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DRUG DISCOVERY PROGRAMME

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Drug Discovery continues to utilise structure-based methodologies to advance our projects against some of the most important and challenging targets in cancer today. In particular, our progress targeting KRAS is hugely encouraging. In addition, our expertise in fragment-based hit identification has been recognised through participation in a CRUK Centre Network Accelerator Award that enables us to collaborate more broadly with the wider CRUK community to utilise these techniques to advance their drug discovery efforts.

KRAS

One of the most fundamental and devastating causes of cancer initiation and progression arises from mutations of the membrane signalling protein KRAS. In normal cells, KRAS activation is responsible for a cascade of signalling events through multiple pathways leading to growth and survival of cells. For this reason this 'switch-like' GTPase is tightly controlled through activation of cell surface receptors and on/off regulators of its activity. In cancer, mutations of KRAS result in this master growth control protein being constantly switched on, as the deactivating hydrolysis enzymes are unable to access their binding site and switch off its activity. The result is an excess of growth signals that drive cancer progression in an uncontrollable manner.

A number of drugs on the market and in clinical trials today, target molecules in the signalling pathways downstream of KRAS such as the PI3K/AKT/mTOR and the RAF/MEK/ERK pathways. However, these approaches have been plagued with issues of redundancy on the one hand and toxicity on the other, when treatment combinations have been used. KRAS mutations are so widespread in cancer, particularly in some of the most difficult to treat cancers such as pancreatic, colorectal and lung (98%, 45% and 31% of patients, respectively, have a KRAS mutation), that they represent a huge unmet clinical need (Cox *et al.*, Nature Reviews Drug Discovery 2014; 13: 828-51).

For over 30 years, scientists have tried to block the activity of KRAS without success but importantly they have made crucial discoveries along the way. The new era of fragment-based drug design, which is a cornerstone of our expertise, has raised exciting new possibilities to inhibit this most intransigent target. Together with the application of medicinal and computational chemistry, crystallography, structural biology and state-of-the-art biophysical techniques in surface plasmon resonance (SPR), nuclear magnetic resonance (NMR) and isothermal titration calorimetry (ITC), we have made substantial progress over the last few years.

Following the initial screen of our fragment collection against GDP loaded (inactive form) G12D mutant KRAS using high field NMR, we

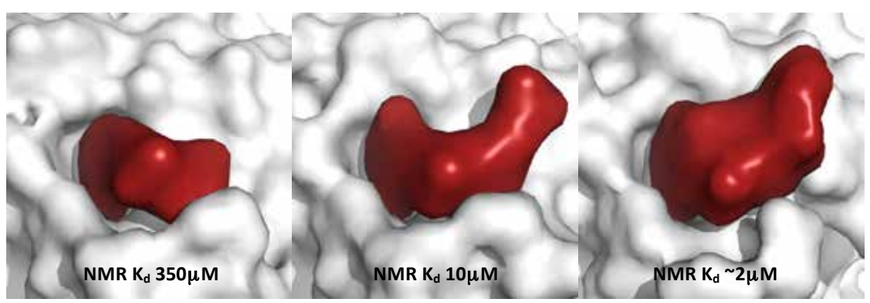
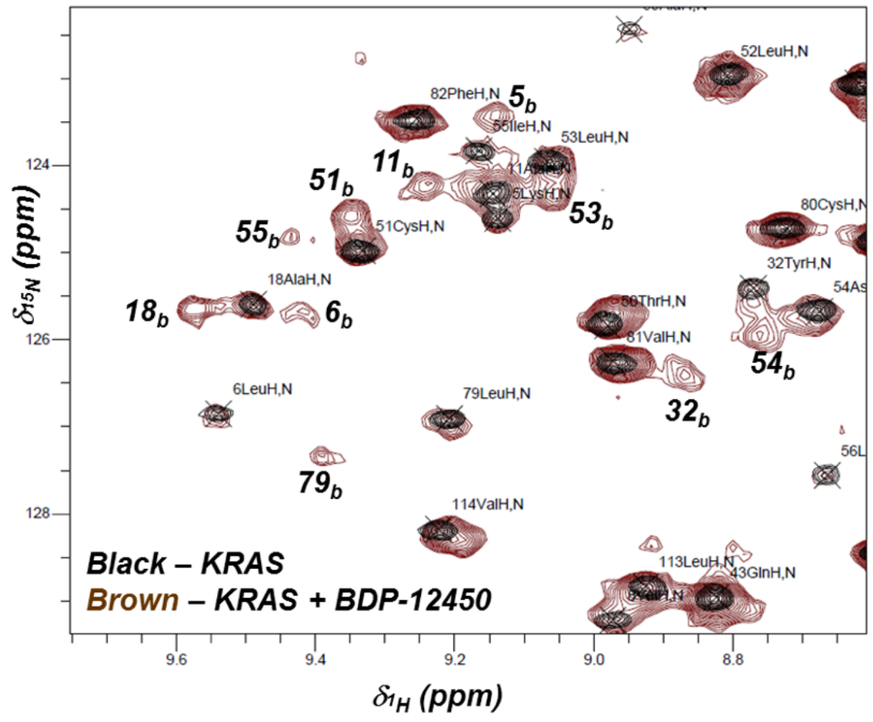


Figure 1
Optimisation of KRAS inhibitors.

Figure 2
^{1}H-^{15}N HSQC of KRAS-G12D.GDP with BDP-00012450.



have been able to optimise these weakly binding starting points into substantially increased affinity compounds. Key to this success has been the ability to generate high quality proteins and their crystallisation to reveal ligand-bound structures at high resolution. The structural insight this has afforded, has allowed us to further develop an optimisation strategy resulting in the design and synthesis of KRAS inhibitors that demonstrate binding affinities in the low µM range (Fig. 1).

The binding affinity of compounds has been determined using NMR against KRAS-G12D.GDP, which has been the primary screen for the project. The K_d values for our best compounds against KRAS-G12D.GDP is now K_d ~2µM, which is a substantial increase from the original µM binding fragment hits. (Fig. 2, showing cross peaks for both free and bound protein, BDP-00012450 estimated K_d ~2µM). This success means that the compounds are now too high affinity for the Kd to be accurately determined using NMR, as a result of a too slow exchange on the NMR timescale. To address this, additional assays have been developed including SPR, ITC and HTRF. We have established two additional biophysical assay formats that allow us to measure K_d values below the 10 µM range, namely SPR and ITC (Fig. 3 overleaf).

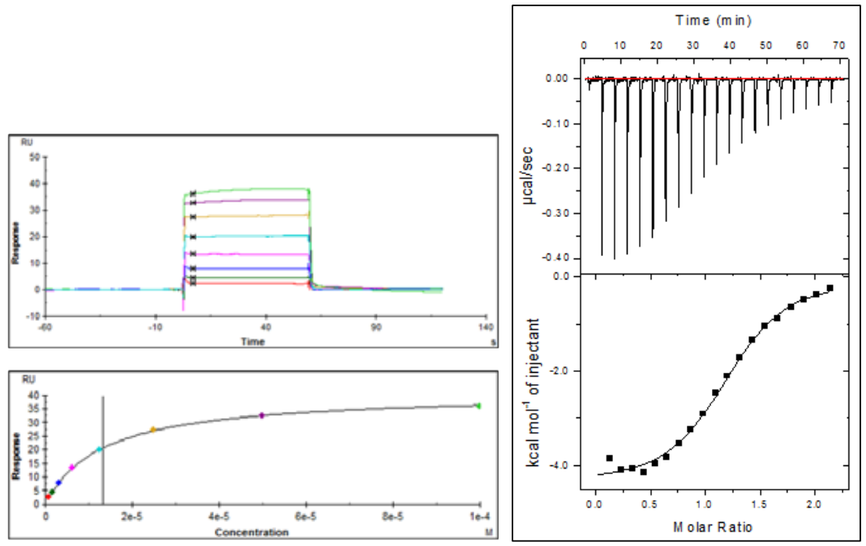
During the last year we have expanded our capability to use the more challenging but more clinically relevant active form of KRAS loaded with the stable GTP analogue GMPPnP. Use of this protein has been implemented across all of the assays including crystallography, NMR, SPR and biochemical assays. Importantly, we have directly addressed one of the most challenging aspects of the project and developed a robust biochemical assay for measuring the potency of our inhibitor compounds against KRAS-effector binding using a homogeneous time resolved fluorescence (HTRF) system. Comparison of data from the HTRF assay with the previously established nucleotide exchange assay (NEA), which measures inhibition of inactive GDP KRAS, has enabled us to assess the ability of compounds to inhibit these two important functions of RAS (Fig. 4 overleaf).

Future strategies will build on the progress made above, leveraging a combination of chemistry, biology and structural biology to develop sub-µM mutant KRAS inhibitors that are effective in cell assays and ultimately be taken into clinical trials. In order to maximise the opportunity for success with this project, the group has initiated key collaborations to boost resource and expertise in this challenging but potentially highly rewarding project.

CRUK Centre Network Accelerator Award: fragment-based drug discovery

In October 2015, CRUK awarded a significant five-year Centre Network Accelerator Award to establish a collaborative network across its drug discovery programmes, enabling challenging target identification and early stage drug discovery through the provision of specialised structural biology resource. Its purpose is to stand

Figure 3
SPR and ITC data for BDP-00013092 (Kd values 13.8mM and 3.7mM respectively).



as a networked, flexible resource that aims to deliver more oncogenic targets leading to rapid development of targeted therapies.

The resource is focused on the following areas (participating centres): Protein production and characterisation (Newcastle and Leicester); Biophysics and fragment screening (CRUK Beatson Institute); Protein structure determination (Leicester); and Computational chemistry (ICR) (Fig. 5 shown right). Participating groups within the network (CRUK Beatson Institute, Leicester, Newcastle, ICR, CRUK Manchester Institute, Cancer Research Technology Discovery Lab (CRT-DL), Belfast and Leeds) are encouraged to submit proposals for resource to undertake work in any of the above areas to a steering committee comprising members from each of the participating centres. The proposal will then be entered into a workflow that for successful projects will result in a fragment screen (Fig. 6 shown right).

As a recognised centre of excellence for fragment screening, biophysics and protein structure determination our group has committed to deliver fragment screens against challenging targets for participating Accelerator Award groups. Fragment screening offers the possibility to discover new potential drugs starting from the binding of a low molecular weight 'fragment' molecule to the target, which is then evolved stepwise increasing its affinity by chemical modification into the final drug. An example of a drug developed by this method is Vemurafenib (Fig. 7 shown right, which targets mutant BRAF malignant melanoma. The simple, low complexity molecular architecture of fragments enables them to access small molecule binding sites on proteins that can, through appropriate choice of assays and structures, be developed further, even against the most challenging, novel drug targets. With this approach we have designed robust and reproducible biophysical/structural biology binding assays using SPR and NMR screening protocols, and identified validated fragment hits for multiple CRUK-funded projects, providing a list of validated hits and a summary report (Fig. 6).

Figure 4
HTRF and NEA dose response curves for BDP 13646, EC50 values 36mM and 6mM respectively.

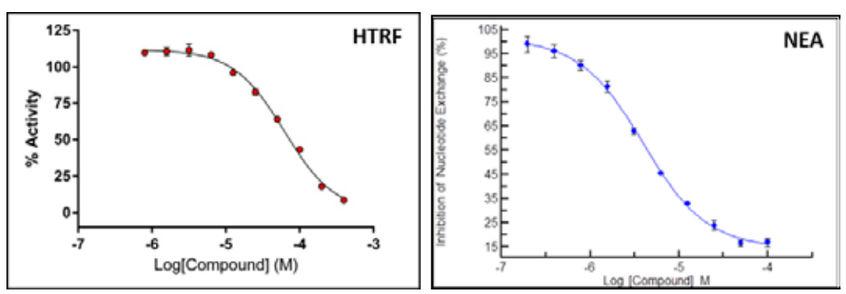
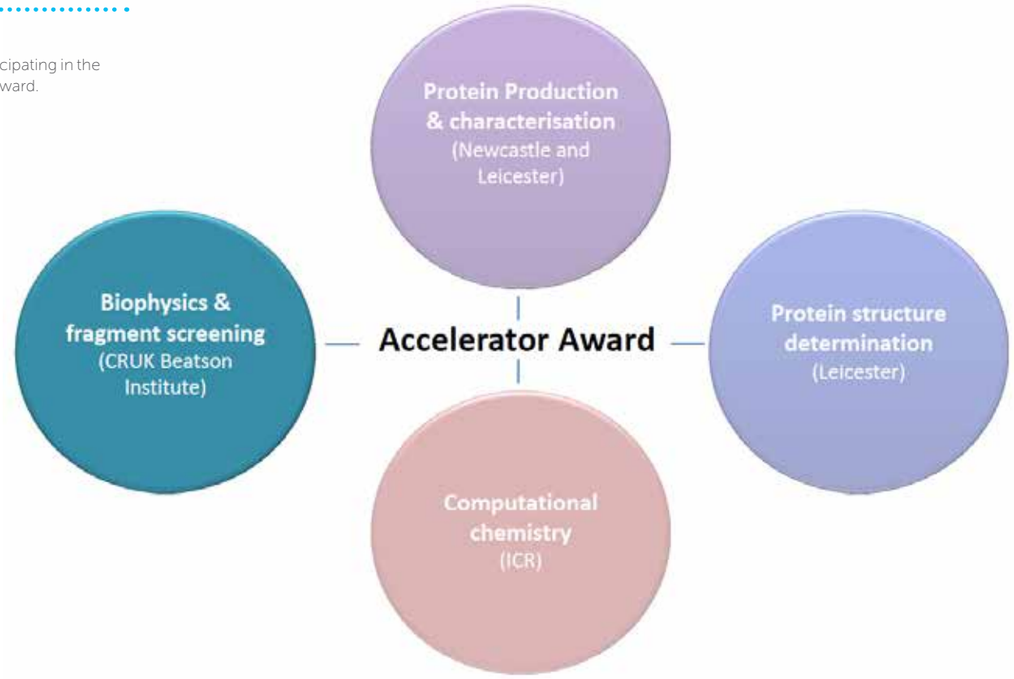


Figure 5
Facilities participating in the Accelerator Award.



The setup of a fragment screen consists of an initial quality control of the protein target followed by a primary screen of ~ 1000 fragments against the target using SPR or NMR. The resulting hits are then validated by a secondary screen (NMR or SPR, respectively) to increase confidence in target engagement. Throughout the screen an ongoing consultation is provided, starting from protein purification up to the further evolvement of the fragment hits after the screen. We also undertake virtual screens in order to provide potential additional start points and enrichment for the fragment screening hits (upon generation of structural information). So far, we have completed three fragment screens within this award, all of which have provided promising starting points for the development of new anti-cancer drugs. For the upcoming year, we are planning to continue to provide our service as a fragment screening facility to members of the Accelerator Award but also to other CRUK-funded projects increasing collaboration across the CRUK network and enabling new project starting points.

Figure 6
Process of fragment screening within the Accelerator Award.

Figure 7
Vemurafenib: Drug created by fragment-based screening (Proc Natl Acad Sci USA 2008; 105: 3041-6, Nature 2010; 467: 596-9).