PHD STUDENTSHIP PROJECT PROPOSAL

PROJECT DETAILS	
Project Title:	Digital proteome mapping of formalin-fixed paraffin embedded (FFPE) biopsies for applications in routine practice and clinical trials.
Short Project Title:	Digital proteome mapping of FFPE biopsies for applications in routine practice and clinical trials.
SUPERVISORY TEAM	
Primary Supervisor(s):	Dr Paul Huang
Additional members of the supervisory team	Dr Khin Thway and Prof Chris Jones
DIVISIONAL AFFILIATION	
Primary Division:	Molecular Pathology
Primary Team:	Protein Networks

PROJECT PROPOSAL

BACKGROUND TO THE PROJECT

Technological developments in next generation sequencing (NGS) has led to the rapid adoption and use of genomic analysis in routine care and clinical trials. However, comprehensive proteomic analysis of tumour specimens has been limited primarily due to technical challenges such as limitations in analytical sensitivity and comparatively high clinical sample requirements for proteomic workflows. Proteomic data provides complementary information for applications in routine care such as guiding treatment choice and in clinical trials for biomarker stratification. In particular, the use of proteomic in formalin-fixed paraffin-embedded (FFPE) tissue has been constrained due to challenges associated with protein modifications which are incompatible with traditional proteomic workflows. Leveraging on highly sensitive and quantitative digital proteome mapping of FFPE tissue developed by the Huang Lab, this project seeks to build on this technological breakthrough by developing its application for core biopsies and extending its capabilities to phosphoproteomics and other post-translational modifications (PTMs).

Through the in-depth profiling of epithelial and mesenchymal tumour specimens from biobanks, this project will employ next generation proteomics based on SWATH mass spectrometry to develop sensitive and highly reproducible assays for measuring the proteome and phosphoproteome from limiting amounts of FFPE tissue material such as in core biopsies. These optimised proteomic protocols will subsequently be deployed on clinical tissue from routine care/clinical trials and integrated with genomic information currently being use in molecular diagnostics (e.g. RMH200 pan-cancer targeted sequencing gene panel from NIHR Centre for Molecular Pathology) and Nanostring-based gene expression analysis (e.g. PAM50) to evaluate if integrated molecular and bioinformatics analysis provides useful additional information that guides clinical decision making.

This translational project will accelerate the application of proteomics into routine clinical care with potential for future utility for biomarker stratification in clinical trials.

PROJECT AIMS

- **Optimising digital proteome mapping for FFPE biopsies using SWATH mass spectrometry**
- **Developing applications in digital proteome mapping for phosphoproteomic analysis**
- **Application of digital proteomic mapping to core biopsies from clinical trials/routine care**
- **Integration of proteomic data with genomic data by pathway analysis to aid patient treatment decisions and biomarker discovery**

RESEARCH PROPOSAL

Digital proteome mapping by mass spectrometry (MS) employs sequential window acquisition of all theoretical fragment ion spectra (SWATH)/data independent acquisition (DIA) MS to convert specimens into a single digital file. This next generation proteomic strategy has thus far only been applied to frozen tissue and not formalin-fixation and paraffin-embedding of tissue. The Huang lab has pioneered the use of digital proteomic mapping of FFPE tissue and in the project, the successful candidate will optimise this technology for use in core biopsies with the intention for future integration with other molecular diagnostic platforms for use in routine care and biomarker stratification in clinical trials.

Aim 1: Optimising digital proteome mapping for FFPE biopsies using SWATH mass spectrometry

The Huang lab has already developed robust protocols for proteomic profiling of FFPE tissue and are one of the few laboratories able to quantitatively measure the proteome from FFPE specimens. The goal of this aim is to extend the capabilities of this approach to core biopsies which would require highly sensitive detection due to limiting sample amounts. Further method development on the SWATH-MS platform will be undertaken to achieve this aim using core cuts from both epithelial (archival breast tissue from the Canadian Ontario Tumour Bank) and mesenchymal (archival sarcoma tissue from the Royal Marsden Sarcoma Unit Bank) cancers to improve sensitivity of this platform. Tissue from both biobanks have clinically annotated data including patient outcome, clinicopathological variables as well as gene expression data. Proteomic data obtained from these studies will be orthogonally validated using immunohistochemistry assessment or gene expression analysis.

Aim 2: Developing applications in digital proteome mapping for phosphoproteomic analysis

Digital proteome mapping by SWATH-MS has not yet been applied to assess the phosphoproteome and other post-translational modifications in FFPE tissue. Leveraging on the expertise of the Huang lab in phosphoprotemics, the student will develop the digital proteome mapping technology for this application. Core cuts from FFPE tissue will first be subjected to dewaxing and reversal of crosslinks. Phosphopepetides will be enriched using TiO_2 as well as phosphotyrosine enrichment with immunoprecipitation prior to SWATH-MS analysis using established protocols from the Huang Lab. This will include generation of spectral libraries for phosphopeptides which will enable rapid retrospective *in silico* interrogation of digital proteome maps.

Aim 3: Application of digital proteomic mapping to core biopsies from clinical trials/routine care

With the optimise protocols developed in previous aims, here the student will apply digital proteomic and phosphoproteomic mapping to tissue biopsies from both breast cancer and sarcoma cohorts. We have access to clinical trial material from both national and international breast cancer studies (in collaboration with Dr Maggie Cheang, ICR-Clinical Trial and Statistics Unit) and sarcoma studies (in collaboration with Dr Robin Jones, Head of the Royal Marsden Sarcoma Unit). This goal of this aim is to evaluate the ability of digital proteomic profiling to obtain comprehensive proteomic profiles of FFPE biopsies in the “real world” setting.

Aim 4. Integration of proteomic data with genomic data by pathway analysis to aid patient treatment decisions and biomarker discovery

We will evaluate if integration of proteomic profiling data generated in Aim 3 with other genomic-based molecular diagnostic information (e.g. RMH200 pan-cancer targeted sequencing gene panel from NIHR Centre for Molecular Pathology or Nanostring-based gene expression assays such as PAM50) using biological pathway analysis will provide additional information that guides clinical decision making. This can include providing additional information in relation to treatment choice or discovery of integrated biomarkers of prognostic or predictive utility. This integrated approach may address some of the shortcomings of current precision trials (e.g. NCI MATCH or MOSCATO) where only a small number of genomic aberrations are clinically actionable by widening the pool of patients eligible for targeted therapies based on pathway information as opposed to relying solely on genetic mutations or gene expression profiles.

Training and development

The PhD student will be integrated into the multi-disciplinary Protein Networks Team in the Division of Molecular Pathology. The student will benefit from mentorship and training from other members of the Huang laboratory within a collaborative and supportive environment. There will be close collaborations with other Team Leaders at the ICR and The Royal Marsden, including Dr Khin Thway, Dr Maggie Cheang and Dr Robin Jones. The student will gain hands-on experience in molecular profiling strategies. The student will be exposed to Cancer Biology, Analytical Chemistry, Molecular Pathology, Translational Research and Precision Medicine.

EXPLAIN BRIEFLY HOW THIS PROJECT WILL PROVIDE A TRANSLATIONAL TRAINING EXPERIENCE FOR THE STUDENT.

This project focuses on the development of proteomic approaches for application in clinical specimens with the longer-term goal of integration with other molecular diagnostic approaches which are currently being used in the NHS. This project has direct translational implications and the student will learn the importance of how laboratory developments (in this case developing a new proteomic technique) links to applications in routine care and clinical trials. The student will have ample opportunity to interact with clinical colleagues at The Royal Marsden to learn about the important clinical challenges faced in deploying precision medicine in the NHS and go on to develop solutions in the laboratory to address these challenges. Furthermore, collaborations with the ICR-CTSU as well as CMP will ensure that the student will gain experience in the state-of-the-art molecular diagnostic tools and biomarker guidelines currently used both in routine care and in clinical trials. The Huang lab already has a number of staff and trainees involved in translational research with multiple Units at The Royal Marsden and NIHR BRC projects and the student will benefit from mentorship and training from this collective experience in the lab.

LITERATURE REFERENCES

Guo T, Kouvonen P, Koh CC et al. Rapid mass spectrometric conversion of tissue biopsy samples into permanent quantitative digital proteome maps. Nat Med 2015; 21: 407-413.

Gillet LC, Navarro P, Tate S et al. Targeted data extraction of the MS/MS spectra generated by data-independent acquisition: a new concept for consistent and accurate proteome analysis. Mol Cell Proteomics 2012; 11: O111 016717.

Gustafsson OJ, Arentz G, Hoffmann P. Proteomic developments in the analysis of formalin-fixed tissue. Biochim Biophys Acta 2015; 1854: 559-580.

Krasny, L., et al., *SWATH mass spectrometry as a tool for quantitative profiling of the matrisome.* J Proteomics, 2018 doi: j.jprot.2018.02.026.

<table>
<tr><td colspan="2">Wong, J.P., et al., Dual Targeting of PDGFRalpha and FGFR1 Displays Synergistic Efficacy in Malignant Rhabdoid Tumors. Cell Rep, 2016. 17: 1265-1275.

Huang, P.H., et al., Quantitative analysis of EGFRvIII cellular signaling networks reveals a combinatorial therapeutic strategy for glioblastoma. Proc Natl Acad Sci U S A, 2007. 104: 12867-72.

Lima, N et al., Progress and impact of clinical phosphoproteomics on precision oncology. Transl Cancer Res. 2017. 6(Suppl 6): S1108-14</td></tr>
<tr><td colspan="2">CANDIDATE PROFILE</td></tr>
<tr><td colspan="2">Note: the ICR’s standard minimum entry requirement is a relevant undergraduate Honours degree (First or 2:1)</td></tr>
<tr><td>Pre-requisite qualifications of applicants:
e.g. BSc or equivalent in specific subject area(s)</td><td>• Candidates must have a First or 2:1 Honours degree or a Masters in biology/ biochemistry/ cancer biology/ analytical chemistry or a related discipline.
• Academic knowledge in cancer biology, cell biology, or analytical chemistry
• Previous laboratory experience
• Good presentation and communication</td></tr>
<tr><td>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.</td><td>• Knowledge in proteomics, translational cancer research, molecular diagnostics
• Experimental skills in biochemical, protein chemistry, proteomic techniques
• Ability to design, manage and progress a defined scientific project
• Scientific writing, presenting and communication skills. Ability to read and process relevant literature.</td></tr>
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