Abstract for the general public

A critical cellular surveillance mechanism that recognizes and eliminates aberrant RNAs containing premature termination codons (PTC) is termed as nonsense-mediated mRNA decay (NMD). PTCs containing mRNAs are rapidly degraded by the NMD machinery. Active NMD renders many dominant mutations recessive by degrading transcripts encoding abnormal proteins with dominant activities. By this mechanism, NMD can exacerbate the phenotypes of many disorders by preventing the synthesis of truncated protein products with normal functions. Therefore, there is an intense interest in identifying NMD inhibitors (small-molecule) for treating certain diseases where the protein products of the corresponding nonsense mRNAs are fully or partially functional. Small-molecule inhibitors that can modulate NMD activity offer critical tools for understanding the mechanism and physiological functions of the NMD pathway, and they also have the potential for treating certain genetic diseases and cancer.

The NMD pathway, including UPF1, UPF2, UPF3a, and UPF3b, is over-produced in human cancers and can stimulate immune escape. Thus, the NMD pathway is a recognized drug target for cancer therapy, but there are no current clinical tools to validate this for use in human populations. Our approach to the treatment of diseases caused by PTC mutations is the inhibition of NMD, which is regulated by UPF1. The UPF1 gene, also termed as a master regulator of NMD pathway offers a new target that has not been successfully explored until now. We will focus on the core functions of UPF1 to find new small molecule ATP mimetic that can be used as a chemical tool to define the function and drugability of the pathway. Such novel compounds targeting UPF1 functions can increase stop-codon readthrough to enable the production of mutated full-length proteins and mutant MHC class I peptides derived from these premature stop codon readthroughs.

Inhibiting hUPF1 with a small molecule, which will generate translation readthrough, can create neoantigens by virtue of the amino acid which is added at a stop codon. In this sense, NMD (UPF1) inhibitors stimulate the tumour cell to make its own vaccine. Most cancer vaccines in clinical trials exploit mutated proteins; these vaccines include peptide products, viral assembly of genomic encoded mutated peptides, or mRNA synthesis that encodes mutated peptides in a patient specific manner. However, our approach to stimulate PTC readthrough with a small molecule, that drives the tumour to synthesize its own novel mutated peptides, is itself a novel approach for developing cancer vaccines.

A set of appropriate methodologies will be applied to ensure that robust data will be obtained and the results from the previous stage substantially influencing the next phase. The specific aims include; identification of novel molecules that act as inhibitors of UPF1 applying computer-aided drug design, synthesis, and biological evaluation approaches, understanding the network between UPF proteins *in vitro* by applying a novel FRET assay, and develop cell based assays with the optimized molecules to determine whether UPF1 inhibition can stimulate mutated immunopeptidome production. Together, these approaches aim to develop biochemical and *in vivo* data to establish the potential of the NMD system as a cancer drug target for use in tumour rejection.

The advantage of NMD inhibition over standard vaccine approaches is that this chemical tool that inhibits UPF1 can be used in a wide range of patients without the need to use the current vaccine pipeline-the current vaccine pipeline is; sequencing patients genomic DNA; creating the vaccine based on mutated proteins that dock into MHC Class I; and injecting the vaccine into the patient. Furthermore, the investigation of the interaction network of UPF (UPF1, UPF2, UPF3a, and UPF3b) genes by experimental assays within the project could bring novel insights into the assembly of this multi-protein complex and other inhibitors of NMD that could impact on cancer vaccinology. Cancer incidence increases worldwide due to aging of the population in developed countries, and improving healthcare and rising awareness in developing and underdeveloped countries. Consequently, novel anticancer drug candidates need to be constantly developed and always draw the attention of researchers. Cancer represents a significant burden on society and any new therapeutic drug that impacts patient prognosis is directly beneficial for society. Personalized medicine requires an increasing repertoire of drugs able to target more exclusive subpopulations of cancer patients, which improves the efficiency and safety of treatment for cancer patients.