

Breast cancer is one of the most common cancers worldwide and although there have been significant advancements in detection and treatment of the primary tumor, once the cancer has spread to other areas of the body (metastasis), patient outcome is poor. Therefore, research needs to be focused on eliminating and inhibiting further spread of metastases. Particularly, metastasis to the brain is proving to be a difficult clinical problem, partly due to limited penetration of therapeutics to the brain. Extracellular vesicles (EVs) are very small containers of information which naturally come from all types of cells. EVs have been shown to contribute to many aspects of tumor growth and spread, and are likely key players in breast cancer metastases. Here they naturally carry cellular information but we can also take advantage of them to carry an imaging agent to visualize them within the body, or use them to carry a treatment to a metastatic site. EVs transfer their cargo to other cells, making their role in treating metastases even more convincing. Importantly, they are able to cross into the brain, and therefore would be of great benefit in treating brain metastases.

Imaging is a common procedure which can be used to visualize parts of the body (anatomy) or further, to investigate molecular processes (to understand what is happening at the cellular level). In the latter, we can use a new imaging modality called Magnetic Particle Imaging (MPI) which directly detects iron with high sensitivity (can detect small amounts), specificity (detects only the iron) and quantitatively. We can label EVs with an iron imaging agent (Fe-EVs) and use this to track their movement to metastatic sites. This information will help us understand timing and accumulation of Fe-EVs in metastases. We will investigate this process in mouse models of spontaneous and brain breast cancer metastases. Fe-EVs will be tracked and quantified by whole mouse body MPI, in conjunction with bioluminescent imaging (BLI) which will provide a measure of metastatic tumor burden and computed tomography (CT) for anatomical reference of the MPI and BLI signals. Histology will be performed to investigate the metastatic tumors by microscopy; here, we will identify the iron and/or the EVs as well as differentiate between cells of the tumor microenvironment.

Using this multi-modal imaging model, **our goal** is to understand how to use EVs as therapeutic carriers to treat established metastases or inhibit further spread. We imagine arming the EVs with a therapeutic and imaging agent, ie. a *theranostic*, and subsequently visualizing their accumulation in a metastatic site prior to initiating therapy. This would prove to be beneficial for patients with established metastatic spread – a treatment option with little immune response, access to the brain and natural tumor targeting ability could improve the outcomes of patients with late stage breast cancer metastases.