aiming the safety and the efficacy of this mobilization regimen is open, and four patients have been already recruited.

Validation of preclinical gene therapy studies with the therapeutic clinical-grade LV: A clinical lot of the *FANCA*-LV has been already produced. The batch is used for the validation of the transduction protocol under GMP conditions, as well as for the validation of all the quality controls required by the regulatory agency. To investigate the biosafety of insertion sites of the therapeutic LV, bone marrow cells from *Fanca*^{-/-} mice were transduced with the therapeutic LV and then transplanted into primary and secondary *Fanca*^{-/-} mice recipients. The analysis of the dynamics of LV-transduced *Fanca*^{-/-} repopulating clones showed a highly polyclonal repertoire and a healthy genetic pattern of clonal turnover in transplanted FA mice.

Expected Outcome

The expected final result of EUROFANCOLEN is the development of a new therapy for the bone marrow failure of FA patients. The proposed gene therapy approach would constitute a new therapeutic opportunity for patients lacking a suitable donor, which may additionally circumvent some of the undesired effects of allogeneic bone marrow transplantation.

Major Publications

- Bogliolo M, Surrallés J. Fanconi anemia: from novel genes to advanced therapies. *Curr Opin Genet Dev* 2015 (submitted, invited review).
- Bueren JA. Towards the lentiviral gene therapy of mobilized CD34+ cells from Fanconi anemia patients. *Hum Gene Ther* 2014;25:A12.
- Molina-Estevez FJ, Nowrouzi A, Lozano ML, et al. Lentiviral gene therapy in Fanconi anemia A mice reveals long-term engraftment and continuous turnover of corrected HSCs. 2015 (submitted).
- Trujillo JP, Surrallés J. Savior siblings and Fanconi anemia: analysis of success rates from the family's perspective. *Genet Med* 2015 (in press).

Coordinator: Prof. Juan A. Bueren, juan.bueren@ciemat.es

Partners: The EuroFancolen consortium brings together 11 partners from 4 countries.

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DOI: 10.1089/humc.2015.2523

Does Vascular Endothelial Growth Factor Gene Therapy Safely Improve Outcome in Severe Early-Onset Fetal Growth Restriction? (EVERREST)

Contract No.: 305823; EU contribution: € 5,998,984;
 Total costs: € 7,766,109.60; Starting date: 01/01/2013;
 Duration: 72 months

Background and Objectives

SEVERE, EARLY-ONSET fetal growth restriction (FGR) affects 11,000 babies annually in the European Union (EU). In most cases, reduced uterine blood flow restricts substrate delivery to the fetus, a condition called placental insufficiency. Fetal growth slows or can cease well before the time of normal birth. There are no therapies that improve fetal growth *in utero*. Current management of severe early-onset FGR involves extremely preterm delivery of the fetus (before 28 weeks of gestation), before death occurs *in utero*, with the aim of minimizing irreversible organ damage, particularly to the brain. This adds additional risks to the baby from extremely preterm birth, with its own attendant short- and long-term complications; affected neonates suffer intracranial hemorrhage, chronic lung disease, and cerebral palsy, with heart disease and diabetes as adults. Furthermore, FGR may be detected while the fetal weight is far below 500 g, a situation considered by many to be nonviable. From cohort studies and local data, we estimate that the cumulative stillbirth/morbidity/neonatal mortality rate in pregnancies affected by severe early-onset FGR is between 65% and 80%.

Recent improvements in the care of premature growth-restricted neonates mean that more neonates survive delivery, but at great cost. Survival studies suggest, however, that even modest increases in birth weight (e.g., from 500 to 600 g) and gestation at delivery (e.g., from 26 to 27 weeks) are associated with significant improvements in mortality and morbidity. Improving uterine blood flow is key and vascular endothelial growth factor (VEGF) is important to achieve this. In preclinical animal models, EVERREST consortium partners have shown that local VEGF gene transfer to the uteroplacental circulation using adenovirus vectors increases uterine blood flow, attenuates constriction of uterine arteries, and increases angiogenesis; these changes result in improved growth of severely growth-restricted fetuses.

The aim of the EVERREST project is to translate these findings from bench to bedside by developing the first

clinically applicable evidence-based therapy that could improve perinatal outcome in severe early-onset FGR.

Approach and Methodology

In order to meet this overarching aim, three key streams of work are ongoing. At the outset, we undertook a thorough study of the bioethics of the EVERREST trial and intervention. The study included a review of the literature on gene therapy, experimental treatment of pregnant women and fetal medicine research to clarify relevant ethical, legal, and regulatory issues, and a qualitative interview study with stakeholders and parents of babies affected by severe early-onset FGR. The second work stream involves completion of a reproductive toxicology program: first, in perfused human placentas *ex vivo* and, second, in rabbits to study the biodistribution of the adenoviral vector after injection into the maternal uterine artery and the effect on the mother and offspring.

On receipt of ethical and regulatory approval, we then aim to perform an uncontrolled, open-label, dose-finding phase I/II (to mother and baby) study in 15 and up to 27 pregnancies affected by severe early-onset FGR of maternal VEGF growth factor therapy using an adenoviral vector, delivered to the maternal uterine artery using minimally invasive techniques. The primary endpoint of the study will be safety and tolerability. Secondary endpoints relate to efficacy by measuring improvements in uterine volume blood flow, fetal growth and gestational age at delivery, and a composite outcome for fetal and neonatal mortality. Outcomes and morbidity will be compared with data from untreated affected pregnancies; these cohort data are currently being generated in the prospective case study that has ethical approval at EVERREST clinical sites in the United Kingdom, Germany, Spain, and Sweden.

Main Findings

Bioethics study

A literature review investigated two key questions: first, whether it is ethical to treat a pregnant woman with a potentially risky treatment when she herself has no benefit from the treatment but it may improve the health and survival prospects of the unborn baby. The review concluded that treatment of the unborn for its own benefit is permitted. The second question considered was whether it is ethical to treat this condition of the unborn baby who may otherwise have died but, with the treatment, may be born with a serious disability. It also addressed the psychological burden of the woman when making her decision in favor of or against the new treatment. The literature review concluded that there was a need to interview women and their partners about their experiences when faced with the diagnosis of FGR.

Key stakeholders from 34 patient or disability organizations and medical stakeholders across Europe were interviewed. Two main topics emerged from the interviews: the problem of obtaining informed consent from the pregnant woman on whether or not to participate in the trial, and the psychological burden facing the woman making the decision when she has just been given the diagnosis of severe early-onset FGR. The former will be addressed in the design of the clinical trial; the latter was discussed in patient interviews.

Interviews in the EVERREST clinical sites with 24 women/couples with experience of a pregnancy complicated by severe

early-onset FGR did not identify any fundamental ethical objections to the use of maternal VEGF growth factor gene therapy. Respondents had a generally favorable view of the ethical and social acceptability of EVERREST. Most pregnant women view their unborn child as a person early on in pregnancy and would generally “do anything to help this child.” While decision making in a pregnancy affected by severe early-onset FGR was difficult, women did not feel incapable of arriving at a decision regarding any treatment options but would listen to the advice of healthcare professionals and discuss the options with their partners. Women were also generally interested in participating in clinical trials particularly where the trial conferred a potential benefit to the unborn child.

The conclusions of the stakeholders and the former patients are broadly consistent and positive. Both empirical studies support the view that a trial would be useful and beneficial, provided that the consent considerations can be managed effectively. These conclusions were endorsed by the EVERREST Independent Ethics Advisory Committee.

Reproductive toxicology

Studies in two different human placental model systems have been used to investigate the effect of the EVERREST vector (Ad.VEGF-D^{ANAC}) on placental function, gene transfer to the trophoblast, and vector cross-over to the fetal circulation: the *in vitro* human placental villous explant culture model and the *ex vivo* dual perfusion model of the human placenta. These human placental experimental data have demonstrated that, while the human placenta has a very low capacity to take up Ad.VEGF-D^{ANAC}, access by the viral vector is very limited to the maternal-facing epithelium and transfer to the fetoplacental circulation is unlikely to occur. Optimization is completed of a quantitative polymerase chain reaction study to detect and quantify the presence of VEGF adenoviral vector on the fetal side of samples of human placental tissue perfusates from normal and FGR pregnancies. The quantification studies are being completed.

Clinical trial design

Development of the EVERREST Clinical Trial protocol has been a multinational and multidisciplinary exercise of fetal medicine, obstetric, neonatology, and interventional radiology experts from the EVERREST consortium. The Independent Ethics Advisory Committee, Data Safety Monitoring Board, and Trial Steering Committee have met and provided their input. Twenty women have been recruited to the prospective study cohort by clinical sites in the United Kingdom and Sweden (to end January 2015).

The EVERREST intervention was granted Orphan Medicinal Product Designation from the European Medicines Agency (number: EU/3/14/1415) in January 2015, and the consortium is seeking scientific advice on the clinical trial protocol from the European Medicines Agency.

Expected Outcome

The EVERREST clinical trial will aim to demonstrate the safety and tolerability of maternal uterine artery administration of VEGF in an adenoviral vector in women with pregnancies affected by severe early-onset FGR. In addition, it will aim to gain initial insights into the efficacy of the

product. Together, these data will enable further development of the therapy through clinical trials to demonstrate efficacy, and will support regulatory approval and commercial launch. Our aspiration for this therapy, should we find that it is safe and effective, is for it to become the standard of care in the EU within the next 15–20 years.

The prospective study that is collecting data and samples from women who experience severe early-onset FGR will be the first database/biobank of its kind to concentrate on this condition from when women first have a diagnosis made. The study will be a rich source of data to explore both short- and long-term maternal and neonatal morbidities of this condition. It may also generate novel biomarkers that could be used to predict prognosis of severe early-onset FGR at the time of diagnosis.

Major Publication

Spencer RN, Carr DJ, David AL. Treatment of poor placentation and the prevention of associated adverse outcomes—what does the future hold? *Prenat Diagn* 2014;34:677–684.

Coordinator: Dr. Anna David, a.david@ucl.ac.uk

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DOI: 10.1089/humc.2015.2524

Mutanome Engineered RNA Immunotherapy (MERIT)

Contract No.: 601939; EU contribution: € 5,964,250;
Total costs: € 7,794,995.47; Starting date: 01/06/2013;
Duration: 36 months

Background and Objectives

THE MUTANOME ENGINEERED RNA IMMUNO-THERAPY (MERIT) project aims to clinically and industrially validate a pioneering RNA-based immunotherapy concept that targets individually expressed tumor antigens and tu-

mor-specific mutations in patients suffering from triple-negative breast cancer (TNBC). This biomarker-guided, personalized therapy is a collaborative effort of five partners from academia and industry, funded by the European Union's Seventh Framework Programme (FP7) and led by BioNTech AG.

TNBC is an aggressive cancer that accounts for 15–20% of all breast cancer patients and is defined by the lack of estrogen receptor and progesterone receptor and the absence of HER-2 overexpression.¹ The 5-year survival rate is less than 80%.² The molecular heterogeneity across TNBCs results in a lack of common targetable molecular alterations, and thus targeted therapies frequently fail to provide clinical benefit. Encouragingly, recent studies report a high immunogenicity of TNBC tumors.³ Moreover, TNBC tumors express various numbers of specific antigens and are characterized by a moderate frequency of tumor mutations.⁴ The MERIT consortium therefore proposes to meet the unmet medical need of TNBC patients by inducing an integrated antitumoral immune response directed against both shared and mutated tumor antigens on a single-patient basis.

The major objective of the MERIT project is to assess in a phase I/II trial, the clinical safety, feasibility, and biological efficacy of a completely novel next-generation sequencing (NGS)-guided, individually engineered, and on-demand-manufactured RNA vaccine approach to treat cancer.

Secondary objectives are (1) to develop a computational medicine platform to allow for rapid identification of immunogenic shared and mutated antigens in patients with TNBC, (2) to set up an RNA vaccine warehouse of shared tumor antigens addressing >95% of TNBC patients and a manufacturing process for timely on-demand manufacturing of a personalized RNA vaccine targeting multiple tumor-specific mutations, (3) to study associated biomarkers to identify molecular and immunological signatures that correlate with clinical events following treatment, and (4) to identify synergistic compounds and optimized protocols of MERIT vaccines.

Approach and Methodology

The personalized treatment consists of (1) a vaccine containing “off-the-shelf” mRNAs selected from a presynthesized mRNA vaccine warehouse (MERIT WAREHOUSE) that encode tumor-specific antigens expressed in the respective patient's tumor and (2) a vaccine containing mRNAs engineered on-demand that encode patient-specific sequence stretches incorporating nonsynonymous mutations (MERIT MUTANOME). Before treatment, every patient's tumor will be profiled to select the suitable shared tumor antigens and identify mutations. To this end, we apply exome sequencing by NGS and qPCR in a clinical-grade processes for the rapid determination of individual tumor antigen signatures and mutations. The output of the profiling will be a patient-specific list of confirmed expressed antigens and immunogenic mutations. Based on this list, a cutting-edge platform technology will be used for the rapid design, manufacture, and release of fully tailored, pharmacologically optimized, GMP-grade RNA vaccines.

The mRNAs are administered intravenously as a nanoparticulate lipoplex formulation and are selectively delivered to splenic antigen-presenting cells (APCs). The