1 Title: Precision-medicine platform trials to improve the care of sick and preterm newborn babies.

2 Summary of research: Our goal over many years has been continuous improvement in the evidence base for personalised care pathways for sick and preterm infants through efficient, cost-effective, collaborative clinical research. The primary aim of this application is to build on our prior work and **accelerate completion** of a detailed **master protocol**, agreed by parent-patient groups, clinicians, and academic/industry partners, for a **precision-medicine platform trial** in the UK and other high- and low-income settings, to be submitted as a **Stage 1 application in May 2023**, with anticipated stage 2 application in September 2023, **planned start date of April 2024**, and end-date in 2029. The platform trial will test the **efficacy of nutritional interventions** in very (below 32 weeks gestation) preterm neonates, and hypotheses regarding **effector mechanisms**. It will **advance generalisable learning** around **platform study design**, UK and international **regulation**, and best practice in relation to **parent-patient-clinician-industry involvement**. Specific objectives for the accelerated development phase, which we will deliver in 7 work-packages, are to finalise i) the selection of interventions, and ii) the research team; iii) conduct a detailed simulation study to inform platform design; complete iv) mechanistic study protocols, v) a stakeholder involvement, engagement, and communications plan, vi) research ethics application/s for UK and non-UK sites; and vii) secure operational readiness.

We will test novel agents and interventions in variable clinical use. Each will have proof of concept, but uncertain definitive proof of efficacy, safety, effect sizes, and precision medicine effects. Primary measures of efficacy relate to survival without severe necrotising enterocolitis (NEC) assessed at 36 weeks postconceptional age, and survival without severe cognitive impairment at age two-years. We have selected these measures because NEC, an acquired acute inflammatory condition of the preterm gastrointestinal tract and major cause of death and long-term impairment, is influenced by feeding interventions, and early nutrition is a major determinant of cognitive outcome (1,2). Precision medicine factors (sex, gestational age, degree of intrauterine growth restriction) are evidenced to be of primary relevance to the efficacy of interventions and effect sizes. Mechanistic studies will test the hypotheses that the mechanism of action of each intervention is through effects on intestinal maturation and brain development. We will also consider testing hypotheses relating to the effect of interventions on body composition and biomarkers of senescence, as early nutrition is also a prime candidate determinant of long-term adult risk of chronic non-communicable diseases associated with ageing, to which the preterm population has increased vulnerability though mechanisms are uncertain. We propose to test 3-4 interventions but will include ability to incorporate additional arms in future. We have identified considerable interest from **academic, public-sector**, and **industry partners**; hence impact will be high and **position the UK as a global leader** in neonatal research.

3 Background: Around 6000 **very** preterm babies are born each year in the UK and around 5 million world-wide. Early nutrition is an important, universal preterm intervention with effects that extend beyond growth, widely considered a prime candidate determinant of life-long health and longevity in addition to an essential requirement for short-term survival and development. Preterm nutrition is influenced by innate factors chief among which are immaturity of gastrointestinal functions, and inability to self-regulate intake. Preterm infants are particularly vulnerable to impaired enteral tolerance, increasing risk of nutritional insufficiency, intestinal bacterial translocation leading to systemic sepsis, and the feared, acute acquired intestinal inflammatory disease, NEC, that has up to 50% mortality and up to 60% likelihood of long-term impairment in survivors (5).

4 Need: Preterm and newborn research is an area of great global need. Preterm births and survival are rising worldwide. In high income countries, over 95% of very preterm infants survive to go home. The greatest burden of preterm births and deaths (over 80%) is in low-income countries, especially sub-Saharan Africa, and Asia (6). However, only 2.5% of trials in the Cochrane Central Register involve neonates (7), over two-thirds of Cochrane neonatal reviews are inconclusive because included trials are too small and/or methodologically weak (8), large parts of the world, especially low- and middle-income countries are not represented, and the number of neonatal trials is diminishing (9). Only one medicine, surfactant, has ever been developed specifically for neonates (10) and over 90% of medicines for neonates are prescribed off-label or off-license, increasing the risk of adverse effects as efficacy, safety and dose data are inadequate (11). Commercial considerations all too often drive decision-making; hence the last two years have seen the development of promising new neonatal therapies halted at phase 2/3 stage until alternative

sponsors could be identified. Instances such as this add considerably to the time, burden, and cost of getting products to market, and to the mistrust in which many parents and clinicians hold commercially sponsored studies. Notably, the development of newborn medicines remains limited despite US Food and Drug Administration and European Medicines Agency incentives to pharmaceutical industries (12).

5 Relevance/importance: Though preterm survival has improved over the years, **neurocognitive** outcomes have not (13). It has also become apparent in recent years that preterm infants are at higher risk of **early onset** of a range of **chronic non-communicable diseases** associated with **ageing**, including, cardiovascular, renal, and respiratory disorders, and types 1 and 2 diabetes, with typical odds ratios ranging from 1.5 to 3.0 (14-18). Preterm nutritional stratagems have potential to improve cognitive development, the principal determinant of adult educational, economic, and societal attainments, as well as influence the risk of future chronic non-communicable disorders that are the major determinant of healthy life expectancy. However, despite this importance, optimal nutritional regimens following very preterm birth, and mechanisms of action of nutritional interventions, are largely unknown (19). Efficacy and safety are also likely to differ in relation to precision medicine factors and high- and low-income settings. Given the rise in the world prevalence of chronic non-communicable disorders, a better understanding of mechanisms action of early nutrition would likely have important scientific relevance beyond preterm care.

6 Knowledge gaps: Maternal milk is the bedrock of early nutrition but mothers who deliver very preterm are on average only able to provide 50% of the volume required, hence infants may also receive cow-milk-based formula or pasteurised human donor milk (19). Human milk contains many hundreds of biologically active molecules including growth promoters, immune regulators, and anti-infective agents specific to the individual mother, but nutrient content is very variable, leading to promotion of macronutrient fortification despite lack of evidence of safety and efficacy (20). Formula has consistent composition and energy density, but uncertain non-nutritive efficacy. Human donor milk is expensive (average cost from UK milk bank: £90-£150/litre; commercial cost £200-£2400/litre), has low nutrient density and pasteurisation inactivates or reduces non-nutrient biologically active components. Practice is very variable arising from concerns regarding over nutrition (from routine fortification), under-nutrition (from avoidance of cow-milk-derived products), and increased risk of NEC (from exposure to cow-milk-derived products). Though standard of care in some countries, in the UK only 20% of babies born below 29 weeks' gestation receive pasteurised human donor milk and 40% fortification of human milk (21). Evidence of efficacy is also needed for new **commercial macronutrient fortifiers prepared from pooled human milk** from paid donors, **new pasteurisation methods** aimed at better preservation of the non-nutritive biological properties of human milk, and **novel therapeutic agents** aimed at enhancing gut maturation, defences against bacterial invasion, and regeneration following injury. The NIHR James Lind Alliance Preterm Birth Priority Setting Partnership involving parents, the public and clinicians ranked "*Which interventions are most effective to prevent NEC in premature babies?*" and "*What is the optimum milk feeding regimen for preterm infants including use of donor and formula milks, and use of milk fortifier*?" 2nd and 6th among the top ten research questions (22).

7 Challenges in preterm research/Justification for our approach: Our approach addresses many long-standing challenges in preterm research. Neonatal populations can be small; hence to detect effects reliably our study will be **collaborative and multi-centre**. Important effects may not become apparent for **many years**; hence we will evaluate of **biomarkers** of outcome and **follow-up at age two-years**. Parental anxieties, clinician bias, and mistrust of industry sponsored studies pose additional barriers to successful research, highlighting the need for **strong stakeholder involvement, engagement, and collaboration**. There are good data indicating that the efficacy and effect-sizes of interventions will differ by sex, degree of immaturity and intrauterine growth restriction, justifying our choice of these **precision-medicine** factors (3, 4, 15, 23, 24). A **platform study** is justified because the traditional approach of conducting sequential studies is highly inefficient given the large number of interventions requiring evaluation. Platform trials offer a cost-effective, efficient means to test multiple interventions simultaneously and incrementally as shown by the landmark UK Covid-19 RECOVERY trial (25). **Mechanistic evaluation** is justified because the biological pathways affected by early nutritional interventions and the causes of differing responses are unknown, and insights may provide better measures of efficacy and identify new

therapeutic targets. Research costs are high, and rising, and participation can be burdensome for participants, families, and clinical teams; hence we will use a **real-world data** approach (see 8.2).

8 Work completed to-date: We have completed substantial work, summarised below.

8.1 Core outcome measures: We have developed a set of core neonatal outcomes comprising measures considered important to parents, former patients, clinicians, and researchers (26).

8.2 Efficient, cost-effective trial data: We have developed processes for data to be gathered electronically throughout studies, primarily from our mature, award-winning National Neonatal Research Database (NNRD) developed and managed at Imperial (27). The NNRD is a globally unique UK Data Asset, a source of real-world health data, established in 2007, that currently supports UK and international research funded to a total of approximately £25M. Data (the Neonatal Data Set, an approved NHS Information Standard comprising approximately 450 variables, many recorded daily throughout the in-patient stay, including exposure to medicines and other interventions and core neonatal outcomes) are submitted by all NHS trusts in England, Wales, and Scotland (n=181; >1.3 million patients to-date; around 100,000 new patients each year). The NNRD is population-based as neonatal specialised care is almost exclusively delivered in NHS neonatal units. Data are received as regular extracts from electronic patient record systems; completeness is high, and we quality-assure, and curate these to a research standard (28, 29). We are therefore well-positioned to meet the stringent real-world data standards required by regulators.

In neonatal trials, patient recruitment is generally carried out by members of the clinical team; hence, with the Imperial Clinical Trials Unit we have also developed a secure, web-based **point-of-care portal** for capture of ancillary UK and international data, including e-consent. These processes reduce need for specific research staff, eliminate lengthy data transcription and validation, and post-recruitment acquisition of missing data, traditional approaches that have substantially added to the expense and burden of data collection, widely acknowledged to be a major determinant of trial costs.

8.3 Low-cost, digital assessment of cognitive and language development at age two-years: With the Imperial Clinical Trials Unit, we have developed and tested an electronic version of a validated parent questionnaire for standardised, low-cost assessment of cognitive and language development at age two-years, previously only available in paper format (PARCA-R; Parent Reported Cognitive Assessment-Revised) (30). The process is fully automated with parents receiving prompts and reminders to complete the questionnaire on a mobile phone, tablet, or desk-top, when their child reaches the appropriate age window. The questionnaire produces a standardised score predictive of long-term development. The system is fully operational and managed by the Imperial Clinical Trials Unit. The PARCA-R score will be a platform trial outcome.

8.4 Parent-public involvement and engagement: This is discussed in the section “Please describe how patients/service user, carers and the public have been involved in developing this proposal”.

8.5 Clinician involvement and engagement: We have held webinars, conducted surveys, and identified prioritised interventions for a large-scale preterm nutrition study. We found most UK neonatal clinicians are highly supportive of addressing uncertainties around the benefits of routine macronutrient fortification of human milk, use of pasteurised human donor milk and the need to develop new specific therapies for preterm neonates. A minority who lack equipoise about a particular intervention would participate in other study arms.

8.6 Evidence reviews/proof-of-concept in man: We have reviewed the literature to identify precision-medicine factors. We have searched ClinicalTrials.gov and identified no other relevant current or planned studies. There is existing proof of concept for all milk products and enteral insulin, one of the experimental interventions under consideration, and anticipated proof for the novel allogeneic amniotic fluid-derived stem cell conditioned media product (see Elgan and Micregen letters of collaboration). The current Cochrane review and meta-analysis including 12 RCT and a total of 1879 infants, concludes evidence is inadequate to show whether pasteurised human donor milk or preterm formula is the optimum supplement to a shortfall in own mother’s milk (all-cause mortality: risk ratio 1.02, 95%CI [0.73,1.44]; necrotising enterocolitis: 1.56 [0.98,2.47]; neurodevelopmental disability at 18 months: 0.92 [0.4,2.1]) (31). The current Cochrane review of fortified versus unfortified human milk for preterm infants identified 18 trials and 1456 participants with no convincing evidence for an effect on necrotising enterocolitis (n=1110; risk ratio1.37 [95%CI

0.72, 2.63]); only a single trial (N=245) in 1996 assessed neurodevelopment at 18 months (mental development index: mean difference 2.20, [95%CI -3.35, 7.75]; psychomotor development index: 2.40 [-1.90, 6.70]; none evaluated metabolic health (32).

9 Objectives/gaps to be filled: This one-year accelerated development application will enable us to tackle remaining gaps in the development of the platform trial. We will undertake this in 7 work-packages (WP) described below. By study end we will have submitted **a full, Stage 1 application**.

9.1 WP1 Final selection of interventions: We are in advanced discussion with Micregen and Elgan Pharma respectively regarding the evaluation of their novel products, respectively a therapeutic agent for NEC, and enhanced gut maturation (see letters of collaboration). We are in early discussion with LactaLogics, manufacturers of pooled human milk derived products and colleagues in the Netherlands to evaluate a new method of pasteurisation aimed at better preservation of biologically active non-nutrient components of human milk. We have previously identified pressing uncertainties in relation to milk products (efficacy of routine addition of cow-milk-derived macronutrient fortifier to human milk (comparator: no routine use), and Holder pasteurised human donor milk (the pasteurisation method currently employed in the UK (comparator; preterm formula) to supplement a shortfall in maternal milk. We are additionally in discussion with colleagues in Kenya as the major burden of preterm birth is in low resource settings where intrauterine growth restriction, a key precision-medicine factor is more prevalent and the balance of risk to benefit likely to be different from high resource locations (e.g., exposure to cow-milk based products may increase NEC rate but improve cognitive outcomes in survivors). The final selection of interventions will be contingent upon these discussions, design considerations, and the feasibility of testing the interventions in the proposed settings.

9.2 WP2 Consolidation of research team: This award will enable us to accelerate dialogue with NHS, commercial and academic collaborators/co-investigators with a clear focus, to a defined time-frame. Supported by the Imperial College Industry Partners and Commercialisation team, we will develop collaborative agreements to cover scope of work, confidentiality, intellectual property rights and technology transfer issues. We will consult with methodology colleagues in the UK and US with practical experience in adult platform trials. Our team includes a co-investigator lead on public-patient and stakeholder involvement and engagement (BM). We have expressions of collaboration and willingness to be co-investigators in the planned full platform trial application from academic colleagues (Ozanne; Boardman) and an expert (Boyd) in neonatal biopharmaceutical development (see Letters of Collaboration). We are in discussion with other potential academic colleagues in the Netherlands and/or UK with expertise in studies of intestinal maturation (intestinal permeability and perfusion, development of the intestinal microbiome), a key candidate effector pathway.

9.3 WP3 Research design: We will develop a master protocol (33) applicable across all locations, for an adaptive precision-medicine platform trial. Sub-protocols will cover evaluation of the efficacy of each intervention and quantify effect-sizes. We will construct this with reference to guidance proposed by the FDA (34) and establish Data Monitoring and Trial Steering Committees to provide oversight and guidance. Preterm infants with no condition precluding enteral feeding (e.g., gastrointestinal malformation) will be eligible to participate. As preterm infants will be eligible for more than one intervention, each occurring at differing times during their care pathway the platform trial will be structured into domains each testing a specific hypothesis. This is based on an approach used in the successful REMAP-CAP study (35). We propose initiating 3-4 domains, with the potential to add further domains, or arms within domains, over time. Parents will be invited to provide informed consent to each domain and will be free to select any or all. We will examine two domain designs based on whether the control arm is an active comparator or a placebo (see simulation study below).

Principal functional outcomes are short- and medium-term measures of primary importance in the context of preterm nutrition. We will use ordinal outcomes to increase statistical efficiency. **Primary**: NEC (none; medical treatment and survived; surgical treatment and survived; died). **Secondary**: systemic sepsis (none; survived; died); cognitive and language development at age two-years (no impairment; mild-moderate; severe impairment). NEC and systemic sepsis will be assessed at 36 weeks post-conceptional age or discharge whichever comes earlier as by this time the risks of both conditions are negligible, most very preterm babies have been discharged, and the timing will support Response Adaptive Randomisation. **Precision medicine factors** are sex (male/female), degree of immaturity (gestational age), and degree of intrauterine growth restriction (birth weight z-

score). We will conduct a causal inference analysis of NNRD data to define precision medicine category thresholds. We will also conduct a simulation study to determine i) optimal domain design for active or placebo control arms; ii) adaptation rules for Response Adaptive Randomisation; stopping thresholds; and target sample sizes. Design parameters will optimise the correct decision for stopping, while minimising total sample size and participants exposed to ineffective interventions. Thresholds will be based on simulation results and investigator and stakeholder perspectives.

We will review published methods and discuss design complexities with colleagues (e.g., Response Adaptive Randomisation on ordinal outcomes; parameter setting for group-sequential designs; hierarchical Bayesian models). Response Adaptive Randomisation will be based on the primary ordinal outcome and maximise enrolment to the most effective intervention (36, 37). For domains with an active comparator, we will examine a fixed sample size design with Response Adaptive Randomisation compared with a Bayesian analysis incorporating periodic assessment of early stopping for efficacy or harm. For domains with a placebo comparator will use a Bayesian framework and incorporate early stopping for efficacy, harm, or futility. We will use hierarchical Bayesian models to determine intervention effects across precision medicine factors.

9.4 WP4 Mechanistic studies: We propose to test the principal hypotheses that each intervention affects i) the risk of NEC through effects on intestinal maturation, and ii) cognitive outcome through effects on brain development. Intestinal immaturity is widely considered the principal cause of the vulnerability of the preterm infant to NEC with mediating pathways involving endothelial damage and/or alterations to the intestinal microbiome resulting from exposure to cow-milk-based products and/or non-exposure to human milk (1, 38). Nutrient adequacy and the effects of non-nutrient human milk growth promoters are plausible pathways to brain development and cognition, e.g., high intake of human milk during neonatal care is associated with enhanced structural connectivity of developing neural networks and cerebral cortical maturation that more closely resembles that of healthy infants born at full term (39). The effects of nutritional interventions on cognitive outcomes may also be indirect e.g., by reducing systemic sepsis that often results in brain injury in the very preterm neonate.

We will also consider testing the hypotheses that nutritional interventions promoting rapid growth affect the risk of early onset of metabolic and cardiovascular disease through effects on body composition and accelerated senescence. We have previously shown differences in postnatal weight in boys and girls (40), that adults born preterm have altered body composition compared to full-term counterparts (41), that short-term dietary manipulations affect body composition (42) and relationships between postnatal growth and accelerated senescence (43). The possibility that early nutritional interventions affect adult health is highly important, but the length of follow-up to detect clinical effects has largely precluded effective causal investigation.

Studies will potentially involve biomarkers of brain development, intestinal maturation, cellular aging, and body composition. Computational MRI for investigating brain development and whole-body MRI for body composition require data to be acquired at sites with requisite expertise. During the accelerator stage, we will explore opportunities to combine multisite data using image analysis pipelines that do not require data harmonisation at acquisition. A final decision regarding mechanistic studies will be based on the feasibility of recruiting sufficient infants at sites within practicable travel distance of research imaging facilities and the practicability and costs of sample acquisition, preparation, transport, and analysis. Deliverables will be precise mechanistic protocols.

9.5 WP5 Regulator readiness: Early engagement with regulators is important to ensure research data meet requirements for licensing. CI Modi has strong links with the UK Medicines and Healthcare Regulatory Agency, European Medicines Agency, and US Food and Drug Administration. We particularly aim to obtain regulator views on adverse event and safety reporting, and acceptable end-points (44, 45). An additional key deliverable of the accelerated development programme is completion of research ethics and other regulatory requirements for the UK and international sites.

9.6 WP6 Stakeholder involvement, engagement, and communications strategy: This is discussed in the section “Please describe the ways in which patients/service users, carers and the public will be actively involved in the proposed research”.

9.7 WP7 Operational readiness: The platform trial will be delivered through the Imperial Clinical Trials Unit. A Clinical Trials Unit manager will engage with the study team during the development of the Master Protocol to ensure that operational aspects are fully considered, costs accurately

ascertained, and the NHS SoECAT processes are completed. They will ensure NHS approvals are obtained and will support international colleagues to complete equivalent processes.

10 Investigators: We are a team that has previously worked together. We will be able to work quickly and efficiently over the 12-months and expand or reduce the team as the proposal develops. Modi and Cornelius are joint co-investigators; they bring strong and complementary expertise to the proposal. Modi is a clinician with internationally recognised expertise in neonatal nutrition, and head of a multidisciplinary neonatal research group. She has over 30 years' experience in neonatal research and real-world data having led the establishment of the NNRD in 2007 and its subsequent development. Cornelius is Director of the Imperial Clinical Trials Unit and a statistician with expertise in novel trial designs. She established the NIHR Statistics Group and leads the MRC Working Group for Adverse Events in Trials. Moss is a social scientist and qualitative researcher, with strong expertise in stakeholder engagement and communications. Uthaya (Imperial) is a clinician with expertise in preterm nutrition trials and neonatal body composition. Collaborator Ozanne (MRC Metabolic Diseases Unit, Wellcome-MRC Institute of Metabolic Science, University of Cambridge) has over 25 years' research experience in early life nutritional mechanisms underlying adult health and disease. Collaborator Boardman (MRC Reproductive Biology Unit, University of Edinburgh) has 15 years' experience researching neonatal brain development and determinants of brain health.

11 Environment: Imperial College London, one of the top ten universities in the world, ranked in lead position in REF 2021 for research outputs and environment, offers a comprehensive range of research, social, and pastoral support facilities. The UKCRC registered Imperial Clinical Trials Unit is committed to supporting this platform study and making know-how available to other trials units.

12 Impact: The platform study will provide new knowledge in preterm nutrition and neonatal platform trials, areas of cardinal clinical and research need. Outputs will add value to the current research landscape and usher in a new era of opportunity for newborn research. Neonatal platform trials have not previously been conducted anywhere in the world and will support UK government ambitions to position the UK as a major international research location.

13 Dissemination: The seniority and experience of the research team, and their strong national and international connections provide multiple dissemination routes (e.g., NM is president-elect of the European Association of Perinatal Medicine; member of Conect4Children (pan-European Clinical Trials Network), MHRA and US-based International Neonatal Consortium working groups and committees, and the Health Data Research UK Alliance Board; VC is Director of the Imperial Clinical Trials Unit and member of NIHR boards). All project outputs including simulation studies will be published or otherwise placed in the public domain except when covered by IP agreements with industry partners. We will also disseminate outputs to the European Medicines Agency, Drug Information Agency, European Federation of Pharmaceutical Industries and Associations, Association of the British Pharmaceutical Industry, at speciality conferences (e.g., Neonatal Society; International Paediatric Association; US Paediatrics Academic Societies) and other trial units.

14 Gantt chart