Project Details	
Project Code	MRCIIAR26Ex Scholpp
Title	Establishing a humanised zebrafish model for studying the genetic resilience in Neurofibromatosis Type 1 (NF1)
Research Theme	IIAR
Project Type	Wet lab
Summary	This PhD project explores the phenomenon of "genetic resilience" in the context of Malignant Peripheral Nerve Sheath Tumours (MPNSTs), aggressive cancers linked to Neurofibromatosis Type 1 (NF1). Although individuals with NF1 are more likely to develop MPNSTs, the varying incidence indicates that certain cellular mechanisms provide protection against malignant transformation. This research seeks to uncover the molecular pathways behind this resilience and, therefore, identify new therapeutic targets that could boost cellular defences, opening new possibilities for prevention against MPNSTs. By using zebrafish as an in vivo model for NF1, this PhD project aims to investigate innovative strategies for disease prevention.
Description	Malignant Peripheral Nerve Sheath Tumours (MPNSTs) are highly aggressive and often fatal cancers, affecting about 10% of patients with Neurofibromatosis Type 1 (NF1). NF1 is a genetic disorder caused by mutations in the NF1 gene, which encodes neurofibromin, a vital negative regulator of Ras activity. While individuals with a single mutated NF1 copy are predisposed to tumour formation, the occurrence of MPNSTs is not universal. The progression from benign neurofibromas to MPNSTs typically involves additional mutations in key tumour suppressor genes such as TP53. Current treatment options for MPNSTs are very limited, with poor responses to chemotherapy and radiotherapy, and surgical removal is often unfeasible, resulting in a dismal 5-year survival rate of 20-50%. Since MPNSTs are classified as a rare cancer, affecting roughly 3 in 100,000 individuals worldwide, there is an urgent need for robust in vivo models to understand the fundamental biological pathways driving MPNST development and to aid in the discovery of new therapeutic interventions. This PhD project aims to establish and thoroughly characterise a humanised zebrafish model for Neurofibromatosis Type 1, which will serve as a valuable tool to elucidate the biological pathways involved in MPNST progression and provide a platform for future drug screening efforts. Objective 1: Establish and Characterise Humanised NF1 Zebrafish Models with MPNST-associated Mutations. This objective will focus on the generation and detailed characterisation of novel humanised zebrafish models that accurately reflect the genetic landscape of NF1 and MPNST. First, we will establish a humanised zebrafish model where the endogenous zebrafish nf1 genes are functionally replaced with the human NF1 gene. This will be achieved through advanced CRISPR/Cas9 editing to generate a stable nf1a/b background, followed by the introduction and stable integration of the human NF1 gene using a long knock-in construct. Within this humanised background, we will then employ an adapted prime editing strategy to precisely insert single-nucleotide variants or small indels that mimic identified human NF1 mutations, specifically focusing on notable missense mutations affecting

	codons 844-848 within the cysteine-serine-rich domain of NF1. Crucially, we will subsequently introduce a knockout of the TP53 (p53) gene in these humanised NF1 mutant backgrounds using CRISPR/Cas9, as TP53 mutations are frequently observed in MPNST progression. We will then perform single-cell sequencing on Schwann Cells from these NF1 mutant and NF1/TP53 double mutant "humanised MPNST-CRISPants" (CRISPR-induced MPNST-like zebrafish) at early developmental stages (e.g., 5dpf larvae) and in adult zebrafish (e.g., 3-month-old). This transcriptional analysis will allow us to characterise the molecular consequences of these specific genetic alterations in peripheral nerve sheath cells and identify key biological pathways perturbed during early MPNST development. Objective 2: Functionally Analyse MPNST Progression in Humanised Zebrafish Models. Using this zebrafish model, we will then focus on the functional characterisation of MPNST progression in vivo. We will phenotypically assess tumour formation and growth in the humanised NF1 mutant and NF1/TP53 double mutant zebrafish. This will involve detailed histological analysis, imaging techniques (e.g., fluorescence microscopy in Tg(sox10-GFP) background to visualise Schwann cells), and quantification of tumour incidence, size, and growth rate over time in both larvae and adult fish. Additionally, we will explore the cellular microenvironment and immune cell infiltration within developing tumours to gain a comprehensive understanding of the in vivo disease pathology. This objective will validate the humanised zebrafish model as a relevant system for studying MPNST biology. Objective 3: Identify the Underlying Mechanism for Genetic Resilience in MPNST Progression. This objective aims to utilise the established humanised zebrafish model to identify factors that influence MPNST progression, laying the foundation for therapeutic development. We will conduct pooled in vivo CRISPant screens in the humanised NF1/TP53 zebrafish models. This screen will target a curated gene library, including those identified as key players in the biological pathways described in Objective 1, to find specific gene knockouts that either speed up or slow down tumour development within the peripheral nerve sheath. This will uncover genetic dependencies that could be targeted for intervention. This objective will offer key insights into potential therapeutic targets and candidate compounds for future drug development. Student Benefits and Skill Development. This PhD studentship provides an excellent training opportunity for a highly motivated life sciences student. The student will gain extensive expertise in cutting-edge molecular biology techniques, including advanced CRISPR/Cas9 and prime editing for precise genetic manipulation in vivo. A key part of the project involves developing and using sophisticated humanised zebrafish models, offering invaluable hands-on experience in in vivo disease modelling—a highly sought-after skill in both academia and industry. The student will become proficient in high-throughput genomic techniques such as single-cell sequencing, along with the necessary computational and bioinformatics skills for analysing large datasets. The student will also develop critical thinking,

	experimental design, data interpretation, and scientific communication skills, preparing them for a successful career in biomedical research.
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