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HEMACURE: APPLICATION OF COMBINED GENE AND CELL THERAPY WITHIN AN IMPLANTABLE THERAPEUTIC DEVICE FOR THE TREATMENT OF SEVERE HAEMOPHILIA A

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Introduction: Our consortium aims to develop a novel *ex vivo* cell-based therapy to treat haemophilia A (HA) that should ultimately lead to improved patient quality of life. Blood outgrowth endothelial cells (BOECs), a production source of factor VIII (FVIII) will be isolated from HA patients and genetically corrected with a lentiviral vector (LV) encoding for functional FVIII. Corrected cells will be expanded to guarantee a long-lasting therapy or cure. The cells will be tested for safety, efficacy and endothelial characteristics and transplanted into a subcutaneously implanted medical device for sustained secretion of FVIII in NOD/SCID γ-null (NSG) HA mice for preclinical studies. All processes will be performed under Good Manufacturing Practice (GMP) conditions, to ensure safety and quality according to the highest standards.

Material & Methods: BOECs were isolated from non-haemophilia donors' and patients' blood. Cells were efficiently transduced by LVs containing the B domain deleted form of human FVIII under control of the Vascular Endothelial Cadherin promoter (VEC). BOECs were characterized by FACS for endothelial phenotype and FVIII evaluated by APTT and ELISA. Ten million LV-VEC.hFVIII-BOECs were transplanted intraperitoneally on cytodex 3 microcarrier beads in NSG-HA mice (n=3). In addition corrected BOECS from HA patients will be transplanted into the medical device in NSG-HA mice for optimization of sustained FVIII secretion.

Results: The number of integrated LV copies/cell was ~3 for LV-VEC.hFVIII transduced cells, yielding in 67% of cells expressing FVIII determined by FACS. We confirmed endothelial phenotype of corrected cells by FACS detecting endothelial specific markers. BOECs survived and secreted FVIII at therapeutic levels (with a peak of 15% FVIII activity) for up to 13 weeks in mice.

Conclusion: With our strategy, we achieved secretion of functional FVIII at therapeutic levels up to 13 weeks in NSG-HA mice. Furthermore, we established a GMP compliant process for isolation of BOECs and currently establishing GMP compliance for the LV-transduction and expansion of BOECs as well. Based on the results we will accomplish GMP compliance for the whole process chain, we plan to transplant GMP produced, autologous gene corrected and safe BOECs for future clinical testing in HA patients.