SHORT TERM SCIENTIFIC MISSION (STSM)
SCIENTIFIC REPORT

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

Action number: BM1402 – Development of a European network for preclinical testing of interventions in mouse models
STSM title: Inflammation and oxidative stress in age-related hearing loss and its association to IGF-1 deficiency
STSM start and end date: 01/09/2018 to 31/10/2018
Grantee name: Angela Garcia Mato

PURPOSE OF THE STSM:
(max.200 words)

Hearing loss affects 1/3 people older than 65 years, being a disease that is associated with the progression of cognitive decline in the elderly. Similar to other age-related diseases, redox stress and chronic inflammation are well-known underlying mechanisms.
Insulin-like growth factor 1 (IGF-1) circulating levels are reduced with ageing, a trend that has been associated with hearing loss and onset of neurodegenerative diseases. IGF-1 deficiency leads to increased inflammation, to the failure of cellular metabolic homeostasis and apoptosis. This is critical in the auditory receptor because hair cells and hearing neurons do not regenerate in mammals.
As a part of my PhD project at Prof. Isabel Varela-Nieto's group, I'm studying cell lines representative of the main types that form the auditory receptor by using genetically modified clones by CRISPR-Cas technology of these lines that we have generated and that are cellular models of IGF-1 deficiency.
The purpose of the STSM was to investigate the relationship between inflammation and oxidative stress in age-related hearing loss and its association to IGF-1 deficiency by using these genetically modified clones with partial or complete absence of *Igf1*. The best way to achieve our goal was through a STSM in Prof. Roosmarijn Vandenbroucke's group, an experienced lab in studying inflammation.

DESCRIPTION OF WORK CARRIED OUT DURING THE STSM:
(max.500 words)

During the STSM, I have learned different techniques to evaluate and compare the inflammatory status of the original Neuro2a cell line and the genetically modified clones (named 4A10 and 2G3). In a first group of experiments, I wanted to study the basal inflammatory status of these cell lines without any treatment. With this objective in mind, I carried out a RT-qPCR, first a Reverse Transcription (RT) and with the resulting cDNA, I carried out a Quantitative-Polymerase Chain Reaction (qPCR) to analyze *Il1β*, *Il6*, *Tnfα*

and *Ifnγ* expression. To do that, I plated the cells and after 2 days of culture, I isolated the RNA to perform the cDNA synthesis with RT and the cDNA synthetized was used to perform the qPCR. The same cells were used to measure cytokines secreted to the culture medium, as IL1β, IL6, IL10, TNFα and IFNγ, as well as the levels of IGF-1, by a multiplex assay. To do that, I collected the supernatant, centrifuged it to discard cellular debris, and carried out the multiplex assay with the free-cell supernatant for measuring cytokines in one hand, and for analyzing IGF-1 levels in the other.
Next step in the STSM was to evaluate the response of the cell lines to treatment with different inflammatory stimuli, like LPS, TNFα and IFNγ. After consulting in the literature, I decided to treat the cells with the following: LPS at 50 µg/mL during 1 hour, TFNα at 100 ng/mL during 1 hour, and IFNγ at 50 U/mL during 24 hours. After plating and treating the cells, I followed the same steps mentioned above to carry out RT-qPCR to analyze genes involved in inflammation, and the multiplex assay for the detection of cytokines produced by cells.
In order to ensure optimal conditions to obtain better results, an MTT assay was carried out to test cell viability following treatment with the inflammatory stimuli used previously. To do that, I treated the cells with the same concentration of the inflammatory stimuli mentioned above, but with different times of exposure: 1 – 3 – 6 – 24 hours. At the indicated incubation times, I added the MTT solution to the cells so that the viable cells would be able to metabolize this compound into formazan crystals that can be dissolved giving a colored solution that can be measured in a spectrophotometer. The higher the intensity of the color, the higher cell viability is present.

DESCRIPTION OF THE MAIN RESULTS OBTAINED:

From the group of experiments performed to evaluate the basal inflammatory status of the different cell lines, I obtained the following results:

- Some differences in the expression of inflammatory genes after qPCR analysis were observed between Neuro2a original cell line and modified clones. The expression of *Il1β* was higher in the two clones, while the expression of *Il6* was higher only in the 4A10 clone, in comparison with Neuro2a cells. Analysis of the expression of the other two genes included in the qPCR assay (*Tnfα* and *Ifnγ*) was not possible because their abundance was lower than the limit of detection of the assay.
- The levels of the cytokine IL6 were also higher in the two clones when compared with the original cell line. No differences were observed in the levels of TNFα, and the levels of IL1β, IL10 and IFNγ were not included in the analysis because they were out of range of the limit of detection of the system.

Taking into account these first results, it can be concluded that IGF-1 levels can be influencing the inflammatory basal status of the cells.

From the group of experiments performed to analyze the response of the cell lines to treatment with different inflammatory stimuli, the following results were obtained:

- No significant changes in gene expression and levels of produced cytokines were observed after treatment with LPS, TNFα and IFNγ, apart from an increment in *Il6* expression in Neuro2a cells after TNFα and IFNγ treatment, and in IL6 levels in 4A10 cells after exposure to IFNγ.
- Cell viability was mainly reduced after treatment with inflammatory stimuli. Concretely, LPS treatment showed the highest effectiveness in reducing the cell

viability in all cell lines and at all times (1 – 3 – 6 – 24 hours).

- Based on the results of the MTT assay, treatments with inflammatory stimuli were more effective at times longer than 6 hours, especially for IFNγ treatment.
- 4A10 cell line was the more sensitive to the different treatments, shown by the higher reductions in cell viability after the MTT assay.

Taking into account these results, it can be concluded that a proper selection of the optimal conditions of treatment with inflammatory stimuli should be done, in order to analyze the gene expression and the levels of cytokines produced by cells.

FUTURE COLLABORATIONS (if applicable):

The interesting and promising results I have obtained during the STSM in Prof. Vandenbroucke's laboratory, have greatly motivated us to continue working in the same direction. My group wants to deepen knowledge in the role of IGF-1 in inflammation by using the cell lines described above, in order to analyze the biochemical consequences of *Igf1* deletion. This STSM has given to me the opportunity to get hands on training in techniques to study inflammation that I can use in my laboratory to continue the work I have started during the STSM. Therefore, the results of my work have also inspired the potential collaboration between the groups of Profs. Vandenbroucke and Varela-Nieto in the future.