Department of Pathology fully-funded PhD studentships: project proposal form

PART A: PROJECT PROPOSAL	
Division	Cellular and Molecular Pathology (CMP)
Supervisor	Dr Matthew Murray
Second supervisor (If supervisor's contract ends before October 2025)	N/A
Project title	Exploring the role of piwi-interacting RNAs (piRNAs), a class of short non-coding RNAs, in malignant germ cell tumour pathogenesis.
Project abstract for advert (Max 100 words)	Malignant germ-cell-tumours (mGCTs) arise much more commonly in the testis than in the ovary. This study will test the hypothesis that an important factor in testicular mGCT development is disruption of the expression and/or function of piwi-interacting-RNAs (piRNAs) and their associated proteins, which maintain germline integrity in male meiosis by repressing mobile DNA elements (retrotransposons). Our initial profiling of small non-coding-RNAs (ncRNA) in testicular mGCTs has identified dysregulation of piRNAs. Following extension of this work, piRNAs showing altered expression will be mapped bioinformatically to repetitive elements in the genome, including LINE-1 retrotransposons. Resulting hypotheses will be tested functionally, using cell-line and *in-vivo* models already established to investigate microRNA dysregulation in mGCTs.
Keywords Please provide up to five	ncRNA, microRNA, piRNA, germ cell tumour

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Full details (Max 250 words) **Will be published on Departmental website; please do not include confidential information**	Malignant germ-cell-tumours (mGCTs) typically arise in the gonads. However, there is a striking difference in incidence between tumours of the testis (which affect 80-per-million males p.a.) and ovary (3-per-million females). The reasons for this discrepancy are poorly understood. One potentially important factor is the function of 26-31nt non-coding-RNAs (ncRNAs), termed piwi-interacting-RNAs (piRNAs), which maintain germline integrity by repressing mobile DNA elements (retrotransposons) [1]. An intact piRNA pathway in germ cells is essential to prevent mobilisation of long-interspersed-nuclear-element (LINE) retrotransposons in male meiosis. Hence, disruption of this pathway would be predicted to be critical to GCT development in the testis. We hypothesise that deregulation of piRNA expression is a key driver of testicular mGCT and explains the much high incidence of such tumours in male *vs.* female gonads. Using ncRNA profiling, we previously defined the landscape of dysregulated (18-23nt) microRNA expression in GCTs [2] and demonstrated the functional consequences of the observed changes [3]. Our current WGS ncRNA profiling study of mGCT tissues, extended with representative GCT cell-line samples and further gonadal (testis/ovary) controls, has been performed in collaboration with Dr Raheleh Rahbari (Sanger Institute, Cambridge) and is now nearing completion. In addition to dysregulation of piRNAs, consistent with limited published observations in testis cases [4,5], this study has identified other novel and important findings to be pursued experimentally. The piRNAs showing altered expression will be bioinformatically matched to repetitive elements in the genome, including LINE-1 retrotransposons. Resulting hypotheses will be tested functionally, using techniques and systems already established to investigate microRNA dysregulation in mGCTs, including: i) Correlating effects of dysregulated piRNA expression using matched mRNA data, interrogating for enrichment/depletion of piRNA nucleotide motifs within regulatory elements or open-reading-frames of protein-coding genes; ii) Depletion of over-expressed piRNAs (siRNA, CRISPR-Cas9) and/or replenishment of under-expressed piRNAs (mimics).

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	iii) Extension to established *in-vivo* murine models, for example using inducible systems for upregulating piRNAs in primary GCTs and lung metastases. *References* [1] Weick & Miska. piRNAs: from biogenesis to function. Development, 2014. PMID:25183868. [2] Palmer RD*, Murray MJ*, Saini HK*, et al. Malignant Germ Cell Tumors Display Common MicroRNA Profiles Resulting in Global Changes in Expression of Messenger RNA Targets. Cancer Research, 2010;70:2911-23. PMID:20332240. [3] Murray MJ, Saini HK, Siegler CA, et al. LIN28 expression in malignant germ cell tumors downregulates let-7 and increases oncogene levels. Cancer Research, 2013;73:4872-84. PMID: 23774216. [4] Rounge et al. Profiling of the Small RNA Populations in Human Testicular Germ Cell Tumors Shows Global Loss of piRNAs. Mol Cancer, 2015. PMID:26265322. [5] Gainetdinov et al. Assessment of piRNA Biogenesis and Function in Testicular Germ Cell Tumors and Their Precursor Germ Cell Neoplasia in Situ. BMC Cancer, 2018. PMID:29301509.
Three of your most important publications in support of the proposed project	[1] Palmer RD*, Murray MJ*, Saini HK*, et al. Malignant Germ Cell Tumors Display Common MicroRNA Profiles Resulting in Global Changes in Expression of Messenger RNA Targets. *Cancer Research,* 2010;70:2911-23. [2] Murray MJ, Saini HK, Siegler CA, et al. LIN28 expression in malignant germ cell tumors downregulates let-7 and increases oncogene levels. *Cancer Research*, 2013;73:4872-84. [3] Murray MJ, Coleman N. Understanding the pathway - MicroRNA dysregulation in malignant germ cell tumors: more than a biomarker? *Journal of Clinical Oncology*, 2019;37:1432-1435.
