

# MYC-INDUCED VULNERABILITIES/THORACIC CANCER RESEARCH



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Oncogenic signalling profoundly alters how cells respond to their environment, typically putting tumour cells under tremendous pressure to reconcile conflicting cues. For example, tumour cells must re-organise their metabolic pathways to balance competing needs for biosynthetic precursors with energetic homeostasis, commonly while surviving in a milieu of limiting oxygen and nutrients.

We use genetically engineered mouse models, primarily of lung cancer and mesothelioma, to understand how developing tumours cope with conflicting cues in their natural environment. Our overarching hypothesis is that oncogene-induced biological perturbations can be exploited for cancer therapy, even in the absence of direct suppression of driver oncogenes. We use deregulated MYC as our paradigm oncogene coupled with a mixture of candidate and RNAi-based approaches to identify induced vulnerabilities *in vivo* and *in vitro*, and are actively exploring several strategies for selective elimination of cells that overexpress MYC.

#### MYC in cancer

Overexpression of the transcription factor MYC occurs in a vast number of human cancers. The overexpression may arise from focal or broad chromosomal amplification, gene translocation, enhanced mRNA and protein stability, or indeed increased signalling through upstream regulatory factors such as Ras, Notch, or  $\beta$ -catenin. In many *in vivo* settings, MYC overexpression is sufficient to initiate or exacerbate tumorigenesis and MYC is moreover typically required to sustain the cancerous phenotype. A successful therapeutic strategy that exploits MYC expression would likely have a tremendous impact on human health. To facilitate investigation of physiologically relevant levels of deregulated MYC expression in any tissue, we have generated and characterised Rosa<sup>26DM-1st</sup>-MYC mice and deposited them with Jaxmice for unrestricted distribution to the broader scientific community.

#### MYC and KRAS drive immune evasion

How tumours evade detection by the immune system defines the underlying principle behind the therapeutic success of immunotherapy across a spectrum of cancer types. MYC is known to induce expression of PD-L1, which

inactivates cytotoxic T cells upon binding to PDL1, but new data from multiple labs, including ours, indicates that PD-L1 expression is not the sole immune evasion strategy deployed by MYC. In 2020, we showed that MYC and KRAS combine to suppress multiple cascades involved in cell communication with the immune system, with downregulation of the Type I Interferon pathway and of MHC I-dependent antigen processing & presentation forefront in these transcriptional responses. The transcriptional changes occur immediately upon acute activation of KRAS or modest overexpression of MYC in cell culture, and importantly, persist throughout tumour progression *in vivo*. Mechanistically, we identified repressive transcriptional complexes comprising MYC and MIZ1 binding directly to multiple key regulators of Type I Interferons in pancreatic ductal adenocarcinoma (PDAC). Genetic suppression of MYC or MIZ1 restore Interferon signalling, enabling PDAC tumours to elicit CXCL13 production in nearby macrophages and thereby recruit anti-tumour effector immune cells to limit tumour progression, resulting in extended survival. In the year since publication, this provocative finding of active suppression of the Type I Interferon cascade by the MYC/KRAS pathway has been reproduced in multiple cancer types, including lymphoma, breast, lung, ovarian and oesophageal cancers, indicating widespread use of this immune evasion strategy across many (all?) cancers. Pharmacological inhibition of MYC transcriptional repressive complexes may thus have benefit as a generic cancer therapy.

#### MYC-induced metabolic vulnerability

As part of a coordinated programme of cell growth required for cell division, MYC engages a number of biosynthetic programmes, such as ribosome assembly and protein translation, placing tremendous energetic demand upon the cell. In order to maintain energetic homeostasis, MYC upregulates glucose

transporters and glycolytic enzymes, promoting the Warburg effect of limited glucose breakdown, and in parallel induces expression of glutamine transporters and exploits this pathway to maintain the citric acid cycle. The energetic strain that MYC deregulation thus places upon the cell is evident in progressive activation of the AMP-activated protein kinase AMPK, which plays a key role in maintaining energetic homeostasis. AMPK in turn inhibits TORC1 to attenuate the rate of macromolecular synthesis, effectively allowing cells to balance the rate of ATP consumption with ATP production. Importantly, the AMPK-related kinase ARK/NUAK1 is also required for maintenance of ATP homeostasis in cells wherein MYC is overexpressed. NUAK1 plays a specific role in MYC-dependent activation of AMPK and also maintains mitochondrial respiratory capacity. Suppression of NUAK1 thus impairs the ability of MYC-overexpressing cells to respond to declining ATP levels while simultaneously depriving cells of ATP-generating capacity, suggesting that suppression of NUAK1 may be an effective means to selectively kill cancer cells with high levels of MYC expression.

#### Oncogene cooperation during lung cancer progression

Lung cancer remains one of the deadliest forms of cancer worldwide, accounting for 18% of all cancer-related deaths, and the incidence of lung cancer is on the rise, especially in the increasingly industrialised and densely populated cities of emerging economies. Poor prognosis arises in large part from the combination of late disease detection and limited matching of patients with emerging targeted therapies. We have found that modestly elevating MYC levels in a KRAS-driven model of lung cancer is sufficient to drive progression to metastatic disease. This progression arises in part through increased transcription of promiscuous ERBB family ligands. We have identified an unexpected requirement for signal transduction through the ERBB receptor tyrosine kinase network for both establishment and maintenance of KRAS-mutant lung cancer. Our data suggest that KRAS-driven tumours actively seek ways to amplify signalling through the RAS pathway to sustain the tumour phenotype.

#### Inflammation and genetics of mesothelioma

Mesothelioma is a lethal cancer of the lining of the chest cavity that arises in people chronically exposed to asbestos. There are no effective therapies and patient survival is typically less than 18 months from diagnosis. Our lab has teamed up with respiratory physician Kevin Blyth to build an international network of clinicians and researchers with the common goal of improving patient outcomes for this dreadful disease. We have developed a new mouse model of mesothelioma that will enable us to investigate the interplay between asbestos-driven chronic inflammation and the major

recurring mutations that are commonly found in human mesothelioma. Significantly, intrapleural injection of asbestos dramatically accelerates onset and severity of mesothelioma in our mice, even after homozygous deletion of 3 major tumour suppressor genes, indicating that chronic inflammation continues to contribute to mesothelioma beyond the acquisition of rate limiting mutations. This startling observation suggests that patients may benefit from interventions that aim to reduce inflammation, in addition to those directly targeting the tumour population.

#### Major developments in 2023

2023 was a year for consolidation of our lab's position as leaders of Mesothelioma research with publication of 2 papers on the subject, one in collaboration with the lab of Olivier Pardo of Imperial College London. In collaboration with Marion MacFarlane, I led a pre-clinical models education session following the bi-annual IMIG (International Mesothelioma Interest Group) worldwide conference, and I also presented invited lectures to the Cambridge Lung Cancer Symposium and the Oxford Early Detection of Cancer Symposium, along with keynote lectures at the Indian Institute of Technology International Cancer Conference, held in Chennai, India, and the Chilean Society for Biochemistry annual conference, held in La Serena, Chile.

Within the National Mouse Genetics Network (NMGN), work continued on development of the Tandem Arrayed Regulator (TAR) mouse that will enable controlled sequential genetic investigation of cancer progression – the allele was successfully integrated into ES cells and the first chimeric mice are expected in Spring '24. Our bid for supplementary funding from the NMGN Business Engagement Fund was approved to support collaboration with OMIC Pharmaceuticals, CA, USA, with work to progress in 2024. Our collaboration with Merck pharmaceuticals and Cancer Research Horizons successfully identified a specific small molecule kinase inhibitor suitable for *in vivo* experimentation.

The work of former PhD student Declan Whyte was published in Molecular Oncology and the lab led or contributed to 4 additional pre-print manuscript released on *BioRxiv* in 2023.

Finally, the lab welcomed new PhD student, Dominika Lubawska, funded through the CRUK Scotland Cancer Centre and commenced recruitment for 2 postdoctoral positions funded by CRUK programme REMIT.

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