**Barbara Mawer Travelling Fellowship Award 2017 - Visiting the University of Ferrara (Italy)**

**Investigating extracellular ATP in the tumour microenvironment of osteosarcoma using the plasma membrane-targeted luciferase (**pmeLUC) **probe.**

**Introduction and background**

My PhD project looks at the role of ATP and P2X7 receptors in osteosarcoma. ATP is now accepted as a prominent extracellular nucleotide modulating the bone microenvironment and plays a role in tumour progression and metastasis. Detection of ATP using standard techniques has been found to be inaccurate and could lead to the misinterpretation of the role of ATP and purinergic signalling in the bone tumour microenvironment.

The pmeLUC probe was developed and optimised at the University of Ferrara (Italy) both *in vitro* and *in vivo*. This probe enables the precise detection and quantification of ATP at the cell membrane, enabling studies to be performed investigating ATP in the tumour microenvironment and has been applied to several experimentally-induced tumours.

The reason I chose this lab to visit for the fellowship was because it would be a unique opportunity for me to benefit from their expertise in ATP detection, generating novel data about the release of ATP from osteosarcoma cells and the effect that P2X7R has on this process in the osteosarcoma tumour microenvironment both *in vitro* in Ferrara and *in vivo* in Sheffield. This data would be otherwise unavailable for my project and is suitable for publication and dissemination at scientific conferences further enhancing my scientific profile. I gained numerous opportunities to expand my own network and gain new collaborations. Additionally, an international visit where I applied for funding and gained experience of grant writing has benefitted my CV greatly for future employment, as has the experience of working in Ferrara at a different laboratory environment with different cultures.

**Results obtained, and skills gained**

In Ferrara, I transfected the pmeLUC probe into my existing Te85-P2X7R cell lines, previously developed thanks to an ongoing collaboration between the Gartland group (Sheffield, UK) and the Adinolfi group (University of Ferrara, Italy). These cells were transfected with the pmeLUC plasmid. I also transfected a second osteosarcoma cell line called MNNG-HOS for future *in vivo* use. I then learned how to obtain single cell clones by serial dilution and how to test if the cells were expressing pmeLUC with a luminometer. These skills enabled me to detect and quantify ATP in my cells of interest using pmeLUC.

I additionally assisted Dr. Adinolfi’s group with their *in vivo* experiments performed with cancer cell lines expressing pmeLUC already available in the laboratory. Following injection of cancer cells in to syngeneic mice, extracellular ATP was measured in live mice by pmeLUC luminescence emission with an IVIS lumina total body imaging system. Due to lack of authorization to work with these specific animal models in Italy, I was not able to perform the experiments myself, but I was taught all the procedure going from tumour induction in mice to luminescence detection, photon analysis and final interpretation of the data. This allowed me to acquire the required techniques to perform *in vivo* experiments on my return to Sheffield.

**How the new skills/knowledge will be incorporated into your own research**

The pmeLUC probe is now available to be used to detect and quantify ATP in the osteosarcoma tumour microenvironment, both *in vitro* and *in vivo*. I can now use this probe in Sheffield for many different experiments. The results will be used in my final PhD thesis and for future publications of my research.