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Immuno**g**lobuli**N** **i**n the **T**reatment of **E**ncephalitis (IgNiTE): Protocol for a multicentre randomised controlled trial

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Abstract

Introduction

Infectious and immune-mediated encephalitides are important but under-recognised causes of morbidity and mortality in childhood, with a 7% case-fatality rate and up to 50% morbidity after prolonged follow up. There is a theoretical basis for ameliorating the immune response with intravenous immunoglobulin (IVIG), which is supported by empirical evidence of a beneficial response following its use in the treatment of viral and autoimmune encephalitis. In immune-mediated conditions, IVIG is often used after a delay (by weeks in some cases) while diagnosis is confirmed. Wider use of IVIG in infectious encephalitis and earlier use in immune-mediated encephalitis could improve outcome for these conditions. We describe the protocol for the first ever randomised control trial of IVIG treatment for children with all-cause encephalitis.

Methods and analysis

308 children (6 months to 16 years) with a diagnosis of acute/sub acute encephalitis will be recruited in approximately 30 UK hospitals and randomised to receive 2 doses (1g/kg/dose) of either IVIG or matching placebo, in addition to standard treatment. Recruitment will be over a 42-month period and follow up for 12 months after randomisation. The primary outcome is "good recovery" (score of 2 or lower on the Glasgow Outcome Score Extended - paediatric version), at 12 months after randomisation. Additional secondary neurological measures will be collected at 4-6 weeks after discharge from acute care and at 6 and 12 months after randomisation. Safety, radiological, other autoimmune and tertiary outcomes will also be assessed.

Ethics and Dissemination

This trial has been approved by the UK National Research Ethics committee (South Central - Oxford A; REC 14/SC/1416). Current protocol: v3.0 (04/11/2015). The findings will be presented at both national and international meetings and conferences and published in peer-reviewed journals.

Trial registration

This trial is registered with clinicaltrials.gov (Ref: NCT02308982), EudraCT (Ref: 2014-002997-35 and ISRCTN (Ref: 15791925).

Strengths and limitations of this trial

- This will be the first randomised controlled trial to evaluate the effect of early IVIG treatment in encephalitis from any cause in children, aiming to recruit a large sample size (N=308) across 30 hospitals
- Outcome measures will utilise robust validated and internationally accepted assessment tools and all trial data will be assessed by blinded investigators
- The trial is expected to provide data on the role of IVIG in reducing poor outcomes following encephalitis from any cause, which would impact on care pathways and individual patient decisions within the health services community, both in the UK and internationally and will also inform on health and social care costs
- Expected recruitment has been based on the reported UK incidence of encephalitis and a high and consistent recruitment rate is required across all centres due to the low disease incidence. While the trial is expected to recruit well at all sites, it is possible that there could be unexpected under-recruitment at one or more sites which would be a barrier to timely completion
- Given that patients with all forms of encephalitis will be enrolled to the trial, a statistically significant effect may be masked if there is a benefit from IVIG in only one or some aetiological sub-groups

INTRODUCTION

Background and rationale

Encephalitis is inflammation of the brain parenchyma and manifests as a clinical syndrome characterised by a combination of encephalopathy, behavioural changes, fever, seizure, and focal neurological deficits.[1] In England, the population incidence for all-cause encephalitis is estimated at 5.23–8.66/100,000/year, [2] with infants and adults >65 years being the most affected.[2] Diagnosis is typically made by a combination of clinical, laboratory, neuroimaging, and electrophysiological findings using an internationally agreed consensus definition.[1 3] Infections, usually viral, are the most common cause of acute encephalitis, where the cause is identified. Immune mediated forms of encephalitis, usually characterised by the detection of neuronal antibodies in serum and/or cerebrospinal fluid (CSF) have been described, although the proportion is not yet clear. [4 5]

Encephalitis causes significant morbidity and mortality with up 7-20% case-fatality rate for certain types[6-8] and up to 50% of survivors reporting deficits such as memory loss, seizures, learning disability and functional impairment after prolonged follow up.[9-13] The significant burden of the disease despite the current standard treatment highlights the need to identify strategies to reduce poor outcomes in patients with encephalitis. Encephalitis also imposes a substantial economic and resource burden on healthcare services. A review of encephalitis admissions to Paediatric Intensive Care Units (PICUs) showed an average length of stay of 4.3 days, with 75% of children requiring ventilation, and some requiring cardiovascular support (17%) and renal dialysis (6.5%).[14] A UK study of encephalitis hospitalisations reported a mean length of stay of 34 days and a cost to the National Health Service of >£40 million per year.[2]

Notwithstanding the aetiology, the common pathophysiological process in infectious and autoimmune encephalitis is brain inflammation. There is evidence that IVIG has a beneficial role in encephalitis from both its therapeutic and prophylactic use in enteroviral encephalitis in the immunocompromised and in outbreaks of enterovirus-71 infections in Asia,[15] as well as other infectious causes of encephalitis.[16-18] Acute immune treatment including IVIG also appears to benefit both adults and children with autoimmune encephalitis. [19] Randomised controlled trials have demonstrated IVIG efficacy in a number of neurological conditions that share similar underlying inflammatory mechanisms to encephalitis even if different aetiologies.[20] IVIG appears to inhibit complement binding, neutralise pathogenic cytokines, down regulate antibody production, and modulate phagocytosis and T-cell function. [21]

In clinical practice, the use of IVIG in encephalitis varies. In the immune mediated forms of encephalitis, IVIG is often used after a period of delay (by weeks in some cases) while the diagnosis is being confirmed. In other cases, IVIG is used as a last treatment option, usually after several days from hospital admission, where clinical improvement is slow. This delay may limit its benefit due to the brain inflammation, which has already occurred. The variation in practice is due to a lack of class 1 evidence to support the use of IVIG in encephalitis and it is currently unknown whether wider use of IVIG in

infectious encephalitis and earlier use in immune-mediated encephalitis could alter the outcome of this group of conditions. There is therefore the need to fill this evidence gap.

At present, there are no robust controlled trials in children to inform on the optimal treatment of encephalitis. Given the available evidence of possible beneficial role of IVIG, it is therefore important to undertake a trial to investigate the effect of IVIG for all children presenting with encephalitis, and optimise use of this expensive and limited resource.

Trial Objectives and Design:

The **I**mmunoglobuli**N** **i**n the **T**reatment of **E**ncephalitis (IgNiTE) trial is a multi-centre, double blind, placebo controlled, parallel arm, randomised controlled trial (RCT) that will evaluate whether early treatment with IVIG provides benefit for children with a diagnosis of encephalitis, when compared with standard therapy alone. In the context of the IgNiTE trial, 'early treatment' is defined as administration of IVIG within 120 hours from presentation to hospital or, for transferred patients, within 72 hours from admission to a recruiting hospital even if >120 hours since initial hospital presentation.

It is expected that the IgNiTE trial will generate first class evidence to inform clinical decisions regarding the use of IVIG for children with acute and sub acute forms of both infectious and inflammatory encephalitis.

Primary Objective:

To compare neurological outcomes of children with encephalitis who have been treated with either IVIG or placebo, in addition to standard therapy

Secondary Objectives:

(a) To compare (i) clinical and (ii) further neurological outcomes of children with encephalitis who have been treated with IVIG or placebo, in addition to standard therapy

(b) To confirm the safety of IVIG treatment for children with encephalitis

(c) To identify the proportion of children with immune mediated encephalitis

(d) To determine the effect of IVIG treatment on neuronal antibody levels in children with immune mediated encephalitis

Tertiary objectives:

(a) To explore clinically relevant neuroimaging predictors of childhood encephalitis

(b) To explore predictors of neurological outcomes in children with encephalitis

(c) To explore radiological patterns associated with different types of encephalitis

(d) To understand the host inflammatory pathways in encephalitis and the relationship with clinical parameters and the effect of IVIG treatment on these pathways

METHODS AND ANALYSIS

Trial Setting

The trial is planned to be conducted in approximately 30 UK hospitals (both tertiary and district general) (**Table 1 of the Supplementary file**).

Eligibility Criteria

Inclusion Criteria (based on the International Encephalitis Consortium consensus case definition)[1]

1) Age 6 weeks to 16 years old AND

2) Acute (within 24 hours) or sub-acute (24 hours to 4 weeks) onset of altered mental state (reduced or altered conscious level, irritability, altered personality or behaviour, lethargy) not attributable to a metabolic cause AND

3) At least two of:

(a) Fever >38^{o}C within 72 hours before or after presentation to hospital

(b) New or acute onset brain imaging consistent with encephalitis or immune-mediated encephalopathy

(c) CSF white blood cells (WBCs) >4/microlitre

(d) Generalised or partial seizures not fully attributable to a pre-existing seizure disorder

(e) New onset focal neurological signs (including movement disorders) for >6 hours

(f) Electroencephalogram (EEG) abnormality that is consistent with encephalitis and not clearly attributable to another cause

AND

4) Parent/guardian/legal representative consent to the patient participating in the trial

Exclusion Criteria

The patient will not be enrolled to the trial if any of the following apply, in addition to failure to meet all the inclusion criteria:

- High clinical suspicion of bacterial meningitis or TB meningitis (for example: presence of frankly purulent CSF; CSF WBCs >1000/microlitre; bacteria on Gram stain and/or culture)
- Prior receipt of any IVIG product during the index admission
- Traumatic brain injury
- Known metabolic encephalopathy
- Toxic encephalopathy
- Hypertensive encephalopathy/posterior reversible encephalopathy syndrome
- Pre-existing demyelinating disorder; pre-existing antibody mediated CNS disorder; pre-existing CSF diversion
- Ischaemic or haemorrhagic stroke
- Children with a contra-indication to IVIG or albumin
- Known hypercoagulable state
- Significant renal impairment defined as GFR of 29mls/min/1.73m2 and below (Chronic Kidney Disease Stage 4)
- Known hyperprolinaemia
- Known to be pregnant

- Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the trial, or may influence the result of the trial, or the participant's ability to participate in the trial
- Participants who are being actively followed up in another research trial involving an investigational medicinal product
- Administration of trial treatment not feasible within 120 hours from presentation to any hospital OR, for transferred patients, 72 hours from admission to a recruiting hospital even if this is >120 hours from presentation to initial hospital as determined by the trial team
- Any other condition which, in the opinion of the investigator, may interfere with the ability to fulfil trial requirements, especially relating to the primary objective of the trial (this includes plans to be outside the UK for more than 12 months after enrolment)

In addition, any patient who, in the judgement of the clinician and prior to enrolment, is thought will benefit from IVIG will not be enrolled.

Interventions

Participants will be randomised to receive two doses of either human immunoglobulin (intervention group) or placebo (control group), in addition to standard therapy (see Methods: assignment of intervention). There will be no set trial definition of standard therapy and this may vary between hospitals since there are currently no established national clinical care pathways for these. Participants will receive 1g/kg/dose, in weight-based dosing bands (**Table 2 of the Supplementary file**). The IVIG product is Privigen (CSL Behring), supplied in unlabelled as 10g/100ml vials. The placebo is 0.1% Human Albumin Solution in 0.9% Sodium Chloride solution which will be manufactured in the Aseptic Production Unit (APU), Pharmacy department, Royal Liverpool & Broadgreen Hospital, Liverpool, UK under cGMP conditions, under its MIA (IMP) license and also supplied as 100ml vials. Packaging and labelling of both trial treatments will also take place at the same location. Labelling, which is identical for both trial treatments, has been approved by the Medicines and Healthcare products Regulatory Authority (MHRA) and conform to Annexe 13 of Good Manufacturing Practice standards and Article 13.3 of Directive 2001/20/EC (http://ec.europa.eu/health/files/eudralex/vol-4/2009_06_annex13.pdf). The APU will provide Qualified Persons services and distribute both trial treatments to the Clinical Trials Pharmacy at each recruiting site where they will be stored under controlled conditions and from where they will be dispensed.

The trial treatment will be prescribed on the participant's drug chart by a clinician who has been delegated for this task and using the suggested wording 'Immunoglobulin/Placebo for the IgNiTE trial'. In addition, a clinical trials prescription form will be completed. For effective management of the trial treatment stock, and to minimise wastage, individual doses may vary slightly. A dosing guide for participants ≥ 13.5kg is provided in a Clinical Study plan and is shown in **Table 2 of the Supplementary file.** Participants <13.5kg will receive 1g/kg, rounded to the nearest whole gram.

Both trial treatments will be administered intravenously by a nurse who has received relevant trial

specific and Good Clinical Practice (GCP) training, is trained to give intravenous infusions and trained in the recognition and treatment of anaphylaxis. The first dose will be given as soon as possible after enrolment, within the defined timelines (see Trial objectives and design). The second dose will be given 24-36 hours after the first dose. The administration rate for the trial treatment will be in line with the guidance outlined in the Summary of Product Characteristics (SmPc) for Privigen (https://www.medicines.org.uk/emc/medicine/21359) and local hospital practices for Privigen administration.

Blood and CSF samples will be obtained before and after administration of the study treatment (see section on data collection methods)

Co-enrolment

Participants in the IgNiTE trial may be co-enrolled to another study where:

a) The study does not involve an investigational medicinal product (IMP)
b) The study involves an IMP, which is not thought to have a potential immunomodulatory, or neuroprotective effect, as judged by the investigator.

Patients on the following treatment(s) may not be enrolled to the IgNiTE trial:

• Long-term maintenance immunotherapy (defined as 14 days or more) or within 3 months of stopping. This includes (but not limited to) the following: steroids (>1mg/kg/day), Azathioprine, Mycophenolate Mofetil, Methotrexate, Monoclonal anti-inflammatory treatment e.g. Rituximab, infliximab (or within 1 year of discontinuing such treatment).

Outcomes

There are currently no established European core outcomes for encephalitis or acquired brain injury in existence (COMET Initiative website: www.cometinitiative.org, searched 22/02/2016). The selected outcome measures reflect recommendations by The American Academy of Neurology Common Data Elements Project for neurological assessment post traumatic brain injury in children (accessible @ www.commondataelements.ninds.nih.gov). The secondary outcome measures will support the data obtained from the primary outcome.

Primary Outcome □

The primary efficacy outcome is “good recovery”, defined as a score of 2 or lower on the Paediatric version of the Glasgow Outcome Score-Extended (GOSE-Peds), at 12 months after randomisation. The GOS-E Peds is a modified version of the GOSE, a gold standard for measuring traumatic brain injury outcome in adults. The GOS-E Peds provides a developmentally appropriate structured interview necessary to evaluate children across different age groups, and it provides a valid measure of outcome in infants, toddlers, children and adolescents. Its use has been validated and found to be sensitive to both severity of injury and to recovery over time, at least 6 months after brain injury and has been suggested as useful in guiding treatment in the early phases of recovery from brain injury.[22] A strong correlation is also seen with parent report of functional outcomes and also with most performance based cognitive

tests for both younger and older children. A 6-month assessment has also been chosen (see secondary objectives) as this has the advantage of improved trial retention, and earlier impact assessment.

Secondary and tertiary outcomes

These are outlined in **Table 1**.

Table 1 Secondary and Tertiary outcomes

	Data collection time point	Outcome measure
Secondary outcomes		
Clinical and neurological	During hospital inpatient stay	• Glasgow coma score • Neurological examination findings as documented by the clinical team • Duration of invasive ventilation (if ventilated) • Length of intensive care unit (ICU) stay in a subset of children admitted to ICU. • Length of hospitalisation
	Around 4-6 weeks after discharge from acute care	• Strength and Difficulties Questionnaire (SDQ) • Adaptive Behaviors Assessment System-Second Edition (ABAS-II) • Peds Quality of Life scoring algorithm • Liverpool Outcome Score • Gross Motor Function Classification System (GMFCS)
	Around 6 months (+/- 4 weeks) after randomisation	• GOSE-Peds
	Around 12 months (+/- 4 weeks) after randomisation	• New diagnosis of epilepsy • Use of anti-epileptic treatment • Strength and Difficulties Questionnaire (SDQ) • Adaptive Behaviors Assessment System-Second Edition (ABAS-II)

		• Peds Quality of Life (PedsQoL) scoring algorithm • Liverpool Outcome Score (LOS) • Gross Motor Function Classification System (GMFCS) • Blinded neuropsychologist assessment of cognitive functioning using age appropriate developmental scales (Bayley Scales for Infant Development (BSID-III)/Wechsler preschool and Primary Scale of Intelligence III (WPPSI-III)/Wechsler Intelligence Scale for Children IV (WISC-IV)
	12 months after randomisation	Proportion of deaths occurring in participants
Radiological	Around 6 months after randomisation	Brain magnetic resonance imaging (MRI) to assess lesion resolution, presence of new lesions and distribution of persisting disease
Safety	24-48 hours after the second IMP dose	Full blood count check to monitor for haemolysis
	First five days after each dose of trial treatment	Adverse events of special interest (AESIs)
	Up to 6 months after randomisation	Serious adverse events (SAEs)
	Up to 12 months after randomisation	Serious adverse reactions (SARs) Suspected Unexpected Serious Adverse Reactions (SUSARs)
Autoimmune		Presence of and comparison of levels of specific neuronal antibodies in serum and/or CSF samples (where lumbar puncture is performed as part of routine care) before and after administration of trial treatment
Tertiary Outcomes		
		(i) Correlate MRI findings with neurological outcomes

		(ii) Correlate clinical and laboratory parameters with neurological outcomes (iii) Comparison of brain MRI findings with aetiological diagnosis (iv) Identification of specific DNA sequence and structural genetic variants in patients with encephalitis (v) The following will be assessed before and after receipt of trial treatment: • Comparison of inflammatory cytokines • Assessment of regulatory T cell frequency and function in blood and/or CSF • Measurement of inflammatory markers in blood and/or CSF • Analysis of gene expression in whole blood • Comparison of the host inflammatory pathways and correlation with clinical parameters

Participant timeline

Time schedule for enrolment, interventions, assessment and visits for participants is shown in Table 2 (**Schedule of trial procedures**)

Table 2 Schedule of trial procedures

	T0: As soon as possible after identification of a potential participant and to allow timely administration of study treatment	T1: As soon as possible after enrolment †	T1+24hours: 24 hours after first dose of trial treatment	T2: 24-36 hours after first dose of trial treatment	T2+ 24-48 hours: 24-48 hours after second dose of trial treatment	T2+7: 7 days after second dose of trial treatment	T3: On the day of discharge from acute care and up to 48 hours prior	T4: 4-6 weeks after discharge from acute care	T5: 6 months (+/- 4 weeks) after randomisation	T6: 12 months (+/- 4 weeks) after randomisation
Eligibility assessment	X									
Informed consent and assent (where appropriate)^	X						X[@]		X[@]	X[@]
Enrolment	X									
Obtain relevant clinical data~	X	X	X	X	X	X	X	X	X	X

Randomisation	X	X[b]								
Scavenged samples~	X	X	X	X	X	X	X	X	X	X
Additional (research sample) where consent is given	X (baseline sample, prior to receipt of trial treatment: neuronal antibody testing, cytokine and DNA analysis*, cellular immunology**)	X (where baseline sample not previously obtained and before administration of trial treatment)	X (functional genomics, DNA analysis*)			X (cellular immunology**, functional genomics, DNA analysis*)			X*** (convalescent sample: neuronal antibody testing, cellular immunology** and cytokine analysis, functional genomics)	
Mandatory full blood count check					X					
Administration of trial treatment and monitoring		X		X						

Completion of Data Capture Form and eCRF[~]	X	X	X	X	X	X	X	X	X	X
Adverse event assessment (AESIs, SARs, SUSARs and SAEs		X	X	X	X	X	X	X	X	X[c]
Questionnaire completion (ABAS-II, SDQ, GMFCS, Peds QL)								X	X	X
Liverpool Outcome Score								X		X
GOSE-Peds									X	X[d]
Research MRI (where consent is given) [c]									X[e]	
Neuropsychology assessment										X

Key: ^ Participant consent if 16 years and assent if < 16 years); [@]where consent/assent (as appropriate) has not been previously obtained; ~ Continuous process throughout the study; [b] First dose of trial treatment may be given on same day as randomisation; *Where DNA sample not previously obtained. Only one DNA sample is required; ** selected centres only; †Visit must be 120 hours from presentation to any hospital OR, for transferred patients, 72 hours from admission to a recruiting hospital even if >120 hours has elapsed since presentation to the initial (referring) hospital; ***To avoid an extra visit solely for this purpose, the '6 month research sample' can be obtained at any routine follow up clinical appointments that occur after the participant has been discharged from acute care; [c] Only deaths or where a serious adverse event is judged to be directly related to the trial treatment; [d] Primary outcome measure; [e] Where consent obtained. May not be required if having routine clinical MRI scan ≥ 3 months after randomisation.

Trial duration

The trial is planned to last 5 years which includes a 42 months for recruitment, 12 month follow up period for each participant and 6 months for data analysis.

Sample size

There is a near paucity of RCT data from previous studies to estimate sample size for this trial. The sample size calculation is based on the assumption that detection of at least 20% difference from 43% in the "good recovery" rate (i.e. GOS-E-Peds score 2 or lower) by 12 months after randomisation is likely to be clinically significant. This is similar to a large observational study on autoimmune encephalitis. [19] Based on this assumption, a total of 308 participants (154 per group), which takes into account an attrition rate of approximately 10%, will provide 90% power and 5% level of significance for a 2- sided test.

Recruitment plan

A flow chart showing the process of patient recruitment is shown in **Figure 1**. Eligible patients will be identified through various routes: by (i) clinicians reviewing medical handover lists and clinical records of new admissions; (ii) research team contacting relevant hospital wards; (iii) microbiologists and/or virologists identifying children who have had a lumbar puncture performed for suspected central nervous system infection, (iv) radiologist identifying a brain MRI scan suggestive of encephalitis, (v) neurophysiologist identifying an EEG suggestive of encephalitis.

Following identification of a potential patient through any of the above routes, a member of the clinical team will approach the parent/ guardian/legal representative to seek their interest in knowing more about the trial and verbal consent will be sought for a member of their details to be passed on to the trial team. Only if consent for this is granted will a member of the trial team contact the family. A member of the trial team will check the patient's eligibility with the parent/ guardian/legal representative, after which they will be provided with the participant information sheet, if the patient is eligible, and given sufficient time to read this and make a decision regarding participation in the trial. The investigator must obtain informed consent and assent (where applicable and obtainable) before the patient undergoes any trial procedure(s). Once appropriate consent and assent (where applicable and obtainable) have been obtained, the patient will be enrolled to the trial by assigning them a participant number using the next available number from the pre-populated enrolment log.

To maximise achievability of the sample size, we have included mostly tertiary paediatric units that are well placed to recruit rapidly a high number of participants. Potential barriers to recruitment will be identified during the pilot phase of the trial, and close support will be provided to sites with recruitment difficulties. A robust system will be put in place to monitor recruitment to ensure that this is on target. A contingency plan will also be put in place to allow for opening of additional sites in the unlikely event of a less than expected recruitment.

A 'Screening log' of all screened patients will be kept and will include patients with a diagnosis of encephalitis but are not eligible, eligible patients who refuse to be approached or may not be suitable to be approached, as well as those for whom consent was declined. The reason(s) why a patient is not enrolled will be clearly documented in the screening log, including reasons for declined consent, where this is provided.

Randomisation

After eligibility is confirmed and consent (and assent where applicable) obtained, enrolled participants will be randomised as soon as possible to allow early administration of the trial treatment in line with the protocol. Randomisation will be performed using a fully validated online randomisation system developed by the Primary Care and Vaccines Collaborative Clinical Trials Unit, University of Oxford and during working hours when the trial treatment are available. Participants will be randomised in a 1:1 ratio to either an intervention or control group. Only trained research staff with appropriate access and who are on the IgNiTE trial delegation log will be able to randomise patients. The incidence of encephalitis is higher in infants and some forms of encephalitis are more prevalent in certain age groups. In addition, as part of standard care, patients with inflammatory encephalopathy may receive steroid treatment, which may have a beneficial effect. Therefore, to ensure balance between the trial groups, and account for steroid use as confounding variable, randomisation will be stratified by age group (n= 4: <1, 1-4, 5-9, 10-16 years), and steroid use (yes/no) at the time of enrolment, and using randomly varying block sizes. A computer-generated randomisation code at the time of randomisation will ensure concealment of allocation.

Withdrawal from trial treatment

The participant will be discontinued from the trial treatment at any time if the investigator considers it necessary for any reason including:

- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening) □
- Significant protocol deviation □
- Significant non-compliance with treatment regimen or trial requirements □
- An adverse event which requires discontinuation of the trial treatment or results in inability to continue to comply with trial procedures □
- Disease progression which requires discontinuation of the trial treatment or results in inability to continue to comply with trial procedures □

A participant may also voluntarily withdraw from the trial treatment due to what he or she perceives as an intolerable adverse event (AE), or for other reasons if they wish.

Blinding

IgNiTE is a double blind trial and a rigid blinding process will be in place throughout the trial to ensure validity of the data collected. Participants and their parents/guardians/legal representatives as well as all research staff involved in any aspect of the trial conduct including recruitment, administration of trial treatment, carrying out trial assessments, data collection and entry, sample and statistical analyses will be blinded to treatment allocation throughout the entire trial period. There will be separate monitors for blinded and unblinded data. The active treatment and placebo will be visually identical (packaged and labelled in the same manner) and administered at the same dose and infusion rate to maintain blinding. To be able to manage trial treatment stock effectively and minimise wastage, the clinical trials pharmacists at each recruiting site who are independent of

the trial will be unblinded. The tear off section of the label will inform the dispensing pharmacist of the true nature of the contents (IVIG or placebo). At dispending this section of the label will be removed to maintain blinding.

Unblinding of treatment allocation will occur only in exceptional circumstances when knowledge of the actual treatment received is absolutely essential for further management of the participant. Unblinding will be done via the online randomisation system. The decision to unblind a participant's treatment allocation will be solely that of the site investigator. Only individuals given access to unblind will be able to do this and will include the site pharmacist, principal investigator and co-investigators. Where there is a problem with Sortition, unblinding will be available via either the site pharmacist (during work hours) or an unblinded staff member in Oxford (out of hours) who is independent of the trial, both of whom will have secure access to the master randomisation list for this purpose.

Data collection methods

Trial data will be collected by delegated research staff with appropriate training using two methods: (i) a paper-data capture form and (ii) an electronic case report form (CRF), OpenClinica™ which is a password protected, web based database with accountability records that is stored on a secure sever within the UK. Trial data will be obtained from various sources including patient medical notes, parent interview, laboratory reports, brain scan pictures and reports, electroencephalogram (EEG) reports, pharmacy records, drug charts, questionnaires, and any correspondences relating to the participants involvement in the trial.

Different data types will be collected throughout the trial period:

Clinical data

These will include information regarding patient demographics, clinical findings, treatment, investigation results, length of hospital stay and intensive care management. These data will be obtained during admission, and around 6 and 12 months after randomisation (**Table 1: Secondary and Tertiary outcomes).**

Questionnaires and outcome measures

Validated questionnaires assessing behaviour, motor and adaptive functioning and quality of life will be completed by the participant and/or by their parent/guardian/authorised legal representative at: (i) 4-6 weeks following discharge from acute care and (ii) 12 months after randomisation:

(i) Adaptive Behavior Assessment System, second edition[23 24]

(ii) Gross Motor Function Classification System[25]

(iii) Strength and Difficulties Questionnaire [26]

(iv) Paediatric Quality of Life Inventory [27 28]

Outcome scores will be assessed using the (i) Paediatric version of the Glasgow Outcome Score Extended (GOSE Peds) at 6 and 12 months after randomisation and (ii) Liverpool Outcome Score (LOS) at 4-6 weeks after discharge from acute care and 12 months after randomisation.

Various measures to obtain complete follow up data will be implemented: (i) blinded research staff or the

participant's clinician can assist with questionnaires, (ii) pre-paid envelopes will be provided for return of questionnaires and families will be reminded by telephone, post and/or email, (iii) the primary outcome (GOSE Peds) assessment will be completed by the neuropsychologist at the 12-month visit. The neuropsychologist may also assist with questionnaire completion.

Laboratory data

Blood and CSF samples (only obtained at the same time as routine lumbar puncture) will be obtained from participants at different time points for neuronal antibodies, cytokine, functional genomics, DNA and cellular immunology evaluation are optional (**Table 2: Schedule of trial procedures**). A mandatory blood sample will be obtained at 24-48 hours following the second dose of trial treatment to assess full blood count levels as a risk mitigation measure to monitor for haemolysis, which is a reported side effect of high dose IVIG treatment. Blood sample volumes will be in line with the Medicines for Children Research Network recommendation.[29] CSF volumes will be in line with British Infection Society TB guideline.[30] All samples will be anonymised. A sample collection and processing guide will be made available to all recruiting sites.

Radiological data

All brain scans (and reports) performed as part of routine clinical care during the study period will be collected. An optional research MRI scan will be performed around 6 months after randomisation for participants who consent to this and where a routine follow up clinical scan is not being done. Where a clinical scan is planned for ≥ 3 months after randomisation, this will be used. All scans will be anonymised and sent to Great Ormond Street Hospital, London for analyses by the blinded study neuroradiologist (WKC) and imaging scientist (CC).

Neuropsychology assessment

This will be done at 12 months after randomisation by a blinded trial neuropsychologist using age appropriate, validated scales of developmental assessment (see Table 1: Secondary and Tertiary outcomes)

Adverse events

Information on adverse events will be collected throughout the trial (see section on Harms)

Withdrawal

Participants may withdraw from the trial at any time. No further data will be collected. Data collected up until the point of withdrawal from the trial will be analysed unless the parent/participant decide against this. If a participant is withdrawn due to an adverse event (AE), the investigator will follow this up until it resolves or stabilises. All participants who are withdrawn from trial treatment (see Withdrawal from trial treatment) will remain in the trial and followed up as per the trial protocol, but will not have any invasive procedures performed. The trial data will be analysed on an intention-to-treat basis therefore all withdrawals, either from the trial or from the trial treatment, will be reported and included in the data analyses. All protocol deviations will also be reported.

Study data

Data management

Data management will be via the OpenClinica™ database. All relevant data recorded elsewhere (see data collection methods) that are required to achieve the trial objectives will be transferred on to the OpenClinica™ database from where they can be downloaded for analysis. To maintain a high quality standard of data entry, the database will be tested and validated prior to use. In addition, research staff will receive appropriate level training on data collection and entry and there will be regular monitoring of trial data throughout the trial. Furthermore, prior to data analysis, the database will be locked for cleaning to ensure that data are complete and reliable. Research staff at the various recruiting sites will be contacted to provide information on any missing data and to clarify any errors identified. All trial documents will be retained and stored securely in accordance with GCP after the completion or discontinuation of the trial for 3 years after the youngest participant turns 18 years.

Statistical methods

The primary statistical analysis will be carried out on the basis of intention-to-treat (ITT). After randomisation, participants will be analysed according to their allocated treatment group irrespective of what treatment they actually receive. Data analysis will be performed using a mixed effect model for repeated measures, i.e. to incorporate all outcome data collected during the 12 months follow-up, in order to apply the intention-to-treat principle as far as possible and to account for potential biases arising from loss to follow-up. The model will include treatment group, time, treatment-by-time interaction, and baseline covariates. An unstructured correlation matrix will be used to model the within-participant error correlation structure. An appropriate contrast will be specified to test for treatment efficacy between randomised groups at 12 months. Various sensitivity analyses will be performed using other imputation methods, as well as analysis of 12-month data cross-sectionally, to test whether the results are robust to different assumptions about the missing data. The primary intention-to-treat analysis will account for steroid use before randomisation as a covariate. Appropriate methods will be used to investigate the treatment effect accounting for the use of steroid after randomisation as an exploratory analysis. The results from the trial will be prepared as comparative summary statistics (difference in response rate or means) with 95% confidence intervals. All the tests will be done at a 5% two-sided significance level. A full detailed analysis plan (including plans for any interim analysis, subgroup analysis, and sensitivity analysis) will be prepared and finalised before the first interim analysis.

Primary analysis

The primary efficacy end point in this trial is “good recovery”, defined by GOS-E-Peds score 2 or lower, at 12 months from randomisation. This will be analysed using a Generalised Linear Mixed Effect model, utilising data collected at discharge, 6 and 12 months from randomisation. An interaction between time and randomised group will be fitted to allow estimation of treatment effect at each time point. The model will adjust for baseline values and other stratification factors (e.g. age and steroid treatment at the time of randomisation).

Secondary and tertiary analyses

As far as possible, we will use similar method for secondary and tertiary continuous outcomes collected at multiple time points or analysis of covariance (ANCOVA) for those collected at 12 months only, adjusting for

baseline measures (if collected) and any stratification variables. Otherwise, an equivalent nonparametric method will be used for outcomes that violate the normal distribution assumption. A log-binomial regression will be performed on binary outcomes with similar adjustment of baseline covariates. Chi-squared or Fisher's exact test will be used to analyse adverse events and non- adherence.

Reporting of the trial findings will be in line with Consolidated Standards of Reporting Trials (CONSORT) guidelines

Interim analysis

Analysis for the DSMC will be performed in accordance with the DSMC Charter. Interim reports containing safety and outcome data, along with any other analyses that the committee may request, will be sent to the DSMC in strict confidence. Close monitoring to assess practical aspects of delivering the trial interventions and recruitment will also be undertaken.

Data Monitoring

The Data Safety Monitoring Committee (DSMC) is responsible for safeguarding the interests of trial patients, monitoring the accumulating data and making recommendations to the Trial Steering Committee (TSC) on whether the trial should continue as planned. The DSMC will comprise of a clinical chair, clinicians, and a statistician, all of whom will be independent of the trial, the sponsor and funders. The role of the TSC is to provide overall supervision for the IgNiTE trial on behalf of the Trial Sponsor and the Trial Funder and to ensure that the IgNiTE trial is conducted according to the guidelines for GCP, Research Governance Framework for Health and Social Care and all relevant regulations and local policies. The TSC will comprise an independent chair, the CI, paediatricians and patient representatives. In discharging its safety role, the TSC will work in conjunction with the Data Safety and Monitoring Committee (DSMC) for the IgNiTE trial. Both the DSMC and TSC will meet prior to trial start and 6 months thereafter. Increased frequency of meetings will be arranged depending on the requirements of the trial, DSMC and TSC recommendations.

Stopping guidelines

This trial may be suspended or prematurely terminated by the sponsor, CI, regulatory authority or funder if there is sufficient reason to think that that the safety of participants is affected by the trial procedures. Written notification, documenting the reason for trial suspension or termination, will be provided by the suspending or terminating party to the investigator, funders, and regulatory authorities. If the trial is prematurely terminated or suspended, the PI will promptly inform the REC, MHRA, and CSL Behring and will provide the reason(s) for the termination or suspension.

Harms

International Conference on Harmonisation (ICH) definitions are used for AEs, AESIs, adverse reactions (ARs), SAEs, Serious adverse events (SARs) and suspected unexpected SARs (SUSARs). IVIG has a well-established side effect profile. All participants will be monitored for (i) AESIs, (includes anaphylaxis, haemolysis, new

onset seizure or abnormal movements not thought to be due to the encephalitis illness, thromboembolism, aseptic meningitis unrelated to the encephalitis illness, acute renal failure, and any other medically significant events as determined by the investigator), in the first five days following receipt of trial treatment, (ii) serious adverse events up to 6 months after randomisation, or up to 12 months after randomisation where the event is judged directly related to the trial treatment and (iii) deaths up to 12 months after randomisation.

Monitoring and reporting of adverse events will be performed by the site PI and research team, and will be recorded on the data capture form and uploaded to the eCRF (OpenClinica™). The nature and severity of each adverse event, and the relationship to trial treatment will be documented. The expectedness of an AE will be determined by whether or not it is listed in the SmPC for Privigen or Human Albumin Solution. AESIs and SAEs will be reported to the CI, CSL Behring and the DSMC. This will be expedited (within 24 hours of the research staff becoming aware), for all AESIs and for all SAEs that are judged related to the trial treatment. SAEs that are judged to be unrelated to the IMP will be discussed with the PI and CI but do not require expedited reporting.

The CI will report all relevant information about a Suspected Unexpected Adverse Reaction (SUSAR) that occurs during the course of the trial to the MHRA, CSL Behring, the relevant ethics committee and the DSMC. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days. The CI or delegate will also inform all principal investigators concerned of relevant information about SARs that could adversely affect the safety of participants.

A summary list of all SAEs (including those unrelated to the trial treatment), AESIs, and SUSARs will be provided in a safety report to the DSMC, which will be submitted at regular interval as specified in the DSMC Charter. In addition, a strict data sheet will be kept by CSL Behring, which will include the randomisation code aligned to the batch number of assigned IVIG product and in order to maintain a link between the participant and the batch of the product.

Pregnancy

Although not AEs, pregnancies are reportable events. Should a participant become pregnant during the trial the study treatment will be discontinued. Any pregnancy occurring during the clinical trial will be reported to the CI and CSL Behring within 24 hours of the investigator becoming aware and will be followed up for an outcome, which will be recorded. If a congenital abnormality or birth defect is identified this would fall within the definition of an SAE and will be reported as such.

Auditing

Regular monitoring by the trial sponsor or delegate will ensure compliance with GCP. The investigator sites will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The Quality Assurance manager will also maintain an internal audit program, which will supplement the external

monitoring process to ensure that systems relating to trial conduct, data recording, analysis and reporting are functional are in compliance with the protocol, GCP and the applicable regulatory requirements. The audit program also includes laboratory activities taking into consideration the MHRA and EMA guidelines for GCP in the laboratory. The Sponsor may carry out audit to ensure compliance with the protocol, GCP and appropriate regulations. GCP inspections may also be undertaken by the MHRA to ensure compliance with protocol and the Medicines for Human Use (Clinical Trials) Regulations 2004.

ETHICS AND DISSEMINATION

Ethical and safety considerations

This trial has been approved by the United Kingdom National Research Ethics Service (NRES) committee (South Central - Oxford A; REC 14/SC/1416). Clinical trial authorisation has been granted via the Medicines and Healthcare Products Regulatory Agency (MHRA) notification scheme (Ref: 21584/0337/001-0001). Current protocol: v3.0 (04/11/2015). Written approval from the respective Research and Development (R&D) departments will be obtained for each participating site prior to recruitment.

The Chief Investigator (CI) will ensure that this trial (and all subsequent approved amendments) is conducted in accordance with the principles of the Declaration of Helsinki (1996), in full conformity with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidelines for GCP (CPMP/ICH/135/95 July 1996), the Research Governance Framework, and the Medicines for Human Use (Clinical Trial) Regulations 2004. The CI will monitor pharmacovigilance and will report to the Research Ethics Committee (REC), MHRA and funders during and at the end of the trial. All protocol modifications will be disseminated to all relevant parties. The findings of the trial will be presented at both national and international meetings and conferences and published in peer-reviewed journals.

Informed consent and assent

Following identification of a potentially eligible participant by the clinical team, a Participant Information Sheet (PIS) explaining the trial (including the rationale, aims and objectives, treatment assignation), potential risks and benefits, and all the trial procedures will be provided. Parents/guardians/legal representatives or patients, where appropriate (i.e. if the patient has capacity), will be allowed sufficient time to consider the information in the PIS, to seek independent advice and to consider participation in the trial. Informed consent (patients aged 16 years and above) and assent (patients below 16 years) will be obtained by trained research staff using an appropriately signed and dated informed consent/assent form, before any trial specific procedures are performed. Given that children with encephalitis will be unwell and may be confused during the acute illness, it is likely that eligible patients would be unable to provide consent/assent prior to enrolment. Therefore, for patients aged 16 years and above, informed consent will be obtained from their parent/guardian/legal representative. Once capacity is regained, appropriate consent/assent will be sought from all participants at follow up time points and if this is not granted, they will be withdrawn from the trial. Participants who previously provided assent but turn 16 years while still in the trial will be required to provide consent for ongoing participation in the trial and will be withdrawn if this is not granted.

Parents/guardians/legally authorised representatives/participants may be approached about a separate, ethically approved, Biobank study and asked if they would like to consent to this study using a separate consent form.

Participation in the Biobank is optional and samples will only be stored where appropriate consent has been obtained.

Confidentiality

Data will be stored securely in line with the Data Protection Act 1998. The randomisation system, data capture form and eCRF have been designed so as to protect participant information and to maintain confidentiality. It will be the responsibility of the local investigators to ensure that the data is password protected and held on local trust computer systems. The research staff will ensure that the participants' anonymity is maintained. Participants will be identified only by initials and participant number on the research notes and eCRF. All investigation results and blood samples will be anonymised. All trial documents will be stored securely and only accessible by research staff and authorised personnel. The CI will be the custodian of the trial data.

Access to data

Direct access will be granted to authorised representatives from the Sponsor or host institution for monitoring and/or audit of the trial to ensure compliance with regulations.

Reimbursement

Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate.

Ancillary and post trial care

There will be no continued provision of treatment available after participants have completed the trial however participants are likely to be followed up by the hospital team as part of routine care. Details of The Encephalitis Society are provided in the PIS, and they can provide ongoing support and information to families.

Dissemination policy

We aim to produce high impact publications of the results of the trial and present the findings to the paediatricians who manage encephalitis in the front line. The Investigators will be involved in preparing drafts of the manuscripts, abstracts, press releases and any other publications arising from the trial. Authors will acknowledge that the trial was funded by the National Institute for Health Research and CSL Behring. Authorship will be determined in accordance with the ICMJE guidelines and other contributors will be acknowledged.

Patient public involvement

The Encephalitis Society provided advice on the clinical problem and need for interventions to address the poor outcomes from encephalitis. The trial proposal was discussed with The Encephalitis Society who affirmed its importance as a priority for evaluation and Dr Ava Easton, the Chief Executive of The Encephalitis Society is a co-applicant on the grant application and a co-author on this paper.

To provide an important patient-centred research perspective, we have engaged members of the public in our PPI programme in both the design and management of the trial, through The Encephalitis Society. The opinion of The Encephalitis Society on the burden of the questionnaire outcome measures on patients was sought at the design stage of the trial. The Encephalitis Society also reviewed and provided comments on patient information

sheets and consent forms. The Encephalitis Society research poster will be provided for use at the respective recruiting centres. Through The Encephalitis Society, we have also recruited two patient representatives as members of the trial steering committee.

We will provide detailed accessible information about the trial outcomes to patients/parents/carers. The Encephalitis Society will drive forward publication and dissemination of the trial findings among lay, therapeutic and health professionals through the use of web materials, newsletters, and guides as well as at conferences and seminars in relation to Encephalitis and related fields. All patients and their parents/carers will be acknowledged in any outputs from the trial. We will also work with The Encephalitis Society on a programme of teaching events and produce guides for healthcare professionals and lay people.

Declaration of interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf

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Study investigators: M Sadarangani, M Absoud, WK Chong, C Clarke, A Easton, V Gray, R Kneen, M Lim, M Pike, T Solomon, A Vincent, L-M Yu, AJ Pollard

Department of Health Disclaimer

The views and opinions expressed herein are those of the authors and do not necessarily reflect those of the MRC, NHS, NIHR or Department of Health.

Authors' contribution

The trial was conceptualised by MP and AJP; with input from ML, MA, RK and TS. MP, MS, RK, TS, WKC, CC, ML, MA, AV, VG, AE, L-M Y, AJP are named investigators on the IgNiTE trial. All authors contributed significantly to the design of the trial, with specific additional contributions from each co-author within their area of expertise; paediatric neurology (MP, RK, ML, MA), paediatric infectious diseases (MS, AJP), paediatric neuropsychology (VG), neuroimaging (CC, WKC), neuroimmunology (TS and AV), statistics (L-MY), and patient group (AE). MAI is the lead doctor for the trial and prepared the first version and all subsequent

revisions of the manuscript. LW is the lead nurse for the trial. All authors contributed manuscript and have approved the final manuscript for publication.

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Competing interests

CSL Behring have provided the study IMP (IVIG) and funded manufacture of placebo and the supply and distribution of IMP and placebo. AJP reports grants from NIHR EME programme, during the conduct of the study. The University of Oxford and AV hold patents for VGKC-complex antibody tests, licenced to Euroimmun AG, and receive royalties. The neuroimmunology work in the described trial is funded through the MRC/NIHR grant. MA serves on the data safety monitoring board for a study sponsored by Neurim Pharmaceuticals and is on the editorial advisory board for the International Journal of Language & Communication Disorders. MAI reports salary from the NIHR EME grant. ML has received consultation fees from CSL Behring, travel grants from Merck Serono, and been awarded educational grants to organise meetings by Novartis, Biogen Idec, Merck Serono and Bayer. MS reports grants from Pfizer, outside the submitted work. TS is supported by the National Institute for Health Research Health Protection Research Unit in Emerging and Zoonotic Infections at Liverpool. AE, LW, L-MY, MP, MS, RK and WKC have nothing to disclose.

Disclaimer

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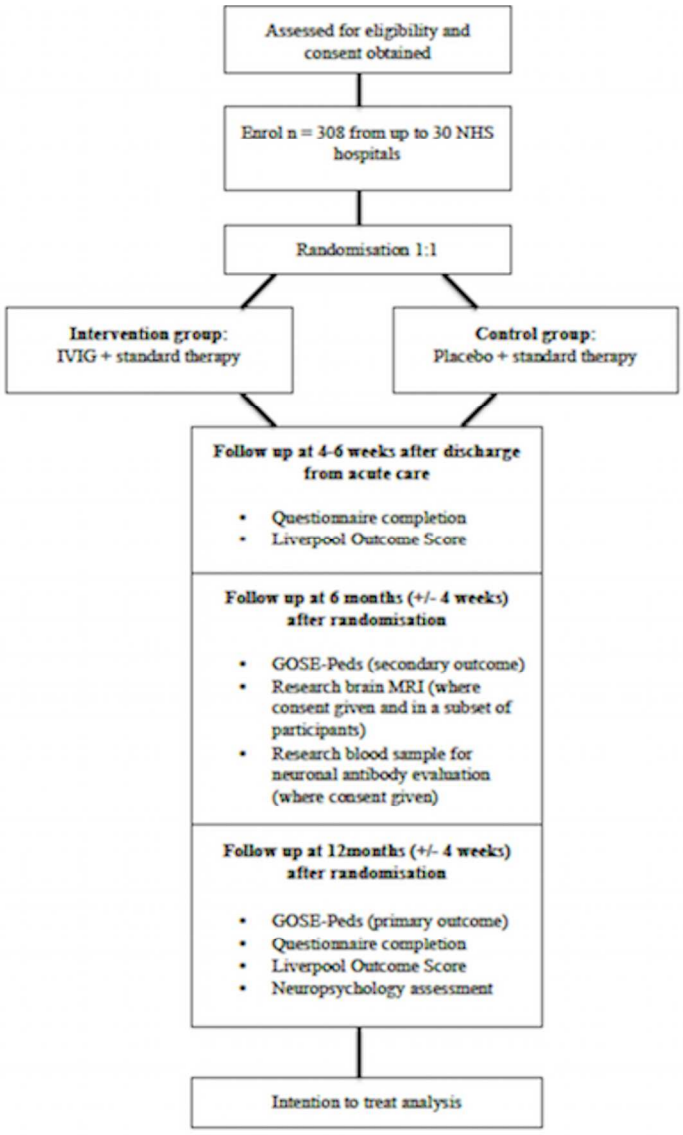

Figure 1: Flow chart showing process of participant recruitment

Flowchart showing process of participant recruitment

144x239mm (300 x 300 DPI)

Supplementary Table 1 List of planned participating sites

1. Addenbrooke's Hospital, Cambridge University Hospitals, Cambridge
2. Alder Hey Children's Hospital NHS Foundation Trust, Liverpool
3. Belfast Hospital for Sick Children, Belfast
4. Birmingham Children's Hospital NHS Foundation Trust, Birmingham
5. Bradford University Hospitals NHS Foundation Trust
6. Evelina Children's Hospital at Guy's and St Thomas' NHS Foundation Trust, London
7. Great Northern Children's Hospital, Newcastle Hospitals NHS Foundation Trust, Newcastle
8. Great Ormond Street Hospital Foundation Trust, London
9. Heartlands Hospital, Heart of England NHS Foundation Trust, Birmingham
10. James Cooke University Hospital, South Tees Hospitals NHS Foundation trust
11. John Radcliffe Hospital, Oxford University Hospitals NHS Trust, Oxford
12. Leeds Teaching Hospital NHS Trust
13. Morriston Hospital, Health in Wales
14. Ninewells Hospital, NHS Tayside
15. Nottingham University Hospitals NHS Trust, Nottingham
16. Pennine Acute Hospital NHS Trust, Manchester
17. Royal Aberdeen Children's Hospital, NHS Grampian (Aberdeen)
18. Royal Cornwall Hospitals NHS Trust
19. Royal Hospital For Children, NHS Greater Glasgow
20. Royal Manchester Children's Hospital, Manchester
21. Sheffield Children's Hospital NHS Foundation Trust
22. St George's University Hospitals NHS Foundation Trust
23. St Mary's Hospital, Imperial College Healthcare NHS Trust
24. University Hospital Bristol NHS Foundation Trust
25. University Hospital of Wales, Cardiff and Vale NHS Trust, Cardiff
26. University Hospitals of Leicester NHS Trust
27. University of Edinburgh, NHS Lothian
28. University of London and Bart's Health NHS Trust, London
29. University Southampton NHS Trust, Southampton

Supplementary Table 2: Dosing guide for trial treatment based on weight band

Weight band (kg)	Dose 1(g)	Dose 2(g)	Total dose (g) to be received by participant
13.5 - 17.4	15	15	30
17.5 - 23.4	20	20	40
23.5 - 27.4	25	25	50
27.5 - 33.4	30	30	60
33.5 - 35.4	35	35	70
35.5 - 45.4	40	40	80
45.5 - 55.4	50	50	100
55.5 - 65.4	60	60	120
65.5 - 75.4	70	70	140
75.5 - 85.4	80	80	160
85.5 - 95.4	90	90	180
95.5 - 105.4	100	100	200
105.5 - 115.4	110	110	220
115.5 - 125.4	120	120	240

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Immuno**g**lobuli**N** **i**n the **T**reatment of **E**ncephalitis (IgNiTE): Protocol for a multicentre randomised controlled trial

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ABSTRACT

Introduction

Infectious and immune-mediated encephalitides are important but under-recognised causes of morbidity and mortality in childhood, with a 7% case-fatality rate and up to 50% morbidity after prolonged follow up. There is a theoretical basis for ameliorating the immune response with intravenous immunoglobulin (IVIG), which is supported by empirical evidence of a beneficial response following its use in the treatment of viral and autoimmune encephalitis. In immune-mediated conditions, IVIG is often used after a delay (by weeks in some cases) while diagnosis is confirmed. Wider use of IVIG in infectious encephalitis and earlier use in immune-mediated encephalitis could improve outcome for these conditions. We describe the protocol for the first ever randomised control trial of IVIG treatment for children with all-cause encephalitis.

Methods and analysis

308 children (6 months to 16 years) with a diagnosis of acute/sub acute encephalitis will be recruited in approximately 30 UK hospitals and randomised to receive 2 doses (1g/kg/dose) of either IVIG or matching placebo, in addition to standard treatment. Recruitment will be over a 42-month period and follow up for 12 months after randomisation. The primary outcome is "good recovery" (score of 2 or lower on the Glasgow Outcome Score Extended - paediatric version), at 12 months after randomisation. Additional secondary neurological measures will be collected at 4-6 weeks after discharge from acute care and at 6 and 12 months after randomisation. Safety, radiological, other autoimmune and tertiary outcomes will also be assessed.

Ethics and Dissemination

This trial has been approved by the UK National Research Ethics committee (South Central - Oxford A; REC 14/SC/1416). Current protocol: v3.0 (04/11/2015). The findings will be presented at both national and international meetings and conferences and published in peer-reviewed journals.

Trial registration

This trial is registered with clinicaltrials.gov (Ref: NCT02308982), EudraCT (Ref: 2014-002997-35 and ISRCTN (Ref: 15791925).

Strengths and limitations of this trial

- This will be the first randomised controlled trial to evaluate the effect of early IVIG treatment in encephalitis from any cause in children, aiming to recruit a large sample size (N=308) across 30 hospitals
- Outcome measures will utilise robust validated and internationally accepted assessment tools and all trial data will be assessed by blinded investigators

- The trial is expected to provide data on the role of IVIG in reducing poor outcomes following encephalitis from any cause, which would impact on care pathways and individual patient decisions within the health services community, both in the UK and internationally and will also inform on health and social care costs
- Expected recruitment has been based on the reported UK incidence of encephalitis and a high and consistent recruitment rate is required across all centres due to the low disease incidence. While the trial is expected to recruit well at all sites, it is possible that there could be unexpected under-recruitment at one or more sites which would be a barrier to timely completion
- Given that patients with all forms of encephalitis will be enrolled to the trial, a statistically significant effect may be masked if there is a benefit from IVIG in only one or some aetiological sub-groups

Keywords: ADEM, autoimmune, encephalitides, immune- mediated, GOSE-Peds

INTRODUCTION

Background and rationale

Encephalitis is inflammation of the brain parenchyma and manifests as a clinical syndrome characterised by a combination of encephalopathy, behavioural changes, fever, seizure, and focal neurological deficits.[1] In England, the population incidence for all-cause encephalitis is estimated at 5.23–8.66/100,000/year, [2] with infants and adults >65 years being the most affected.[2] Diagnosis is typically made by a combination of clinical, laboratory, neuroimaging, and electrophysiological findings using an internationally agreed consensus definition.[1 3] Infections, usually viral, are the most common cause of acute encephalitis, where the cause is identified. Immune mediated forms of encephalitis, usually characterised by the detection of neuronal antibodies in serum and/or cerebrospinal fluid (CSF) have been described, although the proportion is not yet clear. [4 5]

Encephalitis causes significant morbidity and mortality with up 7-20% case-fatality rate for certain types[6-8] and up to 50% of survivors reporting deficits such as memory loss, seizures, learning disability and functional impairment after prolonged follow up.[9-13] The significant burden of the disease despite the current standard treatment highlights the need to identify strategies to reduce poor outcomes in patients with encephalitis. Encephalitis also imposes a substantial economic and resource burden on healthcare services. A review of encephalitis admissions to Paediatric Intensive Care Units (PICUs) showed an average length of stay of 4.3 days, with 75% of children requiring ventilation, and some requiring cardiovascular support (17%) and renal dialysis (6.5%).[14] A UK study of encephalitis hospitalisations reported a mean length of stay of 34 days and a cost to the National Health Service of >£40 million per year.[2]

Notwithstanding the aetiology, the common pathophysiological process in infectious and autoimmune encephalitis is brain inflammation. There is evidence that IVIG has a beneficial role in encephalitis from both its therapeutic and prophylactic use in enteroviral encephalitis in the immunocompromised and in outbreaks of enterovirus-71 infections in Asia,[15] as well as other infectious causes of encephalitis.[16-18] Acute immune treatment including IVIG also appears to benefit both adults and children with autoimmune encephalitis. [19] Randomised controlled trials have demonstrated IVIG efficacy in a number of neurological conditions that share similar underlying inflammatory mechanisms to encephalitis even if different aetiologies.[20] IVIG appears to inhibit complement binding, neutralise pathogenic cytokines, down regulate antibody production, and modulate phagocytosis and T-cell function. [21]

In clinical practice, the use of IVIG in encephalitis varies. In the immune mediated forms of encephalitis, IVIG is often used after a period of delay (by weeks in some cases) while the diagnosis is being confirmed. In other cases, IVIG is used as a last treatment option, usually after several days from hospital admission, where clinical improvement is slow. This delay may limit its benefit due to the brain inflammation, which has already occurred. The variation in practice is due to a lack of class 1 evidence to support the use of IVIG in encephalitis and it is currently unknown whether wider use of IVIG in

infectious encephalitis and earlier use in immune-mediated encephalitis could alter the outcome of this group of conditions. There is therefore the need to fill this evidence gap.

At present, there are no robust controlled trials in children to inform on the optimal treatment of encephalitis. Given the available evidence of possible beneficial role of IVIG, it is therefore important to undertake a trial to investigate the effect of IVIG for all children presenting with encephalitis, and optimise use of this expensive and limited resource.

Trial Objectives and Design:

The **I**mmunoglobuli**N** **i**n the **T**reatment of **E**ncephalitis (IgNiTE) trial is a multi-centre, double blind, placebo controlled, parallel arm, randomised controlled trial (RCT) that will evaluate whether early treatment with IVIG provides benefit for children with a diagnosis of encephalitis, when compared with standard therapy alone. In the context of the IgNiTE trial, 'early treatment' is defined as administration of IVIG within 120 hours from presentation to hospital or, for transferred patients, within 72 hours from admission to a recruiting hospital even if >120 hours since initial hospital presentation.

It is expected that the IgNiTE trial will generate first class evidence to inform clinical decisions regarding the use of IVIG for children with acute and sub acute forms of both infectious and inflammatory encephalitis.

Primary Objective:

To compare neurological outcomes of children with encephalitis who have been treated with either IVIG or placebo, in addition to standard therapy

Secondary Objectives:

(a) To compare (i) clinical and (ii) further neurological outcomes of children with encephalitis who have been treated with IVIG or placebo, in addition to standard therapy

(b) To confirm the safety of IVIG treatment for children with encephalitis

(c) To identify the proportion of children with immune mediated encephalitis

(d) To determine the effect of IVIG treatment on neuronal antibody levels in children with immune mediated encephalitis

Tertiary objectives:

(a) To explore clinically relevant neuroimaging predictors of childhood encephalitis

(b) To explore predictors of neurological outcomes in children with encephalitis

(c) To explore radiological patterns associated with different types of encephalitis

(d) To understand the host inflammatory pathways in encephalitis and the relationship with clinical parameters and the effect of IVIG treatment on these pathways

METHODS

Trial Setting

The trial is planned to be conducted in approximately 30 UK hospitals (both tertiary and district general) (**Supplementary Table 1**).

Eligibility Criteria

Inclusion Criteria (based on the International Encephalitis Consortium consensus case definition)[1]

1) Age 6 weeks to 16 years old AND

2) Acute (within 24 hours) or sub-acute (24 hours to 4 weeks) onset of altered mental state (reduced or altered conscious level, irritability, altered personality or behaviour, lethargy) not attributable to a metabolic cause AND

3) At least two of:

(a) Fever >38^{o}C within 72 hours before or after presentation to hospital

(b) New or acute onset brain imaging consistent with encephalitis or immune-mediated encephalopathy

(c) CSF white blood cells (WBCs) >4/microlitre

(d) Generalised or partial seizures not fully attributable to a pre-existing seizure disorder

(e) New onset focal neurological signs (including movement disorders) for >6 hours

(f) Electroencephalogram (EEG) abnormality that is consistent with encephalitis and not clearly attributable to another cause

AND

4) Parent/guardian/legal representative consent to the patient participating in the trial

Exclusion Criteria

The patient will not be enrolled to the trial if any of the following apply, in addition to failure to meet all the inclusion criteria:

- High clinical suspicion of bacterial meningitis or TB meningitis (for example: presence of frankly purulent CSF; CSF WBCs >1000/microlitre; bacteria on Gram stain and/or culture)
- Prior receipt of any IVIG product during the index admission
- Traumatic brain injury
- Known metabolic encephalopathy
- Toxic encephalopathy
- Hypertensive encephalopathy/posterior reversible encephalopathy syndrome
- Pre-existing demyelinating disorder; pre-existing antibody mediated CNS disorder; pre-existing CSF diversion
- Ischaemic or haemorrhagic stroke
- Children with a contra-indication to IVIG or albumin
- Known hypercoagulable state
- Significant renal impairment defined as GFR of 29mls/min/1.73m2 and below (Chronic Kidney Disease Stage 4)
- Known hyperprolinaemia
- Known to be pregnant

- Any other significant disease or disorder which, in the opinion of the Investigator, may either put the participants at risk because of participation in the trial, or may influence the result of the trial, or the participant's ability to participate in the trial
- Participants who are being actively followed up in another research trial involving an investigational medicinal product
- Administration of trial treatment not feasible within 120 hours from presentation to any hospital OR, for transferred patients, 72 hours from admission to a recruiting hospital even if this is >120 hours from presentation to initial hospital as determined by the trial team
- Any other condition which, in the opinion of the investigator, may interfere with the ability to fulfil trial requirements, especially relating to the primary objective of the trial (this includes plans to be outside the UK for more than 12 months after enrolment)

In addition, any patient who, in the judgement of the clinician and prior to enrolment, is thought will benefit from IVIG will not be enrolled.

Interventions

Participants will be randomised to receive two doses of either human immunoglobulin (intervention group) or placebo (control group), in addition to standard therapy (see Methods: assignment of intervention). There will be no set trial definition of standard therapy and this may vary between hospitals since there are currently no established national clinical care pathways for these. Participants will receive 1g/kg/dose, in weight-based dosing bands (**Supplementary Table 2**). The IVIG product is Privigen (CSL Behring), supplied in unlabelled as 10g/100ml vials. Privigen is a licensed product, further details are outlined in the Product Information[22] and the Summary of Product Characteristics (https://www.medicines.org.uk/emc/medicine/21359). The placebo is 0.1% Human Albumin Solution (HAS) in 0.9% Sodium Chloride solution which will be manufactured in the Aseptic Production Unit (APU), Pharmacy department, Royal Liverpool & Broadgreen Hospital, Liverpool, UK under cGMP conditions, under its MIA (IMP) license and also supplied as 100ml vials. The placebo has been constituted using HAS so as to prevent unblinding.

Packaging and labelling of both trial treatments will also take place at the same location. Labelling, which is identical for both trial treatments, has been approved by the Medicines and Healthcare products Regulatory Authority (MHRA) and conform to Annexe 13 of Good Manufacturing Practice standards and Article 13.3 of Directive 2001/20/EC (http://ec.europa.eu/health/files/eudralex/vol-4/2009_06_annex13.pdf). The APU will provide Qualified Persons services and distribute both trial treatments to the Clinical Trials Pharmacy at each recruiting site where they will be stored under controlled conditions and from where they will be dispensed.

The trial treatment will be prescribed on the participant's drug chart by a clinician who has been delegated for this task and using the suggested wording 'Immunoglobulin/Placebo for the IgNiTE trial'. In addition, a clinical trials prescription form will be completed. For effective management of the trial treatment stock, and to minimise wastage, individual doses may vary slightly. A dosing guide for participants ≥ 13.5kg is provided in a Clinical Study plan and is shown in **Supplementary Table 2.**

Participants <13.5kg will receive 1g/kg, rounded to the nearest whole gram.

Both trial treatments will be administered intravenously by a nurse who has received relevant trial specific and Good Clinical Practice (GCP) training, is trained to give intravenous infusions and trained in the recognition and treatment of anaphylaxis. The first dose will be given as soon as possible after enrolment, within the defined timelines (see Trial objectives and design). The second dose will be given 24-36 hours after the first dose. The administration rate for the trial treatment will be in line with the guidance outlined in the Summary of Product Characteristics (SmPc) for Privigen and local hospital practices for Privigen administration.

Blood and CSF samples will be obtained before and after administration of the study treatment (see section on data collection methods)

Co-enrolment

Participants in the IgNiTE trial may be co-enrolled to another study where:

(a) The study does not involve an investigational medicinal product (IMP)

(b) The study involves an IMP, which is not thought to have a potential immunomodulatory, or neuroprotective effect, as judged by the investigator.

Patients on the following treatment(s) may not be enrolled to the IgNiTE trial:

• Long-term maintenance immunotherapy (defined as 14 days or more) or within 3 months of stopping. This includes (but not limited to) the following: steroids (>1mg/kg/day), Azathioprine, Mycophenolate Mofetil, Methotrexate, Monoclonal anti-inflammatory treatment e.g. Rituximab, infliximab (or within 1 year of discontinuing such treatment).

Outcomes

There are currently no established European core outcomes for encephalitis or acquired brain injury in existence (COMET Initiative website: www.cometinitiative.org, searched 22/02/2016). The selected outcome measures reflect recommendations by The American Academy of Neurology Common Data Elements Project for neurological assessment post traumatic brain injury in children (accessible @ www.commondataelements.ninds.nih.gov). The secondary outcome measures will support the data obtained from the primary outcome.

Primary Outcome □

The primary efficacy outcome is "good recovery", defined as a score of 2 or lower on the Paediatric version of the Glasgow Outcome Score-Extended (GOSE-Peds), at 12 months after randomisation. The GOS-E Peds is a modified version of the GOSE, a gold standard for measuring traumatic brain injury outcome in adults. The GOS-E Peds provides a developmentally appropriate structured interview necessary to evaluate children across different age groups, and it provides a valid measure of outcome in infants, toddlers, children and adolescents. Its use has been validated and found to be sensitive to both severity of injury and to recovery over time, at least 6 months after brain injury and has been suggested

as useful in guiding treatment in the early phases of recovery from brain injury.[23] A strong correlation is also seen with parent report of functional outcomes and also with most performance based cognitive tests for both younger and older children. A 6-month assessment has also been chosen (see secondary objectives) as this has the advantage of improved trial retention, and earlier impact assessment.

Secondary and tertiary outcomes

These are outlined in **Table 1**.

Table 1 Secondary and Tertiary outcomes

	Data collection time point	Outcome measure
Secondary outcomes		
Clinical and neurological	During hospital inpatient stay	• Glasgow coma score • Neurological examination findings as documented by the clinical team • Duration of invasive ventilation (if ventilated) • Length of intensive care unit (ICU) stay in a subset of children admitted to ICU. • Length of hospitalisation
	Around 4-6 weeks after discharge from acute care	• Strength and Difficulties Questionnaire (SDQ) • Adaptive Behaviours Assessment System-Second Edition (ABAS-II) • Peds Quality of Life scoring algorithm • Liverpool Outcome Score • Gross Motor Function Classification System (GMFCS)
	Around 6 months (+/- 4 weeks) after randomisation	• GOSE-Peds
	Around 12 months (+/- 4 weeks) after randomisation	• New diagnosis of epilepsy • Use of anti-epileptic treatment • Strength and Difficulties Questionnaire (SDQ) • Adaptive Behaviours Assessment System-Second Edition (ABAS-II)

		• Peds Quality of Life (PedsQoL) scoring algorithm • Liverpool Outcome Score (LOS) • Gross Motor Function Classification System (GMFCS) • Blinded neuropsychologist assessment of cognitive functioning using age appropriate developmental scales (Bayley Scales for Infant Development (BSID-III)/Wechsler preschool and Primary Scale of Intelligence III (WPPSI-III)/Wechsler Intelligence Scale for Children IV (WISC-IV)
	12 months after randomisation	Proportion of deaths occurring in participants
Radiological	Around 6 months after randomisation	Brain magnetic resonance imaging (MRI) to assess lesion resolution, presence of new lesions and distribution of persisting disease
Safety	24-48 hours after the second IMP dose	Full blood count check to monitor for haemolysis
	First five days after each dose of trial treatment	Adverse events of special interest (AESIs)
	Up to 6 months after randomisation	Serious adverse events (SAEs)
	Up to 12 months after randomisation	Serious adverse reactions (SARs) Suspected Unexpected Serious Adverse Reactions (SUSARs)
Autoimmune		Presence of and comparison of levels of specific neuronal antibodies in serum and/or CSF samples (where lumbar puncture is performed as part of routine care) before and after administration of trial treatment
Tertiary Outcomes		
		(i) Correlate MRI findings with neurological outcomes

		(ii) Correlate clinical and laboratory parameters with neurological outcomes (iii) Comparison of brain MRI findings with aetiological diagnosis (iv) Identification of specific DNA sequence and structural genetic variants in patients with encephalitis (v) The following will be assessed before and after receipt of trial treatment: • Comparison of inflammatory cytokines • Assessment of regulatory T cell frequency and function in blood and/or CSF • Measurement of inflammatory markers in blood and/or CSF • Analysis of gene expression in whole blood • Comparison of the host inflammatory pathways and correlation with clinical parameters

Participant timeline

Time schedule for enrolment, interventions, assessment and visits for participants is shown in Table 2 (**Schedule of trial procedures**)

Table 2 Schedule of trial procedures

	T0: As soon as possible after identification of a potential participant and to allow timely administration of study treatment	T1: As soon as possible after enrolment †	T1+24hours: 24 hours after first dose of trial treatment	T2: 24-36 hours after first dose of trial treatment	T2+ 24-48 hours: 24-48 hours after second dose of trial treatment	T2+7: 7 days after second dose of trial treatment	T3: On the day of discharge from acute care and up to 48 hours prior	T4: 4-6 weeks after discharge from acute care	T5: 6 months (+/- 4 weeks) after randomisation	T6: 12 months (+/- 4 weeks) after randomisation
Eligibility assessment	X									
Informed consent and assent (where appropriate)^	X						X[@]		X[@]	X[@]
Enrolment	X									
Obtain relevant clinical data~	X	X	X	X	X	X	X	X	X	X

Randomisation	X	X[b]								
Scavenged samples~	X	X	X	X	X	X	X	X	X	X
Additional (research sample) where consent is given	X (baseline sample, prior to receipt of trial treatment: neuronal antibody testing, cytokine and DNA analysis*, cellular immunology**)	X (where baseline sample not previously obtained and before administration of trial treatment)	X (functional genomics, DNA analysis*)			X (cellular immunology**, functional genomics, DNA analysis*)			X*** (convalescent sample: neuronal antibody testing, cellular immunology** and cytokine analysis, functional genomics)	
Mandatory full blood count check					X					
Administration of trial treatment and monitoring		X		X						

Completion of Data Capture Form and eCRF[~]	X	X	X	X	X	X	X	X	X	X
Adverse event assessment (AESIs, SARs, SUSARs and SAEs		X	X	X	X	X	X	X	X	X[c]
Questionnaire completion (ABAS-II, SDQ, GMFCS, Peds QL)								X	X	X
Liverpool Outcome Score								X		X
GOSE-Peds									X	X[d]
Research MRI (where consent is given) [c]									X[e]	
Neuropsychology assessment										X

Key: ^ Participant consent if 16 years and assent if < 16 years); [@]where consent/assent (as appropriate) has not been previously obtained; ~ Continuous process throughout the study; [b] First dose of trial treatment may be given on same day as randomisation; *Where DNA sample not previously obtained. Only one DNA sample is required; ** selected centres only; †Visit must be 120 hours from presentation to any hospital OR, for transferred patients, 72 hours from admission to a recruiting hospital even if >120 hours has elapsed since presentation to the initial (referring) hospital; ***To avoid an extra visit solely for this purpose, the '6 month research sample' can be obtained at any routine follow up clinical appointments that occur after the participant has been discharged from acute care; [c] Only deaths or where a serious adverse event is judged to be directly related to the trial treatment; [d] Primary outcome measure; [e] Where consent obtained. May not be required if having routine clinical MRI scan ≥ 3 months after randomisation.

Trial duration

The trial is planned to last 5 years which includes a 42 months for recruitment, 12 month follow up period for each participant and 6 months for data analysis.

Sample size

There is a near paucity of RCT data from previous studies to estimate sample size for this trial. The sample size calculation is based on the assumption that detection of at least 20% difference from 43% in the "good recovery" rate (i.e. GOS-E-Peds score 2 or lower) by 12 months after randomisation is likely to be clinically significant. This is similar to a large observational study on autoimmune encephalitis. [19] Based on this assumption, a total of 308 participants (154 per group), which takes into account an attrition rate of approximately 10%, will provide 90% power and 5% level of significance for a 2- sided test.

Recruitment plan

A flow chart showing the process of patient recruitment is shown in **Figure 1**. Eligible patients will be identified through various routes: by (i) clinicians reviewing medical handover lists and clinical records of new admissions; (ii) research team contacting relevant hospital wards; (iii) microbiologists and/or virologists identifying children who have had a lumbar puncture performed for suspected central nervous system infection, (iv) radiologist identifying a brain MRI scan suggestive of encephalitis, (v) neurophysiologist identifying an EEG suggestive of encephalitis.

Following identification of a potential patient through any of the above routes, a member of the clinical team will approach the parent/ guardian/legal representative to seek their interest in knowing more about the trial and verbal consent will be sought for a member of their details to be passed on to the trial team. Only if consent for this is granted will a member of the trial team contact the family. A member of the trial team will check the patient's eligibility with the parent/ guardian/legal representative, after which they will be provided with the participant information sheet, if the patient is eligible, and given sufficient time to read this and make a decision regarding participation in the trial. The investigator must obtain informed consent and assent (where applicable and obtainable) before the patient undergoes any trial procedure(s). Once appropriate consent and assent (where applicable and obtainable) have been obtained, the patient will be enrolled to the trial by assigning them a participant number using the next available number from the pre-populated enrolment log.

To maximise achievability of the sample size, we have included mostly tertiary paediatric units that are well placed to recruit rapidly a high number of participants. Potential barriers to recruitment will be identified during the pilot phase of the trial, and close support will be provided to sites with recruitment difficulties. A robust system will be put in place to monitor recruitment to ensure that this is on target. A contingency plan will also be put in place to allow for opening of additional sites in the unlikely event of a less than expected recruitment. A 'Screening log' of all screened patients will be kept and will include patients with a diagnosis of encephalitis but are not eligible, eligible patients who refuse to be approached or may not be suitable to be approached, as well as those for whom consent was declined. The reason(s) why a patient is not enrolled will be clearly documented in the screening log, including reasons for declined consent, where this is provided.

Randomisation

After eligibility is confirmed and consent (and assent where applicable) obtained, enrolled participants will be randomised as soon as possible to allow early administration of the trial treatment in line with the protocol. Randomisation will be performed using a fully validated online randomisation system developed by the Primary Care and Vaccines Collaborative Clinical Trials Unit, University of Oxford and during working hours when the trial treatment are available. Participants will be randomised in a 1:1 ratio to either an intervention or control group. Only trained research staff with appropriate access and who are on the IgNiTE trial delegation log will be able to randomise patients. The incidence of encephalitis is higher in infants and some forms of encephalitis are more prevalent in certain age groups. In addition, as part of standard care, patients with inflammatory encephalopathy may receive steroid treatment, which may have a beneficial effect. Therefore, to ensure balance between the trial groups, and account for steroid use as confounding variable, randomisation will be stratified by age group (n= 4: <1, 1-4, 5-9, 10-16 years), and steroid use (yes/no) at the time of enrolment, and using randomly varying block sizes. A computer-generated randomisation code at the time of randomisation will ensure concealment of allocation.

Withdrawal from trial treatment

The participant will be discontinued from the trial treatment at any time if the investigator considers it necessary for any reason including:

- Ineligibility (either arising during the trial or retrospectively having been overlooked at screening) □
- Significant protocol deviation □
- Significant non-compliance with treatment regimen or trial requirements □
- An adverse event which requires discontinuation of the trial treatment or results in inability to continue to comply with trial procedures □
- Disease progression which requires discontinuation of the trial treatment or results in inability to continue to comply with trial procedures □

A participant may also voluntarily withdraw from the trial treatment due to what he or she perceives as an intolerable adverse event (AE), or for other reasons if they wish.

Blinding

IgNiTE is a double blind trial and a rigid blinding process will be in place throughout the trial to ensure validity of the data collected. Participants and their parents/guardians/legal representatives as well as all research staff involved in any aspect of the trial conduct including recruitment, administration of trial treatment, carrying out trial assessments, data collection and entry, sample and statistical analyses will be blinded to treatment allocation throughout the entire trial period. There will be separate monitors for blinded and unblinded data. The active treatment and placebo will be visually identical (packaged and labelled in the same manner) and administered at the same dose and infusion rate to maintain blinding. To be able to manage trial treatment stock effectively and minimise wastage, the clinical trials pharmacists at each recruiting site who are independent of the trial will be unblinded. The tear off section of the label will inform the dispensing pharmacist of the true nature of the contents (IVIG or placebo). At dispending this section of the label will be removed to maintain

blinding.

Unblinding of treatment allocation will occur only in exceptional circumstances when knowledge of the actual treatment received is absolutely essential for further management of the participant. Unblinding will be done via the online randomisation system. The decision to unblind a participant's treatment allocation will be solely that of the site investigator. Only individuals given access to unblind will be able to do this and will include the site pharmacist, principal investigator and co-investigators. Where there is a problem with Sortition, unblinding will be available via either the site pharmacist (during work hours) or an unblinded staff member in Oxford (out of hours) who is independent of the trial, both of whom will have secure access to the master randomisation list for this purpose.

Data collection methods

Trial data will be collected by delegated research staff with appropriate training using two methods: (i) a paper-data capture form and (ii) an electronic case report form (CRF), OpenClinica™ which is a password protected, web based database with accountability records that is stored on a secure sever within the UK. Trial data will be obtained from various sources including patient medical notes, parent interview, laboratory reports, brain scan pictures and reports, electroencephalogram (EEG) reports, pharmacy records, drug charts, questionnaires, and any correspondences relating to the participants involvement in the trial.

Different data types will be collected throughout the trial period:

Clinical data

These will include information regarding patient demographics, clinical findings, treatment, investigation results, length of hospital stay and intensive care management. These data will be obtained during admission, and around 6 and 12 months after randomisation (**Table 1: Secondary and Tertiary outcomes).**

Questionnaires and outcome measures

Validated questionnaires assessing behaviour, motor and adaptive functioning and quality of life will be completed by the participant and/or by their parent/guardian/authorised legal representative at: (i) 4-6 weeks following discharge from acute care and (ii) 12 months after randomisation:

(i) Adaptive Behaviour Assessment System, second edition[24 25]

(ii) Gross Motor Function Classification System[26]

(iii) Strength and Difficulties Questionnaire [27]

(iv) Paediatric Quality of Life Inventory [28 29]

Outcome scores will be assessed using the (i) Paediatric version of the Glasgow Outcome Score Extended (GOSE Peds) at 6 and 12 months after randomisation and (ii) Liverpool Outcome Score (LOS) at 4-6 weeks after discharge from acute care and 12 months after randomisation.

Various measures to obtain complete follow up data will be implemented: (i) blinded research staff or the participant's clinician can assist with questionnaires, (ii) pre-paid envelopes will be provided for return of questionnaires and families will be reminded by telephone, post and/or email, (iii) the primary outcome (GOSE

Peds) assessment will be completed by the neuropsychologist at the 12-month visit. The neuropsychologist may also assist with questionnaire completion.

Laboratory data

Blood and CSF samples (only obtained at the same time as routine lumbar puncture) will be obtained from participants at different time points for neuronal antibodies, cytokine, functional genomics, DNA and cellular immunology evaluation are optional (**Table 2: Schedule of trial procedures**). A mandatory blood sample will be obtained at 24-48 hours following the second dose of trial treatment to assess full blood count levels as a risk mitigation measure to monitor for haemolysis, which is a reported side effect of high dose IVIG treatment. Blood sample volumes will be in line with the Medicines for Children Research Network recommendation.[30] CSF volumes will be in line with British Infection Society TB guideline.[31] All samples will be anonymised. A sample collection and processing guide will be made available to all recruiting sites.

Radiological data

All brain scans (and reports) performed as part of routine clinical care during the study period will be collected. An optional research MRI scan will be performed around 6 months after randomisation for participants who consent to this and where a routine follow up clinical scan is not being done. Where a clinical scan is planned for ≥ 3 months after randomisation, this will be used. All scans will be anonymised and sent to Great Ormond Street Hospital, London for analyses by the blinded study neuroradiologist (WKC) and imaging scientist (CC). MRI findings will be correlated with the primary neurological outcome assessed at 12 months post randomisation. Further exploratory correlations with other neurological outcomes assessed at the different study time points will also be performed.

Neuropsychology assessment

This will be done at 12 months after randomisation by a blinded trial neuropsychologist using age appropriate, validated scales of developmental assessment (see Table 1: Secondary and Tertiary outcomes)

Adverse events

Information on adverse events will be collected throughout the trial (see section on Harms)

Withdrawal

Participants may withdraw from the trial at any time. No further data will be collected. Data collected up until the point of withdrawal from the trial will be analysed unless the parent/participant decide against this. If a participant is withdrawn due to an adverse event (AE), the investigator will follow this up until it resolves or stabilises. All participants who are withdrawn from trial treatment (see Withdrawal from trial treatment) will remain in the trial and followed up as per the trial protocol, but will not have any invasive procedures performed. The trial data will be analysed on an intention-to-treat basis therefore all withdrawals, either from the trial or from the trial treatment, will be reported and included in the data analyses. All protocol deviations will also be reported.

Study data

Data management

Data management will be via the OpenClinica™ database. All relevant data recorded elsewhere (see data collection methods) that are required to achieve the trial objectives will be transferred on to the OpenClinica™ database from where they can be downloaded for analysis. To maintain a high quality standard of data entry, the database will be tested and validated prior to use. In addition, research staff will receive appropriate level training on data collection and entry and there will be regular monitoring of trial data throughout the trial. Furthermore, prior to data analysis, the database will be locked for cleaning to ensure that data are complete and reliable. Research staff at the various recruiting sites will be contacted to provide information on any missing data and to clarify any errors identified. All trial documents will be retained and stored securely in accordance with GCP after the completion or discontinuation of the trial for 3 years after the youngest participant turns 18 years.

Statistical methods

The primary statistical analysis will be carried out on the basis of intention-to-treat (ITT). After randomisation, participants will be analysed according to their allocated treatment group irrespective of what treatment they actually receive. However a further modified ITT analysis will be performed excluding participants found to be ineligible in retrospect.

Data analysis will be performed using a mixed effect model for repeated measures, i.e. to incorporate all outcome data collected during the 12 months follow-up, in order to apply the intention-to-treat principle as far as possible and to account for potential biases arising from loss to follow-up. The model will include treatment group, time, treatment-by-time interaction, and baseline covariates. An unstructured correlation matrix will be used to model the within-participant error correlation structure. An appropriate contrast will be specified to test for treatment efficacy between randomised groups at 12 months. Various sensitivity analyses will be performed using other imputation methods, as well as analysis of 12-month data cross-sectionally, to test whether the results are robust to different assumptions about the missing data. The primary intention-to-treat analysis will account for steroid use before randomisation as a covariate. As required, the impact of post-hospitalisation course including the use of concomitant and/or different immune treatments and period of neurorehabilitation on the primary outcome will be investigated in an exploratory analysis.

The results from the trial will be prepared as comparative summary statistics (difference in response rate or means) with 95% confidence intervals. All the tests will be done at a 5% two-sided significance level. A full detailed analysis plan (including plans for any interim analysis, subgroup analysis, and sensitivity analysis) will be prepared and finalised before the first interim analysis.

Primary analysis

The primary efficacy end point in this trial is “good recovery”, defined by GOS-E-Peds score 2 or lower, at 12 months from randomisation. This will be analysed using a Generalised Linear Mixed Effect model, utilising data collected at discharge, 6 and 12 months from randomisation. An interaction between time and randomised group will be fitted to allow estimation of treatment effect at each time point. The model will adjust for baseline values

and other stratification factors (e.g. age and steroid treatment at the time of randomisation).

Secondary and tertiary analyses

As far as possible, we will use similar method for secondary and tertiary continuous outcomes collected at multiple time points or analysis of covariance (ANCOVA) for those collected at 12 months only, adjusting for baseline measures (if collected) and any stratification variables. Otherwise, an equivalent nonparametric method will be used for outcomes that violate the normal distribution assumption. A log-binomial regression will be performed on binary outcomes with similar adjustment of baseline covariates. Chi-squared or Fisher's exact test will be used to analyse adverse events and non- adherence.

Reporting of the trial findings will be in line with Consolidated Standards of Reporting Trials (CONSORT) guidelines

Interim analysis

Analysis for the DSMC will be performed in accordance with the DSMC Charter. No interim efficacy analysis will be performed. Interim reports containing safety data, along with any other analyses that the committee may request, will be sent to the DSMC in strict confidence. Close monitoring to assess practical aspects of delivering the trial interventions and recruitment will also be undertaken.

Data Monitoring

The Data Safety Monitoring Committee (DSMC) is responsible for safeguarding the interests of trial patients, monitoring the accumulating data and making recommendations to the Trial Steering Committee (TSC) on whether the trial should continue as planned. The DSMC will comprise of a clinical chair, clinicians, and a statistician, all of whom will be independent of the trial, the sponsor and funders. The role of the TSC is to provide overall supervision for the IgNiTE trial on behalf of the Trial Sponsor and the Trial Funder and to ensure that the IgNiTE trial is conducted according to the guidelines for GCP, Research Governance Framework for Health and Social Care and all relevant regulations and local policies. The TSC will comprise an independent chair, the CI, paediatricians and patient representatives. In discharging its safety role, the TSC will work in conjunction with the Data Safety and Monitoring Committee (DSMC) for the IgNiTE trial. Both the DSMC and TSC will meet prior to trial start and 6 months thereafter. Increased frequency of meetings will be arranged depending on the requirements of the trial, DSMC and TSC recommendations.

Stopping guidelines

This trial may be suspended or prematurely terminated by the sponsor, CI, regulatory authority or funder if there is sufficient reason to think that that the safety of participants is affected by the trial procedures. Written notification, documenting the reason for trial suspension or termination, will be provided by the suspending or terminating party to the investigator, funders, and regulatory authorities. If the trial is prematurely terminated or suspended, the PI will promptly inform the REC, MHRA, and CSL Behring and will provide the reason(s) for

the termination or suspension.

Harms

International Conference on Harmonisation (ICH) definitions are used for AEs, AESIs, adverse reactions (ARs), SAEs, Serious adverse events (SARs) and suspected unexpected SARs (SUSARs). IVIG has a well-established side effect profile. All participants will be monitored for (i) AESIs, (includes anaphylaxis, haemolysis, new onset seizure or abnormal movements not thought to be due to the encephalitis illness, thromboembolism, aseptic meningitis unrelated to the encephalitis illness, acute renal failure, and any other medically significant events as determined by the investigator), in the first five days following receipt of trial treatment, (ii) serious adverse events up to 6 months after randomisation, or up to 12 months after randomisation where the event is judged directly related to the trial treatment and (iii) deaths up to 12 months after randomisation.

Monitoring and reporting of adverse events will be performed by the site PI and research team, and will be recorded on the data capture form and uploaded to the eCRF (OpenClinica^{TM}). The nature and severity of each adverse event, and the relationship to trial treatment will be documented. The expectedness of an AE will be determined by whether or not it is listed in the SmPC for Privigen or Human Albumin Solution. AESIs and SAEs will be reported to the CI, CSL Behring and the DSMC. This will be expedited (within 24 hours of the research staff becoming aware), for all AESIs and for all SAEs that are judged related to the trial treatment. SAEs that are judged to be unrelated to the IMP will be discussed with the PI and CI but do not require expedited reporting.

The CI will report all relevant information about a Suspected Unexpected Adverse Reaction (SUSAR) that occurs during the course of the trial to the MHRA, CSL Behring, the relevant ethics committee and the DSMC. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days. The CI or delegate will also inform all principal investigators concerned of relevant information about SARs that could adversely affect the safety of participants.

A summary list of all SAEs (including those unrelated to the trial treatment), AESIs, and SUSARs will be provided in a safety report to the DSMC, which will be submitted at regular interval as specified in the DSMC Charter. In addition, a strict data sheet will be kept by CSL Behring, which will include the randomisation code aligned to the batch number of assigned IVIG product and in order to maintain a link between the participant and the batch of the product.

Pregnancy

Although not AEs, pregnancies are reportable events. Should a participant become pregnant during the trial the study treatment will be discontinued. Any pregnancy occurring during the clinical trial will be reported to the CI and CSL Behring within 24 hours of the investigator becoming aware and will be followed up for an outcome, which will be recorded. If a congenital abnormality or birth defect is identified this would fall within the definition of an SAE and will be reported as such.

Auditing

Regular monitoring by the trial sponsor or delegate will ensure compliance with GCP. The investigator sites will

provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities. Data will be evaluated for compliance with the protocol and accuracy in relation to source documents. Following written standard operating procedures, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The Quality Assurance manager will also maintain an internal audit program, which will supplement the external monitoring process to ensure that systems relating to trial conduct, data recording, analysis and reporting are functional are in compliance with the protocol, GCP and the applicable regulatory requirements. The audit program also includes laboratory activities taking into consideration the MHRA and EMA guidelines for GCP in the laboratory. The Sponsor may carry out audit to ensure compliance with the protocol, GCP and appropriate regulations. GCP inspections may also be undertaken by the MHRA to ensure compliance with protocol and the Medicines for Human Use (Clinical Trials) Regulations 2004.

ETHICS AND DISSEMINATION

Ethical and safety considerations

This trial has been approved by the United Kingdom National Research Ethics Service (NRES) committee (South Central - Oxford A; REC 14/SC/1416). Clinical trial authorisation has been granted via the Medicines and Healthcare Products Regulatory Agency (MHRA) notification scheme (Ref: 21584/0337/001-0001). Current protocol: v3.0 (04/11/2015). Written approval from the respective Research and Development (R&D) departments will be obtained for each participating site prior to recruitment.

The Chief Investigator (CI) will ensure that this trial (and all subsequent approved amendments) is conducted in accordance with the principles of the Declaration of Helsinki (1996), in full conformity with the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Guidelines for GCP (CPMP/ICH/135/95 July 1996), the Research Governance Framework, and the Medicines for Human Use (Clinical Trial) Regulations 2004. The CI will monitor pharmacovigilance and will report to the Research Ethics Committee (REC), MHRA and funders during and at the end of the trial. All protocol modifications will be disseminated to all relevant parties. The findings of the trial will be presented at both national and international meetings and conferences and published in peer-reviewed journals.

Informed consent and assent

Following identification of a potentially eligible participant by the clinical team, a Participant Information Sheet (PIS) explaining the trial (including the rationale, aims and objectives, treatment assignation), potential risks and benefits, and all the trial procedures will be provided. Parents/guardians/legal representatives or patients, where appropriate (i.e. if the patient has capacity), will be allowed sufficient time to consider the information in the PIS, to seek independent advice and to consider participation in the trial. Informed consent (patients aged 16 years and above) and assent (patients below 16 years) will be obtained by trained research staff using an appropriately signed and dated informed consent/assent form, before any trial specific procedures are performed. Given that children with encephalitis will be unwell and may be confused during the acute illness, it is likely that eligible patients would be unable to provide consent/assent prior to enrolment. Therefore, for patients aged 16 years and above, informed consent will be obtained from their parent/guardian/legal

representative. Once capacity is regained, appropriate consent/assent will be sought from all participants at follow up time points and if this is not granted, they will be withdrawn from the trial. Participants who previously provided assent but turn 16 years while still in the trial will be required to provide consent for ongoing participation in the trial and will be withdrawn if this is not granted.

Parents/guardians/legally authorised representatives/participants may be approached about a separate, ethically approved, Biobank study and asked if they would like to consent to this study using a separate consent form. Participation in the Biobank is optional and samples will only be stored where appropriate consent has been obtained.

Confidentiality

Data will be stored securely in line with the Data Protection Act 1998. The randomisation system, data capture form and eCRF have been designed so as to protect participant information and to maintain confidentiality. It will be the responsibility of the local investigators to ensure that the data is password protected and held on local trust computer systems. The research staff will ensure that the participants' anonymity is maintained. Participants will be identified only by initials and participant number on the research notes and eCRF. All investigation results and blood samples will be anonymised. All trial documents will be stored securely and only accessible by research staff and authorised personnel. The CI will be the custodian of the trial data.

Access to data

Direct access will be granted to authorised representatives from the Sponsor or host institution for monitoring and/or audit of the trial to ensure compliance with regulations.

Reimbursement

Reasonable travel expenses for any visits additional to normal care will be reimbursed on production of receipts, or a mileage allowance provided as appropriate.

Ancillary and post trial care

There will be no continued provision of treatment available after participants have completed the trial however participants are likely to be followed up by the hospital team as part of routine care. Details of The Encephalitis Society are provided in the PIS, and they can provide ongoing support and information to families.

Dissemination policy

We aim to produce high impact publications of the results of the trial and present the findings to the paediatricians who manage encephalitis in the front line. The Investigators will be involved in preparing drafts of the manuscripts, abstracts, press releases and any other publications arising from the trial. Authors will acknowledge that the trial was funded by the National Institute for Health Research and CSL Behring. Authorship will be determined in accordance with the International Committee of Medical Journal Editors (ICMJE) guidelines and other contributors will be acknowledged. There is no intended use of professional writers.

Patient public involvement

The Encephalitis Society provided advice on the clinical problem and need for interventions to address the poor outcomes from encephalitis. The trial proposal was discussed with The Encephalitis Society who affirmed its importance as a priority for evaluation and Dr Ava Easton, the Chief Executive of The Encephalitis Society is a co-applicant on the grant application and a co-author on this paper.

To provide an important patient-centred research perspective, we have engaged members of the public in our PPI programme in both the design and management of the trial, through The Encephalitis Society. The opinion of The Encephalitis Society on the burden of the questionnaire outcome measures on patients was sought at the design stage of the trial. The Encephalitis Society also reviewed and provided comments on patient information sheets and consent forms. The Encephalitis Society research poster will be provided for use at the respective recruiting centres. Through The Encephalitis Society, we have also recruited two patient representatives as members of the trial steering committee.

We will provide detailed accessible information about the trial outcomes to patients/parents/carers. The Encephalitis Society will drive forward publication and dissemination of the trial findings among lay, therapeutic and health professionals through the use of web materials, newsletters, and guides as well as at conferences and seminars in relation to Encephalitis and related fields. All patients and their parents/carers will be acknowledged in any outputs from the trial. We will also work with The Encephalitis Society on a programme of teaching events and produce guides for healthcare professionals and lay people.

Declaration of interests

All authors have completed the ICMJE uniform disclosure form at www.icmje.org/coi_disclosure.pdf

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Study investigators: M Sadarangani, M Absoud, WK Chong, C Clarke, A Easton, V Gray, R Kneen, M Lim, M Pike, T Solomon, A Vincent, L-M Yu, AJ Pollard

Department of Health Disclaimer

The views and opinions expressed herein are those of the authors and do not necessarily reflect those of the MRC, NHS, NIHR or Department of Health.

Authors' contribution

The trial was conceptualised by MP and AJP; with input from ML, MA, RK and TS. MP, MS, RK, TS, WKC, CC, ML, MA, AV, VG, AE, L-M Y, AJP are named investigators on the IgNiTE trial. All authors contributed significantly to the design of the trial, with specific additional contributions from each co-author within their area of expertise; paediatric neurology (MP, RK, ML, MA), paediatric infectious diseases (MS, AJP), paediatric neuropsychology (VG), neuroimaging (CC, WKC), neuroimmunology (TS and AV), statistics (L-MY), and patient group (AE). MAI is the lead doctor for the trial and prepared the first version and all subsequent revisions of the manuscript. LW is the lead nurse for the trial. All authors contributed manuscript and have approved the final manuscript for publication.

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Competing interests

CSL Behring have provided the study IMP (IVIG) and funded manufacture of placebo and the supply and distribution of IMP and placebo. AJP reports grants from NIHR EME programme, during the conduct of the study. The University of Oxford and AV hold patents for VGKC-complex antibody tests, licenced to Euroimmun AG, and receive royalties. The neuroimmunology work in the described trial is funded through the MRC/NIHR grant. MA serves on the data safety monitoring board for a study sponsored by Neurim Pharmaceuticals and is on the editorial advisory board for the International Journal of Language & Communication Disorders. MAI reports salary from the NIHR EME grant. ML has received consultation fees from CSL Behring, travel grants from Merck Serono, and been awarded educational grants to organise meetings by Novartis, Biogen Idec, Merck Serono and Bayer. MS reports grants from Pfizer, outside the submitted work. TS is supported by the National Institute for Health Research Health Protection Research Unit in Emerging and Zoonotic Infections at Liverpool. AE, LW, L-MY, MP, MS, RK and WKC have nothing to disclose.

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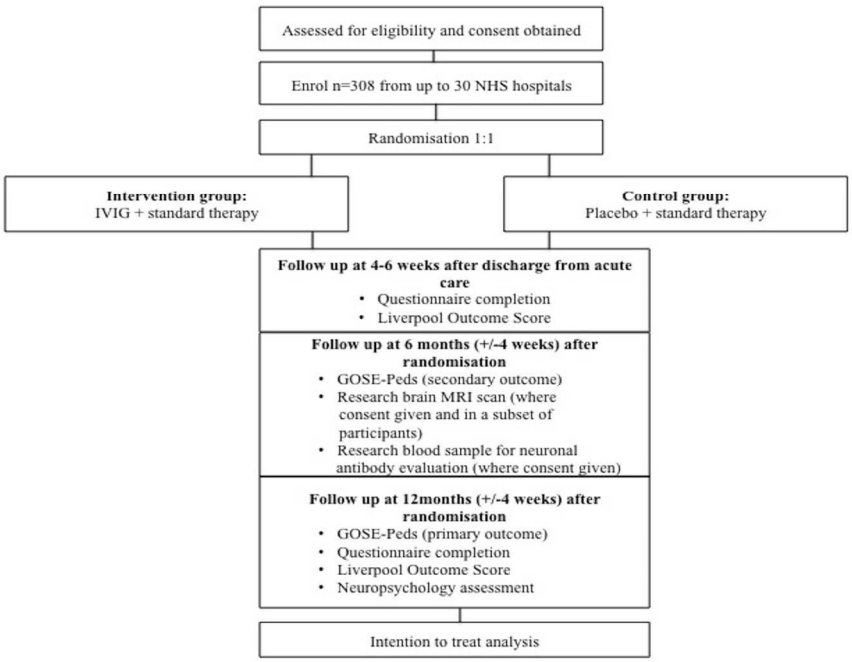

Flowchart showing process of participant recruitment

152x101mm (300 x 300 DPI)

Supplementary Table 1 List of planned participating sites

1. Addenbrooke's Hospital, Cambridge University Hospitals, Cambridge
2. Alder Hey Children's Hospital NHS Foundation Trust, Liverpool
3. Belfast Hospital for Sick Children, Belfast
4. Birmingham Children's Hospital NHS Foundation Trust, Birmingham
5. Bradford University Hospitals NHS Foundation Trust
6. Evelina Children's Hospital at Guy's and St Thomas' NHS Foundation Trust, London
7. Great Northern Children's Hospital, Newcastle Hospitals NHS Foundation Trust, Newcastle
8. Great Ormond Street Hospital Foundation Trust, London
9. Heartlands Hospital, Heart of England NHS Foundation Trust, Birmingham
10. James Cooke University Hospital, South Tees Hospitals NHS Foundation trust
11. John Radcliffe Hospital, Oxford University Hospitals NHS Trust, Oxford
12. Leeds Teaching Hospital NHS Trust
13. Morriston Hospital, Health in Wales
14. Ninewells Hospital, NHS Tayside
15. Nottingham University Hospitals NHS Trust, Nottingham
16. Pennine Acute Hospital NHS Trust, Manchester
17. Royal Aberdeen Children's Hospital, NHS Grampian (Aberdeen)
18. Royal Cornwall Hospitals NHS Trust
19. Royal Hospital For Children, NHS Greater Glasgow
20. Royal Manchester Children's Hospital, Manchester
21. Sheffield Children's Hospital NHS Foundation Trust
22. St George's University Hospitals NHS Foundation Trust
23. St Mary's Hospital, Imperial College Healthcare NHS Trust
24. University Hospital Bristol NHS Foundation Trust
25. University Hospital of Wales, Cardiff and Vale NHS Trust, Cardiff
26. University Hospitals of Leicester NHS Trust
27. University of Edinburgh, NHS Lothian
28. University of London and Bart's Health NHS Trust, London
29. University Southampton NHS Trust, Southampton

Supplementary Table 2: Dosing guide for trial treatment based on weight band

Weight band (kg)	Dose 1(g)	Dose 2(g)	Total dose (g) to be received by participant
13.5 - 17.4	15	15	30
17.5 - 23.4	20	20	40
23.5 - 27.4	25	25	50
27.5 - 33.4	30	30	60
33.5 - 35.4	35	35	70
35.5 - 45.4	40	40	80
45.5 - 55.4	50	50	100
55.5 - 65.4	60	60	120
65.5 - 75.4	70	70	140
75.5 - 85.4	80	80	160
85.5 - 95.4	90	90	180
95.5 - 105.4	100	100	200
105.5 - 115.4	110	110	220
115.5 - 125.4	120	120	240

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

Section/item	Item No	Description	Addressed on page number (marked protocol)
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	2
	2b	All items from the World Health Organization Trial Registration Data Set	2
Protocol version	3	Date and version identifier	2
Funding	4	Sources and types of financial, material, and other support	27
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	1, 27
	5b	Name and contact information for the trial sponsor	27
	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	27
	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)	22

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	4
	6b	Explanation for choice of comparators	7
Objectives	7	Specific objectives or hypotheses	5
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)	5
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	5, 6
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)	6, 7
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	7
	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)	18
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	7, 8
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	8
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	10 (Table 1), 11,12
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)	13,14,15 (Table 2), 18 (Figure 1)

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
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	17
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
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	18
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	18
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	18
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	18
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	19
Methods: Data collection, management, and analysis			
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	19, 20
	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	19, 20

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	21
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	21
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	22
	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)	21
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	22
	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	22
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	23
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	23, 24
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	24
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)	24

Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	24
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	25
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	25
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	26 and completed COI forms
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	25
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	25
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	25
	31b	Authorship eligibility guidelines and any intended use of professional writers	25
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	27
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	Participant information sheet and consent forms attached
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	Sample collection and processing guide attached.

*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.