Fetal and maternal safety considerations for *in utero* therapy clinical trials: iFeTiS consensus statement

Rachel Sagar[1], Graça Almeida-Porada[2], Karin Blakemore[3], Jerry KY Chan[4], Mahesh Choolani[5], Cecilia Götherström[6], Agnes Jaulent[7], Tippi C. MacKenzie[8], Citra Mattar[5], Christopher D. Porada[2], William H Peranteau[9], Holm Schneider[10], Steven W Shaw[11], Simon N Waddington[1,12], Magnus Westgren[6], Anna L David[1, 13, 14]*

1. Prenatal Cell and Gene Therapy Group, Elizabeth Garrett Anderson Institute for Women's Health, University College London, London, WC1E 6HX, UK.

2. Institute for Regenerative Medicine, Wake Forest School of Medicine, Winston- Salem, North Carolina 27101, USA.

3. Division of Maternal-Fetal Medicine, John's Hopkins, Baltimore, Maryland 21218, USA.

4. KK Women's and Children's Hospital, Duke-NUS Medical School, Singapore 229899.

5. Department of Obstetrics and Gynaecology, Yong Loo Lin School of Medicine, National University of Singapore, Singapore 119228.

6. Department of Clinical Science, Intervention and Technology, Division of Obstetrics and Gynecology, ANA Futura, Karolinska Institutet, 141 52 Huddinge, Sweden.

7. EspeRare Foundation, Avenue de Secheron 15, Geneva 1202, Switzerland.

8. Department of Surgery and the Center for Maternal-Fetal Precision Medicine, University of California, San Francisco, USA.

9. Children's Hospital of Philadelphia, Philadelphia 19104, USA.

10. Department of Paediatrics and Center for Ectodermal Dysplasias, University Hospital Erlangen, Germany.

11. Department of Obstetrics and Gynecology, Taipei Chang Gung Memorial Hospital, Taipei, Taiwan.

12. Wits/SAMRC Antiviral Gene Therapy Research Unit, Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, South Africa.

13. NIHR University College London Hospitals Biomedical Research Centre, 149 Tottenham Court Road, London W1T 7DN, UK.

14. Department of Development and Regeneration, Katholieke Universiteit, Leuven, Belgium.

***Correspondence should be addressed to ALD:** a.david@ucl.ac.uk

Anna L David FRCOG, PhD, MBChB, Professor and Consultant in Obstetrics and Maternal Fetal Medicine, Elizabeth Garrett Anderson Institute for Women's Health, University College London, London, WC1E 6HX, UK

Tel: +44 20 7679 6651

Mob: +44 7852 220385

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High-resolution ultrasound scanning and rapid advances in prenatal genetics enabled the diagnosis of life-threatening and severe congenital disorders early in gestation. *In utero* interventions with advanced therapy products, such as gene or stem cell transplantation, hold great promise to ameliorate *in utero* damage, induce immune tolerance and improve outome. *In utero* treatment must not only present advantages over postnatal treatment but also offer a favourable risk/benefit balance for both mother and fetus. *In utero* application of stem cells and proteins has already happened on a case-by-case basis and clinical trials of *in utero* stem cell transplantation (IUSCT) have begun. The International Fetal Transplantation and Immunology Society (IFeTIS, https://www.fetaltherapies.org) facilitated a panel discussion of international experts in 2019 to define best practice and to consider the key safety aspects of such clinical trials, including recommendations for patient monitoring and managing ethical dilemmas.

Current *in utero* therapy clinical trials

In utero stem cell transplantation and protein therapies are now entering collaborative clinical trials. A trial of *in utero* haematopoietic stem cell (HSC) transplantation for fetuses with alpha thalassemia major has reported the successful birth of the first two participating infants (https://fetus.ucsf.edu/node/406). Here, bone marrow HSCs retrieved from the mother during pregnancy are used for transplantation; these are delivered once during the pregnancy coinciding with an ultrasound-guided intrauterine blood transfusion for fetal anaemia. Based on promising results from individual compassionate cases[1], the BOOSTB4 clinical trial of postnatal or pre- and postnatal mesenchymal stem cell (MSC) transplantation for severe osteogenesis imperfecta (OI) has received regulatory and ethical approval in Sweden and the UK, and has treated three postnatal referrals in Sweden (www.boostb4.eu). First trimester human fetal liver-derived MSCs are administered once under ultrasound guidance into the

umbilical vein, with postnatal booster doses after birth. Intra-amniotic administration of a recombinant ectodysplasin A protein for *in utero* prevention of X-linked hypohidrotic ectodermal dysplasia (XLHED) also looks promising. In three affected infants treated *in utero* at 26 weeks gestation, now between three and four years old, no ectodermal dysplasia-related illnesses have been observed. The children are able to sweat normally, in contrast to untreated XLHED-affected children who do not sweat[2]. Regulatory and ethical applications for a pivotal clinical trial are in development.

Consensus statement

The requirements for optimal assessment of safety for *in utero* interventions are becoming clear through discussions of clinical trial protocols with regulatory and ethical authorities during scientific advice and approvals. Safety evaluations must consider the risks of both the mode of administration and the product itself to the fetus and to the mother. Monitoring strategies aimed at detecting potential adverse events have been developed.

Minimally invasive, ultrasound-guided injection into the umbilical vein is being used in currently approved IUSCT clinical trials. Technically, IUSCT via umbilical vein injection is similar to fetal blood transfusion, a procedure performed worldwide for decades. In a series of 937 fetal blood transfusions for fetal anaemia (2001-2015) there was per procedure a 1.2% complication risk and a 0.6% rate of fetal loss[3]. Notably these procedures were performed in anaemic fetuses. The risk of complications may be even lower when the fetal circulation is accessed in fetuses without anaemia or before manifestation of other congenital pathology. Fetal blood transfusion complications include emergency Caesarean section (0.4%), fetal bradycardia (0.3%), preterm rupture of the membranes (0.1%), infection (0.1%) and preterm birth (0.1%)[3]. Having experienced operators perform the transfusion, administering fetal

paralysis and avoiding administration into free umbilical cord loops reduces the complication rate[3]. The risk of fetal bleeding falls almost forty-fold by accessing the umbilical vein within its transit of the fetal liver as compared to cordocentesis[4]. Avoiding transplacental needling decreases the risk of fetal-maternal bleeding four-fold. This is an important consideration in IUSCT with cells from a non-maternal donor, to reduce maternal exposure to the advanced therapy product. The iFetis panel recommended that the targeted primary intravascular injection site for IUSCT was to be the intrahepatic umbilical vein. Ultrasound-guided intraperitoneal injection and intracardiac injection are alternative systemic routes of delivery that have been safely used in individual cases of IUSCT. Ultrasound guided administration of recombinant proteins or enzymes to correct metabolic disorders are likely to carry similar procedural risks. For XLHED, which requires intra-amniotic administration of medication, the complications are likely to be similar to those of ultrasound-guided amniocentesis, a commonly performed prenatal diagnostic procedure, although the risk of this procedure is not actually known. A recent meta-analysis found the risk of pregnancy loss following amniocentesis at 15-24 weeks to be around 0.11%[5].

Any invasive procedure in pregnancy carries a risk of materno-fetal transfer of infectious disease. Transmission of hepatitis B virus to the fetus after amniocentesis in women who are carriers is low; there is no evidence that transmission of hepatitis C is increased following amniocentesis in seropositive mothers. Likewise, the rate of transmission of human immunodeficiency virus (HIV) in women on highly active antiretroviral therapy (HAART) undergoing amniocentesis is similar to HIV positive women who do not undergo invasive prenatal diagnosis [6]. For diagnostic or therapeutic invasive procedures other than amniocentesis, there is little data on the risk of vertical transmission of hepatitis B, hepatitis C or HIV, and patients should be counselled to this effect prior to their use[7]. Particularly in

IUSCT the potential of fetal/neonatal liver damage due to active hepatitis or immune downregulation from active HIV infection is important. Excluding women positive for hepatitis and HIV infection from current IUSCT clinical trial protocols seems sensible until specific risk/benefit information is available. For patients undergoing HSC transplantation with maternal cells (such as in the ongoing UCSF clinical trial for alpha thalassemia), standard donor criteria also mandates exclusion of such patients.

To reduce maternal and fetal pain or discomfort the procedure is performed under local anaesthetic, and similar needling procedures for fetal blood transfusion are generally well tolerated. Severe maternal complications such as maternal bowel injury are uncommon probably because the procedure is ultrasound-guided (<0.1%)[13]. Maternal deaths are rarely reported after amniocentesis, mainly associated with Escherichia coli bacterial infection. As *in utero* drug product interventions administer only a small volume of stem cells or protein, procedures are short, lasting only a few minutes. Recovery time will not be prolonged, and venous thromboembolism secondary to immobility is unlikely to occur. Maternal exposure to fetal or donor antigens may cause sensitisation. Development of red cell antigens could place future pregnancies at risk of haemolytic disease of the newborn, and affect maternal blood transfusion. Rho(D) globulin should be given to the 15% of pregnant women who are Rhesus blood group negative to reduce sensitisation risk to 0.35% [10]. Sensitisation to other red cell antigens is rare.

Whilst the risks associated with the product administered will depend upon the exact therapeutic agent, the iFetis panel agreed common considerations for targeted monitoring strategies. Immune, allergic or toxic reactions to the therapeutic product or to a component

part involved in its manufacture warrant monitoring. And despite extensive microbiological testing and production in accordance with Good Manufacturing Practice, an advanced therapy drug product could theoretically transmit an infectious disease from the donor, or itself could be a source of microbiological contamination. Ectopic tissue formation is a specific potential risk often considered with MSC administration. A recent meta-analysis of randomised controlled trials of adult and childhood MSC transplantation did not observe acute toxicity, organ system complications, infection, death or malignancy; the only significant association detected was transient fever[11]. The specific risks of postnatal HSC transplantation are related to the pre-transplant ablative conditioning. This has not been used in IUSCT cases nor in the current alpha-thalassemia trial. Graft-versus-Host Disease remains a potential complication, but the tolerogenic environment of the fetal immune system is believed to increase the threshold for this complication. Where the mother is the stem cell donor, HSCs collection from the maternal bone marrow is associated with additional minor maternal risks such as those due to spinal anaesthesia and maternal anaemia. Given the small size of the fetus, only low volumes of maternal bone marrow are required which makes the chance of maternal anaemia less likely. Paternal bone marrow or a related donor are alternative donors.

Adverse events are most likely occur short-term following fetal injection. The iFetis panel recommended inpatient monitoring of both mother and fetus for 24 hours, to enable detection of both procedure-related complications and toxic or allergic reactions to the drug product administered. Fetal monitoring would be similar to that recommended after standard fetal needling procedures. This includes ultrasonographic visual assessment throughout and immediately following the procedure to enable timely action such as intracardiac

resuscitation. If a suitable gestational age has been reached, emergency Caesarean delivery may be indicated should a prolonged fetal bradycardia occur. In late gestation uterine tocodynamometry with fetal cardiotocography (CTG) should be performed following IUSCT to confirm fetal wellbeing. CTG interpretation in the preterm fetus with congenital disease is difficult due to the lack of standard evaluation criteria, and CTG monitoring plans will need to be individualised. Fetal ultrasound assessment should be performed before hospital discharge to assess fetal haemorrhage, effusions and hydrops, with Doppler evaluation of fetal middle cerebral and umbilical arteries for vascular perfusion and fetal anaemia. A detailed protocol for examination and analysis of the fetus or neonate and placenta is vital if perinatal loss occurs. Adverse event reporting should use the maternal and fetal criteria defined in the Medical Dictionary of Regulatory Activities (MedDRA, www.meddra.org). A Delphi consensus for grading maternal and fetal adverse events is shortly to be available, with neonatal adverse event criteria and grading provided by National Cancer Institute INC Terminology. Admission for routine maternal and fetal monitoring in the absence of pathology is common in pregnancy and should not be documented as an adverse event.

Regular follow-up is mandatory with ultrasound examination to assess fetal growth and wellbeing, fetal Doppler blood flow, organ-specific growth and echotexture, and examination of the pregnant woman for side effects. The timing and mode of delivery such as Caesaean section after *in utero* therapy should depend on the underlying fetal congenital disorder. Umbilical cord blood can be collected at birth for fetal biochemistry, immune reaction to the therapeutic products, cell engraftment, donor-specific immune tolerance and expression of the target protein. Often pregnancies affected by severe life-threatening congenital disease deliver spontaneously preterm, and this should be anticipated.

Long term follow-up of the treated neonate will depend upon the underlying congenital condition and should be planned carefully. Maternal health should also be monitored postpartum to collect information on adverse events. Current clinical trials require long term follow up of both mother and infant, for example 10 years in the BOOSTB4 trial; a registry of *in utero* therapy interventions is under consideration. A maternal peripheral blood sample allows for engraftment studies if the donor was not the mother herself. Testing for donor-specific antibody formation may inform about the maternal immune response to the cell product. Further maternal long-term data collection should coincide with longitudinal neonatal monitoring. Based on outcomes of pregnancies after amniocentesis or cord blood transfusion, it is not expected that future pregnancies will be adversely affected by these minimally invasive fetal interventions. The proposed fetal interventions for cellular, protein, and gene therapies are far less invasive than open fetal surgery for example, where high live birth rates in subsequent pregnancies are seen. But whilst IUSCT is less invasive, the risks from the proposed interventions will relate to the drug rather than the procedure.

Safety should be the primary outcome of initial clinical trials and the wellbeing of all participants (maternal, fetal, cell donor) must be considered. Counselling must be non-directive, in which the options of no intervention versus the experimental intervention—with all possible risks and benefits—are explained without personal bias. The language used to describe the clinical trial must be carefully considered, using the terms “intervention” rather than “therapy”. An independent healthcare professional or patient advocate to review patient understanding will reassure that the patient or couple are not under a “therapeutic misconception” but appreciate the experimental nature of the proposed intervention. The

potential for a lethal disorder to be partially treated, resulting in survival of a neonate with an extremely poor quality of life, is highly relevant to informed consent discussions . Paternal consent and the role of the father in the ongoing care of a neonate treated as a fetus are also important[12]. Interventions that are commenced *in utero* with a further postnatal application, for example a postnatal stem cell booster, will require reconfirmation of parental consent after birth to allow ongoing neonatal participation in the clinical trial.

iFetis panel members endorsed engagement with stakeholder and patient groups for development and ongoing conduct of clinical trials of *in utero* therapy. Patient groups have provided input during protocol development including acceptability and ethical considerations of the proposed interventions, inclusion and exclusion criteria, participant monitoring and outcome measures[13–15]. They are also key to disseminating information about *in utero* trials to potential participants.

In conclusion, newly approved clinical trials have highlighted important safety considerations to enable safe testing of these novel therapies. This consensus should allow *in utero* stem cell, protein and, eventually, gene replacement/modification therapies, to fulfil their great promise to treat many severe congenital diseases.

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