CRUK Cambridge Centre MRes rotation project

Rotation Project Title	Developing nanobodies to stabilise and restore the function of melanoma-associated mutant $p16^{INK4a}$
Head of Laboratory (PI) Name	Prof Laura Itzhaki
Second supervisor if applicable	Dr Heike Laman
Programme	CMB
Supervisor's Email	lsi10@cam.ac.uk, hl316@cam.ac.uk
Laboratory Location	Department of Pharmacology, Department of Pathology

Project Outline	**Aims and objectives** Somatic alterations affecting the *CDKN2A* gene occur in more than 50% of human tumours. The *CDKN2A* gene encodes the cyclin-dependent kinase CDK4/6 inhibitor $p16^{INK4a}$ (p16), and more than 150 missense mutations have been identified in primary tumours (melanoma and others) and tumour-derived cell lines. These mutations are located throughout the sequence, without any hot spots, and the majority cause loss of function by severely destabilising the protein's structure. Nanobodies are single-domain antibodies derived from naturally occurring Heavy-chain antibodies in camelids and have many advantages compared to conventional antibody molecules in fundamental research as well as in biotechnological and clinical applications. Nanobodies have properties that make them extremely useful as therapeutic and diagnostic agents, including low immunogenicity due to high sequence identity with human VH family III, the ability to bind non-conventional epitopes, such as enzyme active sites and their soluble and functional expression inside the cell, allowing their use as intrabodies. The aim here to investigate the effects of nanobodies that bind to p16 and stabilise the protein's structure. Such a molecule that restores the structural stability of mutant p16 to the wild-type level should reactivate the protein and is expected to induce apoptosis either alone or in combination with DNA-damaging agents. With a previous CCC studentship, we succeeded in identifying a number of p16-binding nanobodies. We showed that several of these nanobodies have a large stabilising effect on mutant p16. We determined the structure of one of the nanobodies in complex with p16 and showed that the nanobody bound on the opposite surface of p16 to CDK4/6 and therefore should not disrupt p16 function. This work will lead to new insights into the underlying molecular origins of p16-related pathologies and will point the way to new therapeutic approaches for the treatment of melanoma and other cancers.
Experimental plan	The effects of the nanobodies will be evaluated in melanoma cell lines with known destabilising mutant p16 proteins. Our hypothesis is that the expression of nanobodies using a tetracycline-inducible system in these cancer cell lines should induce an irreversible state of cellular senescence. We will assay for the rate of cell proliferation, measure the phosphorylation status of pRb, a principal substrate of Cyclin D/CDK4/6, and also assay for the induction of senescence by the expression of SA-beta-galactosidase. We will additionally assess the specificity of various nanobody constructs for different INK4A mutant alleles. Promising candidates will be taken forward for testing in xenograft models, in which nanobodies will be tested as potential therapeutics.

Main Techniques	• Cell biology (proliferation and senescence assay) • Biochemistry (immunoblotting, protein expression and purification and delivery) • In vivo tumour modelling
Key References	Serrano, M., Lee, C.W., Chin, L., Cordon-Cardo, C., Beach, D., DePinho, R. A. Role of the INK4A locus in tumour suppression and cell mortality. *Cell* 85: 27-37 (1996). Steeland,S., Vandenbroucke, R.E., Libert, C. Drug Disc. Today 21: 1076-1113 (2016). Tang, K.S., Guralnick, B.J., Wang, W.K., Fersht, A.R., Itzhaki, L.S. Stability and folding of the tumour suppressor protein p16. *J. Mol. Biol*. 285: 1869-1886 (1999).