SHORT TERM SCIENTIFIC MISSION (STSM) SCIENTIFIC REPORT

**Action number: CA17103 - Delivery of Antisense RNA Therapeutics**
**STSM title: Validation of a pig cell culture to evaluate antisense therapies for DMD**
**STSM start and end date: 01/03/2019-31/03/2019**
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The Friedrich-Baur-Institute, Department of Neurology, in collaboration with other groups at the Ludwig-Maximilians-University, Munich, Germany has generated DMD exon 52 deficient pigs, to establish a large animal model for Duchenne muscular dystrophy (DMD). As we are working on a project with gene editing and antisense therapies applied to animal model cultures we found it very interesting to learn some protocols to work with porcine myoblast cell culture derived from the DMD pig.

The aim of this STSM was to validate exon skipping efficiency using an antisense oligonucleotide (AON) to skip exon 51 (the same we have tried before in DMD human myoblasts) and to test dystrophin expression comparing control and DMD pig cultures before and after being treated with the AON.

While doing this, I was interested in learning as many techniques as possible related with this pig model like quantitative western blot measurements and various myoblast differentiation and immunostaining procedures. I also wanted to train the staff at the host institution in the in-cell western techniques developed by our laboratory at Biocruces Bizkaia Research Institute, adapting our current protocol to their cell culture model, analyzer and reagents.

My main interest during the STSM were the proliferation and differentiation conditions of porcine myoblast cell cultures (wild type and DMD cells). We tried different coatings to improve differentiation both in microplates for future in-cell western assays and also to improve immunostaining on coverslips and chamber slides.

I have also learned the complete exon skipping protocol of exon 51 in DMD porcine myoblasts carrying an exon 52 deletion, including AON transfection, RNA extraction and quality control, RT-PCR reaction and finally nested PCR and gel electrophoresis.

Together with a PhD student from the institute, I have been learning their western blotting protocol to measure dystrophin in cells and muscle samples. Moreover, I have been trained in the isolation of primary pig myoblasts from muscle biopsies using a method that has been developed by the Munich laboratory for this kind of cells and I have also learned the protocol for human myoblast isolation from diagnostic muscle biopsies.

As DMD porcine myoblasts are difficult to grow in microplates and that is the preferred format to carry out an in-cell western assay, we first tried different coatings in P96 well plates to improve differentiation.

The Odyssey® scanner available at the Friedrich-Baur-Institute, is a different model from the one at Biocruces and our usual 96-well-microplates do not fit and scanning in individual 3.5 cm dishes (array like in a 6-well plate) was not feasible. Using conventional techniques, we saw very low dystrophin expression in DMD compared to wild type and carrier.

Moreover, I received training on the exon skipping protocol in porcine myoblasts. Desmin expression was high in all the cell types which indicates good myogenicity of the cultures and dystrophin expression was differently distributed depending on the cell type and stage of myotube formation.

This STSM has been very interesting and we would be very happy to collaborate in the future. We will adapt some Friedrich Baur methods in the Biocruces lab and also finally optimize the in-cell western analysis in porcine myoblasts in our lab to complement their western blot results and to allow for quantitative dystrophin measurement, in particular to determine the efficiency of dystrophin restoration in exon skipping experiments.