**CONTROL OF OSTEOSARCOMA CELL MIGRATION AND INVASION BY THE SCAFFOLD PROTEIN AFAP1L1**

## OVERVIEW

Sarcomas are a highly diverse group of soft tissue cancers comprising of osteosarcoma (bone cancer), liposarcoma (fat cell cancer) and liomyosarcoma (muscle cell cancer), thought to arise from mesodermal cells. Osteosarcoma is the most commonly occurring primary bone tumour and is prominent in adolescents and young adults, but also has an incidence peak in those over 50 years of age[1,2]. Current best clinical practise for treating osteosarcoma often involves high dose cytotoxic drugs and surgical resection, which have led to improved prognosis over the last 20 years for this cancer, with sustained survivorship rates approaching 70% for patients with localized (non- metastatic) cancer at diagnosis. However, up to 20% of patients present with metastatic disease, and when patients develop relapse, these cancers are predominantly metastatic. For these patients with metastatic osteosarcoma the survivorship remains poor with less than 20% showing long-term survival, which has not changed over the last 30 years indicating that there is a substantial unmet need for advances in understanding the fundamental biology, and targeting, of metastatic osteosarcoma.

We have recently identified a new and important regulator of osteosarcoma cell migration and invasion (**AFAP1L1: Actin Filament Associating Protein-1-Like-1**) that shows a strong association with malignant/metastatic disease[3]. We have detailed much of the molecular pathway that AFAP1L1 mediates at the cellular level[3] to promote osteosarcoma migration and invasion through specialized subcellular protrusions called invadopodia (**Fig-1**), which are actin-rich structures that facilitate delivery of metalloproteases to mediate extracellular matrix (ECM) degradation, promoting cancer cell migration and invasion, a hallmark of metastatic disease. AFAP1L1 is a scaffold protein that provides direct links between multiple critical pathway components of the invadopodia, with inputs from growth factors and integrins, to regulate cytoskeletal components of the invadopodia. There is great potential for AFAP1L1 to have prognostic and predictive diagnostic applications for metastatic osteosarcoma as well as the possibility of targeting AFAP1L1 for therapeutic benefit in malignant disease. The overall aim of this application is to investigate the role of AFAP1L1 in metastatic osteosarcoma through focusing on *in vitro* and *in vivo* models of this disease, correlating its activity status with disease progression and identifying regions of AFAP1L1 amenable for potential therapeutic targeting.

**HYPOTHESES**

1. AFAP1L1 plays a critical direct role in and is a marker of osteosarcoma metastasis through acting as a scaffold for mediating invadopodia promotion of cell migration and invasion.

2. Manipulating *AFAP1L1* in osteosarcoma cells and in animal models of osteosarcoma will establish this gene as an important potential therapeutic target for metastatic disease.

3. Identifying critical regions of AFAP1L1 required for its function may uncover potential avenues to developing targeted therapies for metastatic osteosarcoma.

**AIMS**

1. To inactivate *AFAP1L1* in cell and animal models of osteosarcoma and demonstrate its importance for tumour development, and regulation of metastasis through controlling cell migration and invasion *in vitro* and *in vivo*.

2. To correlate the expression level and activity status of AFAP1L1 (phosphorylation) with osteosarcoma development and metastasis; providing strong evidence for its potential as a diagnostic and or predictive marker.

3. To identify minimal critical regions of AFAP1L1 that regulate osteosarcoma migration, invasion and metastasis *in vitro* and *in vivo*) and can act as dominant negative moieties; potentially identifying targetable motifs for therapeutic development.

**SIGNIFICANCE AND OUTCOMES OF THE PROJECT.**

The successful completion of this project will firmly establish *AFAP1L1* as an important gene in controlling osteosarcoma metastasis not only *in vitro* but also *in vivo* through the use of appropriate murine osteosarcoma models, with supporting correlative data from human tumour analysis. Further, we will identify critical regions of AFAP1L1 that can act as competitive inhibitors of AFAP1L1 function and osteosarcoma cell metastasis *in vitro* and *in vivo*. These will be important findings that will provide essential new knowledge in the fight against devastating metastatic osteosarcoma, which deserves major attention to address its unmet clinical need.