Project Details	
Project Code	MRC23IIARCa Parker
Title	Defining the adenoviral “interactome” to develop safer and more efficacious virus-based therapies.
Research Theme	Infection, Immunity, Antimicrobial Resistance and Repair
Summary	The AZ adenovirus-based coronavirus vaccine has prevented ~6M COVID deaths worldwide. Nevertheless, interactions with host proteins have resulted in rare adverse blood clotting events, harming vaccine confidence. This highlights the need to define proteins interacting with adenoviruses prior to clinical role out. This project will develop methodologies to achieve this, defining the viral “interactome” to develop safer platforms for therapeutic applications.
Description	Background: Adenoviruses (Ad) are critical platform vectors for vaccine and oncology applications. The deployment of Ad based vaccines against SARS-CoV2 during the COVID-19 pandemic is estimated to have saved many millions of lives worldwide. Their success in protecting against severe COVID has not been without controversy, and rare cases of fatal blood clotting events post vaccination have damaged public confidence in these vaccines. Our laboratory has long-standing expertise in defining how Ad: host protein interactions influence viral infection and dose limiting toxicities. We defined how Vaccine Induced Thrombosis with Thrombocytopaenia (VITT) initiates, providing evidence that a low abundance blood protein, PF4, binds the Ad capsid, promoting a misplaced immune response (see https://www.bbc.co.uk/news/health-59418123). Production of anti-PF4 antibodies drives platelet activation and thrombus formation, causing blood clots. Further, we defined how Ad interact with blood clotting factors, in particular FX, to drive efficient “off-target” infection of the liver. A complete understanding of the full repertoire of Ad: host protein interactions (the "interactome") would help to proactively generate safer and more efficacious virotherapies of the future. The key research question this project addresses is: what blood proteins bind to adenoviral vector capsids and how do they influence viral infection and dose limiting toxicities? Objectives: The researcher will develop methodologies to immobilise adenoviral vectors and pull down protein associated with the capsid. Proteomic analysis will be performed using the GW4 Bristol proteomics facility. Initial methodologies have been developed which the recruited researcher will develop and lead. These include chemical approaches using cyanogen bromide, used in an initial study by Matthews (Bristol) to pull away proteins associating with the Ad5 capsid, whilst the Parker lab has developed methodologies to chemically or genetically biotinylate Ad vectors. Chemical biotinylation will use kit-based methods, whilst genetic approaches have been developed in house using viruses containing AviTAG (GLNDIFEAQKIEWHE) or biotin acceptor peptides incorporated at site specific locations in the capsid. The researcher will examine how these genetic and chemical alterations to the Ad capsid impact viral fitness using biological assays in the Parker lab, and structurally, how these alterations impact the viral protein structure and integrity, using state-of-the-art protein analytical technologies available in the Pudney lab (Bath). Initial studies involve optimising capsid labelling and immobilisation and comparing the proteins associated with

	the capsid using differing techniques. The researcher will lead on comparing these techniques, preparing the pull down proteins for proteomic analysis, as well as analysing the output from the proteomic studies. It is envisaged these initial studies will use well-established vectors, Ad5, as well as ChAdOx1, the platform of AZD1222 vaccine, and using healthy donor serum. Once optimised, the researcher will compare the viral interactome of healthy serum to that of serum from patients who developed VITT (available through the UK TTS consortium, on which Parker is a partner) to identify changes observed that mark out VITT patient serum as different to healthy control serum. These studies will be extended to encompass novel recombinant Ad serotype vectors developed in house. Once identified, novel Ad: protein interactions will be further validated by SPR, and the nature of the binding will be probed both biologically and by cryoEM with collaborating labs (Glasgow and Arizona). Recombinant Ad virions can then be engineered which are ablated for these interactions, and the resultant vectors will be assessed for efficacy in vivo. This project will ultimately develop safer and more efficacious Ad based therapies for translational applications.
Supervisory Team	
Lead Supervisor	
Name	Professor Alan Parker
Affiliation	Cardiff
College/Faculty	Biomedical and Life Science
Department/School	School of Medicine
Email Address	ParkerAL@Cardiff.ac.uk
Co-Supervisor 1	
Name	Professor David Matthews
Affiliation	Bristol
College/Faculty	Favulty of Life Sciences
Department/School	School of Cellular and Molecular Medicine
Co-Supervisor 2	
Name	Dr Christopher Pudney
Affiliation	Bath
College/Faculty	Faculty of Science
Department/School	Department of Biology & Biochemistry
Co-Supervisor 3	
Name	Dr Rebecca Bayliss
Affiliation	Cardiff
College/Faculty	College of Biomedical and Life Sciences
Department/School	School of Medicine