# PhD Project Proposal

## Funder details

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| **Studentship funded by:** | iCASE with AstraZeneca |

## Project details

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| **Project title:** | Deciphering the immunopeptidome in chromatin regulator driven cancers |

## Supervisory team

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| **Primary Supervisor:** | Prof. Jyoti Choudhary |
| **Associate Supervisor(s):** | Dr. Zuza Kozik |
| **Secondary Supervisor:** | Prof. Jessica Downs |

## Divisional affiliation

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| **Primary Division:** | Cancer Biology |
| **Primary Team:** | Functional Proteomics |
| **Site:** | Chelsea |

## Project background

The display of human leukocyte antigen (HLA) bound T-cell antigens is central to anti-tumour immunity and has important therapeutic consequences. Chromatin regulators are often mutated in cancer, and these types of tumours have been shown to respond to immune checkpoint therapy, implicating the presence of cancer-specific antigens. Here we propose to investigate the consequences of chromatin regulator gene deficiency on T-cell antigen presentation, which will be used to guide a rational approach for the selection of combination therapies, as well as to identify neoantigens for designing vaccines and CAR-T based therapies in these cancers.

## Project aims

- To establish a pipeline for large scale non-canonical transcriptome analys
- To map the immunopeptidome landscape of chromatin regulator deficient biological models
- To evaluate the impact of IR/drug combinations on the immunopeptidome dynamics
- To evaluate immunogenic potential of radiotherapy/drug combinations
- To assess the interplay between gene deficiency and the immunopeptidome in clinical samples

## Research proposal

The presentation of HLA-bound antigens on the cell surface is an important component of the immunogenicity. The repertoire of presented antigens is collectively referred to as the immunopeptidome. The immunopeptidome landscape can be influenced by several factors, such as the activity of the interferon response pathway that directly regulates many components of the antigen presentation pathway (Yewdell, 2022; Shapiro and Bassani-Sternberg, 2023). In addition, antigen composition reflects cellular protein translation and degradation processes, but gaps in current knowledge restrict our ability to predict the peptide repertoire. Mass spectrometry has gained prominence as a key technology for identifying the immunopeptidome and has contributed to the discovery of antigens from mutant proteins and novel post-translational modifications that represent the neoantigen immunopeptidome of cancer cells. Recent immunopeptidomic studies have also revealed expression of the non-canonical “dark proteome” events in many cancers, suggesting that deregulation of protein transcription and translation leads to cancer specific antigens derived from non-canonical protein expression (Prensner et al., 2023).

The cancer-specific antigens represent attractive targets for multiple immunotherapy approaches; however, in most cases the antigens are not known and cannot be predicted. This proposal

aims to fill this gap, by investigating the immunopeptidome landscape of chromatin deficient cells and by studying the dynamics of neoantigens upon exposure to clinically relevant irradiation and drug

combinations. We hypothesise chromatin miss-regulation can generate cancer specific T-cell antigens from non-canonical proteins that arise as a consequence of loss of chromatin regulation (Soldi et al., 2020; Leruste et al., 2021). We will map changes across molecular layers from the genome, transcriptome, translatome, proteome and immunopeptidome in isogenic cell models. These data will enable a detailed characterisation of cellular pathways and non-canonical events, that will be interrogated against clinical immunotherapy studies to identify the key predictors of therapy response/insensitivity.

Aim 1: To establish a pipeline for large scale non-canonical transcriptome analysis

Nextflow pipelines will be developed for the analysis of proteogenomics data. We have generated and validated isogenic cell models in a variety of different cell types (Feng et al., 2022; Schiavoni et al., 2022). Whole genome (WGS) and transcriptome (RNA seq) will be generated for these models. We will use genome mapping and de novo assembly reveal changes in expression, diverse non-canonical events will be explored including splicing as well as gene fusions and expression of the dark genome elements. This will result in the identification of common or subunit specific non-canonical events that can be used to predict neoantigens expression candidates. In addition, these data will also be used to generate personal protein databases for the analysis of immunopeptidomes (Weisser et al., 2016).

Aim 2: To map the immunopeptidome landscape of gene deficient isogenic models

Established methods for affinity capture of HLA molecules will be used to enrich the immunopeptidome, which will then be characterised by quantitative mass spectrometry. We will use isobaric labelling and label-free data-independent mass spectrometry to deeply analyse the peptidomes across the cell models. Personalised protein databases will be used to discover non-canonical expression. These events will be validated using Riboseq, a method that captures the ribosomes and for sequencing the engaged translating mRNAs. Comparative analysis will enable the assignment of non-canonical antigens associated with SWI/SNF gene deficiency states. The output will be candidate immunogenic antigens for that can be explored for tumour immunogenicity.

Aim 3: To evaluate the impact of RT/drug combinations on the immunopeptidome dynamics

We and others have screened and monitored changes in the proteome upon drug treatments. Our analyses identified several drugs and irradiation combinations that culminate in changes to the antigen process pathway. For example, irradiation of PBRM1 deficient cell lines results in an increase in interferon signalling and the expression of HLAs, as well as upregulation of the immunoproteasome. Changes in the immunopeptidome landscape have not been studied. In this project, we will characterise the effects of single agent and combination treatments on the immunopeptidomic landscape. For the selected conditions, we will also capture the proteomes, transcriptomes and translatomes to comprehensively assess the effects across multiple molecular layers. The output will be to identify conditions that enhance or inhibit the expression of neoantigens and inflammatory signalling.

Aim 4: To evaluate immunogenic potential of IR/drug combinations

Selected conditions from Aim 3 will be evaluated in animal models, which will be performed in partnership with the Melcher/Harrington labs. We will generate tumours in immune-competent mouse models and characterise their immunopeptidomes. Targeted mass spectrometry will be used to follow the expression of candidate neoantigens at primary, nodal, and metastatic sites. Candidate antigens will also be monitored following irradiation and in combination with drug treatments. Human cytotoxic T cell (CTL) priming assays will be used to investigate the impact of selected candidates (Jennings et al 2019). This assay, which uses whole tumour cells (with or without IR /

drug treatments) or peptides as an antigen source against which CTL can be primed, exploits intracellular interferon gamma staining in primed CD8 T cells as a readout of antigen recognition.

Aim 5: To assess the interplay between gene deficiency and the immunopeptidome in clinical samples

We will use available data from clinical trials to evaluate the effect of key dependencies on predicting immunotherapy response. Proteins and pathways revealed from proteomics datasets generated by this study will be correlated with the outcome data to reveal relationships that may contribute to immunogenicity. We will use small-molecule and genetic intervention approaches to test the contribution of selected factors on modulating the immunopeptidome complement. The expression of the selected recurrent neoantigen candidates will also be evaluated in clinical samples. These analyses will improve our understanding of the key molecular processes regulating neoantigen expression and provide new insights to guide the use of immunotherapy in the clinic.

## Literature references


[1] Feng, H. et al. (2022) 'PBAF loss leads to DNA damage-induced inflammatory signaling through defective G2/M checkpoint maintenance', Genes & Development, 36(13–14), pp. 790–806. Available at: https://doi.org/10.1101/GAD.349249.121.

[2] Leruste, A. et al. (2021) 'Immune responses in genomically simple SWI/SNF–deficient cancers', Cancer, 127(2), pp. 172–180. Available at: https://doi.org/10.1002/CNCR.33172.

[3] Prensner, J.R. et al. (2023) 'What can Ribo-seq, immunopeptidomics, and proteomics tell us about the non-canonical proteome?', Molecular & Cellular Proteomics, 0(0), p. 100631. Available at: https://doi.org/10.1016/J.MCPRO.2023.100631.

[4] Schiavoni, F. et al. (2022) 'Aneuploidy tolerance caused by BRG1 loss allows chromosome gains and recovery of fitness', Nature Communications 2022 13:1, 13(1), pp. 1–15. Available at: https://doi.org/10.1038/s41467-022-29420-3.

[5] Shapiro, I.E. and Bassani-Sternberg, M. (2023) 'The impact of immunopeptidomics: From basic research to clinical implementation', Seminars in immunology, 66. Available at: https://doi.org/10.1016/J.SMIM.2023.101727.

[6] Soldi, R. et al. (2020) 'The novel reversible LSD1 inhibitor SP-2577 promotes anti-tumor immunity in SWItch/Sucrose-NonFermentable (SWI/SNF) complex mutated ovarian cancer', PLoS ONE, 15(7). Available at: https://doi.org/10.1371/JOURNAL.PONE.0235705.

[7] Weisser, H. et al. (2016) 'Flexible Data Analysis Pipeline for High-Confidence Proteogenomics', Journal of proteome research, 15(12), pp. 4686–4695. Available at: https://doi.org/10.1021/ACS.JPROTEOME.6B00765.

[8] Yewdell, J.W. (2022) 'MHC Class I Immunopeptidome: Past, Present, and Future', Molecular & Cellular Proteomics : MCP, 21(7), p. 100230. Available at: https://doi.org/10.1016/J.MCPRO.2022.100230.

[9] Yu, C. et al. (2022) 'ARID1A loss derepresses a group of human endogenous retrovirus-H loci to modulate BRD4-dependent transcription', Nature Communications 2022 13:1, 13(1), pp. 1–16. Available at: https://doi.org/10.1038/s41467-022-31197-4.

[10] Zhou, M. et al. (2021) 'Emerging role of SWI/SNF complex deficiency as a target of immune checkpoint blockade in human cancers'. Available at: https://doi.org/10.1038/s41389-020-00296-6.


## 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:** BSc (Hons) Biological Sciences

**Intended learning outcomes:**

- Quantitative immunopeptidomics
- Genome biology
- Bioinformatics
- Immunology
- Clinical multiomics analysis
- Translation cancer biology

## Advertising details

**Project suitable for a student with a background in:**

- [x] Biological Sciences
- [ ] Physics or Engineering
- [ ] Chemistry
- [ ] Maths, Statistics or Epidemiology
- [ ] Computer Science