm their
25
26 333 eligibility to participate. If deemed eligible, participants will be invited to an appointment to
27
28 334 complete a full eligibility assessment.
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32 33533
34 336 **Eligibility assessment**

35
36 337 Assessments will be carried out to establish eligibility and to obtain informed consent.
37
38 338 Patients will complete the MINI to confirm ICD-10 diagnosis of schizophrenia and related
39
40 339 psychoses, the PANSS to confirm the presence of positive symptoms of psychosis, and the
41
42 340 TEPS to confirm eligibility based on anticipatory and consummatory pleasure sum scores. A
43
44 341 blood sample will be collected from patients for serum IL-6 measurement. An MRI screening
45
46 342 questionnaire will be administered to those willing to give informed consent for
47
48 343 neuroimaging.
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52 34453
54 345 **Baseline assessment**

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56 346 All participants (60 inflamed psychosis, 30 non-inflamed psychosis, and 30 HCs) will attend
57
58 347 a baseline assessment comprising psychiatric measures, cognitive tasks, blood sampling, and
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3 348 neuroimaging (optional). This will be the final study contact for patients without evidence of
4
5 349 inflammation and HCs. Patients with evidence of inflammation will undergo further tests to
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7
8 350 establish safety/eligibility to receive tocilizumab, including a chest X-ray and blood tests to
9
10 351 exclude pregnancy and certain infections, such as TB, HIV, and COVID-19. Eligible
11
12 352 participants will be randomised and invited for infusion.
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15 353

16 17 354 **Intervention**

18
19 355 Intravenous infusion of tocilizumab or normal saline will be given continuously over one
20
21 356 hour at CRFs in Birmingham, Bristol, and Cambridge by trained clinical staff under the
22
23
24 357 supervision of a designated study doctor. Participants will remain under clinical observation
25
26 358 for a further 1-hour period after the end of infusion.
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30 31 360 **Follow-up assessments**

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33 361 Follow-up assessments will take place approximately 7-, 14-, and 28-days post-infusion, and
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35 362 will collect similar data to the baseline assessment. Cognitive tasks and neuroimaging
36
37 363 (optional) will be administered only on day 14. Around 42 days post-infusion, participants
38
39 364 will be contacted by phone to provide a final debrief; at which point they will exit the study.
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43 44 366 **RISK MANAGEMENT**

45 46 47 367 **Psychosis-related risks**

48
49 368 All patients will be under the care of a specialist NHS psychosis EI service. Participation will
50
51 369 not involve any treatment modifications or significant delays in receiving treatment. If a
52
53 370 patient becomes distressed during an assessment, or does not wish to continue for any reason,
54
55 371 the researcher will stop the assessment. Participants may withdraw at any time without giving
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3 372 a reason. If there is any concern for the participant's safety, the research team will liaise with
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5 373 participant's GP and/or mental health team as needed.
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10 375 **Procedure-related risks**
11

12 376 *Venepuncture*
13

14 377 Blood taking is associated with mild discomfort and other side effects are rare. Efforts will be
15
16 378 made to minimise discomfort. Blood taking will be performed by a nurse, doctor, or research
17
18 379 team member trained in venepuncture.
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21 380

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23
24 381 *Chest X-ray*
25

26 382 This study will use a typical effective radiation dose of 0.014 mSv; equivalent to 2.5 days of
27
28 383 average natural background radiation in the UK. The risk of developing cancer as a
29
30 384 consequence of participating in this study is 0.0001%. Only non-pregnant, adult participants
31
32 385 will be included.
33
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35 386

36
37 387 *Neuroimaging*
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39
40 388 Discomfort during MRI will be minimised by using mirrors to allow participants to view
41
42 389 outside of the machine, providing ear plugs and a panic button, and allowing participants to
43
44 390 communicate with the researcher and scan operator throughout. Mild transient vertigo may be
45
46 391 experienced when being moved into the MRI machine. Risk of dislodgement or malfunction
47
48 392 of medical implants or metallic foreign objects will be minimised by screening participants to
49
50 393 ensure no metal is present on or within the body.
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56 395 *IL-6 levels*
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3 396 We expect some 30-50% of patient participants to have evidence of inflammation in the
4
5 397 blood (IL-6 ≥ 0.7 pg/ml). This is not a cause for concern. Reasons for elevated IL-6 in the
6
7 398 absence of an acute infection or chronic inflammatory illness could include obesity, smoking,
8
9 399 alcohol use, and lack of exercise, so knowledge of 'inflammation status' may prompt
10
11 400 participants to adopt a healthier lifestyle. If serum IL-6 level is high (i.e., IL-6 ≥ 0.7 pg/ml)
12
13 401 along with elevated CRP (>20 mg/L) without any apparent explanation, such as infection or
14
15 402 chronic inflammatory illness, we will inform the participant's GP and the participant will be
16
17 403 excluded from the study.
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405 *Risk to research staff*

406 Staff will follow local safety procedures when lone working. No other risks are anticipated.

407

408 **Safety considerations for infusion and monitoring of adverse reaction**

409 *Before infusion*

410 Participants will be selected based on strict inclusion and exclusion criteria. Additionally, we
411 will carry out tests for TB, HIV, VZV antibody, and Hepatitis B and C because, though
412 unlikely after a single dose, tocilizumab could make these infections worse. Female
413 participants of childbearing age will be given a pregnancy test, which must be negative.
414 Participants who are sexually active will be asked to use at least one form of effective
415 contraception for six weeks post-infusion. Male participants will also be asked not to donate
416 sperm samples for six weeks post-infusion.

417

418 *During infusion*

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3 419 Infusions will be given under supervision of a designated study doctor. Participants will be
4
5 420 monitored for possible side effects, which will be managed in line with use of tocilizumab for
6
7 421 treating patients with RA.
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11
12 423 *After infusion*

13
14 424 Participants will remain under observation for one-hour post-infusion. Participants will be
15
16 425 advised to seek help if they feel unwell after leaving the assessment centre and will be given
17
18 426 an information sheet containing a telephone number their health professionals can call. If
19
20 427 necessary, we will unblind the participant and inform their health professional whether they
21
22 428 received tocilizumab or normal saline. Adverse reactions will be recorded at each follow-up
23
24 429 visit. Additional, safety blood tests will be done at second follow-up (e.g., WBC count, liver
25
26 430 function, lipids).
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33 432 ETHICS AND DISSEMINATION

34
35 433 The study will be conducted in accordance with the REC, Health Research Authority (HRA),
36
37 434 and local Research and Development (R&D) department approvals and guidelines (REC
38
39 435 reference: 22/EE/0010). The study team will prepare protocol amendments as required and
40
41 436 ethics approval will be sought before implementing any changes to the approved protocol.
42
43 437 The ISRCTN Trial Registry and the Research Governance Office will be informed of any
44
45 438 amendments to the protocol.
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51 440 **Consent**

52
53 441 Informed consent will be obtained prior to eligibility assessments for participation in the
54
55 442 study (Appendix I, II, and II). This will include consent to randomise, for contact with their
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57 443 GP to inform them about participation, access GP/psychiatric records to verify medical
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3 444 history to establish eligibility, and to inform the participant's GP any results/outcomes as
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5 445 necessary. Consent for additional tests to establish safety for tocilizumab infusion and for
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7
8 446 storing biological samples will also be obtained.
9

10 447

11 448 **Study management**

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13
14 449 The study is sponsored by the University of Bristol. The sponsor, the Chief Investigator
15
16 450 (GMK), and the co-Lead (RU) will have overall responsibility for the study. A named
17
18 451 principal investigator will take clinical responsibility for study activities at each site. The
19
20 452 study does not require the formal arrangement of a steering committee because, according to
21
22 453 the HRA, it is not a Clinical Trial of an Investigational Medicinal Product. However, to
23
24 454 enhance monitoring of the study, a study management group will be established, comprising
25
26 455 academic and clinical experts in psychiatry, rheumatology, neuroscience, and immunology.
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31 457 **Data management and retention of samples**

32
33 458 All potential participants will be assigned a unique study-specific participant ID number. All
34
35 459 data will be subject to good practice as laid down in the Data Protection Act. Each study
36
37 460 stage is tracked so that participant's (de-identified) status within the study is known, and
38
39 461 assessment and other appointment dates are forecasted. This information is held on a secure,
40
41 462 password-protected database. Anonymised data from assessments will be uploaded to a
42
43 463 secure, password-protected database using secure web-based data entry systems. Minimal
44
45 464 personal data (age, sex) will be indexed by each participant's unique ID number. Blood
46
47 465 samples collected in this study may be stored for up to 10 years after the completion for
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49 466 additional research. Stored samples will be coded throughout the sample storage and analysis
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51 467 process and will not be labelled with personal identifiers. Participants may withdraw their
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53 468 consent for their samples to be stored for future research.
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5 470 **Dissemination plan**

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8 471 Study results will be published in peer-review journals and will conform to the guidelines of
9
10 472 the International Committee of Medical Journal Editors. Findings will be disseminated at
11
12 473 conferences, departmental talks, and via social and traditional media.
13
14

15 474

16
17 475 **AUTHORS CONTRIBUTIONS**

18
19 476 ÉMF wrote first draft of the PIMS trial protocol and of this manuscript. SLG, MK, GKM,
20
21 477 BD, DJ, JS, and NMB contributed to study design and protocol development and revised
22
23 478 manuscript drafts. RU contributed to study design and study protocol, and revised manuscript
24
25 479 drafts. GMK devised study design and trial protocol, and revised drafts. ÉMF and SLG
26
27 480 developed study materials and liaised with REC and HRA regarding approvals. AM, JR,
28
29 481 FCZ, HH, EW, and MW contributed to the revision of the manuscript and validation of
30
31 482 operating procedures and mechanistic protocols. RU and GMK co-lead the MRC grant that
32
33 483 funds the PIMS trial and provide overall supervision and oversight for the project.
34
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36

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39
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18
19 502 study.
20
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26 504 COMPETING INTERESTS STATEMENT

27
28 505 ÉMF, SLG, AM, JR, FCZ, HH, EW, MW, MK, GKM, BD, DJ, JS, RU, and GMK have no
29
30 506 conflicts of interest to report. NMB holds shares and is a Director of Celentyx Ltd.
31
32
33
34

35 508 PIMS COLLABORATION

36
37 509 Members of the PIMS Collaboration include Golam Khandaker, Rachel Upthegrove, Alice
38
39 510 Egerton, Anthony David, Bill Deakin, Carmine Pariante, David Cotter, Ed Bullmore, Eva
40
41 511 Meisenzahl, Gary Donohoe, Georgios Gkoutos, Jack Rogers, James MacCabe, Joanna Neill,
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43 512 John Suckling, Neil Harrison, Nicholas Barnes, Nikos Koutsouleris, Paola Dazzan, Peter
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45 513 Jones, Stephen Burgess, Stephen Wood, Valeria Mondelli.
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683 FIGURE LEGEND

684 Figure 1. Overview of study design

For peer review only

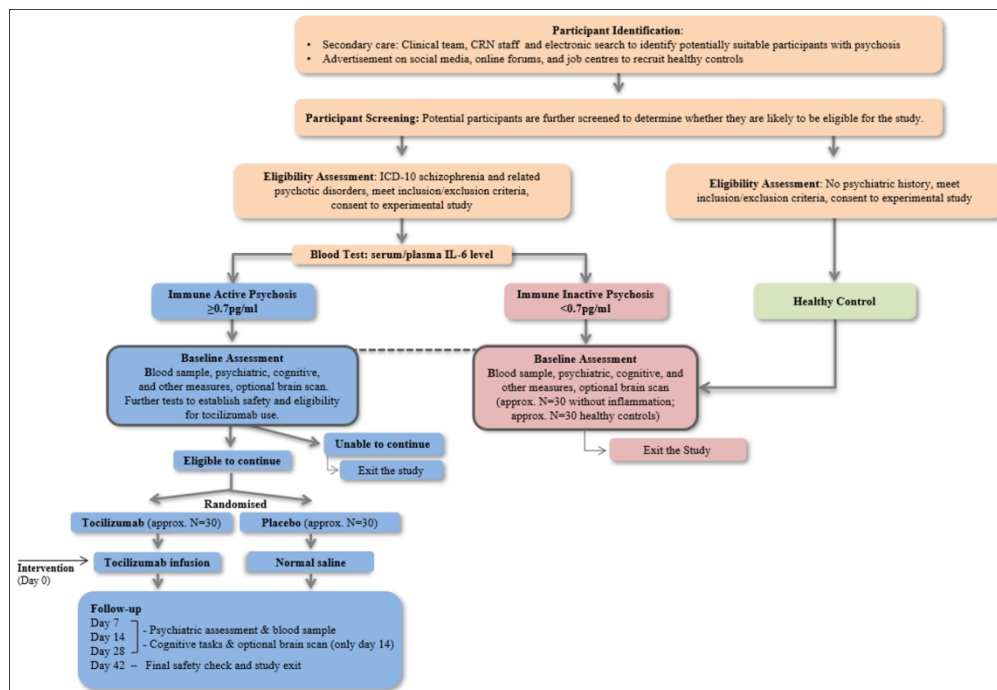


Figure 1. Overview of study design

240x165mm (144 x 144 DPI)

Supplementary Material

Foley et al. Protocol for the Psychosis Immune Mechanism Stratified Medicine (PIMS) trial: A randomised double-blind placebo-controlled trial of single dose tocilizumab in patients with psychosis.

Table of Contents

eTable1: SPIRIT 2013 Checklist..... 2

Appendix I – Consent Form for Screening: All Participants..... 8

Appendix II – Informed Consent Form for Study Participation: Patients 10

Appendix III – Informed Consent Form for Study Participation: Healthy Controls..... 13

For peer review only

eTable1: SPIRIT 2013 Checklist – Recommended items to address in a clinical trial protocol and related documents*

| Section/item | Item No | Description | Addressed on page number |
|-----------------------------------|---------|--|---------------------------|
| Administrative information | | | |
| Title | 1 | Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym | 1 |
| Trial registration | 2a | Trial identifier and registry name. If not yet registered, name of intended registry | 4 |
| | 2b | All items from the World Health Organization Trial Registration Data Set | 4 (ISRCTN 23256704) |
| Protocol version | 3 | Date and version identifier | - |
| Funding | 4 | Sources and types of financial, material, and other support | 24 - 25 |
| Roles and responsibilities | 5a | Names, affiliations, and roles of protocol contributors | 1, 24 |
| | 5b | Name and contact information for the trial sponsor | 23 |
| | 5c | Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities | 23-25 |

| | | | | |
|----|---|-----|--|-------------------------|
| 1 | | 5d | Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee) | 23 - 25 |
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| 9 | Introduction | | | |
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| 11 | Background and rationale | 6a | Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention | 6-9 |
| 12 | | | | |
| 13 | | 6b | Explanation for choice of comparators | 6-9 |
| 14 | | | | |
| 15 | Objectives | 7 | Specific objectives or hypotheses | 9-10 |
| 16 | | | | |
| 17 | Trial design | 8 | Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory) | 9 |
| 18 | | | | |
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| 20 | | | | |
| 21 | | | | |
| 22 | Methods: Participants, interventions, and outcomes | | | |
| 23 | | | | |
| 24 | Study setting | 9 | Description of study settings (e.g., community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained | 9, 11 |
| 25 | | | | |
| 26 | Eligibility criteria | 10 | Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists) | 12, Table 1 |
| 27 | | | | |
| 28 | Interventions | 11a | Interventions for each group with sufficient detail to allow replication, including how and when they will be administered | 11, 19, 21-22, Figure 1 |
| 29 | | | | |
| 30 | | 11b | Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease) | 22 |
| 31 | | | | |
| 32 | | 11c | Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests) | - |
| 33 | | | | |
| 34 | | 11d | Relevant concomitant care and interventions that are permitted or prohibited during the trial | 12, Table 1 |
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| 1 | Outcomes | 12 | Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended | 9-10, Figure 1, Table 2 |
| 2 | | | | |
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| 6 | Participant timeline | 13 | Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure) | 18-19, Figure 1 |
| 7 | | | | |
| 8 | | | | |
| 9 | Sample size | 14 | Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations | 17 |
| 10 | | | | |
| 11 | | | | |
| 12 | | | | |
| 13 | Recruitment | 15 | Strategies for achieving adequate participant enrolment to reach target sample size | 18 |
| 14 | | | | |
| 15 | Methods: Assignment of interventions (for controlled trials) | | | |
| 16 | Allocation: | | | |
| 17 | | | | |
| 18 | Sequence generation | 16a | Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions | 17 |
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| 25 | Allocation concealment mechanism | 16b | Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned | 17 |
| 26 | | | | |
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| 29 | Implementation | 16c | Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions | 17 |
| 30 | | | | |
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| 33 | Blinding (masking) | 17a | Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how | 17 |
| 34 | | | | |
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| 36 | | 17b | If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial | 22 |
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46**Methods: Data collection, management, and analysis**

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|----------------------------|-----|--|-----------------------|
| Data collection methods | 18a | Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol | 10-15, 18-19, Table 2 |
| | 18b | Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols | 17-18 |
| Data management | 19 | Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol | 23-24 |
| Statistical methods | 20a | Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol | 17-18 |
| | 20b | Methods for any additional analyses (eg, subgroup and adjusted analyses) | 17-18 |
| | 20c | Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation) | - |
| Methods: Monitoring | | | |
| Data monitoring | 21a | Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed | 23 |
| | 21b | Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial | - |
| Harms | 22 | Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct | 20-22 |

| | | | | |
|----|---------------------------------|-----|---|-------|
| 1 | Auditing | 23 | Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor | - |
| 2 | | | | |
| 3 | | | | |
| 4 | Ethics and dissemination | | | |
| 5 | | | | |
| 6 | Research ethics approval | 24 | Plans for seeking research ethics committee/institutional review board (REC/IRB) approval | 22 |
| 7 | | | | |
| 8 | | | | |
| 9 | Protocol amendments | 25 | Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators) | 22 |
| 10 | | | | |
| 11 | | | | |
| 12 | | | | |
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| 14 | Consent or assent | 26a | Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32) | 22-23 |
| 15 | | | | |
| 16 | | | | |
| 17 | | 26b | Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable | 22-23 |
| 18 | | | | |
| 19 | | | | |
| 20 | Confidentiality | 27 | How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial | 23-24 |
| 21 | | | | |
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| 24 | Declaration of interests | 28 | Financial and other competing interests for principal investigators for the overall trial and each study site | 25 |
| 25 | | | | |
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| 27 | Access to data | 29 | Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators | 23 |
| 28 | | | | |
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| 30 | Ancillary and post-trial care | 30 | Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation | 22 |
| 31 | | | | |
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| 33 | Dissemination policy | 31a | Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions | 24 |
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| 38 | | 31b | Authorship eligibility guidelines and any intended use of professional writers | 24 |
| 39 | | | | |
| 40 | | 31c | Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code | - |
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|----------------------------|----|--|------------------------|
| Appendices | | | |
| Informed consent materials | 32 | Model consent form and other related documentation given to participants and authorised surrogates | Appendix I, II and III |
| Biological specimens | 33 | Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable | 23-24 |

*It is strongly recommended that this checklist be read in conjunction with the SPIRIT 2013 Explanation & Elaboration for important clarification on the items. Amendments to the protocol should be tracked and dated. The SPIRIT checklist is copyrighted by the SPIRIT Group under the Creative Commons [“Attribution-NonCommercial-NoDerivs 3.0 Unported”](#) license.

Appendix I – Consent Form for Screening: All Participants

Informed Consent Form for Screening

REC No. 22/EE/0010, Date: 08.02.2022, Version: 1.2

Psychosis Immune Mechanism Stratified Medicine Trial: The PIMS Trial

PARTICIPANT ID: _____

Thank you for considering taking part in the PIMS Trial. The research team must explain the eligibility assessment of the study to you before you agree to take part. If you have any questions arising from the Participant Information Sheet or from the explanation already given to you, please ask a member of the research team before you decide to participate. You will be given a copy of this Informed Consent Form to keep for future reference.

Please read the statements below and insert your initial in the box next to each statement if you agree with them:

| Statement | Initial Here | |
|--|--------------|----|
| 1. I have read and understood the information sheet version XX, DD.MM.YYYY and have had the opportunity to ask questions. | | |
| 2. I understand that my participation is voluntary and I am free to withdraw at any time without giving a reason, without my current or future medical care or legal rights being affected. | | |
| 3. I agree to provide blood samples for eligibility screening. I understand my blood samples will be analysed to test for evidence of immune activation. The purposes and possible risks of donating these samples have been explained to me. I understand that donated samples will be considered a gift but I will have the right to withdraw permission for analysis. | | |
| 4. I agree that my GP can be told that I am participating in the eligibility assessment of the PIMS Trial, and can be informed if any unexpected results are found pertaining specifically to my health. | | |
| 5. I consent for my GP/Psychiatrist to share information from my medical record in order to confirm my eligibility to take part in this study. The study team may access my GP/Psychiatrist records if necessary. | | |
| <i>Optional (Not agreeing to these will not exclude you from this study). Please tick Yes / No (as appropriate)</i> | Yes | No |
| 6. I agree that the samples can be stored after completion of the screening analysis, for use in future, ethically approved, non-genetic studies, even if I am deemed non-eligible to partake in the PIMS study. | | |
| 7. I agree that data and samples can be stored after completion of the PIMS Trial for use in future, ethically approved, genetic studies. This includes the main stocks of any genetic material collected, such as DNA and RNA. | | |

If you want to participate in the screening session of the PIMS Trial, please sign your name below:

Participant Signature _____

Participant Full Name _____

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5 Date ____ / ____ / ____
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10
11 **The researcher who has explained this study to you also needs to sign this form:**
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14
15 Staff Signature _____
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17
18 Staff Full Name _____
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20
21 Date ____ / ____ / ____
22
23

24 **Thank you for your help.**
25

26 By completing and returning this form, you are giving us your consent that the personal
27 information you provide will be treated as strictly confidential and handled in accordance with
28 the provisions of the UK Data Protection Act 2018.
29

30 **When completed: 1 for participant; and 1 for researcher site file.*
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Appendix II – Informed Consent Form for Study Participation: Patients

Informed Consent Form for Study Participation

REC No. 22/EE/0010, Date: 24.08.2022, Version: 1.5

Psychosis Immune Mechanism Stratified Medicine Trial: The PIMS Trial

PARTICIPANT ID: _____

Thank you for taking part in the PIMS Trial eligibility assessment. Based on this assessment, you are eligible to take part in the PIMS Trial. Before you agree to take part, the research team must explain the study to you. If you have any questions arising from the Participant Information Sheet or from the explanation already given to you, please ask a member of the research team before you decide to participate. You will be given a copy of this Informed Consent Form to keep for future reference.

Please read the statements below and insert your initial in the box next to each statement if you agree with them:

| Statement | Initial Here |
|---|--------------|
| 1. I have read and understood the information sheet version XX dated DD.MM.YYYY and have had the opportunity to ask questions. | |
| 2. I agree to take part in the PIMS Trial. I understand that my participation is voluntary and I am free to withdraw at any time without giving a reason, without my current or future medical care or legal rights being affected. | |
| 3. I understand that confidentiality and anonymity will be maintained and it will not be possible to identify me in any publications. | |
| 4. I agree to partake in interviews, complete questionnaires, and cognitive tests as part of this study. I understand what will happen during the study assessments. | |
| 5. I agree to provide blood samples. The purposes and possible risks of donating these samples have been explained to me. I understand that donated samples will be considered a gift but I will have the right to withdraw permission for analysis. | |
| 6. I understand that blood samples collected from me will be used to measure non-genetic factors such as biochemical changes in the blood. | |
| 7. I agree that the samples and information I provide can be stored, used and shared between PIMS Trial sites and with collaborators/contractors for the purpose of the study. | |
| 8. I understand that blood samples collected will be stored at PIMS Trial centres. | |
| 9. I understand that any of my samples (labelled with an anonymous ID only), or any information obtained from them, including the sequence of my genetic material, may be sent to specialist research laboratories in the UK and abroad for analyses and the results returned to PIMS Trial centres. Researchers at these laboratories have no access to personal information about study participants. | |
| 10. I agree, if necessary, to provide blood/urine samples to test for pregnancy, COVID-19 immunity, Hepatitis B, Hepatitis C, HIV, VZV and Tuberculosis, and to undergo a chest X ray. | |
| 11. I agree to being randomised into the tocilizumab or placebo group if deemed eligible to take part. | |

| | | |
|--|-----|----|
| 12. I understand that taking part will involve the administration of a single intravenous infusion of the anti-inflammatory drug tocilizumab or normal saline. | | |
| 13. I agree that my GP can be told that I am participating in this study, and about any findings that require further attention. | | |
| 14. I understand that information related to my participation in this study may be accessed by responsible individuals from the sponsors of this study for quality control purposes. I give permission for these individuals to have access to this data. | | |
| 15. I understand that relevant sections of my medical notes and data collected during the study, may be looked at by individuals from research team, from regulatory authorities or from the NHS Trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. | | |
| <i>Optional (Not agreeing to these will not exclude you from this study). Please tick Yes / No (as appropriate)</i> | Yes | No |
| 16. I agree to undergo brain scans as part of the PIMS Trial. | | |
| 17. I agree to be contacted in future by researchers to participate in follow up studies to this project, or in future studies of a similar nature. | | |
| 18. I understand that researchers may use the blood samples for genetic analysis. | | |
| 19. I agree that the samples can be stored after completion of the PIMS Trial for use in future, ethically approved, non-genetic studies. | | |
| 20. I agree that the information I give can be stored after completion of the PIMS Trial for use in future, ethically approved, non-genetic studies. | | |
| 21. I agree that data and samples can be stored after completion of the PIMS Trial for use in future, ethically approved, genetic studies. This includes the main stocks of any genetic material collected, such as DNA and RNA. | | |

If you want to participate in the PIMS Trial, please sign your name below:

Participant Signature _____

Participant Full Name _____

Date ____/____/____

Research staff who has explained this study to you also needs to sign this form:

Staff Signature _____

Staff Full Name _____

Date ____/____/____

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5 **Thank you for your help.**
6

7 By completing and returning this form, you are giving us your consent that the personal
8 information you provide will be treated as strictly confidential and handled in accordance with
9 the provisions of the UK Data Protection Act 2018.
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11 **When completed: 1 for participant; and 1 for researcher site file.*
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Appendix III – Informed Consent Form for Study Participation: Healthy Controls

Healthy Controls Informed Consent Form for Study Participation

REC No. 22/EE/0010, Date: 17.02.2022, Version: 1

Psychosis Immune Mechanism Stratified Medicine Trial: The PIMS Trial

PARTICIPANT ID: _____

Thank you for taking part in the PIMS Trial eligibility assessment. Based on this assessment, you are eligible to take part in the PIMS Trial. Before you agree to take part, the research team must explain the study to you. If you have any questions arising from the Participant Information Sheet or from the explanation already given to you, please ask a member of the research team before you decide to participate. You will be given a copy of this Informed Consent Form to keep for future reference.

Please read the statements below and insert your initial in the box next to each statement if you agree with them:

| Statement | Initial Here | |
|---|--------------|----|
| 1. I have read and understood the information sheet version XX dated DD.MM.YYYY and have had the opportunity to ask questions. | | |
| 2. I agree to take part in the PIMS Trial. I understand that my participation is voluntary, and I am free to withdraw at any time without giving a reason, without my current or future medical care or legal rights being affected. | | |
| 3. I understand that confidentiality and anonymity will be maintained, and it will not be possible to identify me in any publications. | | |
| 4. I agree to partake in interviews, complete questionnaires, and cognitive tests as part of this study. I understand what will happen during the study assessments. | | |
| 5. I agree to provide blood samples. The purposes and possible risks of donating these samples have been explained to me. I understand that donated samples will be considered a gift, but I will have the right to withdraw permission for analysis. | | |
| 6. I understand that my blood samples collected will be stored at PIMS Trial centres. | | |
| 7. I understand that blood samples collected from me will be used to measure non-genetic factors such as biochemical changes in the blood. | | |
| 8. I understand that any of my samples (labelled with an anonymous ID only), or any information obtained from them, including the sequence of my genetic material, may be sent to specialist research laboratories in the UK and abroad for analyses and the results returned to PIMS Trial centres. Researchers at these laboratories have no access to personal information about study participants. | | |
| 9. I agree that the samples and information I provide can be stored, used and shared between PIMS Trial sites and with collaborators/contractors for the purpose of the study. | | |
| 10. I understand that information related to my participation in this study may be accessed by responsible individuals from the sponsors of this study for quality control purposes. I give permission for these individuals to have access to this data. | | |
| <i>Optional (Not agreeing to these will not exclude you from this study). Please tick Yes / No (as appropriate)</i> | Yes | No |

| | | |
|--|--|--|
| 11. I agree to undergo brain scans as part of the PIMS Trial. | | |
| 12. I agree to be contacted in future by researchers to participate in follow up studies to this project, or in future studies of a similar nature. | | |
| 13. I understand that researchers may use the blood samples for genetic analysis. | | |
| 14. I agree that the samples can be stored after completion of the PIMS Trial for use in future, ethically approved, non-genetic studies. | | |
| 15. I agree that the information I give can be stored after completion of the PIMS Trial for use in future, ethically approved, non-genetic studies. | | |
| 16. I agree that data and samples can be stored after completion of the PIMS Trial for use in future, ethically approved, genetic studies. This includes the main stocks of any genetic material collected, such as DNA and RNA. | | |

If you want to participate in the PIMS Trial, please sign your name below:

Participant Signature _____

Participant Full Name _____

Date ____ / ____ / _____

Research staff who has explained this study to you also needs to sign this form:

Staff Signature _____

Staff Full Name _____

Date ____ / ____ / _____

Thank you for your help.

By completing and returning this form, you are giving us your consent that the personal information you provide will be treated as strictly confidential and handled in accordance with the provisions of the UK Data Protection Act 2018.

**When completed: 1 for participant; and 1 for researcher site file.*