The Drug Discovery Unit continues to make progress across its portfolio of exciting drug discovery projects that not only mirrors the focus of the outstanding research undertaken within the Institute, but also the CRUK focus of working on high-risk but potentially high-reward targets. In particular, our continued progress against KRAS is very exciting, and through our collaboration with the Frederick National Laboratory for Cancer Research in the US, we look forward to progressing to the next stage. In addition, through existing collaborations with Professor Mike Olson (Beatson Institute) and Professor Anthony Chalmers (University of Glasgow), our MRCK inhibitors are showing great promise in two independent in vivo models, one of skin squamous cell carcinoma and another of radiation-induced glioblastoma invasion.

KRAS
KRAS mutations are associated with many human cancers, and in particular are associated with the vast majority of pancreatic ductal adenocarcinomas (PDAC), and a significant number of other tumour types including colon and lung (30–40%). In the GTP-bound active state, KRAS signals from the plasma membrane through a functionally diverse set of downstream effectors proteins (including PI3K, RAF and RALGDS) to pathways that control cellular growth, apoptosis, survival and differentiation. We aim to block the interaction between KRAS and its effector proteins to inhibit oncogenic KRAS signalling.

Our KRAS project maintains a highly competitive position within the small molecule inhibitor field. Signing a two-year agreement with the USA National Cancer Institute’s RAS Initiative (at the Frederick National Lab) has enhanced our resource in multiple areas of the project, enabling us to progress our main chemical series more aggressively whilst also working on additional back-up series, presenting different opportunities and molecule profiles. Resource embedded within the Frederick Labs in biochemistry, cell biology, biophysics and protein production provides support for the development of novel assays, enabling us to explore the most effective approaches to measuring activity in a clinically relevant setting. Another key benefit of the collaboration is the direct access to the network of RAS cancer research across the USA, through this central hub of the RAS Initiative. This collaboration is funded jointly by CRUK and the CTR Pioneer Fund (CPF).

We have developed a medicinal chemistry strategy to optimise (Fig. 1) our existing KRAS ligands, resulting in new, significantly more active compounds. The team have made full use of our in-house X-ray crystallography data and computational chemistry expertise to effectively design and prioritise compounds for synthesis.

Optimisation has lead to compounds with increased affinity as they fit better with the KRAS protein and make additional key interactions with amino acid residues in the pocket. This binding affinity has been improved significantly against KRAS G12D and KRAS G12V/P12A with very tight binding observed and 33nM in our KRAS G12D GMP/Pi and KRAS G12V GDP/surface plasmon resonance (SPR) binding assays. Importantly, tight binding has also been confirmed in additional, orthogonal binding assays, providing highly valuable information useful for further optimisation. Multiple, parallel approaches have been employed to develop biochemical assays for S250-mediated nucleotide exchange (N2A) and effector binding (HTRF, FRET and pulldown). Significant improvements in potency were demonstrated across all biochemical assay formats as a result of the optimisation strategy and in line with our expectation. Excitingly, data for our best compounds show an effect on downstream signalling in cells at low micromolar concentrations.

We have characterised appropriate cell assay systems (both in wild-type, G12C/G12D mutant colorectal and pancreatic cancer cell lines and engineered RAS-less MEF cells) to measure target modulation and efficacy readouts. Assays looking at RAS/RAF interaction or levels of downstream signalling markers of the MAP kinase pathway (e.g. pERK) suggest that our current compounds are permeable and able to bind to RAS with low micromolar IC50 potency in cells. Importantly, we are in the process of developing 3D cell culture systems, where we have observed improved responses to our compounds when compared to cells grown in monolayers, in terms of cell survival and proliferation.

Figure 1
KRAS x-ray crystal structure, highlighting small molecule binding site (red) and areas of focus for small molecule optimisation (purple and blue).

MRCK
In close partnership with Professors Mike Olson and Anthony Chalmers, the Drug Discovery Unit has made excellent progress in its aim of developing and characterising inhibitors of MRCK. The myotonic dystrophy kinase-related CDC42-binding kinases, MRCKα and MRCKβ, regulate actin–myosin contractility and have been implicated in cancer invasion and metastasis. In concert with the closely related ROCK1/ROCK2 kinases, MRCK is known to phosphorylate downstream substrates such as MLC and MYPT1 to facilitate the cytoskeletal changes which contribute to cancer cell motility and invasion. Previous studies in which MRCK knockdown was shown to reduce invasion of cancer cells in vitro suggest there are likely to be clinical areas in which MRCK inhibitors would have therapeutic benefits.

Using a focused fragment-based MRCK biochemical screen, in combination with MRCK structural biology, our lab has developed selective and potent MRCK inhibitors. In particular, our lead compound BDP-00009066 (MRCKβ Kd = 23pM) was designed starting from a ligand-efficient fragment BDP-0003246 (MRCKβ Kd = 4.49uM). Iterative rounds of medicinal chemistry and structure-based design using BDP-0003246 led to the identification of BDP-00009066, a potent and selective MRCK inhibitor with sufficient pharmacokinetic properties to enable its use to further explore the role of MRCK as a cancer drug target. Generation of such inhibitors has allowed us to validate the hypothesis that MRCK is involved in cancer cell invasion in different indications. First, we have studied the effects of MRCK inhibition in glioblastoma (GBM). GBM is an aggressive, incurable primary tumour which is characterised by highly infiltrative cells. Patients are treated with surgery, radiotherapy and chemotherapy, but due to the invasive nature of the disease, outcomes remain poor and recurrence rates high. Whilst radiotherapy extends life expectancy, recent research has indicated that it can also promote a more invasive phenotype in cells which survive treatment. Our studies have shown that MRCK activity is upregulated by irradiation at the invasive edges of GBM tumours. Using BDP-00009066, it has been demonstrated that inhibiting MRCK activity is effective at reducing radiation-induced migration of GBM cells in vitro and in vivo. In a clinically relevant intracranial G7 cell mouse model (Fig. 2), BDP-00009066 was shown to prevent the invasion of GBM cells into the contralateral brain hemisphere (Birch et al., in preparation), and studies are ongoing to determine whether such effects of this compound lead to improved survival rates.
In addition to studies in glioblastoma, BDP-00009066 has also been used to show that MRCK inhibition has utility in squamous cell carcinoma (SCC). BDP-00009066 treatment of SCC cells led to reduced cell motility and 3D invasion at sub-micromolar levels in vitro, and MRCK activity was elevated in mouse skin tumours in a chemical carcinogenesis model. When evaluating the in vivo efficacy of BDP-00009066 in this SCC mouse model, we found that topical application of the compound resulted in a significant reduction in papilloma size (Unbekandi et al., Cancer Res. 2018; 78: 2096–114), highlighting further therapeutic action of this inhibitor.

These studies have revealed exciting opportunities for BDP-00009066 as a potential chemotherapeutic agent. As a consequence, two patent applications for our MRCK inhibitors were filed in 2017, adding further support that our compounds represent considerable advances in probing the effects of MRCK inhibition in in vivo models of cancer cell invasion.

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