Project Details	
Project Code	MRCIIAR24Ca Bowen
Title	Investigating long noncoding RNAs as novel therapies in chronic kidney disease
Research Theme	Infection, Immunity, Antimicrobial Resistance & Repair
Summary	Chronic kidney disease (CKD) affects 15% of the global population and is associated with significant multimorbidity and high mortality, but has no cure. This project will use cell culture models of tissue regeneration, animal models of kidney disease and systems bioinformatic approaches to investigate modulation of long noncoding RNA expression as a novel therapeutic approach to improve CKD patient outcomes.
Description	BACKGROUND This project will provide skills and facilitate knowledge transfer to address the global clinical challenge of chronic kidney disease (CKD), which is associated with high morbidity and mortality, by focusing on understanding mechanisms of tissue repair. Kidney fibrosis is a key determinant of CKD progression and correlates strongly with increased synthesis of the extracellular matrix glycosaminoglycan hyaluronan (HA). Expression of the enzyme HA synthase 2 (HAS2), which produces HA, is significantly upregulated in CKD, promoting progressive fibrosis and poor patient outcomes. However, since HAS2 knockout mice die before birth, indirect methods are required to modulate its biological effects. Advanced sequencing techniques have revealed that most human gene expression transcribes RNAs that do not code for proteins. Analysis of these noncoding RNAs, or genomic “dark matter”, is revolutionising our understanding of the mechanisms underpinning human disease. We have detected that HAS2 has a naturally occurring antisense long noncoding (lnc)RNA termed HAS2-AS1. HAS2-AS1 is transcribed from the opposite genomic DNA strand to HAS2. HAS2 messenger RNA and HAS2-AS1 lncRNA share a region of sequence complementarity that varies in length, and therefore potential function, due to formation of two HAS2-AS1 variants by alternative splicing. We have detected these HAS2-AS1 splice variants and HAS2-AS1:HAS2 heteroduplexes in kidney cells. We have also demonstrated increased HAS2-AS1 expression in animal models of kidney fibrosis, and that functional HAS2 expression and activity in vitro is dependent on HAS2-AS1. HYPOTHESIS Long noncoding RNAs (lncRNAs) are important regulators in fibrotic disease in the kidney, and manipulation of the lncRNA HAS2-AS1 is a novel therapeutic approach that can be used to improve CKD outcomes. PLAN OF INVESTIGATION 1. REGULATION OF HAS2-AS1 EXPRESSION AND SPLICE VARIATION The WKRU host lab at Cardiff University has identified molecular factors that regulate HAS2-AS1 transcription, including HA-degrading enzyme hyaluronidase 2 (HYAL2) and transcription factor GATA binding protein 3 (GATA3). HYAL2 and GATA3 are also markers for distinct subpopulations of kidney fibroblasts and regulate stromal cell heterogeneity. The student will learn how to characterise transcriptional regulation of HAS2-AS1. The regulatory elements governing HAS2-AS1 splice variation will be identified by bioinformatic mapping. Expression of selected variants will be enforced using splice-switching oligonucleotides, and effects on differentiation and proliferation analysed. 2. MANIPULATING HA SYNTHESIS IN ANIMAL MODELS OF CKD The student will use next-generation

	biocompatible kidney-targeting nanocarriers in interventional animal CKD models to modulate expression of HAS2-AS1 splice variants in the renal stroma, determining the therapeutic efficacy of each variant by analysing attenuation of CKD progression, kidney regeneration and functional recovery. 3. ANALYSIS OF NONCODING RNAS IN RENAL FIBROSIS AND REGULATION OF HAS2-DRIVEN HA SYNTHESIS Using historical RNA sequencing data, the student will employ bioinformatics analysis to i) identify other lncRNA transcripts that are differentially expressed during kidney fibrosis and ii) examine the interactions between microRNAs, HAS2 mRNA and HAS-AS1 lncRNA. Time permitting, selected lncRNA transcripts will be analysed as above. The student will revise the research objectives according to their areas of specific interest and reference to data generated.
Supervisory Team	
Lead Supervisor	
Name	Dr Timothy Bowen
Affiliation	Cardiff
College/Faculty	College of Biomedical and Life Sciences
Department/School	School of Medicine
Email Address	bowent@cardiff.ac.uk
Co-Supervisor 1	
Name	Dr Soma Meran
Affiliation	Cardiff
College/Faculty	College of Biomedical and Life Sciences
Department/School	School of Medicine
Co-Supervisor 2	
Name	Professor Lorna Harries
Affiliation	Exeter
College/Faculty	College of Medicine and Health
Department/School	School of Medicine
Co-Supervisor 3	
Name	Professor Mark Lindsay
Affiliation	Bath
College/Faculty	Faculty of Science
Department/School	Department of Life Sciences