SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

This report is submitted for approval by the STSM applicant to the STSM coordinator

Action number: CA17103
STSM title: Ex vivo force measurement of the diaphragm muscle to assess dystrophin restoration efficacy
STSM start and end date: 24/02/2020 to 28/02/2020
Grantee name: Dr. Maaike van Putten

PURPOSE OF THE STSM:

Duchenne muscular dystrophy is a chronic devastating disorders affecting 1:5000 new born males. It is caused by a lack of dystrophin protein, which is normally expressed in muscle fibers and prevents them from damage during contractions. One of the most promising therapeutic strategies is restoring the disrupted open reading frame of the dystrophin gene through antisense oligonucleotide-(AON) mediated exon skipping. The efficacy of this therapeutic approach significantly depends on optimal delivery; all muscles need to be targeted. In my research, I aim to improve AON delivery through either conjugation of peptides to AONs or the use of modified chemistries. In addition to pharmacokinetic and dynamic studies, I assess muscle performance to determine treatment efficacy.

Since the diaphragm is one of the most severely affected muscles in DMD mice, it is one of the preferred muscles to study the influence of antisense mediated exon skipping on muscle functionality and pathology. Performing *ex vivo* physiology on the diaphragm is therefore a valuable physiological outcome measure.

The aim of this STSM was to learn how to perform *ex vivo* physiology on the diaphragm to assess muscle functionality.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSMS

During my visit, I joined a post-doc of Prof. De Luca, Paola Mantuano. On the first few days she showed me how to isolate the extensor digitorum longus and diaphragm from a mouse, prepare them for and execute ex vivo physiology measurements on them. Thereafter, I used the contralateral muscle and spare pieces of diaphragm to practice myself.
On the following day, I received a surplus animal to isolate and prepare the EDL and diaphragm for physiology myself, while Paola gave me advise, tips and tricks. We also performed *in vivo* torque of the hindlimb together. On the last day, we focused on data analyses and interpretation of the results.

I would like to stress that the *ex vivo* physiology of the EDL and the *in vivo* torque measurements on the hindlimb were not part of the orginal plan. However, as time permitted, and these analyses are performed utilizing the same equipment, this was a nice occacion to learn. With only a few adaptations, this can also be set-up in the LUMC relatively easily. I therefore allowed time to also learn these techniques.
Lastly, Paola also showed me around in the animal facility and we discussed technical details of several techiniques we both regularly perform to assess muscle functionality in vivo.

DESCRIPTION OF THE MAIN RESULTS OBTAINED

At the end of the visit, I acquired the skills to not only perform *ex vivo* physiology measurements of the diaphragm strips, but also of the EDL muscle. In addition, I learned how to measure *in vivo* torgue of the hindlimbs. This can be used as a longitudinal measure to assess muscle performance in contrast to the two *ex vivo* techniques.
I am currently in contact with Aurora Scientific to acquire the *ex vivo* physiology bath required for the analyses of the diaphragm and EDL muscles, and the foot pedal and hind limb clamp required for the torgue measurements. When I have obtained the equipment from Aurora Scientific, I will set-up these techniques within the LUMC and also teach research technicians to perform these analyses.

FUTURE COLLABORATIONS (if applicable)

I will continue my close collaboration with prof. Annamaria De Luca. Being able to perform these techniques in a standardized manner between our two labs will also facilitate future collaborations, in which RNA delivery methods can be compared and validated in both labs.

In addition, I strongly believe that the three techniques I learned will be widely used within the LUMC. As such, I will also collaborate internally with many research groups currently focusing on RNA therapies for multiple muscular disorders.