Report on the outcomes of a Short-Term Scientific Mission[1]

Action number: CA17103 (E-COST-GRANT-CA17103-05889eac)

Grantee name: Konstantinos Kafetzis

Details of the STSM

Title: **miR29-b for the prevention of postoperative fibrosis after glaucoma surgery – miRNA research techniques**

Start and end date: 19/09/2022 to 23/09/2022

Description of the work carried out during the STSM

Description of the activities carried out during the STSM. Any deviations from the initial working plan shall also be described in this section.

(max. 500 words)

During my 5-day visit at DKFZ, Germany, and in accordance with the working plan submitted for the current STSM, I was introduced to the basic principles of microRNA therapeutics, miRNA experimental design, and studying the effect of miRNAs on the protein and mRNA levels. In detail, a variety of commercial transfection reagents (i.e., Lipofectamine™ RNAiMAX, DharmaFECT™ 2) or LNPs developed by Dr. Eichmüller's and our (Dr. Tagalakis') group were used to encapsulate miR-1285 and deliver it in human (MDA-MB-231) and mouse (EO771) cancer cells. Later, total RNA was isolated from the treated cells with QIAGEN's miRNeasy Kit (in order to also isolate high quality miRNAs) and 2 different types of qPCR (SYBR® Green and TaqMan®) were performed in order to study the effects of miRNA overexpression on *NT5E* gene expression, a target of miR-1285. This experiment was also used to compare the different delivery systems and formulation methodologies (different LNP formulation buffers and lipid molar ratios were tested), since Dr. Eichmüller's group is currently searching for a non-cytotoxic and highly efficient delivery vector, in order to move their anti-cancer miRNA therapies *in vivo*. Furthermore, I was introduced to the principles of and carried out a 3' UTR-reporter assay, in order to investigate whether miR-1285 recognises and binds to the 3' UTR region of *NT5E* to exert its regulatory role. In detail, the commercial transfection reagent DharmaFECT™ Duo was used to co-transfect MDA-MB-231 cells with miR-1285 and a plasmid carrying the luciferase gene with the 3' UTR region of *NT5E*. This technique is extremely useful during the initial stages of miRNA studies, as it helps verify *in vitro* whether a specific gene is targeted by the tested miRNA.

[1] This report is submitted by the grantee to the Action MC for approval and for claiming payment of the awarded grant. The Grant Awarding Coordinator coordinates the evaluation of this report on behalf of the Action MC and instructs the GH for payment of the Grant.

Unfortunately, due to delayed delivery of some reagents and time restrictions we were unable to perform the QuickChange site-directed mutagenesis during my short time in DKFZ, as it was outlined in the initial working plan. However, detailed protocols of the technique and a full introduction to the theoretical and scientific background of the method was provided instead.

Description of the STSM main achievements and planned follow-up activities

Description and assessment of whether the STSM achieved its planned goals and expected outcomes, including specific contribution to Action objective and deliverables, or publications resulting from the STSM. Agreed plans for future follow-up collaborations shall also be described in this section.

(max. 500 words)

During this STSM I received training in techniques essential for the study of miRNAs and the development and research of miRNA therapeutics. More importantly, I had extensive conversations with members of Dr. Eichmüller's group, who are experts in the field of miRNA therapeutics, pertaining my own PhD project. Specifically, they offered me invaluable protocols and insights on how to identify and evaluate potential targets of my miRNA, and warned me about common pitfalls of miRNA research, and how to easily overcome them. Furthermore, since our (Dr. Tagalakis') group is more experienced with the development and study of non-viral delivery vectors, and more specifically LNPs, I reviewed Dr. Eichmüller's formulations and offered some suggestions on steps they can change or add in their manufacturing process, in order to improve the quality and purity of their nanoparticles and increase their effectiveness.

Overall, the main goal of this STSM, which was the exchange of protocols and expertise between our 2 groups, has been achieved and proved to be very rewarding. Moreover, as an Early Career Researcher this short mission not only offered me the opportunity to network with other young researchers working in the field of RNA therapeutics, but it also allowed me to look at my own project from a different point of view, and helped me identify new objectives and research avenues. Finally, this short scientific mission also opened up new opportunities, to both groups, for future collaborations and publications. For example, the 2 group leaders have now met several times online and decided to cement this new collaboration with a collaborative grant application that will be submitted to the German Research Foundation (DFG).