The Institute of Cancer Research

PHD STUDENTSHIP PROJECT PROPOSAL:

PROJECT DETAILS	
Project Title:	**Role and Therapeutic Potential of Histone Demethylases in Synovial Sarcomas that affect Young Adults**
Short Project Title:	**Histone Demethylases in Synovial Sarcomas**

SUPERVISORY TEAM	
Primary Supervisor:	Professor Janet Shipley
Other supervisory team members:	Dr Joanna Selfe and Professor Richard Houlston

DIVISIONAL AFFILIATION	
Primary Division:	Division of Molecular Pathology
Primary Team:	Sarcoma Molecular Pathology Team

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Soft tissue sarcomas are a heterogenous group of mesenchymal tumours that resemble tissues such as muscle or appear undifferentiated. Sarcomas are blocked in their ability to terminally differentiate.[1] Synovial sarcomas are a type of undifferentiated sarcoma that have a pluripotent ability to differentiate into many cell types of mesenchymal origin and can even have an epithelial/glandular component. They frequently arise in joints and occur mainly in young adults. Metastatic synovial sarcomas have overall survival rates of <50% (www.ncin.org.uk/databriefings). There is therefore an unmet clinical need to identify more effective, targeted, less toxic therapies for patients with synovial sarcomas.

All synovial sarcomas are associated with chromosome translocations that fuse *SS18* and *SSX* genes to produce an aberrant fusion protein. The SS18-SSX fusion protein re-programmes cells through epigenetic and gene expression changes and has been shown to involve the SWI/SNF (BAF) and polycomb chromatin remodeling complexes in maintaining the stem cell-like properties of synovial sarcomas.[2] Epigenetic changes include histone modifications, such as methylation, that can alter chromatin conformation, gene transcription, DNA repair and genetic stability. Histone methylation is a tightly regulated process in normal cellular development and differentiation and its deregulation can significantly influence the malignant progression of cancers.[3] The genes encoding the enzymes that add or remove methyl groups from histone tails are known as histone methyltransferases and demethylases, respectively, and are emerging as promising therapeutic targets.[4]

Our preliminary data strongly supports involvement of histone demethylases in the malignant behaviour of synovial sarcomas although it is currently not known which of these enzymes are key events in driving synovial sarcoma progression or how they impact on synovial sarcoma cells at the molecular level.

PROJECT AIMS

The overall aim is to identify and determine how therapeutically targetable histone demethylase enzymes play a role in the malignant phenotype of synovial sarcomas in order to address the unmet clinical for more effective treatments for young adults who have this type of sarcoma. The more specific aims are to:

- **Identify which histone demethylases impact most on growth and viability of synovial sarcoma cells and assess whether this is dependant on the oncogenic SS18-SSX fusion protein associated with synovial sarcomas.**
- **Determine the molecular mechanisms underlying the activity of the histone demethylase(s).**
- **Provide pre-clinical evidence to support use of histone demethylase inhibitors in the treatment of synovial sarcomas.**

RESEARCH PROPOSAL

Identification and validation of key histone demethylase(s) involved in synovial sarcomas and dependency on the SS18-SSX fusion protein

An siRNA screen will be designed for histone demethylases that are potentially targetable with available drugs. The siRNAs will specifically reduce expression of histone demethylases in several synovial sarcoma cell lines and an available cell line model we developed that is plus and minus expression of the SS18-SSX fusion protein.[5] This will identify effects of histone demethylases on cell proliferation using a metabolic assay (MTS) and dependency on the fusion protein. Using siRNAs/shRNAs for the histone demethylases that had the greatest effect on cell growth in the screening phase, will be validated for effects on cell proliferation and cell death, including cell counts, cell cycle analyses using FACs and caspase/annexinV analyses for cell death.[5] These features will be assessed in further synovial sarcoma cell lines and in 3D. This will identify the most significant histone demethylase(s) to investigate further.

To assess presence of the selected histone demethylase in primary patient samples, publicly available genomic and expression data for synovial sarcoma patients will be collated, including those described by Legarde *et al.*[6] With bioinformatics support, this will be mined for mutations, genomic copy number changes and associated expression levels of the candidate histone demethylase(s). The RNA and protein expression levels will also be assessed in primary human synovial sarcomas that we have available from patients collected through the Sarcoma Unit at the Royal Marsden NHS Trust. Genomic copy number changes of the selected histone demethylase(s) will be assessed by NGS, quantitative PCR and FISH analyses in the same samples to correlate with expression levels, assessed by quantitative Reverse Transcription-PCR (qRT-PCR) and immunohistochemistry (IHC). Elevated expression, potentially associated with increased genomic copy number or mutation, will support their role in synovial sarcoma progression in patients and as a potential therapeutic target.

Molecular mechanisms for histone demethylase involvement in synovial sarcoma

Molecular mechanisms underlying the role of the candidate histone demethylase in maintaining the malignant phenotype will be investigated. This will first be considered based on the hypothesis that the activity of the histone demethylase is associated with that of the SS18-SSX fusion protein. Initial analyses will be based upon the

known interactions of SS18-SSX with both SWI/SNF and ATF2/TLE1 complexes.[7,8]

RNA interference experiments will reduce the candidate histone demthylase(s) in SS cell lines and assessment of changes in the expression level of key downstream targets of SS18-SSX-containing complexes, such as SOX2 and EGR1, will be undertaken using qRT-PCR and Western blotting. Results from this will be compared with reducing other specific complex components, such as ATF2 and TLE1. The effect of reducing candidate histone demthylase(s) on the known occupancy of these complexes and changes to relevant histone marks at target gene sites will also be assessed. Co-immunoprecipitation (coIPs) will be used to demonstrate protein interactions using antibodies previously described for SWI/SNF (BAF155), TLE1 and ATF2. This will determine whether the histone demethylase is associated with the previously described complexes that are associated with the SS18-SSX fusion proteins.

If the histone demethylase(s) identified are not involved through these complexes, ChIP-seq analyses will be used to identify the histone demethylase binding sites to DNA and histone mark changes along side RNAseq analyses. Changes to the histone methylation status at specific loci associated with altered gene expression will be determined. Mass spectrometry may be used to identify potential protein binding partners that will be validated through co-IP experiments. These analyses will determine the genes affected by the histone demethylase, the methyl marks involved and the interacting proteins in order to develop a mechanistic molecular model of how the histone modifier interacts to affect malignant behaviour in synovial sarcomas.

Implications for synovial sarcoma treatment

Synovial sarcoma cell lines and the cell line plus and minus expression of *SS18-SSX* will be tested for their sensitivities to available histone demethylase inhibitors/tool compounds, including combining with existing chemotherapy to assess potential for introduction into patients, as previously described.[5] Investigation of 3D model cultures will be used and ultimately promising results taken forward for *in vivo* studies, including patient derived xenografts that are being established by a separate programme of activity in the laboratory.

The molecular mechanisms identified may inform investigating other potential therapeutic targets that could be pursued in addition or as an alternative.

Taken together this project will elucidate epigenetic mechanisms that contribute to the malignant phenotype of synovial sarcomas with potential to lead to improved treatment of patients with synovial sarcomas.

LITERATURE REFERENCES

1. Naka N, Takenaka S, Araki N, Miwa T, Hashimoto N, Yoshioka K, Joyama S, Hamada K, Tsukamoto Y, Tomita Y, Ueda T, Yoshikawa H, Itoh K. (2010). Synovial sarcoma is a stem cell malignancy. *Stem Cells.* **28**(7), 1119-1131.
2. Stacchiotti S, Van Tine BA. (2018) Synovial Sarcoma: Current Concepts and Future Perspectives. *J Clin Oncol.* **36**(2), 180-187.
3. Pedersen MT, Helin K. (2010). Histone demethylases in development and disease. *Trends Cell Biol* **20**, 662-671
4. Wang L, Chang J, Varghese D Dellinger M, Kumar S, Best AM, Ruiz J, Bruick R, Peña-Llopis S, Xu J, Babinski DJ, Frantz DE, Brekken RA, Quinn AM, Simeonov A, Easmon J, Martinez ED. (2013). A small molecule modulates Jumonji histone demethylase activity and selectively inhibits cancer growth. *Nat Commun* **4**,

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6. Lagarde P, Przybyl J, Brulard C, Pérot G, Pierron G, Delattre O, Sciot R, Wozniak A, Schöffski P, Terrier P, Neuville A, Coindre JM, Italiano A, Orbach D, Debiec-Rychter M, Chibon F. (2013) Chromosome instability accounts for reverse metastatic outcomes of pediatric and adult synovial sarcomas. *J Clin Oncol* **31**, 608-15
7. Su L, Sampaio AV, Jones KB Pacheco M, Goytain A, Lin S, Poulin N, Yi L, Rossi FM, Kast J, Capecchi MR, Underhill TM, Nielsen TO. (2012) Deconstruction of the SS18-SSX fusion oncoprotein complex: insights into disease etiology and therapeutics. *Cancer Cell* **21**, 333-47.
8. Kadoch C, Crabtree GR. (2012) Reversible disruption of mSWI/SNF (BAF) complexes by the SS18-SSX oncogenic fusion in synovial sarcoma. *Cell* **153**, 71-85.

CANDIDATE PROFILE

Note: the ICR's standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)

Pre-requisite qualifications of applicants: e.g. BSc or equivalent in specific subject area(s)	BSc in relevant Biological Science
Intended learning outcomes: Please provide a bullet point list (maximum of seven) of the knowledge and skills you expect the student to have attained on completion of the project.	• Understanding of genetic and molecular basis of synovial sarcomas, sarcomas and cancers more generally • Learning about the roles for histone modifications in sarcomas and other cancers • Deep understanding of how to perform cellular and molecular investigations of cancer cells • Learning how to interpret and present data from experiments in the context of current understanding, both orally and written