ICR The Institute of Cancer Research

UKRI Medical Research Council

PhD Project Proposal

Funder details

Studentship funded by: MRC-DTP

Project details

Project title: Developing novel mRNA vaccines against cancer associated mis-splicing events to enhance therapy response

Supervisory team

Primary Supervisor: Rachael Natrajan

Associate Supervisor(s): Esther Arwert

Secondary Supervisor: Jyoti Choudhary

Divisional affiliation

Primary Division: Breast Cancer

Primary Team: Functional Genomics

Site: Chelsea and Sutton

Project background

In cancers such as breast cancer where immunotherapy responses are seen in a small subset of patients, inducing T-cell responses against common mis-splicing events that generate neo-antigens holds promise. This is advantageous compared to costly personalised tumour specific mutation-based vaccines. Cancers harbouring mutations in the spliceosomal component protein SF3B1 generate common neo-antigens that can also be phenocopied in wild-type cancers through inhibition of transcriptional cyclin-dependent kinases via their direct interference with SF3B1-mediated splice-site selection. This project aims to develop mRNA anti-cancer vaccines based on shared frequently mis-spliced aberrations and test whether these are enhanced in combination with other therapies.

Project aims

Aim 1: Identify shared neoantigens induced by aberrant SF3B1 induced mis-splicing

Aim 2: Functionally test the immunogenicity of shared mis-spliced neopeptides

Aim 3: Generation of mRNA based vaccines to assess *in vivo* efficacy

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Research proposal

Dysregulation of mRNA processing is predicted to generate neoantigens that would be both tumour-specific and shared between patients. In cancers where spontaneous responses to immune checkpoint blockade are not seen, inducing T cells to epitopes based on tumour specific mutations requires personalized vaccines, which is costly and may not be therapeutically effective. Generation of vaccines using shared neoantigens produced due to aberrant-splicing are thus an attractive alternative strategy that could be used to further enhance therapeutic response to target agents such as DDR inhibitors that also themselves increase tumour immunogenicity.

We and others have shown that hotspot point mutations of the SF3B1 splicing factor are associated with poor prognosis in multiple cancer types including breast cancer that also that do not conventionally respond to immune checkpoint blockade (ICB)[1]. These lead to alternative 3' splice site selection, resulting in conserved global aberrant splicing alterations across multiple cancer types resulting in insertion of additional amino acids or exonic frameshifts leading to increased neoantigen diversity. Moreover, many of these specific splicing changes can be invoked in SF3B1 wild-type cells through SF3B1 inhibitors or inhibition of transcriptional cyclin dependent kinases such as CDK7 and CDK12 (via their direct interference with SF3B1's role in splice site selection), leading to potential neoantigen diversity[2]. This could expand the patient populations that would benefit from this approach that are refractory to ICB.

Aim 1: Identify shared neoantigens induced by aberrant SF3B1 induced mis-splicing (Yr 1-2)

We have recently identified that although SF3B1 mutant cancers are homologous recombination (HR) proficient, they are selectively sensitivity to PARP1 trapping agents mediated through loss of the ATR replication stress response and show synergistic effects in combination with ATM inhibitors as a consequence[1]. Using SF3B1 mutant cancers as proof-of-principle of our approach, focussing on the most common haplotype HLA-A2, in this aim we will identify shared neoantigens that are generated due to mis-splicing events. Using our well characterised set of SF3B1 mutant and wild-type isogenic cell lines models with known HLA-A2 status harbouring different SF3B1 hotspot mutations, we will characterise the full immunopeptidome by Mass Spectrometry under PARPi exposure. Integration with RNA-sequencing data +/-PARPi already generated in these cells will be used to prioritise a series of candidates that are produced as a direct consequence of aberrant splicing in SF3B1 mutant cells and potentiated with addition of a PARPi.

In tandem we will use computational predictions from proteomics and RNA sequencing data already generated from isogenic cells, patient derived organoids, and primary tumours harbouring naturally occurring SF3B1 hotspot mutations to identify potential neopeptides for functional testing. Using additional computational predictions from RNA sequencing of triple negative breast cancer (TNBC) cells, (for which many patients do not benefit from ICB) +/- SF3B1 modulation via CDK7 and/or CDK12 inhibition, we will also predict neoantigens and the overlap with SF3B1 mutant cells. This will enable generation of a repository of possible neoantigens that we will rank based on clonality/thermal stability/binding affinity from alternative isoforms.

Aim 2: Functionally test the immunogenicity of shared mis-spliced neopeptides (Yr 2-3)

To assess whether SF3B1 mutant-induced neoantigens on tumour cells are recognized by specific CD8 T cells, common candidate neopeptides will be synthesised and tested *ex vivo* using MHC binding and T-cell priming assays. To identify those that are more likely to span multiple HLA-types, we will additionally explore relevant peptide pools based on specific proteins consisting mainly of 15-mer sequences with 11 amino acids overlap and covering the complete sequence of target protein. These will be fed to dendritic cells to be presented to CD8 T cells using PBMCs from healthy donors.

Candidates will be subsequently screened to assess T cell activation in response to the antigen specifically in SF3B1 mutant tumour cells *in vitro* using both isogenic and patient derived SF3B1 mutant and wild-type organoids and if this is further enhanced though the addition of treatment with PARPi.

This approach will be extended to test the immunogenicity of mis-spliced neopeptides in TNBC cell lines and patient derived organoids we have available in house using CDK7 and CDK12 inhibitors to phenocopy SF3B1 aberrant mis-splicing.

Aim 3: Generation of mRNA based vaccines to assess *in vivo* efficacy (Yr 3)

In this aim, we will test whether generation of mRNA vaccines identified from aims 1-2 supresses tumour growth *in vivo* using murine syngeneic models. For instance, multiple studies have demonstrated a significant overlap in splicing alterations in Sf3b1 mutant genetically engineered murine models with human patient data[3-4]. We will additionally define the splicing repertoire and immunopeptidomes using spike-ins from the peptides identified from Aim 1-2. Tumour volume will be measured over time +/- vaccine and in combination with PARPi. This will additionally be tested using wildtype cells injected into mice and treatments with mRNA vaccine with CDK7/CDK12 inhibitors to induce SF3B1 mis-splicing +/- PARPi. Tetramers against aberrantly-spliced candidates will also be generated to follow specific T cell responses against particular peptides.

Literature references

1. Bland, P. et al. SF3B1 hotspot mutations confer sensitivity to PARP inhibition by eliciting a defective replication stress response. Nature Genetics 55, 1311-1323 (2023). https://doi.org:10.1038/s41588-023-01460-5
2. Panzeri V. et al. CDK12/13 promote splicing of proximal introns by enhancing the interaction between RNA polymerase II and the splicing factor SF3B1. Nucleic Acids Res 51(11):5512-5526 (2023). https://doi: 10.1093/nar/gkad258.
3. Obeng E. et al. Physiologic Expression of Sf3b1(K700E) Causes Impaired Erythropoiesis, Aberrant Splicing, and Sensitivity to Therapeutic Spliceosome Modulation. Cancer Cell. 2016 Sep 12;30(3):404-417. doi: 10.1016/j.ccell.2016.08.006.
4. Liu B. et al. Mutant SF3B1 promotes AKT- and NF-κB-driven mammary tumorigenesis. J Clin Invest. 2021 Jan 4;131(1):e138315. doi: 10.1172/JCI138315.

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 or Masters in Biology, Immunology, Genetics, Molecular biology or related disciplines

Intended learning outcomes:

- Knowledge of the molecular basis of cancer

- Advanced skills in a wide range of molecular, cellular and biochemical assays
- Experience in bioinformatics
- Project management skills
- Wide appreciation of cancer research
- Acquisition of skill-set relevant to future as postdoctoral research fellow
- Ability to productively liaise with internal and external collaborators

Advertising details

Project suitable for a student with a background in:

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