NHS
Blood and Transplant

NHSBT CLINICAL TRIALS UNIT

A randomised controlled trial to compare two different platelet count thresholds for prophylactic platelet transfusion to preterm neonates.

Platelets for Neonatal Transfusion - Study 2
PlaNeT-2

ISRCTN: 87736839

CSP: 45968 NIHR CRN: 10305 REC: 10/H0306/61

GRANT REF: BSO6/30

Protocol Version 4.0
Protocol date: 11.01.2016

Authorised by:

Name:	Simon Stanworth	Role:	Lead Chief Investigator
Signature:		Date:	
Name:	Anna Curley	Role:	Joint Chief Investigator
Signature:		Date:	
Name:	Alison Deary	Role:	Head of Clinical Operations NHSBT CTU
Signature:		Date:	

GENERAL INFORMATION

This document describes the *Platelets for Neonatal Transfusion – Study 2 (PlaNeT-2)* trial. It provides general information, and contains the procedures that are to be followed by those involved in the study. It also acts as a written record for audit purposes. Every care has been taken to ensure that the protocol is complete and current. Corrections or amendments will be circulated to all investigators, and in the event of substantial changes the protocol will be updated and re-issued with a new protocol version number. Before entering neonates into the trial the investigator should contact the trial manager to ensure that the most up-to-date information and documentation is held relating to the trial. The study is being coordinated by the NHSBT Clinical Trials Unit.

This protocol has been based on the MRC Clinical Trials Unit protocol template version 3.15

Contacts
If you have any queries regarding this protocol, or the general conduct of the trial, please contact the Trial Manager: Karen Willoughby Email: kw369@medschl.cam.ac.uk

Compliance
This trial will be conducted in compliance with the protocol, the principles of GCP, UK Data Protection Act, NHS research governance, and the Medicines for Human Use Regulation as appropriate.

Sponsor and Funder
The NHSBT is the trial sponsor and has delegated responsibility for the overall management of the Planet 2 trial to the NHSBT CTU.

Queries relating to the NHSBT sponsorship of the trial should be addressed to:
Research and Development Manager
R&D Office,
NHSBT,
500 North Bristol Park, Northway, Filton, Bristol, BS34 7QH
Email (research.office@nhsbt.nhs.uk or via the trial team.

Trial Management Group:

Lead Chief investigator
Dr Simon Stanworth
Consultant Haematologist
NHSBT Oxford Centre, John Radcliffe Hospital – Level 2
Headington, Oxford. OX3 9DU
Tel. 01865 447917/7976 Email: simon.stanworth@nhsbt.nhs.uk

Joint Chief Investigator
Dr Anna Curley
Consultant Neonatologist
National Maternity Hospital
Dublin 2 Email: anna.curley@nmh.ie

Trial Manager:
Karen Willoughby
C/o NHSBT CTU,
Long Road, Cambridge Email: kw369@cam.ac.uk

Medical experts
Dr Paul Clarke

Consultant Neonatologist
Norfolk and Norwich University Hospitals NHS Foundation Trust
Telephone: 01603 286337 Email: paul.clarke@nnuh.nhs.uk

Dr Tim Watts
Consultant Neonatologist
St Thomas Hospital
Guys and St Thomas Hospitals NHS Foundation Trust
Telephone: 020 7188 3944 Email: timothy.watts@gstt.nhs.uk

Dr Helen New
Consultant Paediatric Haematologist
NHS Blood and Transplant
Colindale
Charcot Road
London NW9 5BG
Telephone: 020 8957 2928 Email: helen.new@nhsbt.nhs.uk

Dr Priya Muthukumar
Consultant Neonatologist
Norfolk and Norwich University Hospitals NHS Foundation Trust
Telephone: 01603 286337 Email: priya.muthukumar@nnuh.nhs.uk

Research Fellows
Dr Vidheya Venkatesh
Consultant Neonatologist
Box 226 Neonatal Unit
Cambridge University Hospitals NHS Foundation Trust
Hills Road, Cambridge CB2 2QQ
Tel No: 01223 217006 Email: Vidheya.venkatesh@addenbrookes.nhs.uk

Dr Rizwan Khan
Consultant Paediatrician
Kerry General Hospital
Tralee, Ireland Email: Rizwan.khan@hse.ie

Trial Statistician
Ms Cara Hudson
Senior Statistician
Statistics and Clinical Studies
NHS Blood and Transplant
Tel: 0117 975 7432 (internal: 37432)
Fax: 0117 975 7577 Email: Cara.Hudson@nhsbt.nhs.uk

Head of Clinical Operations
Ms Alison Deary
NHSBT CTU
NHSBT
Long Road, Cambridge. CB2 0PT
Tel. 01223 588004 Fax: 01223 588136 Email: Alison.deary@nhsbt.nhs.uk

Project Manager
Dr Ana Mora
NHSBT CTU
NHSBT
Long Road, Cambridge. CB2 0PT
Tel. 01223 588045 Fax: 01223 588136 Email: Ana.mora@nhsbt.nhs.uk

Senior Data Manager
Ms Renate Hodge
NHSBT
Long Road, Cambridge. CB2 0PT
Tel: 01223 588064 Fax: 01223 588136 Email: Renate.Hodge@nhsbt.nhs.uk

RANDOMISATIONS
Via 24hr Internet-based Randomisation service:

http://www.sealedenvelope.com

If unable to randomise patients online, please contact the trial manager who will be able to do this for you.

Randomisations can also be undertaken through the Planet-2 website:

www.planet-2.com

Click on the red button in the centre of the home page to randomise

DOCUMENT HISTORY

Version	History	Date
1.0	**Original version**	**28 July 2010**
1.1	**General Information Section -** Addition of trial manager name and contact details. Change made to membership of the TMG – New research Fellow Dr Rizwan Khan substituted for Dr Priya Muthukumar who remains a medical expert. **Section 6** - addition of definition of grades of bleeding and references to definitions added to appropriate sections **Section 8-** (safety section) to clarify the collection of episodes of NEC and sepsis as adverse events. **Section 9 -** clarifications to primary outcome description to make consistent with change requested to ethics submission form. **Section 11** - Addition of Dr Anthony Emmerson to the membership of the TSC	**21 October 2010**
2.0	**Abbreviations Table** – addition of "Corrected Gestational Age (CGA), Post Menstrual Age" (PMA) and "Apgar Score" **Throughout protocol**: End of study for each neonate changed from "28 days Corrected gestational Age" to 38 weeks Corrected Gestational Age (CGA) and addition of a phone call to parents of neonates who are discharged home before study day 28. Minor spelling/administrative changes. **Fig 1.0, p12** – revised for accuracy **Section 4.3, p18** : revision of exclusion criterion 2 **Section 5.2 (4)**, **p19** addition of information required by researchers before randomising a neonate **Section 6.4, p21** – revision of bleeding grade definitions **Section 7.5, p26** – revised to clarify information about the use of the BAT **Section 7.9, p27** – clarification of data to be collected from neonates on transfer to another hospital **Section 7.10, p27** – clarification of information required from neonates discharged home before study day 28. **Section 7.17, p29 –** clarification of transfusion data to be collected **Section 8.3, p31** – clarification of SAE definitions **Section 8.4, p32** – addition of definitions of NEC and Sepsis **Section 9.3, p35** – clarification of sample size calculation **Section 10.1.2, p36** – removal of requirement to have a completed database before start of recruitment **Appendix B, p48** – addition of historical risk factors for NEC	

Version	History	Date
3.0	**General Information Section:** Change of contact details for sponsor to Dr Nicholas Watkins; addition of Ms Cara Hudson as Trial Statistician. Amendment of details of Dr Vidheya Venkatesh & Dr Rizwan Khan – now both consultants. Head of clinical Operations now Ms A Deary, project manager now Ms A Mora. Addition of Ms Beatriz Santamaria to TMG. References to NHSBT/MRC deleted since structural re-organisation. **P4** –Addition of details for randomisation process through website **Abbreviations table:** Change of name from Clinical Studies Unit (CSU) to Clinical Trials Unit (CTU). All references to CSU replaced by CTU throughout this document **Section 1.1.3, p11** – Clarification of end of study for neonates developing subsequent episodes of thrombocytopenia **Section 1.1.4, p11** – Clarification of reason for choosing Study Day 28 as primary outcome point **Section 1.1.5, p12** – The following revisions to this section have been made: • For greater clarity, addition of wording for clarification of End of Study for babies recruited after 34 weeks CGA in age (though must be <34 weeks CGA at birth). • Amendment of clause relating to babies discharged home before Study Day 28, allowing possibility to contact parents /GP for basic information. Permission to contact will be added to the Informed Consent Form (ICF) and Patient Information Leaflet (PIL) • Elucidation of details to be requested from units not participating in PlaNeT-2 if a baby is transferred out to such a unit before Study Day 28. Information requested consists of number of platelet transfusions, occurrence of any safety events and the results of a cranial USS at Study Day 28 (+/- 3days). a cranial USS at SD28 (+/- 3 days). Process of relaying data back to NHSBT CTU is described • Clarification that no data will be sought from a non-participating unit if a baby is transferred after the primary outcome point (SD28) • Addition of section relating to permission to contact parents / GP or Health Visitor for the 2 year follow up. • Addition of section relating to accessing national neonatal database for extracting primary outcome and 2 year follow up data in the event that information cannot be obtained directly from non-participating units **Section 1.2,** – Addition of expected dates for End of Trial milestones **Fig 1.0,** – Clarification of end of study **Section 7.1, p27** – Addition of detail regarding filing of ICF **Section 7.2, p28** – Simplification of table regarding schedule of assessments by combining 2 columns to form one representing SD29 to End of Study. Amendment of notes to reflect changes elsewhere and to reference other sections in protocol for more detail **Sections 7.6, 7.7** – addition of wording for clarification of End of Study for babies recruited after 34 weeks CGA in age (though must be <34 weeks CGA at birth) **Section 7.9** – Clarification of process for obtaining data for babies	17.07.2014

transferred to non-participating unit up to SD28
Section 7.10, 7.12 – Addition of wording to allow phone contact to parents / GP / Health Visitor
Section 7.12 – Addition of clause to allow extraction of data from national database from consented babies
Section 9.2.1 - Clarification of reason for choosing Study Day 28 as primary outcome point
Section 10.1.2 – Addition of citation for published paper
Section 18 – Addition of new centres participating in trial
Appendix B – updated NEC definitions

4.0	**History** **General Information Section:** Update of membership contact details **Throughout protocol:** extension of window for SD28 cranial ultrasound from +/- 3 days to any imaging -5 / + 10 days **Section1- Outcome Measures Data Collection** – use of postal questionnaire (amended PARCA-R) has been added to 2 year follow up data collection section. Collection of parent / guardian contact details for use by 2 year follow up co-ordinator also added. **Section 1.4-** New section introduced for parent and public engagement **Fig 1.1** renumbered as Fig 1 **Section 4.4-** Co-enrolment section introduced for clarification **Sectopm 7.12 - Two year neuro-developmental follow up**. Process outlined for obtaining information using postal questionnaires. Insertion of Fig 3 describing proposed pathway **Section 9.2, - Imputing primary outcome.** Section describes how medical experts will use clinical evidence to obtain indirect evidence of primary outcome if no scans within -5 / +10 days of SD28 **Section 11.1 & Section 11.2 -** Amendment of TMG & TSC to reflect involvement of Dutch team and change of original personnel to PlaNeT team. **Section 18 -** Addition of new sites to list of participating units	**07.01.2016**

Date

CONTENTS

ABBREVIATIONS AND GLOSSARY

AE	Adverse event
Apgar Score	A score assigned to a neonate based on heart rate, muscle tone, skin colour, reflex irritability and respiratory effort that describes the condition of the neonate at birth
AR	Adverse reaction
BAPM	British Association of Perinatal Medicine
BAT	Bleeding Assessment Tool
CF	Consent form
CGA	Corrected Gestational Age
CI	Chief Investigator
CPA	Clinical Pathology Accreditation
CRF	Case Report Form
CTU	Clinical Trials Unit
CUSS	Cranial Ultrasound Scan
DM	Data Manager
DMC	Data Monitoring Committee
GA	Gestational Age
GCP	Good Clinical Practice
GLH	Germinal Layer Haemorrhage
HE	Health Economics
Hb	Haemoglobin
DMC	Independent Data Monitoring Committee
IVH	Intraventricular haemorrhage
IUGR	Intrauterine growth retardation
LD	Leucocyte depleted/depletion
LDH	Lactate Dehydrogenase
MCRN	Medicines for Children Research Network
MHRA	Medicines and Healthcare Regulatory Authority, London, UK
MRC	Medical Research Council, London, UK
NBA	National Blood Authority, London, UK
NEC	Necrotising Enterocolitis
NEQAS	National External Quality Assurance Scheme
NHS	National Health Service, UK
NHSLA	NHS Litigation Authority
NHSBT	NHS Blood & Transplant
NICU	Neonatal Intensive Care Unit
NICUS	Neonatal Intensive Care Units
NNAP	National Neonatal Audit Programme
PARCA	Parent Report of Children's abilities for very premature infants
PI	Principal Investigator
PISL	Parent Information Leaflet
PMA	Post Menstrual Age
QL	Quality of life
SAE	Serious adverse event
SCBU	Special Care baby Unit
RN	Research Nurse
SABRE	Serious Adverse Blood Reactions and Events
SEND	Standard Electronic Neonatal Database
SHOT	Serious Hazards of Transfusion
SOP	Standard operating procedure
SSA	Site specific assessment
TMG	Trial Management Group
TRALI	Transfusion Related Acute Lung Injury
TRPG	Thames Regional Perinatal Outcome Group
TSC	Trial Steering Committee
USS	Ultrasound Scan
vCJD	Variant Creutzfeld-Jacob Disease
WHO	World Health Organisation

1 SUMMARY

1.1 Abstract and summary of trial design

A trial assessing clinically relevant outcomes in relation to the platelet count thresholds commonly used as triggers for transfusion has never been undertaken in preterm neonates with severe thrombocytopenia. Practice in many neonatal units in UK has seen the adoption of thresholds for prophylactic platelet transfusions at around 20 to 30 $x10^9$/L, but the effectiveness and safety of any thresholds in preterm neonates has not been established in randomised controlled trials. In the only randomised controlled trial to assess a threshold level for the effectiveness of neonatal prophylactic platelet transfusions, the platelet count thresholds were 50 and 150 $x10^9$/L (Andrew et al, 1993), but this trial excluded neonates with platelet counts < 50 $x10^9$/L.

Platelets for Neonatal Transfusion - study 2, (PlaNeT-2) is a 2-stage, randomised, parallel group, superiority trial, which follows on from a prospective multicentre observational study of platelet transfusion practice in neonates (PlaNeT-1 study; Stanworth et al, 2009). PlaNet-2 compares clinical outcomes in preterm neonates randomised to receive prophylactic platelet transfusions to maintain platelet counts at or above either 25 $x10^9$/L or 50 $x10^9$/L. An interim analysis to assess trial feasibility and re-calculate the sample size will be conducted after 100 patients have completed the study period for the primary outcome.

PlaNeT-2 aims to assess whether a higher prophylactic platelet transfusion threshold is superior to the lower thresholds in current standard practice in reducing the proportion of patients who experience a major bleed or death up to study day 28. This trial will help define optimal platelet transfusion support for severely thrombocytopenic preterm neonates by evaluating the risks and benefits of two different prophylactic neonatal platelet transfusion thresholds highlighted by the PlaNeT-1 study. If superiority is demonstrated then it would have an important influence on clinical practice. If superiority is not demonstrated for a higher platelet count threshold, the potential benefits of the lower threshold in neonatal practice include fewer transfusions, reduced donor exposure, less risk of platelet transfusion related adverse effects, including errors in processing and administration, and reduced costs (see section 2).

1.1.1 Type of design

- 2-stage, randomised, parallel group, superiority trial

1.1.2 Disease/neonates studied

- Thrombocytopenic preterm neonates <34 weeks gestation at birth admitted to UK Level 2 or 3 neonatal intensive care units (NICU's) (level of unit as defined by the British Association of Perinatal Medicine; 2001) that meet the inclusion criteria will be eligible for recruitment.

1.1.3 Trial interventions

- Counselling and procedures for obtaining parental consent for the study will commence when the neonate's platelet counts fall below 100 $x10^9$/L.
- Neonates for whom parental consent has been obtained will be enrolled into the study when their platelet count has fallen below 50 $x10^9$/L and randomised into one of two arms to receive prophylactic platelet transfusions triggered by either a platelet count below 25 $x10^9$/L or below 50 $x10^9$/L.

- Consented neonates with platelet counts below 50 $x10^9$/L will be monitored using an updated version of a bleeding assessment tool, developed following the PlaNeT-1 study (Stanworth et al, 2009).
- Some neonates in each participating hospital may develop a new additional episode of thrombocytopenia (defined as platelet counts falling below 50 $x10^9$/L) after study day 14 during their hospital stay. These neonates will remain allocated to the same intervention arm for their entire length of stay in the participating hospital until discharge, death, 38 weeks Corrected Gestational Age (CGA), or Study Day 28 if this is later. In some cases Study Day 28 will fall after the baby has reached 38 weeks CGA if recruited after 34 weeks CGA.

1.2 Outcome measures

Primary outcome

- The primary outcome measure is the proportion of patients who either die or experience a major bleed up to and including Study Day 28.

This composite outcome was chosen because these endpoints represent significant clinical outcomes of relevance to the trial question.

Observation up to and including Study Day 28 (SD28) was chosen for several reasons. First, in the PlaNet-1 study all major bleeds occurred within the 28 days following onset of thrombocytopenia. Secondly, primary outcome measures in many studies in this patient population are measured at Study Day 28.

Secondary outcomes include:

- Proportion of patients surviving to go home following a major bleed, censoring at discharge
- Proportion of patients surviving to go home without having had a major bleed, censoring at discharge
- Proportion of patients who have died up to Study Day 28
- Proportion of patients who sustain a major bleed up to Study Day 28
- The rate and time from randomisation of minor, moderate and major bleeding derived from the bleeding assessment tool up to study day 14, and for major bleeds up to Study Day 28. Definitions for all grades of bleeds are shown in the bleeding assessment tool; grading will be assigned based on a modified version of the WHO Bleeding Score
- Number of platelet units transfused up to Study Day 28
- Time to discharge home, censoring on death
- Neuro-developmental outcome as assessed by the Thames Regional Perinatal Outcome Group/ Standard Electronic Neonatal Database/ National Neonatal Audit Programme (TRPG/SEND/NNAP) 2- year corrected age outcome form at 2 years corrected postnatal age. This will provide a basis of correlation with the WHO definition of the functional disability score
- Platelet transfusion-related adverse events up to discharge (section 7.6 and 8.3.2)

Data collection for assessment of outcome measures

Evidence of all types of bleeding will initially be recorded in all neonates after randomisation up to Study Day 14 using a daily bleeding assessment tool, adapted for neonates from the WHO bleeding system of grading and the PlaNeT-1 study. In the PlaNeT-1 study, most major bleeds occurred within 14 days after birth, and neonates were more likely to show evidence of minor bleeding with thrombocytopenia in the first 10 days of life, than after 10 days.

- For neonates still in hospital at Study Day 14, daily bleeding assessments will cease and a weekly data collection form will be started and continued until the time of discharge, death, 38 weeks Corrected Gestational Age (CGA), or Study Day 28 if this is later. In some cases Study Day 28 will fall after the baby has reached 38 weeks CGA if recruited after 34 weeks CGA.
- Weekly data collection:
  The weekly data collection form will collect data on new major bleeds, mortality, platelet counts, platelet transfusions and cranial USS.
- Discharge home before Study Day 28:
  For those (occasional) neonates who are discharged home before Study Day 28, a telephone call follow-up by the local site research team to parents or GP will be made to determine if there were any serious adverse events in that time. Prior consent for telephone contact will be sought.
- End of Study:
  An End of Study form will be completed at the time of death, NICU discharge home, 38 weeks CGA or at Study Day 28 if this is later (for those neonates recruited after 34 weeks CGA). For neonates transferred whilst on the study to another unit participating in Planet, data collection will continue to end of study.
- Transfer out to non-participating units before Study Day 28:
  For neonates transferred to a non-participating unit before reaching Study Day 28, basic information will be requested from the non participating (receiving) unit in order to obtain the primary outcome data. A data collection form will be sent with the baby to the receiving unit with a copy of the consent form, requesting information on number of platelet transfusions, occurrence of any safety events and the results of any imaging -5 / +10 days of SD28. The local research nurse from the recruiting site will be responsible for liaising with the staff in the receiving unit. The trial manager will be available for any queries. The receiving unit will be asked to send pseudo anonymised primary outcome data back to the recruiting centre who will in turn pass the information on to the NHSBT CTU. After this point no further information will be requested
- Transfer out to non-participating units after Study Day 28:
  For babies transferred to a non-participating unit after Study Day 28, (post primary outcome), no data will be requested from the receiving unit
- Further episodes of thrombocytopenia:
  Some neonates in hospital may develop a new additional episode of thrombocytopenia after study day 14 during their hospital stay with platelet counts again falling below 50 $x10^9$/L. These neonates will remain allocated to the same intervention arm until the time of discharge, 38 weeks Corrected Gestational Age (CGA), or Study Day 28 if this is later.
- Two year follow up:
  A developmental follow-up will be undertaken using a validated Thames Regional Perinatal Outcome Group/ Standard Electronic Neonatal Database/ National Neonatal Audit Programme (TRPG/SEND/NNAP) 2- year corrected age outcome form at the corrected postnatal age of 2 years.

  As some children will not receive a formal assessment, or in some cases the results will be unobtainable to the trial team, all parents will be sent a self-completion questionnaire by post. This questionnaire will take the form of an amended PARCA-R survey (Parent Report of Children's Abilities for very Premature Infants) with supplementary questions designed specifically for PlaNeT-2. Parent / guardian contact details (address, phone number and email address) will be collected at point of trial entry and at end of study (to confirm no change) and entered on to a detachable portion of the Case Report Form (CRF) which will be returned to the NHSBT CTU. These details will be held centrally on a separate database from that containing clinical data and will be used by the 2 year follow-up co-ordinator to contact parents when their child is 2 years of age (corrected) prior to sending out the postal questionnaire

(see Fig 3). If the questionnaire is not returned the co-ordinator will contact parents to remind them, or if preferred by parents, will conduct the survey over the phone or suggest it is undertaken online.

As a further alternative to obtain this data, agreement will be sought when discussing the initial informed consent to contact parents / GP and Health Visitor at around 2 years CGA.

Using the National Database:

Where primary outcome and 2 year follow-up data cannot be obtained by direct contact with parents or clinicians, it may be possible to extract pseudo-anonymised information from the national neonatal database. The research team at the recruiting centre will be able to access this data.

1.3 End of Trial Date

The expected dates for the following study milestones are as follows:

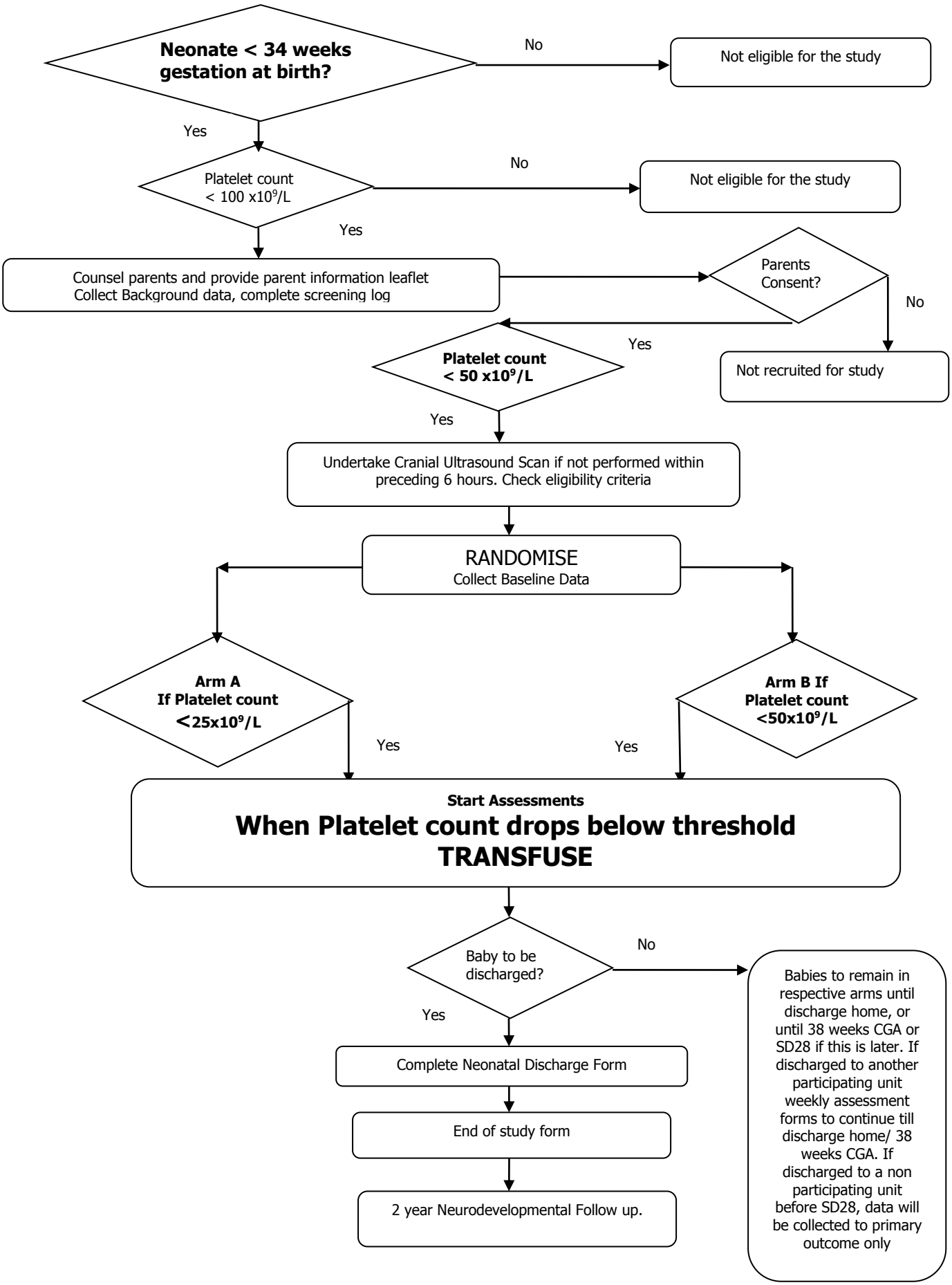
- Last Randomisation Date: 31.12.2017
- Latest possible End of Study date for baby potentially randomised on 31.12.2017 is 31.03.2018
- Latest possible date for 2 year follow-up: 31.12.2019

1.4 Parent and Public Engagement

In line with recommendations produced by INVOLVE and the National Research Ethics Service (NRES) we will encourage greater parent and public engagement in PlaNeT-2. We intend to involve parental perspectives in the design of a parent webpage on the trial website (www.planet-2.com) by consulting with groups such as BLISS and local parent support groups. This page will be used to showcase parent 'stories' about the trial and opinions on research generally. It will also be used to disseminate results as they become available. A separate consent form will be available for this involvement and will cover use of photos and videos for the website. These will all be entirely optional.

Parent 'stories' will also be used in the PlaNeT-2 newsletter which is circulated solely to clinicians. Eventually we would like to develop a separate newsletter for parents and this will be designed in consultation with parent groups. Feedback on parental experience of the trial will be used to improve the experience for other participants where possible.

Figure 1.0: Summary of trial entry, randomisation and platelet treatment



2 BACKGROUND

2.1 Introduction and relevant studies

The rationale for this trial is the need to establish whether the *benefits* of platelet support outweigh the risks, and to better define a safe strategy for platelet transfusion in preterm neonates. Previous studies estimate that 25% of neonates whose platelet counts fall below 150 $x10^9$/L (Del Vecchio et al 2001; Roberts and Murray, 2003) receive one or more transfusions and this increases to 50% in extremely low birth weight (birth weight <1000g) neonates (Baer et al 2007).

Neonatal thrombocytopenia and bleeding are common and important clinical problems for very preterm neonates. Platelet transfusions are frequently used in modern neonatal clinical practice as prophylaxis in thrombocytopenic neonates (Stanworth et al 2009; Strauss, 2008; Murray et al; 2002; Sola 2004). However, policies and protocols for neonatal platelet transfusion therapy vary widely between clinicians and institutions, reflecting the generally broad nature of recommendations in national guidelines, which themselves are based largely on consensus rather than evidence (Calhoun et al, 2000; BCSH, 2004; Saxonhouse et al, 2004 Josephson et al, 2009) and are often extrapolated from adult data. Current national guidance in the UK (BCSH 2004 Handbook of Transfusion Medicine, 2007) recommends platelet transfusion thresholds of 20-30 $x10^9$/L for neonates depending on the clinical situation.

Recent surveys have highlighted the disparities in clinical practice with regard to platelet transfusion in preterm neonates. In the UK, a telephone survey of all tertiary level neonatal units has shown variation in platelet transfusion practice among different neonatal units, but the most common thresholds for transfusion were 25 and 30 $x10^9$/L in well or stable infants at term and preterm, respectively (Chaudhury et al 2008). A large web based survey of neonatologists in Canada and USA has also shown differences in practice with regards to platelet transfusion (Josephson et al, 2008), and some neonatal units reported the adoption of higher thresholds up to 100 $x10^9$/L for routine prophylaxis.

In the only randomised controlled trial to assess a threshold level for the effectiveness of neonatal prophylactic platelet transfusions, moderate thrombocytopenia (defined as 50-150 $x10^9$/L) was not detrimental to short-term neonatal outcome. However, neonates with severe thrombocytopenia and platelet counts less than $50x10^9$/L were excluded from the study (Andrew et al, 1993) because of their perceived high risk of haemorrhage. The study was aimed at evaluating the 'benefit' of maintaining a 'normal' platelet count, to prevent deterioration in unstable neonates, and did not address the issue of lower transfusion thresholds used currently for many preterm neonates.

Until recently, other evidence supporting platelet transfusion practice has been limited and comes from a small number of retrospective case note studies of platelet transfusion practice in neonates which pointed to variable rates of transfusion some as high as 9% of all admissions (Del Vecchio et al, 2001; Garcia et al, 2001, Murray et al, 2002). In all these studies, the majority of platelet transfusions were given prophylactically to non-bleeding neonates. Significantly, Murray et al, (2002) did not demonstrate increased haemorrhage irrespective of whether or not platelets were administered to neonates with severe thrombocytopenia. In this retrospective study 53 (6%) of all neonates admitted to NICU developed severe thrombocytopenia. Twenty-seven neonates received a total of 63 platelet transfusions, the main triggers being: platelet count less than 30 $x10^9$/L, or less than 50 $x10^9$/L in those with previous haemorrhage or clinical instability. No major haemorrhage occurred during severe thrombocytopenia either in neonates in whom platelet transfusions were withheld (26/53) or in neonates given platelets who survived to discharge (22/27). Establishing more evidence-based trigger thresholds for platelet transfusion is complicated by

the clinical diversity in this patient group (e.g. gestational age, post-natal age, presence of coagulopathy, mechanism of thrombocytopenia including consumption vs. production; see Roberts & Murray, 2003).

In order to help clarify current treatment of severe thrombocytopenia in preterm neonates and provide baseline information for a clinical trial, we carried out the first prospective study of neonatal platelet transfusion practices (PlaNeT-1 study, Stanworth et al, 2009), in which we collected detailed information both about indications for transfusion and outcome (including bleeding). In this study we observed platelet transfusion practices in 7 UK neonatal centres. 169 neonates with platelet counts of $<60x10^9$/L were enrolled in this study. In this study, major haemorrhage occurred in 13% of severely thrombocytopenic neonates (intraventricular haemorrhage 53%, pulmonary 26%, renal 11%, 'other' 10%), the vast majority (84%) of neonates who had a major haemorrhage were <30 weeks gestation at birth. The study confirmed previous data that sepsis and necrotising enterocolitis (NEC) are common underlying diagnoses in thrombocytopenic neonates who bleed. In contrast, neonates with thrombocytopenia secondary to intra uterine growth retardation or pregnancy-induced hypertension tend not to have major haemorrhage despite severe thrombocytopenia. There was no clear relationship between major bleeding and platelet counts in the PlaNeT-1 study (Stanworth et al, 2009), and further analysis of all bleeding events (including minor bleeds) indicates no evidence for a significant increase in rates of bleeding with severe thrombocytopenia (data unpublished; manuscript in preparation). Data from the PlaNeT-1 study also showed that 69% of all neonates with a platelet count below 60 $x10^9$/L were given a platelet transfusion. The findings in PlaNeT-1 (platelet thresholds for transfusion illustrated in Figure 1) highlighted the clinical uncertainty and variation in practice but also provided information about the thresholds in place at those NICU's who have indicated a willingness to participate in this trial.

Recent randomised trials addressing red cell transfusion triggers for anaemic neonates (Bell et al 2005 and Kirpalani et al 2006) have contributed to the debate on appropriate neonatal red cell transfusions. However, a comparable platelet trigger trial, assessing clinically relevant outcomes, has never been undertaken in preterm neonates with severe thrombocytopenia (platelet counts<50 $x10^9$/L).

Platelet transfusions also carry risks. These potential risks related to any transfusion are generally well known, including both errors and reactions to blood components. Interpretation of the data from the UK Serious Hazards of Transfusion (SHOT) national haemovigilance scheme against a population based epidemiological study of transfused patients has suggested that a disproportionate number of adverse events occur in paediatric compared to adult transfusion practice, and more so in infants and neonates (Stainsby et al, 2008). In the most recent SHOT report (2008), 31/92 (34%) of paediatric incidents were in infants (babies under 1 year of age), of whom 20/31 (65%) were neonates ≤4 weeks old, and the majority of these events were related to transfusion errors, including incorrect blood component transfused. The lack of reactions to transfusions in this age group reported to SHOT may be due to either patients' immunological immaturity or to non-recognition of reactions in infants already sick from other causes. There have been additional concerns about adverse reactions to platelet transfusions in neonates. There are reports suggesting an association between repeated platelet transfusions and adverse events such as hepatic dysfunction following necrotising enterocolitis (Kenton et al, 2005). Furthermore, one neonatal study demonstrated an increased incidence of bacterial infection from 5% in non-thrombocytopenic neonates to 45% in neonates who received >10 platelet transfusions (Baer et al 2007). The risk of bacterial contamination is estimated at 15 per million donations but platelets are the blood component most likely to be contaminated by bacteria as they are stored at room temperature. This risk has recently been highlighted via a national transfusion alert from the Royal College of Paediatrics and Child Health and NHSBT following an episode of bacterial contamination of platelet concentrates in an infant (www.rcpch.ac.uk/doc.aspx?id_Resource=5111). SHOT (2008) reported a striking increase in

acute transfusion reactions in children, of which 72% were to platelets, and which were disproportionately high in children compared to adults. Only 2/25 of these reports were from infants, with one ≤ 4 weeks old, but again this may be due to lack of recognition in the infant age group.

There are other possible adverse outcomes of transfusion which may be particularly relevant for neonates. Concerns of possible transfusion-transmitted variant CJD (vCJD) may be particularly relevant for neonatal transfusion practice, given the long life expectancy of many of these recipients. Moreover, side effects related to transfusions do not just reflect the adverse events or reactions related to the blood components themselves but also the problems of maintaining vascular access in a NICU with risks of extravasated/infected intravenous cannulae.

2.1.1 Principal clinical research question being addressed

Is a prophylactic platelet transfusion policy for preterm neonates based on a higher threshold blood platelet count of <50 $x10^9$/L superior to one based on a lower threshold platelet count of <25 $x10^9$/L with regard to mortality and major bleed events as measured up to Study day 28 after randomisation?

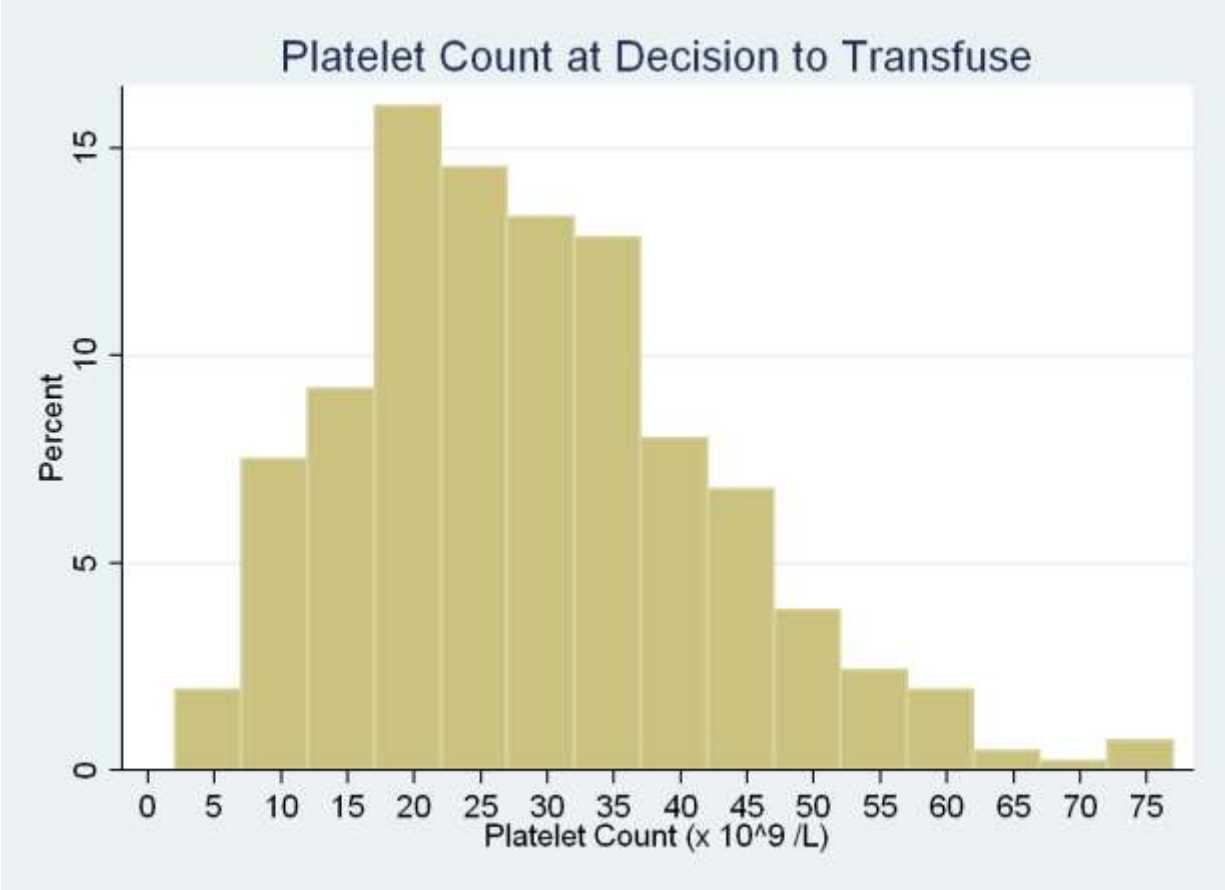
2.2 Justification for platelet count thresholds in this randomised trial

The justification for selection of lower and higher platelet count thresholds at 25 $x10^9$/L and 50 $x10^9$/L for this trial is based on:

1. Current national guidance (BCSH 2004 Handbook of Transfusion Medicine, 2007) recommends platelet transfusion thresholds of 20-30 $x10^9$/L and 50 x 10^9/L for neonates depending on the clinical situation.
2. In a UK survey, the most common stated thresholds for transfusion were 25 and 30 $x10^9$/L in well infants at term and preterm, respectively (Chaudhary et al, 2009).
3. In PlaNet-1 most transfusions were given at platelet counts between 10 and 50 $x10^9$/L (see histogram below). The 50th and 90th centile pre-transfusion platelet count thresholds observed in the PlaNeT-1 study were 27 and 48 $x10^9$/L.
4. In PlaNeT-1, 173 /415 (42%) transfusions were administered at a minimum platelet count of <25 $x10^9$ /L, and 383/415 (92%) transfusions were administered at a minimum platelet count of <50 $x10^9$/L, so there should be clear difference in the intervention between the two arms of this proposed trial
5. The recognition that in the only randomised controlled trial to assess a threshold level for the effectiveness of neonatal prophylactic platelet transfusions, the lower platelet count threshold was 50 $x10^9$/L (Andrew et al, 1993).

The figure below shows the variation of platelet count threshold used for transfusion in the PlaNeT-1 study. This was an observational study and decision to administer a platelet transfusion was at the discretion of the attending clinician.

Figure 2.0 Lowest Platelet Count (x10^9 /L) recorded prior to Transfusion in the PlaNeT-1 Study



The above graph has been truncated to exclude 1 out of 415 transfusions where the platelet count was 266 x 10^9/L but the neonate was given a transfusion due to its clinical condition.

3 SELECTION OF CENTRES/CLINICIANS

3.1 Centre/Clinician eligibility criteria

Centre selection will be based on relevant clinical and research infrastructure, adequate resources and facilities to support recruitment, and adequate qualified staff to conduct the trial properly and safely. Centres are required to have the facility and expertise to perform cranial ultrasound scans around the clock. Centres are expected to care for neonates providing intensive and high dependency care as per British Association of Perinatal Medicine (BAPM) (2001). The TMG will invite eligible centres and will discuss the resources required for conducting the trial, including the importance of training and education to the clinical staff on the use of the bleeding assessment tool.

All UK laboratories should be Clinical Pathology Accreditation accredited, and National External Quality Assurance Scheme participants.

The trial will start at the centres listed in section 18. Additional trial centres across the UK will be approached as required once the trial is successfully up and running at the initial centres.

4 SELECTION OF NEONATES AND CONSENTING

Neonates will be identified by the research team at trial centres and assessed as to their eligibility to enter the trial according to the criteria below. Identification and counselling of parents/guardians will start when the platelet count falls below 100 $x10^9$/L. A trial screening log will be completed for all neonates admitted to selected participating units with platelets <100 $x10^9$/L and gestational age (GA) <34 weeks. This will record data such as GA, post-natal age, gender and main diagnosis. The log will include neonates not approached, and those neonates approached but in whom consent was not obtained for the trial (with reasons) and will define how representative randomised neonates are as a group relative to the group of eligible neonates who were not randomised.

4.1 Obtaining Consent

Parents/ guardians will be counselled when the platelet count is below 100 $x10^9$/L. At this stage a Parent Information Leaflet will be provided. Written, informed consent will be obtained. As part of the consent process, it will be explained to the parents/ guardians that randomisation will occur only if the platelet count falls below 50 $x10^9$/L. It is estimated that platelet count will drop to below 50 $x10^9$/L in about 50% of neonates with platelet counts below 100 $x10^9$/L. If the baby's platelet count does not drop to below 50 $x10^9$/L they will not be randomised and will not participate in the study.
For neonates with an initial platelet count of <50 $x10^9$/L, parents will be approached for consideration of immediate study participation.

4.2 Patient inclusion criteria for randomisation

1. Written informed consent obtained
2. Admission to a participating NICU (includes postnatal transfers)
3. <34 weeks gestational age at birth
4. A platelet count of <50 $x10^9$/L
5. Cranial ultrasound scan must have been undertaken less than 6 hours prior to randomisation in order to rule out recent major Intraventricular Hemorrghage (IVH) (refer to section 6.4)

4.3 Patient exclusion criteria for randomisation

1. Major/life-threatening congenital malformations (e.g. chromosomal anomalies, Fanconi's anaemia, Thrombocytopenia Absent Radius syndrome).
2. The occurrence of a major/ severe bleed within the previous 72 hours. However, the neonate may be eligible for randomisation later, once 72 hours has elapsed, provided there are no further major bleeds and the baby meets all the inclusion criteria (refer to Section 6.4)
3. All foetal intracranial haemorrhages excluding subependymal haemorrhage from any antenatal ultrasound scan.
4. Known immune thrombocytopenia or family history of alloimmune thrombocytopenia or maternal antiplatelet antibodies or maternal idiopathic thrombocytopenic purpura.
5. Neonates judged by the attending neonatologist to be unlikely to survive more than a few hours at the time of proposed randomisation.
6. Neonates who were *not* given parenteral Vitamin K after birth.

4.4 Co-Enrolment

Participation in other neonatal clinical studies is permitted as long as the other studies do not influence the outcomes or end points or compromise the overall study design and delivery of the Planet 2 study.

Babies randomised to the Planet 2 study could also be enrolled in studies such as Baby OSCAR, PREVAIL, PINE and ELFIN as long as the Chief Investigators for these studies are happy with this.

5 RANDOMISATION & ENROLMENT PROCEDURE

5.1 Summary of Randomisation Process

Counselling and consent for the trial will occur when the platelet count falls below 100 $x10^9$/L. Randomisation will occur when the platelet count falls below 50 $x10^9$/L. Neonates will be allocated to one of the two groups by the participating clinicians at each centre using an independent centralised 24 hour web-based randomisation service, as described below. The neonate will be assigned a trial number (6 digits, the first 3 digits will be the centre number and the remaining 3 digits will be the patient number from 001 to 999), and allocated to either Arm A or Arm B. The trial number will also be entered in the screening log. The randomisation service will send confirmation to the PI of the trial number and the allocated treatment policy and inform the Trial Manager. Once the neonate has been randomised, data collection on bleeding will commence. Unless there are other clinical reasons, for neonates in Arm A, a platelet count threshold of below 25 $x10^9$/L will be used as a trigger for platelet transfusion. For neonates in Arm B, a platelet count threshold of below 50 $x10^9$/L will be used as a trigger for platelet transfusion.

5.2 Details of Registration Process and Randomisation

1. The local PI (or designate) will be responsible for identifying and consenting the parents/guardians of eligible neonates once the platelet count has dropped to <100 $x10^9$/L.
2. A screening sheet with a log number will be completed on all neonates admitted to selected participating units with platelets <100 $x10^9$/L and GA <34 weeks. The log will include GA, post-natal age, birth weight and main diagnosis. It will include neonates not approached, and those neonates approached but in whom consent was not obtained for the trial (with reasons) and therefore will define how representative randomised neonates are as a group relative to the group of eligible neonates who were not randomised.
3. Eligibility for randomisation will be assessed with reference to inclusion and exclusion criteria.
4. When the neonate's platelet count falls to <50 $\times 10^9$/L, the PI or designate (the randomiser) will ensure a cranial ultrasound has been undertaken within the last 6 hours, and will then complete the trial registration/randomisation form and access the web based randomisation service at http://www.sealedenvelope.com to obtain a unique trial number and assignment of treatment policy. Before logging onto the website to randomise the neonate, the following pieces of information must be available: the neonate's gestational age at birth (in weeks and days); knowledge of presence or absence of IUGR; the most recent platelet count (which must be below 50$x10^9$/L) and the date and time of the most recent cranial USS, which must have been undertaken within 6 hours before randomisation.
5. Following randomisation, the randomisation service will e-mail confirmation of the allocated treatment policy and trial number to the PI and Trial Manager. The PI will place the emailed allocated treatment policy in the neonate's medical notes.
6. The local PI/designate is responsible for informing the neonate's consultant and the parents of the neonate's treatment allocation, and for placing a label indicating trial participation on the cot and cover of the neonate's medical notes.

7. The local PI/designate will complete the patient's screening sheet adding the patient's trial number to the existing data. The local PI/designate will also be responsible for informing the Blood Bank of the trial number and the trial treatment allocation.
8. The treatment allocation will persist for the length of stay in the centre participating in the study. Neonates who develop repeated episodes of thrombocytopenia of less than 50 $x10^9$/L will remain on the same treatment arm that they were allocated to when first enrolled.
9. Twins will be randomised separately and will be allocated to their individual treatment arm as per randomisation.

RANDOMISATIONS

http//:www.sealedenvelope.com

Randomisations can also be undertaken through the Planet-2 website:

www.planet-2.com

Click on the red button in the centre of the home page to randomise

6.TREATMENT OF NEONATES

6.1 Introduction

Neonates will be allocated to one of two treatment policies (treatment arms) at randomisation. The intervention is a platelet transfusion at two different platelet count thresholds. If there is uncertainty about the accuracy of a platelet count at any time (e.g. spurious due to traumatic venesection, blood sample is reported to show clots) the peripheral blood count should be repeated.

6.2 Arm A Standard (platelet transfusions at platelet counts <25x10^9/L)

Prophylactic platelet transfusions should be given only below platelet counts of 25 x10^9/L, for neonates with no bleeding or with only minor bleeding **(for definitions see Section 6.4 below**)

Additional platelet transfusions may be considered at the discretion of the attending neonatologist, at platelet counts higher than the allocated threshold, under the following circumstances:

- Therapeutically to treat major bleeding, following objective and documented signs of clinically relevant bleeding graded as moderate, major or severe, but **not** for minor bleeding (**for definitions see Section 6.4 below).**
- Prior to planned invasive procedures (including Suprapubic Aspiration and Lumbar Puncture but not percutaneous central lines or arterial line insertion) or major surgery where haemostasis may be critical to outcome.

Specific situations which will **not** generally be considered indications for platelet transfusion outside of the allocated threshold are as follows:

- Prior to planned insertion of percutaneous central lines or arterial lines
- Planned or current indomethacin (or ibuprofen) treatment of patent ductus arteriosus.
- Extreme prematurity without additional risk factors, irrespective of the postnatal age of the infant
- Any platelet count ≥25 x10^9/L, irrespective of previous values or rate of drop, in the absence of the exceptions noted above. 'Pre-emptive' transfusing to keep the platelet count above the allocated threshold will constitute a protocol violation. In situations where the count appears to be falling, clinicians are at liberty to repeat the platelet count earlier than planned if concerned about an impending drop below the threshold.

All instances of platelet transfusion outside this study protocol must be recorded on the daily transfusion data collection form.

6.3 Arm B Intervention (platelet transfusions at platelet counts <50 x109/L)

Prophylactic platelet transfusions should be given only below platelet counts of 50 x10^9/L, for neonates with no bleeding or with only minor bleeding (**for definitions see Section 6.4 below**)

Additional platelet transfusions may be considered at the discretion of the attending neonatologist, under the following circumstances:

- Therapeutically, following objective and documented signs of clinically relevant bleeding graded as moderate, major or severe, but **not** for mild bleeding (**for definitions see Section 6.4 below).**
- Prior to planned invasive procedures (including Suprapubic Aspiration and Lumbar Puncture, but not percutaneous central lines or arterial lines) or major surgery where haemostasis may be critical to outcome.

Specific situations which will **not** generally be considered indications for platelet transfusion outside of the allocated threshold are as follows:

- Prior to planned insertion of percutaneous central lines or arterial lines
- Planned or current indomethacin (or ibuprofen) treatment of patent ductus arteriosus.
- Extreme prematurity without additional risk factors, irrespective of the postnatal age of the infant
- Any platelet count ≥50 $x10^9$/L irrespective of previous values or rate of drop, in the absence of the exceptions noted above. 'Pre-emptive' transfusing to keep the platelet count above the allocated threshold will constitute a protocol violation. In situations where the platelet count appears to be falling, clinicians are at liberty to repeat the platelet count earlier than planned if concerned about an impending drop below the threshold.

All instances of platelet transfusion outside this study protocol must be recorded on the daily transfusion data collection form.

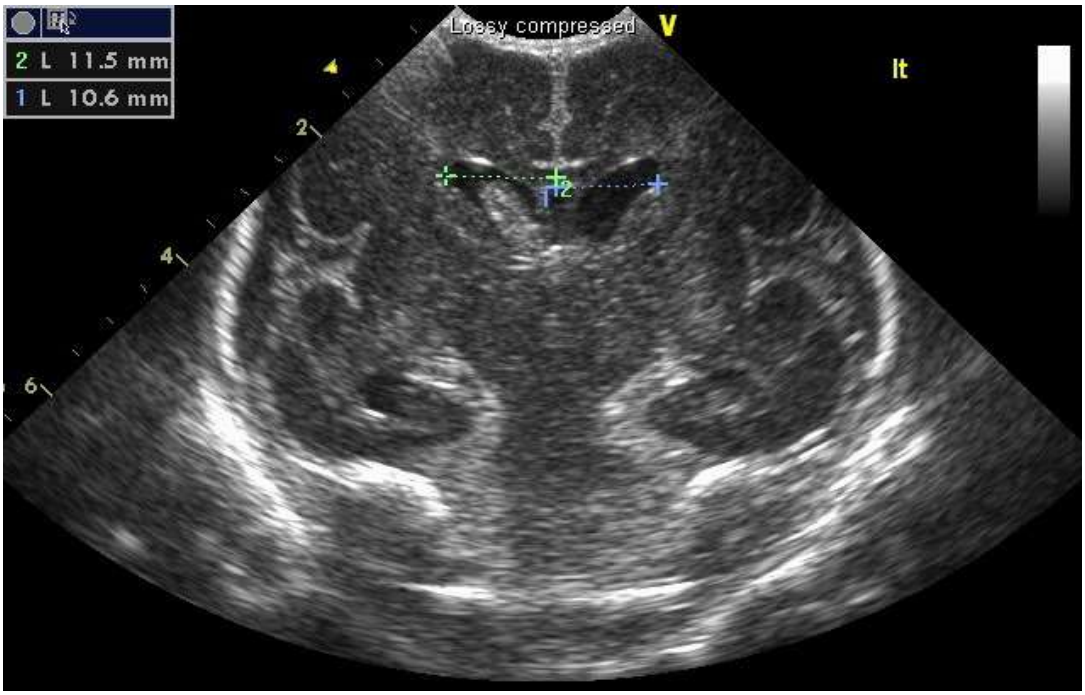
6.4 Definitions of Bleeding Grades including IVH

IVH will be graded as shown in the table below:

Haemorrhage	
H0	No haemorrhage
H1	Germinal Layer Haemorrhage (GLH) which includes subependymal haemorrhage and localized choroid plexus haemorrhage
H2	IVH extent< 50% of the ventricle
H3	IVH extent ≥ 50% of the ventricle, or large intraparenchymal / intracerebral haemorrhage (i.e. > 2cms)
Ventricular size	
V0	No ventricular dilatation/ ventricular dilatation ≤ 12 mm
V1	Dilatation > 12 mm.
Parenchymal injury	
P0	No injury
P1	Echodensity measuring < 1 cm (max diameter in any plane)
P2	Echodensity measuring 1 – 2 cm (max diameter in any plane)
P3	Echodensity measuring > 2 cm (max diameter in any plane)
PC	Porencephalic cyst
PVL	Periventricular Leukomalacia (PVL)

Measurement of ventricular Index:

In order to assess ventricular dilatation, the ventricular index will be measured. The measurement will be taken from each side of the lateral wall of the body of the lateral ventricle to the falx, at the level of foramina of Munro. Measurement has to be in the horizontal plane as illustrated below:



For the purposes of this study, bleeding will be graded according to the following definitions:

I Minor:
Any bleed from skin, umbilical cord, skin around stoma, surgical scar, mucosa. Any pink frothy or old bleed from the ET tube. H1 haemorrhage on cranial US (Germinal Layer Haemorrhage, GLH, which includes subependymal haemorrhage and localised choroid plexus haemorrhage)

II Moderate:
Any frank bleed from the stoma, macroscopic haematuria, or, acute fresh bleed through the endotracheal tube (ETT) without ventilatory changes**.**
Moderate IVH is defined as

- H2 or H3 without dilatation (V0)
- H0, H1, H2, H3 with P2.

III Major:
Frank rectal defined as macroscopic faecal bleed (not if only occult positive). Pulmonary bleed defined as acute fresh bleed through the endotracheal tube (ETT) associated with increased ventilatory requirements or the need for intubation and ventilation.
An intracranial bleed is defined as a major bleed if any of the following apply:
Neurosurgical intervention is required
Radiological imaging showing a midline shift
Clinical signs and symptoms of o deficit with significant derangement of laboratory investigations.
Major IVH is defined as

- H2 or H3 with ventricular dilatation (V1)
- H1, H2, H3 with parenchymal involvement (P3)
- Any evolution of intracranial haemorrhage to H2V1, H3V1, or (H1, H2, H3) with parenchymal involvement (P3)

IV Severe:
Fatal major bleeding or shock is defined as life threatening major bleed associated with hypotension, hypovolaemia or any other haemodynamic instability and/or bleeding requiring volume boluses or red cell transfusion in the same 24 hours.

6.5 Platelet prescribing and dose

The platelet transfusion dose will be 15ml/kg. Platelets for neonates will be issued from the hospital blood bank according to local standard operating procedures.

Current UK specifications for apheresis platelets are a platelet cell content >240 $x10^9$ per unit.

6.6 Other blood components - Dispensing and Accountability

Red cells, fresh frozen plasma, cryoprecipitate and other blood products (e.g. fibrinogen concentrate) will be transfused according to local departmental guidelines. Departmental policies on transfusion will be reviewed from each participating centre. Current local guidelines for vitamin K prophylaxis will be followed.

All neonatal units will have local policies in place describing the administration of blood components including platelets, which will include the management of transfusion side effects. All blood transfusion components in the UK, including red cells and platelets, are standardised and conform to national specifications. From 1999, all allogeneic blood components produced in the UK have been subjected to a pre-storage leukocyte filtration process. Normal blood bank procedures will be followed for stock control and issuing of platelet units and patient details.

6.7 Early stopping of the allocated treatment

If in the clinical judgement of the attending neonatologist, an enrolled neonate cannot follow treatment allocation (e.g. a higher platelet transfusion threshold is clinically warranted), the case should be discussed with the joint chief investigator or one of the medical experts. Irrespective of the clinical decision, data collection should continue, aiming to complete data collection at least up to study day 28. Reasons for deviation from the randomised treatment allocation will be recorded.

6.8 Other Medications

Any clinically-indicated medication is permitted during the time the patient is in the PlaNet-2 trial; there are no restrictions on concomitant medications.

7. ASSESSMENTS AND PROCEDURES

7.1 Introduction

It will be the responsibility of the local researcher(s) to identify eligible neonates with a platelet count below 100 $x10^9$/L, using the patient eligibility checklist. Having identified an eligible neonate, the local PI or designate (e.g. research nurse) will approach the parents or guardians to discuss the trial using the patient information sheet and to seek their consent. Once informed consent has been obtained, a copy of the signed consent form and a copy of the Parent Information Leaflet (PIL) should be given to the parent. The local researcher should keep the original consent document in the site file and a copy must be filed in the neonate's medical notes.

Data collection will be the responsibility of the local clinical team led by the local PI and study co-ordinator at each site. Data will be recorded on paper record Case Report Forms (CRFs) which will be transferred onto MACRO Trial Manager Software at the CTU. CRFs will be used to collect data from study entry. Three patient identifiers (Patient ID allocated at randomisation, initials and date of birth) will be used on all CRFs.

7.2 Schedule of Assessments

Assessment to be performed	**Pre-randomisation (a)**	**At randomisation (b)**	**Study Day**																**SD 29 to End of Study**	**2 year follow up**
			1	2	3	4	5	6	7	8	9	10	11	12	13	14	21	28 (d)		
Registration	X																			
Informed Consent	X																			
Randomisation		X																		
Cranial USS (c)		X							X							X	X	X	X	
Daily bleeding Assessment			X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Transfusion adverse event form			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Weekly assessment form(d)																	X	X	X (f)	
Serious Adverse Events			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
End of Study Form (e)																			X	
TRPG/SEND/NNAP 2-year corrected age outcome form																				X

a: Registration and consent when platelet count falls below $100x10^9/L$

b: Randomisation when platelet count falls below $50x10^9/L$

c: Cranial USS: to be performed no more than 6 hrs pre randomisation and at least weekly thereafter up to STUDY DAY 28. Thereafter scans are at the discretion of the clinicians apart from an End of Study Scan which must be undertaken within 7 days prior to discharge. (refer to s7.11)

d: Weekly assessment will be completed until End of Study. Collect data on major bleeds, mortality, cranial USS, platelet count, platelet transfusion, NEC and sepsis. For babies discharged home before study D28 a telephone call will be made to parents around SD28 in order to monitor any further AEs / SAEs that have occurred since discharge providing prior consent to contact has been given (refer to s.1.1.5 & s.7.6)

e: End of study form: An End of Study form will be completed at the time of death, NICU discharge home, 38 weeks CGA or at Study Day 28 if this is later (for those neonates recruited after 34 weeks CGA). For neonates transferred whilst on the study to another unit participating in Planet, data collection will continue to End of Study (refer to s1.1.5)

f: The first weekly data collection form to be completed after SD 28 is on SD 35.

7.3 Procedures for data collection

Assessments will be performed according to the schedule in Section 7.2. Prime responsibility for the complete collection of data for each centre will reside with the local PI but may be delegated (e.g. research nurse). Overall responsibility for collating data from all centres will reside with the Trial Manager. Local centres will send copies of completed CRFs to the CTU Data Manager, at Study Day 28 and then at End of Study. The procedure for SAE reporting is detailed in Section 9.

7.4 Randomisation

Background data will be collected before or at the time of randomisation including:

- gestational age at birth
- weight at birth
- gender
- postnatal age in days
- weight at enrolment
- co-existing or prior medical conditions/ diagnosis including major bleeds, IUGR, NEC ≥Stage 2 defined as per Bell's criteria (Bell et al; 1978) and sepsis

Once final eligibility is confirmed, the neonate will be randomised into the study according to the procedures described in Section 5

7.5 Data for daily bleeding and outcome assessments up to 14 days

A bleeding assessment form will be completed daily for all study subjects for 14 days after randomisation. The daily bleeding assessment will be performed by the local PI or designate e.g. research nurse (RN) or research staff or other ward staff as agreed locally. The first bleeding assessment form completed within 24 hours after randomisation will be Study Day 1. The PI and designated staff completing the daily bleeding assessments will receive training and have guidance notes to help them collect these data. Particular attention will be given to the repeated training of local designated site staff in the correct use of the bleeding assessment tool.

For the purposes of this trial, the period of observation for daily data collection is defined as:

- From study day 1 (within 24 hours after randomisation; when platelet count below 50 x109/L) and ending at study day 14, or death whichever is earlier

Information on clinical bleeding outcomes and transfusions will be collected prospectively through clinical assessment and interrogation of patient case records undertaken by the local designated site staff on a daily basis for the complete period of observation. The daily CRF for the collection of clinical bleeding outcomes and transfusions is based on a modified version of the bleeding assessment tool adapted for neonates from the WHO bleeding assessment tool and the results of the multi-centred prospective PlaNeT-1 study.

Decisions regarding prophylactic platelet transfusions will be made by ward staff on the basis of the baby's platelet count as outlined in the flow chart for both arms of the trial, shown at start of section 5. There may be situations where clinicians give additional platelet transfusions not according to the trial flow chart and these will be recorded (see 6.2, 6.3 above).

7.6 Data for major bleeding and outcome assessments from study day 14 until discharge: weekly data collection

For neonates still in hospital at study day 14, daily data collection will cease and a weekly data collection form will then be started and continued until the time of death, discharge home, 38 weeks CGA or at Study Day 28 if this is later (for those neonates recruited after 34 weeks CGA). This will be regardless of whether the neonate's thrombocytopenia has resolved or whether the neonate has further episodes of thrombocytopenia (see 7.7). The weekly form will collect data on new major bleeds, mortality, platelet counts, platelet transfusions, NEC, sepsis extravasation injuries and adverse events related to platelet transfusions, including those reported to the UK national haemovigilance reporting schemes (SHOT and SABRE, see 8.3.2)

7.7 Neonates with further episode(s) of thrombocytopenia after study day 14

After randomisation, allocation will apply for the entire inpatient stay, including for neonates that develop new episodes of thrombocytopenia after study day 14, until 38 weeks CGA or at Study Day 28 if this is later. Neonates who have a further episode of thrombocytopenia <50 $x10^9$/L will therefore remain in the same treatment arm that they were allocated to when first enrolled. Daily bleeding assessment forms are not required after Study Day 14 even if thrombocytopenia recurs but weekly assessment forms will be completed (see Figure 7.2).

7.8 Neonates with platelet counts that remain <50 x109/L after study day 14

Some neonates will have platelet counts that remain <50 $x10^9$/L at the end of the 14 day daily data collection period. It is envisaged that there will be sufficient daily bleeding assessment data on these neonates for detailed analysis and comparison with other patient groups. Therefore daily data collection will stop at this point as with neonates whose platelet count has recovered. Data collection will continue weekly until End of Study (Section 7.6).

7.9 Neonates transferred out of the participating unit

A log will be kept of neonates transferred out of a participating unit to another unit.
For neonates transferred to a non-participating (receiving) unit before reaching Study Day 28, basic information will be requested in order to obtain the primary outcome data. A data collection form will be sent with the baby to the receiving unit with a copy of the consent form, requesting information on number of platelet transfusions, occurrence of any safety events and the results of any imaging -5 / +10 days of SD28 The local research nurse from the recruiting site will be responsible for liaising with the staff in the receiving unit. The trial manager will be available for any queries. The receiving unit will be asked to send pseudo-anonymised primary outcome data back to the recruiting centre who will in turn pass the information on to the NHSBT CTU. After this point no further information will be requested

For babies transferred to a non-participating unit after Study Day 28, (post primary outcome), no data will be requested from the receiving unit

7.10 Neonates discharged home from the participating unit

Data collection will cease and an End of Study Form will be completed at the time of death, discharge home, 38 weeks CGA or at Study Day 28 if this is later (for those neonates recruited after 34 weeks CGA). For those occasional neonates who are discharged home before Study

Day 28 a telephone call follow up by the local research team will be made to determine if there were any serious adverse events at Study Day 28. Prior consent will be sought to contact the parents / GP/ Health Visitor for this information

7.11 Cranial ultrasound scans

Each centre must have the capability to conduct cranial USS at any time, 24 hours a day. Cranial USS required are as follows:

a) Within 6 hours prior to randomisation
b) Thereafter, a minimum of weekly USS (+/-3 days) should be performed until study day 28 or prior to discharge if earlier
c) For neonates who remain in hospital longer than 28 days, any scan performed should be recorded in the weekly assessment form and a final USS should be done prior to discharge.

The data to be collected from the USS reports and recorded in the bleeding assessment tool / weekly assessment forms (to be filled in as per the guidance of the bleeding assessment record guidance notes) are as follows:

- Evidence of intracranial haemorrhage and its extent
- Ventricular Size
- Parenchymal injury and grade.

7.12 Two year neuro-developmental follow-up.

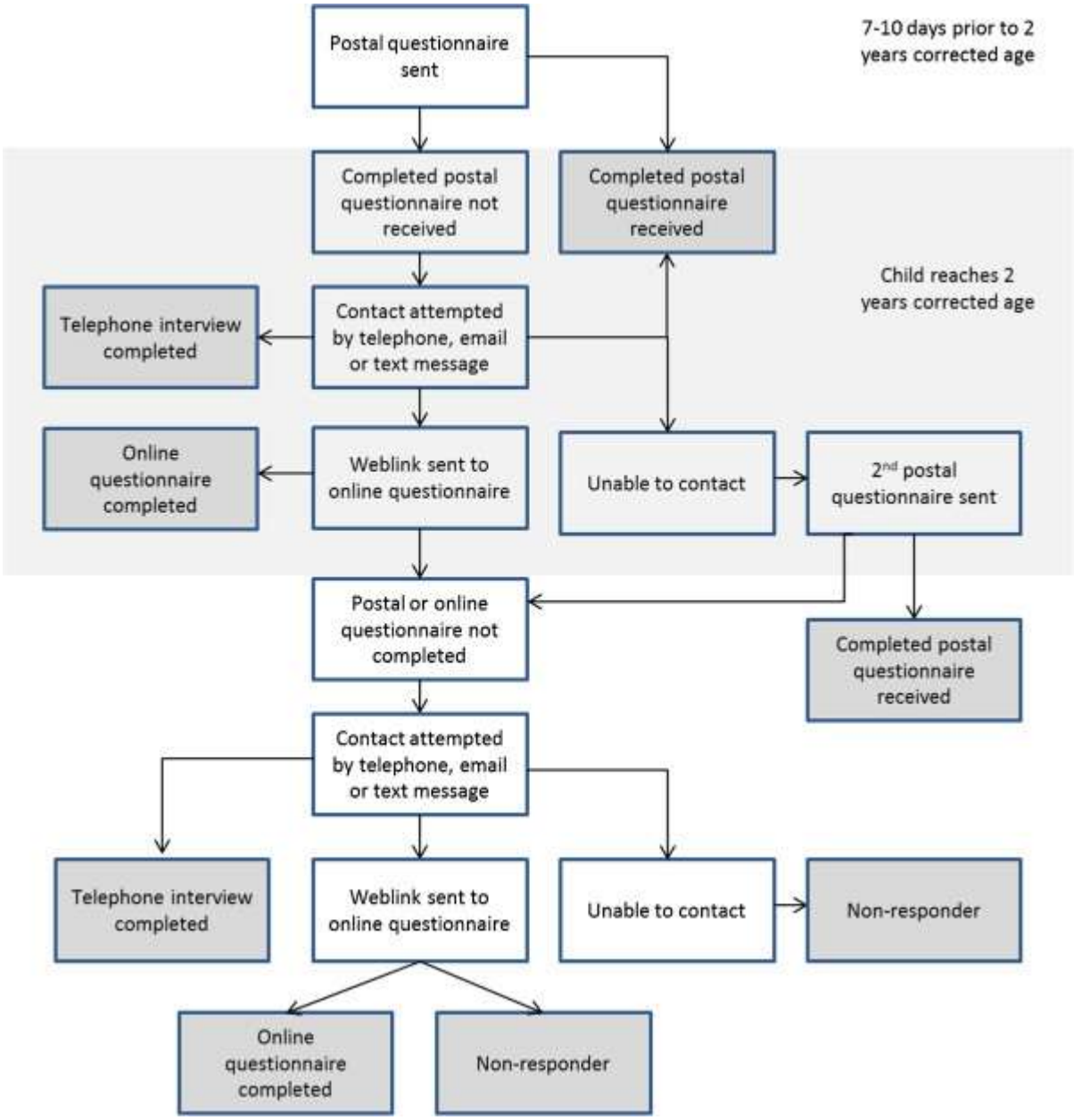
Neuro-developmental follow-up will be undertaken by validated Thames Regional Perinatal Outcome Group/ Standard Electronic Neonatal Database/ National Neonatal Audit Programme (TRPG/SEND/NNAP) 2- year corrected age outcome form and will be administered at 2 years of corrected gestational age. The results of the WHO functional disability criteria and any disability will be classified into 3 groups: mild, moderate and severe. A record of all deaths within 2 years after randomisation will be kept.

In order to obtain the 2 year follow-up data, permission will be sought to contact parents at around 2 years CGA when obtaining informed consent for the trial.

Approximately 7 – 10 days before the baby reaches 2 years corrected age a 2 year follow up questionnaire will be posted out by the site research team to the parents/guardians with a self-addressed envelope for completion. The questionnaire is the (PARCA-R) standardised assessment with additional questions adapted from the TRPG survey to obtain information regarding gross and fine motor skills. Permission to contact is outlined in the Informed Consent Form (V3.0). Further contact will be made by the Two year follow up co-ordinator if required, by telephone, email, or text if the questionnaire is not returned. A telephone interview will be offered at this time, or a weblink sent by text or email to complete the questionnaire online if preferred. The process is outlined in Fig 3

Where primary outcome and 2 year follow-up data cannot be obtained by direct contact with parents or clinicians, it may be possible to extract pseudo-anonymised information from the national neonatal database. The research team at the recruiting centre will be able to access this data.

Fig 3 Pathway for Sending out 2 Year Follow-up Questionnaire



Definition for Corrected Gestational Age (CGA)

Corrected Gestational Age is defined as the age of the child from the expected date of delivery when the child is born preterm. This can be calculated as chronological age - the number of weeks the baby was born preterm.

For example, for a baby born at 28 weeks gestation who has celebrated his 2nd birthday today this would mean

24 months - [(40 weeks - 28 weeks) x 1 month/ 4 weeks] months which equates to a corrected gestational age of 21 months.

NB: In this protocol, the term "Corrected Gestational Age" is used, but its' definition is considered synonymous with the term "Post-Menstrual Age" (PMA)

7.13 Other assessments

Overall figures for numbers of admissions to participating units during the active period of the study will be collected. It is expected that these data will be available from locally maintained databases on the unit. A trial screening log will be maintained for all neonates with platelet counts <100 $x10^9$/L and gestational age <34 weeks at birth, to record data to include GA, post-natal age, birth weight, gender and main diagnosis.

Data will be recorded on all-cause mortality, causes of death as recorded on the death certificate, length of hospital stay, and occurrence of medical conditions such as sepsis and NEC (this is because these conditions may develop between multiple episodes of thrombocytopenia). NEC ≥ Stage 2 will be defined as per Bell's criteria (Appendix B). Sepsis will be defined as culture positive sepsis or culture negative sepsis where antibiotics are given for a minimum of 5 complete days.

7.14 Assigning a grade (none, minor, moderate, major and severe) of bleeding using Bleeding Assessment Tool for Analysis

The grade of bleed will be assigned centrally by means of a computer algorithm at the time of data entry. The database's bleeding algorithm will be validated by using data from pre-piloted bleeding assessment tools.

7.15 Withdrawal of consent

Parents or guardians are free to withdraw their baby from the trial at any stage, and for any reason, and without having to provide any reason for their decision. Only data collected up to the time of withdrawal will be included in the analysis. An end of study form will be completed.

7.16 Early stopping of trial treatment allocation

If the parents, guardians or clinicians responsible for clinical care wish to discontinue trial treatment allocation, study personnel should explain to the parents the value of complete trial data collection, and request that they allow collection of trial follow-up data (up to end of study period and 2 year developmental assessment) to continue. In this case, the neonate will not be withdrawn from the trial, but be discontinued from the randomised treatment. The early stopping of trial treatment allocation form must be completed to record this.

7.17 Blood results and transfusion data

Platelet transfusion data and platelet count should be collected on a daily basis up to study day 28 (and then weekly using the weekly assessment form after Study day 14) using a transfusion data form for each patient. This will require close scrutiny of the patient's current prescription chart and liaison with the hospital blood bank. The number of red cell transfusions received from randomisation to study day 28 will also be recorded.

7.18 Demographic data

Demographic data will be gathered on a screening log on all neonates with platelets <100 $x10^9$/L who are cared for in selected participating units. These data will include age, gender, GA, post-natal age and main diagnoses. They will contain no identifying information (initials/date of birth) before being sent to the CTU. The local PI or designate will be responsible for co-ordinating this data collection. These data will be used to identify the proportion of eligible babies that are recruited to the trial, as well as providing valuable

information about the population of very pre-term thrombocytopenic neonates in the participating hospitals.

8 SAFETY REPORTING

8.1 Defining safety reporting

The main *anticipated* complications for safety reporting in this trial are bleeding episodes and transfusion related adverse events. In addition, data on episodes of necrotising enterocolitis and sepsis will be collected. All safety reporting will begin from the time of randomisation and continue until the neonate completes the study.

8.2 Major bleeding events

Major (including severe – see definitions in Section 6.4) bleeding events will not be reported as SAEs as this is the primary outcome measure for this trial. All new major bleeding events will be reported on the specific form as soon as possible and preferably within 24 hours to the CTU using a major bleed form, and without disclosing the allocation arm. Each report will be forwarded to the DMC for review as soon as it is received at the CTU. All bleeding events will continue to be monitored and recorded in all neonates on a daily basis using the bleeding assessment form. In cases of uncertainty by the local research team about the interpretation and recording of any (major) bleed on the bleeding assessment form, the local team may contact the joint CI or one of the neonatal medical experts. The medical experts and the joint CI will form an adjudication panel for any further clarifications if necessary (excluding the local PI).

The DMC will also review regular summary reports on all bleeds produced by the trial statistician. The DMC will have a clear responsibility to advise the TSC on any concerns about these data (see section 11.3).

MAJOR BLEED FORM

Within one working day of becoming aware of an Major Bleed, please fax a completed Major Bleed form to the NHSBT CTU

Fax: 01223 588136

8.3 Serious adverse events (SAEs)

The PI should assess all adverse events collected on the daily and weekly assessment forms for seriousness, expectedness and causality. All adverse events except major bleeds that meet the criteria for seriousness as defined below will be reported as SAEs in this trial.

A SAE is an adverse event that

- results in death
- is life-threatening*
- requires hospitalisation or prolongation of existing hospitalisation (including readmission within 28 study days if discharged home earlier)**

- results in a likelihood of persistent or significant disability or incapacity

Platelet transfusion related adverse events, as defined in 8.3.2, will also be reported as SAEs for this trial

* The term 'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
** Hospitalisation is defined as an inpatient admission, regardless of length of stay, even if the hospitalisation is a precautionary measure for continued observation. Hospitalisations for a pre-existing condition, including elective procedures that have not worsened, do not constitute an SAE.

8.3.1 Reporting of serious adverse events

SAE forms should be completed by the PI or other investigator (who has delegated responsibility for this task) and must be faxed to the CTU within one working day of the investigator becoming aware of the event. The CTU office will log each one and will send the SAE to the CI (Simon Stanworth or delegate) according to CTU procedures. SAEs will include serious platelet transfusion related adverse events as defined in section 8.3.2.

The CI (Simon Stanworth or delegate) will review all SAEs received and record his opinion, which does not overrule the opinion of the PI.

Related and unexpected SAEs must be submitted to the main REC by a CI (or by delegate) within 15 days of the CI being made aware of the SAE.

Other SAEs including related and expected events (platelet transfusion events reported to SHOT or MHRA, see below) will be submitted annually to the main REC that gave the favourable opinion for the study.

SAE NOTIFICATION

Within one working day of becoming aware of an SAE, please
fax a completed SAE form to the NHSBT CTU
Fax: 01223 588136

Or email: serious_adverse_events@nhsbt.nhs,uk

8.3.2. Definitions of platelet transfusion related adverse events

Data collected on transfusion related adverse events, including platelet related transfusion related adverse events, will be based on the current definitions used by hospitals reporting to the UK national haemovigilance reporting scheme (Serious Hazards of Transfusion: SHOT and MHRA). These definitions cover the following: Incorrect Blood Component Transfused, Acute Transfusion Reactions, Transfusion-related Acute Lung Injury, and Transfusion Transmitted Infections, including bacterial transmission. The hospital site should report any platelet transfusion related adverse event to SHOT or MHRA as is normal practice.

8.4 Adverse events

Adverse events (not SAEs) that are specifically of interest to the trial and that are not bleeding episodes (section 8.2) will be reported on a separate form. These are restricted to:

1. Necrotising enterocolitis ≥ Stage 2 defined as per Bells Criteria (Bell et al, 1978) See below for definitions:

NEC Definitions: Only record if category 2 or 3 applies.

Modified Bell's Staging Criteria for Necrotizing Enterocolitis

STAGE	SYSTEM SIGNS	INTESTINAL SIGNS	RADIOLOGIC SIGNS	TREATMENT
II. Definite				
A: Mildly ill	Same as IA	Same as I, plus absent bowl sounds, abdominal tenderness	Ileus, pneumatosis intestinalis	NPO, antibiotics x 7 to 10 days
B: Moderately ill	Same as I, plus mild metabolic acidosis, mild thrombocytopenia	Same as I, Plus absent bowel sounds, definite abdominal tenderness, abdominal cellulitis, right lower quadrant mass	Same as IIA, plus portal vein gas, with or without ascites	NPO, antibiotics x14
III Advanced				
A Severely ill, bowel intact	Same as IIB, plus hypotension, bradycardia, respiratory acidosis, metabolic acidosis, disseminated intravascular coagulation, neutropenia	Same as I and II, plus signs of generalised peritonitis, marked tenderness and distension of abdomen.	Same as IIB, plus definite ascites	NPO, antibiotics x 14 days, fluid resuscitation, inotropic support, ventilator therapy, paracentesis
B Severely ill: bowel perforated	Same as IIA	Same as IIA	Same as IIB, plus pneumoperitoneum	Same as IIA, plus surgery

2. Sepsis, which must be concurrent. Sepsis is defined for the purposes of this study as culture positive sepsis or culture negative sepsis where a course of at least 5 days of antibiotics is to be administered for proven or clinically-suspected sepsis.

Any adverse event meeting the criteria for seriousness will be reported as an SAE. A listing of adverse events (necrotising enterocolitis, sepsis) will be reported six monthly to the Data Monitoring Committee (DMC).

9 OUTCOMES & STATISTICAL CONSIDERATIONS

9.1 Randomisation

A web-based randomisation service provided by (http//:www.sealedenvelope.com) accessible by each participating centre will allocate neonates using minimisation with a random element.

9.2 Outcome measures

9.2.1 Primary outcome

The primary outcome measure is the proportion of patients who either die or develop a major or severe bleed up to and including study day 28.

This composite outcome was chosen because these endpoints represent significant clinical outcomes of relevance to the trial question.

Observation up to and including Study Day 28 was chosen for several reasons. First, in the PlaNet-1 study all major bleeds occurred within the 28 days following onset of thrombocytopenia. Secondly, primary outcome measures in many studies in this patient population are measured at Study Day 28.

In cases where no imaging is performed within -5 / + 10 days of SD28:

- If a baby is discharged home well and not readmitted prior to SD28, we will assume no MB had occurred by SD28.
- For babies discharged home after SD28, we will assume that no new major cranial bleed had taken place by SD28 if other Cranial Ultrasound (CUSS), CT or MRI scans performed on or between SD14 and discharge showed no new major bleeding providing that there was no documented episode of any significant clinical deterioration (e.g proven sepsis, NEC, or collapse) that required transfusion with blood products, or evidence of other new major bleeding in that period.
- In cases where no subsequent pre-discharge CUSS, CT or MRI scan was undertaken, providing that there is, prior to discharge, no documented episode of any significant clinical deterioration (e.g. proven sepsis, NEC, or collapse) that required transfusion with blood products, or evidence of other new major bleeding, then we will also assume that there was no major bleed at SD28.
- All other cases are to be dealt with on a case by case basis
- Review of evidence in the first case by Dr Anna Curley, Joint Chief Investigator. Further review to be undertaken by medical experts within TMG.
- For difficult cases it may be necessary to refer to an independent neonatologist to review.

9.2.2 Secondary outcomes

Secondary outcomes include:

- Proportion of patients surviving to go home following a major bleed, censoring at discharge
- Proportion of patients surviving to go home without having had a major bleed, censoring at discharge
- Proportion of patients who have died up to study day 28
- Proportion of patients who sustain a major bleed up to study day 28
- The rate and time from randomisation of minor, moderate and major bleeding derived from the bleeding assessment tool up to study day 14, and for major bleeds up to study day 28. Definitions for all grades of bleeds are shown in the bleeding assessment tool; grading will be assigned based on a modified version of the WHO Bleeding Score
- Number of platelet units transfused up to study day 28
- Time to discharge home, censoring on death
- Neuro-developmental outcome as assessed by the Thames Regional Perinatal Outcome Group/ Standard Electronic Neonatal Database/ National Neonatal Audit Programme (TRPG/SEND/NNAP) 2- year corrected age outcome form at 2 years corrected postnatal age. This will provide a basis of correlation with the WHO functional disability score.
- Platelet transfusion-related adverse events from the time of randomisation up to end of study (section 7.6 and 8.3.2)

A comprehensive statistical analysis plan will be written before the final data analysis is conducted. Analysis of all outcomes will be conducted on the basis of intention to treat. The primary analysis will be adjusted for the stratifying variables.

9.3 Sample size

This trial (PlaNeT-2) represents the first trial in the field of neonatal platelet transfusion prophylaxis in this population of preterm neonates. This study has been designed as a two stage trial with an interim analysis which will inform the final sample size requirement and trial feasibility (See Section 9.4). Data on the frequency of bleeding outcomes in severely thrombocytopenic neonates is available from the PlaNeT-1 survey (Stanworth et al, 2009), in which 30/169 or 18% of neonates died or experienced a major bleed while on study. However, this proportion applies to neonates of all gestational ages, and only during episodes of significant thrombocytopenia. Overall mortality (and new major bleeds) is likely to be greater than this figure derived from PlaNeT-1 data for the baseline event rate in the arm A of this trial. Assuming a proportion of 20% for the primary outcome for Arm A, in order to detect a difference between proportions of 8% (assuming that the proportion in Arm B is 12%), using a two-sided test, 5% significance level and 80% power, 329 neonates in each group is needed. Rounding this figure up, a total of 660 neonates will be required for this study. Note that this calculation does not take account of withdrawals or loss to follow-up.

The Data Monitoring Committee (DMC) will be closely monitoring the primary outcome (mortality and major bleeds) as well as safety data for any imbalance between the two arms (See Sections 10, 11 for further details).

9.4 Interim analysis

The trial has been designed as a two-stage, superiority randomised trial with an interim analysis performed after 100 neonates have been recruited. The main purpose of the interim analysis is to provide a good estimate of the event rate for the primary outcome in the standard arm A, and to review the final sample size requirement and need for additional recruiting centres. No conclusions based on possible emerging benefits between the two arms will be drawn. Using data from the PlaNeT-1 study, we currently estimate that it will take 18-24 months to recruit 100 patients. Data from the interim analysis will help refine these estimates and help plan the number of centres and length of time needed to reach the required sample size. Additionally, the numbers of platelet transfusions and protocol violations affecting per protocol analysis will help determine the feasibility of this study.

The trial statistician will prepare regular reports of trial progress and serious adverse events for the DMC.

10 TRIAL MONITORING

10.1 Risk assessment

A risk assessment was carried out by the NHSBT CTU and the TMG of the perceived hazards associated with the overall conduct of this trial, taking into consideration hazards for the participating patients, to the conduct of the study and for the organisation (NHSBT) as a whole. This has been used to develop appropriate management strategies to contain these risks and to inform the trial monitoring plan. The risk assessment will be regularly reviewed by the TMG as the trial progresses and management strategies adjusted when necessary. The overall level of risk to conduct the PlaNeT–2 study has been assessed as low to moderate and

deemed to be acceptable for the trial to proceed. The major issues identified and strategies for mitigating them are described below.

10.1.1 Risks to neonates

a) Ensuring only eligible patients are recruited. This will addressed by training research and clinical staff at sites and ensuring the pre randomisation USS is carried out (see section 4).
b) Risk of an excess of severe bleeding events in either arm of the trial will be addressed by close monitoring of all neonates using the pre-piloted bleeding assessment tool by trained clinical staff (see section 7), education to highlight bleeding events and severity, and immediate reporting of all major bleeding events to the IDMC. In addition, the trial will have an interim analysis when 100 neonates are recruited.
c) Risks of adverse events related to platelet transfusions and lack of reporting of SAEs to the DMC will be addressed by education and training including the correct use of the assessment and SAE forms by trained clinical staff (see section 7).
d) The risk of inadequate training and knowledge in the use of the assessment tools will be addressed by training of the clinical staff by the research team at site visits, principal investigator meetings and workshops. Site visits will be conducted regularly, with the initial visit occurring soon after the first neonate is recruited at the site. Monitoring will include ensuring that the cranial ultrasound scans are performed (see section 7), missing collection of data is minimised, and that the follow up for outcomes including neuro-development assessment is undertaken as per protocol (see section 7).

10.1.2 Risks to the conduct of the trial

The risk of poor data quality and integrity will be addressed by the use of a pre-piloted Bleeding Assessment Tool (Venkatesh et al 2012) and conducting regular workshops and provide training at the centres to ensure that the data are recorded accurately. The CRFs, will be designed with input from the data manager and piloted before recruitment to the trial starts. Furthermore the Trial Manager will prepare a monitoring plan to establish the quality and existence of source data during the regular site visits (see sections 7, 10)

The risk of inadequate recruitment to the study will be addressed by conducting a site feasibility questionnaire and the setting of monthly recruitment targets before the trial starts and preparation of progress reports in order to identify any potential problems at the Trial Management Group meetings).

The risk of excessive discontinuation of trial treatment will be addressed by providing training, particularly during the site set up visits, to the principal investigators and clinical staff as additional platelet transfusions can be administered according to the protocol (see section 6).

10.2 Data entry at CTU

Completed Trial CRFs will be sent to the CTU DM and entered on the study database designed on MACRO Trial Manager Software. Routine validation checks will be performed on data entry and a full audit trail of data queries and resolution maintained in accordance with CTU procedures. Missing or inconsistent data values identified by CTU DM will be queried with sites. The DM will send reminders for any overdue and missing data.

10.3 Clinical Site Monitoring

10.3.1 Direct Access to data

Local site monitoring with direct access to data will be arranged by the Trial Manager. Local investigators will permit trial-related monitoring, ethics committee review and regulatory inspections by providing direct access to source data/documents. Parental consent for this will be obtained during registration.

10.3.2 Confidentiality

Data will be held and processed in accordance with the Data Protection Act 1998. All study data will be held securely. It will not be disclosed to third parties. All staff working on the study owe a duty of confidentiality to the participants. Manual records will be held securely (for example in locked filing cabinets). Electronic records will be held on a secure network requiring user ID and password access. Patient names will not be disclosed to CTU staff and a fully data set will be used for data analysis. Individuals will not be identifiable from the results of the trial.

10.3.3 Trial monitoring at site and quality assurance of bleeding outcome data

The trial manager will visit each site initially for a 'set-up visit'. Regular site monitoring visits will then occur as per the monitoring plan. During the set up visit the site will be given all relevant documentation and training in order for them to participate safely and effectively in the trial.

The following measures will be put in place to ensure that neonates receive the treatment that they are randomised to:

- label on front cover of neonates medical notes
- participation in education and training of all individuals involved in the trial as an ongoing process

A monitoring plan will be drawn up and followed. This will include 100% monitoring of parent consent forms and source data verification on a proportion of CRFs (including 100% verification of the data collected from the first few neonates recruited at each site).

The designated site staff will have access to laboratory records from the local blood bank, to cross-check and validate red cell and platelet transfusion requirements up to 28 days for each enrolled patient, and these will be reviewed at site monitoring visits.

Particular attention will be given to the ongoing training and education of local site staff in the inclusion/exclusion criteria, and in the correct use of the bleeding assessment tool. All designated site staff will receive dedicated training from the research team.

11 TRIAL GOVERNANCE

11.1 Trial Management Group (TMG)

The Trial Management Group will be responsible for the daily management of the trial. Members include the Chief Investigators (CI), Principal Investigators (Pis) from representative centres, the Trial Manager, the CTU Project Manager, trial Data Manager and trial statistician.

TMG Committee members:

Chief Investigators for PlaNeT-2: Dr Anna Curley, Dr Simon Stanworth
Chief Investigators for MATISSE: Dr Karin Fijnvandraat, Dr Enrico Lopriore

Medical Experts:

For PlaNeT-2: Dr Paul Clarke, Dr Rizwan Khan, Dr Priya Muthukumar, Dr Helen New, Dr Vidheya Venkatesh, Dr Tim Watts,

Research Nurse: Ms Beatriz Santamaria (Guys and St Thomas's)

NHSBT CTU Head of Clinical Operations: Ms Alison Deary
NHSBT CTU Project Manager: Dr Ana Mora
NHSBT Statistician: Mrs Cara Hudson
NHSBT CTU, Senior Data Manager: Ms Renate Hodge
Amsterdam Medical Center MATISSE Trial Manager: Dr Suzanne Gunnink
NHSBT /University of Cambridge PlaNeT-2 Trial Manager: Ms Karen Willoughby

11.2 Trial Steering Committee (TSC)

A Trial Steering Committee will provide oversight of this trial, which will be conducted according to the principles of ICH Good Clinical Practice. The role of the TSC is to provide overall supervision for the trial and provide advice through its independent consultant neonatologist Chairman. In addition to the Chairman and CI, other members of the TSC will include PIs from representative centres and two/three independent clinicians. The ultimate decision for the continuation of the trial lies with the TSC.

TSC members:

Prof Colin Morley	Independent Chair and Retired Professor of Neonatology
Dr Sandy Calvert	Independent Consultant Neonatologist
Dr Anthony Emmerson	Independent Consultant Neonatologist
Dr Anna Curley	Consultant Neonatologist and joint Chief Investigator PlaNeT-2
Dr Simon Stanworth	Consultant Haematologist and joint Chief Investigator PlaNeT-2
Dr Karin Fijnvandraat	Consultant Haematologist and joint Chief Investigator MATISSE
Dr Enrico Lopriore	Consultant Haematologist and joint Chief Investigator MATISSE
Mrs Cara Hudson	NHSBT Statistician
TBA	Parent Representative(s)
TSC Facilitators:	
Dr Suzanne Gunnink	MATISSE Trial Manager:
Ms Karen Willoughby	NHSBT/University of Cambridge PlaNeT-2 Trial Manager

11.3 Data Monitoring Committee (IDMC)

A core DMC for all CTU trials is chaired by Professor Adrian Newland (Barts Health). Other core members are Dr. Gavin Mruphy (University of Leicester) and Professor Keith Wheatley (Cancer Research UK Clinical Trials Unit, University of Birmingham) and an expert in neonatal medicine (Dr Henry Halliday, Queen's University, Belfast) has been co-opted to the IDMC for this trial. The DMC will monitor recruitment, all severe bleeding events and serious adverse events. The DMC will also review trial outcomes for feasibility.

12 ETHICAL CONSIDERATIONS AND APPROVAL

12.1 Ethical considerations

Approval for the study must be obtained from the National Research Ethics Service (NRES), Healthcare Trust R&D Officers, Data Protection Officers, and Caldicott Guardians, at each trial

site, following application to the Multi Centre Research Ethics Committee (MREC). Each neonate in the study will be given a unique identifying number aside from their hospital number which will be used to identify them on the database together with initials and date of birth. Data will be held and handled according to Caldicott principles and the requirements of the Data Protection Act. The study will abide by the principles of the Declaration of Helsinki and the principles of GCP.

The right of the parent to refuse to participate in the trial without giving reasons will be respected. The parent will remain free to withdraw their baby at any time from the allocated protocol treatment and trial follow-up (see section 7.15). After the baby has entered the trial, the clinician will remain free to give additional platelet transfusions (see section 6.2 and 6.3). If the clinician or the parent/guardian feels it is in the best interest of the patient they may discontinue trial treatment allocation (see section 7.16). However, the reason for doing so will be recorded and the patient will remain within the trial for the purpose of follow-up data collection and analysis.

12.2 Ethical Issues

Good neonatal research is essential if we are to improve the care and outcome of newborn premature babies. Many surveys on parental perspectives of neonatal research have shown that most parents of premature babies felt able to think about and join research studies and appreciate that studies need to be performed to improve the outcome of ill and fragile babies (Stenson *et al*, 2004; Morley *et al*, 2005). These surveys show that most parents do not want us to stop neonatal research.

This study has been thought through and considered very carefully following completion of a prospective observational study (PlaNeT-1). We understand that it is a stressful period for the parents and many of these babies are very fragile and unwell. However it is particularly just such sick babies where the best treatment needs to be defined by research studies. The study has been designed to allow parents time to consider participation by approaching them for consent when the baby's platelet count has fallen below 100 $x10^9$/L although randomisation will not occur until the platelet count has fallen below 50 $x10^9$/L. The parents are under no obligation to join the trial and participation is completely voluntary. The parent information leaflet version (current) specifically deals with this issue.

Randomised trials are considered to be the most reliable form of providing scientific evidence and in order to conduct such trials we will need to keep neonates in 2 groups assigned randomly by a computer programme as described in section 5.2.

This is a superiority trial and is intended to determine whether the effect of transfusing at platelet counts of below 50 $x10^9$/L is better than below 25 $x10^9$/L. The justification for selecting the control arm of 25 $x10^9$/L has been explained in section 2.3. We have described in detail under sections 7, 10 and 11 the monitoring of the trial to ensure that any major bleeding events and discrepancies in major bleeding between the two treatment arms are highlighted to the DMC and that appropriate action is taken.

13 INDEMNITY

13.1 Definitions

The definitions used in the indemnity clause (see 13.2) are as follows;

"Authority" means prior to 1st October 2005 the National Blood Authority established under SI 1993 No 586 and from 1st October 2005, NHS Blood and Transplant (NHSBT) established under SI2005 No 2529

"Entity" means the NHS Body or Company participating in the clinical trial or project."

"Clinical trial or project" means such trial or project in relation to proposed new blood products, blood components and tissues produced or to be produced; in relation to proposed diagnostic, therapeutic services and proposed donation procedures which have been approved by the appropriate ethical committee(s) and the Clinical Trials Authorisation Scheme where appropriate and which are conducted under the Authorities protocols.

"Patients" means patients, healthy volunteers and donors. For the purposes of this trial this also includes neonates.

13.2 Indemnity Clause

The Authority is a Special Health Authority which is a member of the NHSLA risk pooling schemes for clinical negligence and liabilities to third parties. In addition when the Authority undertakes or sponsors Clinical Trials and Research Projects within its functions in England the Authority, through the Department of Health, has under certain circumstances in place indemnity provisions for non-negligent harm to patients participating in these Trials or Projects. The indemnities referred to above do not relieve the participating entities from their duty of care to the patient participating in these Trials or Projects. For the avoidance of doubt the Authority cannot accept any responsibility or liability for any breach of the entities duty of care nor any negligent act or omission committed by the entity, its employees, agents or sub-contractors.

14 FINANCE

The trial is funded by a Project Grant from the NHSBT which is administered by the National Research & Development Committee. The trial has been designed as a two-stage, randomised, superiority trial with an internal pilot after 100 neonates have been recruited, and funding has been extended to cover the whole trial. Payments made to participating centres to cover costs associated with the undertaking of this trial are specified in individual Investigator Site Agreements.

15 PUBLICATION

The data from all sites will be analysed together and the results published as soon as possible after trial completion. Individual PIs at participating sites must not publish or divulge any report or result from the Trial until the main trial results have been published. After discussion with the TSC, a Trial Writing Group will be formed for this purpose by the Chief Investigators, which will include key members of the Trial Management Group. The TSC will oversee the timely analysis, writing up and publication of the main Trial results. Investigators and independent members of the TSC and DMC must be given the opportunity to read and comment on the main trial findings before submission for publication. The members of the TSC and DMC and all PIs should be listed with their affiliations in the Acknowledgements/Appendix of the main publication and the support of the NHSBT Clinical Trials Unit and Funder acknowledged.

16 REFERENCES

Alfaleh K, Smyth JA, Roberts RS, Solimano A, Asztalos EV, Schmidt B; for the Trial of Indomethacin Prophylaxis in Preterms Investigators. Prevention and 18-month outcomes of serious pulmonary haemorrhage in extremely low birth weight infants: results from the trial of indomethacin prophylaxis in preterms. Pediatrics. 2008;121:e233-8.

Andrew M, Castle V, Saigal S, et al. Clinical impact of neonatal thrombocytopenia. J Pediatr. 1987; 110: 457-464

Andrew M, Vegh P, Caco C, et al. A randomized controlled trial of platelet transfusions in thrombocytopenic premature infants. J Pediatr. 1993;123:285-91

Baer VL, Lambert DK, Henry E et. Al. Do platelet transfusions in the NICU adversely affect survival? Analysis of 1600 thrombocytopenic neonates in a multihospital healthcare system. Journal of Perinatology 2007;27(12):790-796

Bassan H, Limperopoulos C, Visconti K, et al. Neurodevelopmental outcome in survivors of periventricular hemorrhagic infarction. Pediatrics. 2007;120:785-92.

BCSH guidelines. Gibson BES, Bolton-Maggs PHB, Pamphilon D, et al. Transfusion guidelines for neonates and older children. British Journal of Haematology. 2004;124:433-453

Bell EF, Strauss RG, Widness JA, Mahoney LT, Mock DM, Seward VJ, Cress GA, Johnson KJ, Kromer IJ, Zimmerman MB. Randomized trial of liberal versus restrictive guidelines for red blood cell transfusion in preterm infants. Pediatrics. 2005; 115:1685–1691. doi: 10.1542/peds.2004-1884

Bell M J, Ternberg JL, Feigin RD, Keating JP, Marshall R, Barton L, and Brotherton T. Neonatal necrotizing enterocolitis. Therapeutic decisions based upon clinical staging. Ann Surg. 1978 January; 187(1): 1–7

Blanchette VS, Kuhne T, Hume H, Hellmann J. Platelet transfusion therapy in newborn infants. Transfus Med Rev. 1995;9(3):215-30

British Association of Perinatal Medicine. Standards for hospitals providing neonatal intensive and high dependency care 2nd edition. 2001 December. Available online on http://www.bapm.org/media/documents/publications/hosp_standards.pdf. Accessed 23rd December 2009

Carr R, Brocklehurst P, Dore CJ, Modi N. Granulocyte-Macrophage colony stimulating factor administered as prophylaxis for the reduction of sepsis in extremely preterm, small for gestational age neonates (the PROGRAMS trial): a single-blind, multicentre, randomised controlled trial. Lancet (2007) 373:226-233.

Castle V, Andrew M, Kelton J, Giron D, Johnston M, Carter C. Frequency and mechanism of neonatal thrombocytopenia. J Pediatr. 1986;108:749-55

Chaudhary R, Clarke P. Current transfusion practices for platelets and fresh, frozen plasma in UK tertiary level neonatal units. Acta Paediatr. 2008 Jan;97(1):135.

Committee on Fetus and Newborn. Age Terminology During the Perinatal period. Pediatrics Vol. 114 No. 5 November 2004, pp. 1362-1364 (doi:10.1542/peds.2004-1915)

Darlene A. Calhoun DO, Robert D. Christensen MD, Cynthia S. Edstrom MD, Sandra E. Juul MD, PhD, Robin K. Ohls MD, Kurt Schibler MD, Martha C. Sola MD and Sandra E. Sullivan MD Consistent Approaches to Procedures and Practices in Neonatal Hematology,Clinics in Perinatology,Volume 27, Issue 3, 1 September 2000, 733-753

Del Vecchio A, Sola MC, Theriaque DW, et.al. Platelet transfusions in the neonatal intensive care unit: factors predicting which neonates will require multiple transfusions. Transfusion. 2001;41:803-8.

Fernandes CJ & O'Donovan DJ. Platelet transfusions in infants with necrotizing enterocolitis. Curr Hematol Rep. 2006;5:76-81

Garcia MG, Duenas E, Sola MC, Houston AD, Theriaque D, Christensen RC. Epidemiologic and outcome studies of patients who received platelet transfusions in the neonatal intensive care unit. Journal of Perinatology, 2001;21:415-420

Jones B, Jarvis P, Lewis JA, et al. Trials to assess equivalence: the importance of rigorous methods. BMJ. 1996;313:36-39

Josephson CD, Su LL, Christensen RD, Hillyer CD, et al. Platelet transfusion practices among neonatologists in the United States and Canada: results of a survey. Pediatrics. 2009 Jan;123:278-85.

Kenton AB, Hegemier S, Smith EO, et al. Platelet transfusions in infants with necrotizing enterocolitis do not lower mortality but may increase morbidity. J Perinatol. 2005;25:173-7.

Kirpalani H, Whyte RK, Andersen C, Asztalos EV, Heddle N, Blajchman MA, Peliowski A, Rios A, LaCorte M, Connelly R, et al. The premature infants in need of transfusion (PINT) study: a randomized controlled trial of a restrictive (low) versus liberal (high) transfusion threshold for extremely low birth weight infants. J Pediatr. 2006;149:301–307. doi: 10.1016/j.jpeds.2006.05.011

Kopko PM and Holland PV. Mechanisms of severe transfusion reactions. Elsevier Science Paris 2001;8:278-281

Morley CJ, Lau R, Davis PG, Morse C. What do parents think about enrolling their premature babies in several research studies? Arch Dis Child Fetal Neonatal Ed 2005;90:F225–F228. doi: 10.1136/adc.2004.061986

Murray NA, Howarth LJ, McCloy MP, Letsky EA, Roberts IA. Platelet transfusion in the management of severe thrombocytopenia in neonatal intensive care unit neonates. Transfus Med. 2002 Feb;12(1):35-41.

Papile LA, Burstein J, Burstein R., Koffler H. Based on the range of "IVH" found, separate grades were done in an attempt to describe the varied natural history of "IVH". J Pediatr. 1978 Apr;92(4):529-34.

Papile LA, Burstein J, Burstein R, Koffler H. Incidence and evolution of subependymal and intraventricular haemorrhage: a study of infants with birth weights less than 1,500 gm. J Pediatr. 1978;92:529-34

Roberts I, Stanworth S, Murray N A. Thrombocytopenia in the Neonate. Blood Rev. 2008;22(4):173-86. Epub 2008 Apr 22.

Roberts IAG, Murray NA. Thrombocytopenia in the newborn. Curr Opin Pediatr 2003;15:17-23

Roland EH, Hill A. Germinal matrix-intraventricular haemorrhage in the premature newborn: management and outcome. Neurol Clin. 2003;21:833-51

Royal College of Paediatrics and Child Health. 2009 Transfusion alert for paediatricians and neonatologists – recognition of bacterial contamination of blood components. www.rcpch.ac.uk/doc.aspx?id_Resource=5111

Saxonhouse M, Slayton W, Sola MC. Platelet transfusions in the infant and child. In: Hillyer CD, Luban NLC & Strauss RG (Eds) Pediatric Handbook of Transfusion Medicine. San Diego. Elsevier 2004 pp253

Serious Hazards of Transfusion Annual Report 2008. Also available at: http://www.shotuk.org.

Sherlock RL, Anderson PJ, Doyle LW; for the Victorian Infant Collaborative Study Group. Neurodevelopmental sequelae of intraventricular haemorrhage at 8 years of age in a regional cohort of ELBW/very preterm infants. Early Hum Dev. 2005;81:909-16.

Sola MC, Evaluation and treatment of prolonged and severe thrombocytopenia, Clinics in Perinatology, Volume 31, Issue 1, March 2004, Pages 1-14

Sola-Visner M, Saxonhouse MA, Brown RE. Neonatal thrombocytopenia: what we do and don't know. Early Hum Dev. 2008;84:499-506

Stainsby D, Jones H, Wells AW, Gibson B, Cohen H. Adverse outcomes of blood transfusion in children analysis of UK reports to the serious hazards of transfusion scheme 1996-2005. Br J Haematol.. 2007 Apr;141(1):73-9

Stanworth SJ, Clarke P, Watts T, Ballard S, Choo L, Morris T, Murphy MF, Roberts I; for the Platelets and Neonatal Transfusion Study Group. Prospective, Observational Study of Outcomes in Neonates With Severe Thrombocytopenia. Pediatrics. 2009 Oct 19. [Epub ahead of print]PMID: 19841111

Stenson BJ, Becher J-C,McIntosh N. Neonatal research: the parental perspective; Arch Dis Child Fetal Neonatal Ed 2004;89:F321-F324 doi:10.1136/adc.2002.021931

Strauss R. How do I transfuse red blood cells and platelets to infants with the anaemia and thrombocytopenia of prematurity. Transfusion. 2008;48:209-217

TRPG-SEND Form AHC February 2010 from http://www.rcpch.ac.uk/doc.aspx?id_Resource=6140

Venkatesh V, Curley A, Khan R, Clarke P, Watts T, Josephson C, Muthukumar P, New H, Seeney F, Morris S, Stanworth S. A novel approach to standardised recording of bleeding in a high risk neonatal population. Arch Dis Child Fetal Neonatal Ed. 2012 Nov 9.

Whyte RK, Kirpalani H et al. Neurodevelopmental Outcome of Extremely Low Birthweight Infants Randomly Assigned to Restrictive or Liberal Haemoglobin Thresholds for Blood Transfusion. Pediatrics Vol 123 No 1 Jan 2009

17. PROTOCOL AMENDMENTS

Protocol Version 1.0 (dated 28 July 2010)
Version submitted to main REC for ethical approval

Protocol Version 1.1 (dated 21 October 2010)
Administrative amendment submitted with revised documentation in response to REC comments on the original aplication. Approved 05 November 2010

Protocol Version 2.0 (dated 13 April 2011)
Revised to reflect changes made to data to be collected agreed during CRF development.

Protocol Version 3.0 (dated 17 July 2014)
Please refer to Document History on Page 5 for details of protocol amendments.

Protocol Version 4.0 (dated 11 January 2016)
Please refer to Document History on Page 5 for details of protocol amendments.

18. PARTICIPATING CENTRES

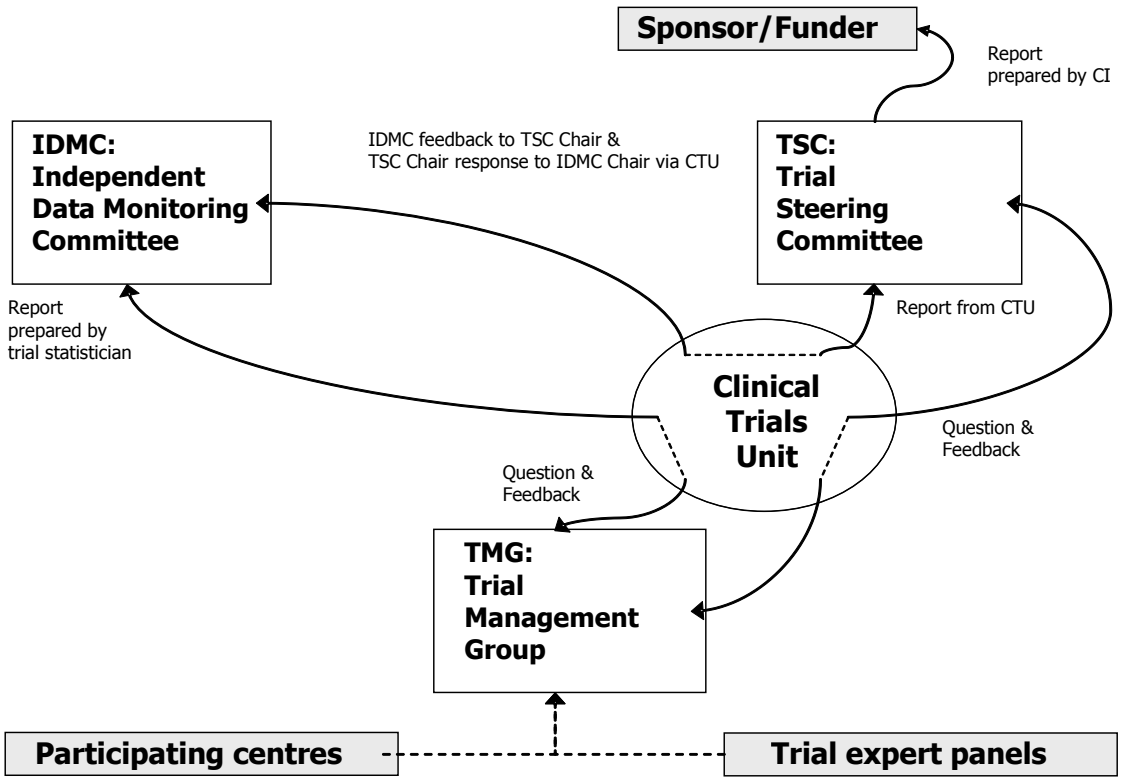
Trial centres currently agreeing to participate in the study are:

- Addenbrooke's Hospital, Cambridge University Hospitals NHS Foundation Trust
- Norfolk and Norwich University Hospital, Norfolk and Norwich University Hospitals NHS Foundation Trust
- St Thomas Hospital, Guys and St Thomas Hospital NHS Foundation Trust
- John Radcliffe Hospital, Oxford Radcliffe Hospitals NHS Trust
- St Mary's & Queen Charlotte's Hospitals, Imperial College Healthcare NHS Trust
- University Hospital of North Tees, North Tees and Hartlepool NHS Trust
- Luton & Dunstable University Hospital NHS Foundation Trust
- Royal Bolton Hospital, Bolton NHS Foundation Trust
- James Cook University Hospital, South Tees Hospitals NHS Foundation Trust
- Bradford Royal Infirmary, Bradford Teaching Hospitals NHS Foundation Trust
- University Hospital of Wales, Cardiff & Vale University Health Board
- Royal Preston Hospital, Lancashire Teaching Hospitals NHS Foundation Trust
- Royal Berkshire Hospital, Royal Berkshire NHS Foundation Trust
- Royal Victoria Infirmary, The Newcastle upon Tyne Hospitals NHS Foundation Trust
- Cork University Maternity Hospital, Ireland
- Royal Jubilee Hospital, Belfast Health & Social Care Trust
- Craigavon Area Hospital, Southern Health & Social Care Trust
- Altnagelvin Area Hospital, Western Health & Social Care Trust
- Royal Gwent Hospital, Aneurin Bevan University Health Board
- Royal Cornwall Hospital, Royal Cornwall Hospitals NHS Trust
- New Cross Hospital, The Royal Wolverhampton NHS Trust
- St Mary's, Central Manchester University Hospitals NHS Foundation Trust
- St George's Hospital, St George's Healthcare NHS Trust
- Sunderland Royal Hospital, City Hospitals Sunderland NHS Foundation Trust
- Burnley General Hospital, East Lancashire Hospitals NHS Trust
- St Michael's, University Hospitals Bristol NHS Foundation Trust
- Royal Oldham Hospital, Pennine Acute Hospitals NHS Trust
- Sheffield Teaching Hospital, Jessop Wing, Sheffield Teaching Hospitals NHS Foundation Trust
- Musgrove Park Hospital NHS Trust
- Birmingham Women's NHS Foundation Trust
- Medway Maritime Hospital, Medway NHS Foundation Trust
- Arrowe Park Hospital, Wirral University Teaching Hospital NHS Foundation Trust

- William Harvey Hospital, East Kent University NHS Foundation Trust
- Princess Royal Hospital, Shrewsbury & Telford NHS Foundation Trust
- Academic Medical Center Amsterdam, Holland
- Isala Klinieken Zwolle (Isala)
- Maxima Medical Center Veldhoven
- Erasmus University Medical Center (Rotterdam), Holland
- Leiden University Medical Center, Holland
- Maxima University Medical Center Veldhoven, Holland
- Radbouid University Nijmegen, Holland
- Maastricht Medical Center, Holland

APPENDIX A

Relationships between trial committees for CTU trials



APPENDIX B

DEFINITIONS OF NEC AND SEPSIS

Bell's Criteria for NEC

NEC Staging system based on historical, clinical and radiographical data (Bell et al 1978)

Modified Bell's Staging Criteria for Necrotizing Enterocolitis
Only tick YES if category II or III

STAGE	SYSTEM SIGNS	INTESTINAL SIGNS	RADIOLOGIC SIGNS	TREATMENT
I. Suspected				
A	Temperature instability, Apnoea, bradycardia	Elevated pregavage residuals, mild abdominal distension, occult blood in stool	Normal or mild ileus	NPO, antibiotics x 3days
B	Same as IA	Same as IA, plus gross blood in stool	Same as IA	Same as IA
II. Definite				
A: Mildly ill	Same as IA	Same as I, plus absent bowl sounds, abdominal tenderness	Ileus, pneumatosis intestinalis	NPO, antibiotics x 7 to 10 days
B: Moderately ill	Same as I, plus mild metabolic acidosis, mild thrombocytopenia	Same as I, Plus absent bowel sounds, definite abdominal tenderness, abdominal cellulitis, right lower quadrant mass	Same as IIA, plus portal vein gas, with or without ascites	NPO, antibiotics x14
III Advanced				
A Severely ill, bowel intact	Same as IIB, plus hypotension, bradycardia, respiratory acidosis, metabolic acidosis, disseminated intravascular coagulation, neutropenia	Same as I and II, plus signs of generalised peritonitis, marked tenderness and distension of abdomen.	Same as IIB, plus definite ascites	NPO, antibiotics x 14 days, fluid resuscitation, inotropic support, ventilator therapy, paracentesis
B Severely ill: bowel perforated	Same as IIA	Same as IIA	Same as IIB, plus pneumoperitoneum	Same as IIA, plus surgery

Sepsis

Sepsis is defined for the purposes of this study as culture positive sepsis or culture negative sepsis where antibiotics are given for a minimum of five days.