Institution: The Institute of Cancer Research

Unit of Assessment: UoA5

Title of case study: Enabling the development of PKB inhibitors as novel cancer therapeutics

1. Summary of the impact

PKB (protein kinase B), also known as AKT, is an enzyme in the PI3 kinase/mTOR intracellular signalling pathway, which is deregulated in many cancers. Professor David Barford's team at the ICR solved the crystal structure of PKBβ using innovative protein engineering. The ICR has licensed six international pharmaceutical companies with reagents to enable them to begin PKB drug discovery programmes. The Barford team has also used their structural biology expertise to advance the ICR's own PKB inhibitor drug discovery programme. Two series of inhibitors were developed that were licensed to AstraZeneca and Astex and are now both in clinical trials.

2. Underpinning research

Professor David Barford (ICR Faculty) and his team published the results of research, conducted between 1999 and 2002 in collaboration with a team at the Friedrich Miescher Institute, on the crystal structure of the enzyme PKBβ (also known as AKT2) (Refs 1 and 2). PKB was known to be a key enzyme in the PI3 kinase/mTOR signalling pathway and is itself mutated, overexpressed or amplified in certain cancers. As such it is a potential target for anti-cancer drugs, but development of inhibitors of PKB had been hindered by the lack of protein structural information. By the early 2000s, it had been established that PKB is activated by two phosphorylation events. While it was known that PDK1 phosphorylates Thr309, the kinase responsible for phosphorylating Ser474 in the hydrophobic motif had not been identified. Thus, there was no procedure for generating activated PKB *in vitro*. The method of introducing a sequence based on 'PIFtide' to mimic the structural consequences of Ser474 phosphorylation circumvented the need to phosphorylate Ser474, allowing the Barford team to produce activated PKB for structural, biochemical and functional studies. The innovative protein engineering used in the design of the phospho-Ser474 mimetic constituted a major breakthrough in the field. The ICR made the expression systems encoding the engineered protein widely available to commercial companies and academic researchers.

The Barford team then collaborated with ICR Cancer Therapeutics teams led by Dr Michelle Garrett (ICR Faculty, UoA1), Professor Paul Workman (ICR Faculty, UoA1), Dr Ian Collins (ICR Faculty, UoA1) as lead chemist and Dr Suzanne Eccles (ICR Faculty, UoA1). They began an in-house drug discovery research programme in 2002, aimed at finding ATP competitive inhibitors of PKB. In 2003, the ICR team began a collaboration with the UK company Astex on this research programme. One innovative approach that the Barford and Astex teams used was fragment based lead discovery (Ref 3). Another innovative approach was a "back-soaking" method for obtaining PKBβ-ligand crystal structures (Ref 4).

Two fragment hits from the PKB screen were elaborated using structure-based design and medicinal chemistry based on the Barford protein structure information. Half of these medicinal chemistry research studies and the majority of the biological research studies were undertaken at the ICR, and these helped to validate PKB as a potential oncology target. Each of the two hits led to the identification of a lead chemical series and a number of publications resulted (Yap et al, 2012, Clin Cancer Res 18, 3812-3823 and McHardy et al, 2010, J Med Chem 53, 2239-2249). One chemical series was licensed to AstraZeneca. The second series was retained by Astex; this chemical series is distinct from the first as it has a broader specificity and inhibits other AGC kinases, which could contribute usefully to anticancer activity. In particular it has potent Rho kinase activity and a biologically distinct profile (Yap et al, 2012, Clin Cancer Res, 18, 3812-3823).

3. References to the research

All ICR authors are in bold and ICR team leaders/Faculty are in bold and underlined.

1. **Yang J**, Cron P, **Thompson V**, **Good VM**, Hess D, Hemmings BA, **Barford D**. 2002, Molecular Mechanism for the Regulation of Protein Kinase B/Akt by Hydrophobic Motif Phosphorylation, Mol Cell. 9 (6), 1227-1240. (http://dx.doi.org/10.1016/S1097-2765(02)00550-6)
2. **Yang J,** Cron P, **Good VM, Thompson V,** Hemmings BA, **Barford D.** 2002, Crystal structure of an activated Akt/protein kinase B ternary complex with GSK3-peptide and AMP-PNP, Nat Struct Biol. 9, 940-944. (http://dx.doi.org/10.1038/nsb870)
3. Saxty G, Woodhead SJ, Berdini V, Davies TG, Verdonk ML, Wyatt PG, Boyle RG, **Barford D**, Downham R, **Garrett MD**, Carr RA. 2007, Identification of Inhibitors of Protein Kinase B Using Fragment-Based Lead Discovery, J Med Chem. 50 (10), 2293-2296. (http://dx.doi.org/10.1021/jm070091b)
4. Davies TG, Verdonk ML, Graham B, Saalau-Bethell S, Hamlett CCF, McHardy T, **Collins I**, **Garrett MD, Workman P**, Woodhead SJ, Jhoti H, **Barford DJ**. 2007, A Structural Comparison of Inhibitor Binding to PKB, PKA and PKA-PKB Chimera, Mol Biol. 367 (3), 882-894. (http://dx.doi.org/10.1016/j.jmb.2007.01.004)

4. Details of the impact

The ICR has made a major impact on the international search for inhibitors of PKB, a key signalling enzyme and a target for the development of cancer therapeutics. This research has enabled a number of pharmaceutical companies to advance their research programmes for the development and commercialisation of novel drugs: currently, seven novel PKB inhibitors are in clinical trials (ClinicalTrials.gov), two of which are from the joint ICR and Astex PKB drug discovery programme.

Impacts on health

Two distinct drugs discovered by ICR and Astex are progressing through clinical trials in the UK and overseas; patients are benefiting by participating in these trials.

The ICR has discovered two series of PKB inhibitors in a collaborative research programme with Astex. As a result, one series was licensed in a commercial agreement with AstraZeneca and the lead drug, AZD5363, is currently undergoing clinical trials at The Royal Marsden NHS Foundation Trust (RM), The Christie (Manchester), the NKI (Netherlands) and in Japan, involving a total of over 400 patients (ClinicalTrials.gov Identifiers: NCT01226316, NCT01353781, NCT01625286, NCT01692262, NCT01895946) [1]. The ICR has led on the first trial of AZD5363 and Dr Udai Banerji (ICR Faculty) gave an oral presentation at AACR 2013 summarising its exciting potential in the clinic in selected patients. Results of the first phase I clinical trial of AZD5363 have reported both partial responses and stable disease in patients harbouring mutations in PIK3CA or AKT1. This therefore identifies these mutations as potential predictive biomarkers of response for AZD5363. The lead compound of the second series, AT13148, which is being developed by Astex, has different properties and could be useful in a distinct patient group. This drug is also in clinical trial at RM, enrolling 40 patients with advanced solid tumours (ClinicalTrials.gov Identifier: NCT01585701) [2].

Impacts on commerce

The international PKB drug discovery effort has been facilitated by ICR's work to solve the crystal structure.

The ICR solved the PKB crystal structure and published this work (Research Refs 1 and 2 above) and in total, over 30 international commercial companies, including many major pharmaceutical companies, have cited the influence of these publications in their own publications (17 commercial companies have cited Ref 2 since 1 January 2008). This shows that this work had an important impact on their in-house research investment and endeavour (data from Web of Science). For

example, since 2008 GSK has made major investment into developing PKB inhibitors as novel therapeutics. Its lead product, GSK2110183, progressed into Phase II clinical trials in 2009. GSK cited Reference 2 in their publications [3, 4].

Two distinct drugs discovered by ICR and Astex are being commercially developed
The drugs AZD5363 and AT13148, derived from chemical series discovered by ICR and Astex, are being developed by AstraZeneca and Astex, respectively. Both are in clinical trial. This has a commercial benefit to both these companies by adding to their development pipeline and therefore increasing shareholder value.

Industry is investing in pre-clinical research and clinical research to develop PKB inhibitors in the UK (including at ICR and RM) and overseas.
AstraZeneca and Astex are investing in the clinical research of AZD5363 and AT13148 respectively by conducting clinical trials. These drugs are based on the chemical series discovered by the ICR. Companies such as GSK that have cited ICR's underpinning research are investing in clinical research worldwide (ClinicalTrials.gov lists several GSK2110183 clinical trials, for example NCT01428492, NCT01531894, NCT01532700 and NCT01653912) and seven novel PKB inhibitors are now being developed.

5. Sources to corroborate the impact

[1] http://investor.astx.com/releasedetail.cfm?ReleaseID=663984

[2] http://investor.astx.com/releasedetail.cfm?ReleaseID=663805

[3] Najafov A et al. 2011, Characterization of GSK2334470, a novel and highly specific inhibitor of PDK1, Biochem J. 433, 357-369. (http://dx.doi.org/10.1042/BJ20101732)

[4] Heerding DA et al. 2008, Identification of 4-(2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-{[(3S)-3-piperidinylmethyl]oxy}-1*H*-imidazo[4,5-*c*]pyridin-4-yl)-2-methyl-3-butyn-2-ol (GSK690693), a Novel Inhibitor of AKT Kinase, J Med Chem. 51 (18), 5663-5679. (http://dx.doi.org/10.1021/jm8004527)