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In vivo base editing of the common Asian IVS1-5G>C mutation causing transfusion-dependent Beta-Thalassaemia in Humanized mice - proof of concept for fetal HSC-targeting gene therapy

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In-vivo gene modification therapy is an important strategy for correction of hereditary conditions in the fetus. The main challenges are safety and efficiency of editing, and delivery of large editing toolkits. The common South-Asian pathogenic IVS1-5G>C causing severe β -thalassaemia intermedia is a suitable candidate for in-vivo base editing of HSC.

We corrected this mutation in-vivo by delivering split-intein cytosine-to-guanine base editor (CGBE) in dual single-strand adeno-associated vector AAV6. Humanized NOD/SCID/IL2r γ null mice (HM) harbouring human CD45⁺ HSC (hCD45, derived from transfusion-dependent thalassaemic patients with IVS1-5G>C) were given 2×10^{13} vg/kg of AAV-split-CGBE. AAV6-transduction affected $4.25 \pm 6.72\%$ – $63.04 \pm 9.63\%$ of hCD45 nested in the spleen.

While in-vitro C>G correct editing frequency was $0.87 \pm 2.20\%$ with AAV6-split-CGBE, the pathogenic mutation was corrected in-vivo in 2/5 treated HM, at frequencies of $3.75 \pm 4.60\%$ – $4.54 \pm 12.26\%$ across all organs; 4/5 HM showed correct editing in hCD45 engrafted in at least one haemopoietic niche. hCD45 cells in lung and peripheral blood showed overall correct editing of $1.35 \pm 0.31\%$ – $13.29 \pm 16.52\%$ respectively, while hCD45 edits in other organs were incorrect. We observed minimal or no bystander A/T substitutions, indels, off-target mutations, and pro-inflammatory activation.

This proof-of-concept study, which can be applied in an affected fetus, demonstrates high-precision correction, and can be optimised to precisely target any HSC mutation.

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Correction of Preeclampsia by Intraplacental Gene Transfer of IGF-1 in the BPH/5 Mouse

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Objective

The BPH/5 is the only spontaneous mouse model that mimics human features of preeclampsia (PE). We hypothesize that placental IGF-1 gene transfer could correct endothelial dysfunction and the maternal PE in pregnant BPH/5.

Study Design

Treatment groups (N): C57 Ad-LacZ (8), C57 Ad-IGF-1 (8), BPH/5 Ad-LacZ (4), and BPH/5 Ad-IGF-1 (8). Laparotomy embryonic day 16, (e16), all placentas received 1×10^8 PFU of adenovirus with human IGF-1 (Ad-IGF-1) OR a control (Ad-LacZ). Delivery at E20-21 allowed for placentas, maternal serum, liver, kidney, urine protein was assessed across gestational timepoints. Placental microvascular density (MVD) was quantified (CD31, ImageJ). Maternal liver and kidney were stained with H&E and PAS Serum sFlt-1 was measured by ELISA.

Results

In BPH/5 mice, SBP ($p=0.0001$), MAP ($p=0.001$), and DBP ($p=0.03$) were significantly lower following Ad-IGF-1 vs. Ad-LacZ. Urine protein differed across BPH/5 groups ($p<0.05$). Glomerulosclerosis was reduced in BPH/5 Ad-IGF-1 ($p=0.0255$; 62.97% decrease, $\eta^2=0.48$). Ad-IGF-1 increased MVD ($p=0.0122$), reduced VC ($p=0.0014$), and increased PS ($p=0.0014$) in BPH/5.

Conclusion

Intraplacental gene transfer of IGF-1 in BPH/5 normalized BP, restored MVD, normalized VC, proteinuria, and glomerulosclerosis without affecting fetal viability. Intraplacental IGF-1 gene transfer corrects PE in the BPH/5 mice.

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Mammalian-Optimized Plasmid DNA (pDNA) as a Novel Agent of Transamniotic Nucleic Acid Therapy (TRANAT): Pharmacokinetics in a Murine Model**Alex Liu Huang**

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Purpose: We sought to determine the feasibility and pharmacokinetics of intra-amniotic mammalian-optimized plasmid DNA (pDNA) delivery.

Methods: Eight dams were divided into four groups based on the content of intra-amniotic injections performed in all their fetuses (n=106) on gestational day 17 (E17; term=E21-E22). Two groups received pDNA containing a firefly luciferase gene (figure) either encapsulated within a lipid nanoparticle (LNP; n=28) or within a semi-synthetic composite (lipopolyplex; n=24) while the other two groups received corresponding encapsulation materials without pDNA. Animals were euthanized at term, when 15 fetal anatomical sites and annexes were screened for luciferase activity via luminometry as surrogate for host translation of functioning protein from the pDNA. Statistical analyses included the Fisher's exact and Wilcoxon rank sum tests ($p < 0.05$).

Results: Fetal survival was 89% (94/106). When controlled by the corresponding empty encapsulations, positive luminescence was detected in the LNP-pDNA group at most fetal anatomical sites (heart, intestines, kidney, liver, lungs, spleen, thymus, and bone marrow $p < 0.001$ - $p = 0.034$) and all fetal annexes (placenta, umbilical cord, and gestational membranes; all $p < 0.001$) (table – abbreviated). Positive luminescence was detected in the lipopolyplex-pDNA group only in the umbilical cord ($p = 0.002$).

Conclusions: pDNA is a viable novel agent of transamniotic nucleic acid therapy (TRANAT).

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Xenogeneic Fetal Kidney Transplantation as a Prenatal Bridge Therapy for Congenital Renal Failure

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Background: Severe congenital kidney diseases, including bilateral renal agenesis, cause oligohydramnios, pulmonary hypoplasia, and fatal neonatal renal failure. Immediate postnatal dialysis is limited by prematurity and gastrointestinal immaturity. We developed xenogeneic fetal kidney transplantation to establish urinary excretion before birth and serve as a prenatal bridge therapy.

Methods: GFP-positive fetal rat or mouse kidneys were transplanted transuterinely into fetal rats. To establish a clinically applicable transplantation technique, porcine fetal kidneys were transplanted under ultrasound guidance into microminipig fetuses with tacrolimus monotherapy. In non-human primates, porcine fetal kidneys were transplanted into fetal common marmosets and cynomolgus monkeys to evaluate feasibility in clinically relevant models.

Results: In rat-to-rat transplantation, grafts developed glomerular and tubular structures, and sustained urine production was confirmed for up to 150 days. In pig-to-pig transplantation, term delivery, graft engraftment, nephron formation, and urine production for 97 days were achieved with mild rejection. In both primate models, offspring survived to term under tacrolimus and steroid treatment; however, porcine graft development was limited.

Conclusion: Xenogeneic fetal kidney transplantation may provide a novel prenatal bridge therapy for lethal congenital kidney diseases. Future studies using genetically modified porcine fetal kidneys may improve graft acceptance and development.

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Isoflavones modulate ER α /PR balance and inhibit preterm labor-associated pathways through PR-B-dependent regulation in human uterine cells**Kyung mi Choi¹, Ki Hoon Ahn¹, Min Ji Lee¹, Akihiro Hasegawa²,
Alireza A Shamshirsaz²**¹ Korea University College of Medicine Department of Obstetrics and Gynecology² Dell Medical School, The University of Texas at Austin, Austin, Tx, USA Division of Fetal Medicine and Therapy, Department of Women's Health

Uterine contraction, inflammation, and extracellular matrix remodeling are key biological processes involved in the transition from uterine quiescence to labor during the initiation of parturition. Isoflavones are known to regulate uterine function through hormone receptor and anti-inflammatory mechanisms. This study investigated whether genistein, genistin, and daidzein modulate the estrogen receptor alpha (ER α)/progesterone receptor (PR) ratio and suppress uterine activation pathways in human uterine cells, with a focus on PR-B-dependent signaling. Primary human myometrial and cervical stromal cells were treated with isoflavones under oxytocin- and lipopolysaccharide- induced conditions. Expression of contraction-associated markers (OXTR, GJA1, PTGS2), inflammatory cytokines, and extracellular matrix (ECM) remodeling markers (MMPs) were analyzed using qRT-PCR, Western blot, and immunofluorescence. PR-B overexpression and inhibition (RU486) were employed to assess causality. Isoflavone reduced contraction- and inflammation-associated gene expression, particularly under stimulated conditions, with more pronounced effects in preterm samples. They also decreased MMP-2 and MMP-9 expression, indicating suppression of ECM remodeling. PR-B overexpression enhanced these inhibitory effects, whereas RU486 treatment reversed them, confirming PR-dependent regulation. These findings demonstrate that isoflavones suppress uterine activation through a PR-B-dependent mechanism and may serve as promising candidates for preventing preterm birth by interrupting key activation pathways. Funded from KHIDI (RS-2025-02263778), NRF (NRF-2018R1D1A1B07046332) and KURILF.

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**AMNIOWELL, A NOVEL SYNTHETIC AMNIOTIC FLUID
DESIGNED TO IMPROVE OUTCOMES IN FETAL THERAPY****Jose L. PEIRO^{1,2}**¹NYU Langone Health NYULH Advanced Fetal Care Center²New York University NYU Grossman School of Medicine

Background and Objectives: Fetal interventions, open or fetoscopic surgery, often are associated with preterm premature rupture of membranes (PPROM). Current replacement fluids, Normal Saline (NS) and Lactated-Ringer's (LR), differ markedly from native amniotic-fluid in pH and nutrient composition. We hypothesized that these fluids injure the non-healing amniotic membrane through oxidative stress and inflammation. These studies aimed to develop a novel synthetic fluid, AmnioWell (AW), that mimics second-trimester amniotic-fluid and compare its effects with NS and LR.

Methods: Human amniotic cells and membrane explants were exposed to NS, LR, or AW in vitro. A novel rat model enabling complete amniotic fluid exchange at E17.5 was then used to assess membrane integrity and fetal organ development after exposure to NS, LR, AW, or growth factor-enriched AW++.

Results: NS and LR reduced amniotic cell viability, increased reactive oxygen species, promoted amnion microfractures, and increased the MMP9/collagen ratio, indicating tissue weakening. AW preserved viability and membrane structure. NS and LR also caused lung edema, macrophage infiltration, and major reductions in surfactant proteins, whereas AW reduced inflammation and improved surfactant expression. GI histology remained largely unchanged.

Conclusions: AW better preserves amniotic membrane integrity and supports fetal lung development than NS or LR in preclinical models.

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PRENATAL ABDOMINAL INTRAMUSCULAR BOTULINUM TOXIN IN FETAL LAMBS: FEASIBILITY, BIO-SAFETY, AND EFFICACY.**Jose L. PEIRO^{1,2}**¹ NYU Langone Health NYULH Advanced Fetal Care Center² New York University NYU Grossman School of Medicine

Background: Botulinum-toxin may be useful in some fetal interventions because of its reversible muscle-relaxing effects, but prenatal maternal-fetal safety data are limited. This study evaluated the feasibility, biosafety, and efficacy of fetal-intramuscular botulinum-toxin administration in a fetal ovine model.

Methods: Ten pregnant ewes underwent ultrasound-guided fetal abdominal intramuscular injection of botulinum-toxin (n=6) or normal saline (n=4). Safety outcomes included fetal survival, intrauterine fetal demise, and procedure-related complications. Amniotic fluid was analyzed for botulinum-neurotoxin type A (BoNT-A) by ELISA. At term, placenta, fetal brain, lungs, thoracic/abdominal wall, kidneys, and liver underwent histological examination in an independent laboratory. Efficacy was assessed by serial ultrasound measurements of fetal abdominal size, analyzed using linear mixed-effects modeling.

Results: All injections were successfully performed, with 100% fetal survival and no maternal, fetal, or procedure-related complications. BoNT-A was undetectable in amniotic fluid. Histological assessments showed normal organ architecture and no evidence of toxicity, inflammation, or structural abnormalities, indicating no systemic distribution or deleterious effects. Botulinum toxin-treated fetuses showed significantly greater abdominal growth than controls, with a significant group-by-time interaction.

Conclusion: Prenatal fetal intramuscular botulinum toxin injection was feasible, showed an excellent biosafety profile, and significantly increased fetal abdominal capacity in this ovine model.