Institution: University of Dundee

Unit of Assessment: 5: Biological Sciences

Title of case study: Uncovering the PKB signalling cascade and the development of PKB inhibitors for the treatment of cancer.

1. Summary of the impact (indicative maximum 100 words)

The protein kinase PKB (also known as Akt) is a key regulator of cell proliferation and survival that is commonly dysregulated in human cancers. Work at the University of Dundee in the late 1990s identified key components of this signaling pathway and established the mechanism by which PKB becomes activated through phosphorylation. Structural studies at the University provided important insights for the design of small molecules permitting targeted inhibition of this enzyme. PKB is a firmly established focus for pharmacological intervention and several clinical trials are underway testing the antineoplastic activity of PKB inhibitors in a variety of cancers.

2. Underpinning research (indicative maximum 500 words)

Intracellular signaling through a cascade involving the phosphoinositide 3-kinase (PI 3-kinase) and Protein kinase B (PKB) is a major regulator of metabolism, cell growth, cell-cycle progression, cell survival, transcription and motility. PKB was first identified in 1991 by its homology to protein kinase A, protein kinase C and the retroviral oncogene, viral Akt. Interest was stimulated in the cancer field by the finding that PKB was overexpressed in a significant percentage of ovarian, pancreatic and breast cancers. In 1995, it was shown that PKB acted downstream of the PI 3-kinase, which generates phosphatidylinositol-(3,4,5)-trisphosphate (PIP_3), an inositol phospholipid that is a key signalling intermediate. Six months later, a collaboration between the laboratory of **Sir Philip Cohen FRS** (Director of the MRC Protein Phosphorylation Unit in Dundee from 1990 to 2012) and Brian Hemmings (Friedrich Miescher Institute) identified glycogen synthase kinase 3 (GSK3) as the first physiological substrate for PKB (1). This publication was highly influential and is the third most highly cited basic research paper in the PKB field. Shortly thereafter, **Prof Dario Alessi FRS** (a postdoctoral fellow then Programme Leader at the MRC Protein Phosphorylation Unit) demonstrated that PKB interacts with PIP_3 with submicromolar affinity (2). A further paper in 1996 by Alessi demonstrated that phosphorylation of PKB at two key sites (Thr308 and Ser473) was required for its maximal activation (3). This work suggested that PKB was activated by an unidentified upstream kinase and in 1997 Alessi reported in a seminal paper the purification and identification of 3-phosphoinositide-dependent protein kinase-1, PDK1, which activates PKB by phosphorylation on Thr308 in the presence of PIP_3 (4). A subsequent paper by Alessi in 1998 revealed that binding of the inositol phospholipids to the pleckstrin homology (PH) domain of PKB was necessary for PDK1 to phosphorylate and activate PKB (5).

Further insight into the mechanism of PKB activation resulted from determination of the PKB crystal structure by **Prof Daan Van Aalten FRSE** (Principal Investigator at the College of Life Sciences) (6 and subsequent papers). These studies have aided small molecule targeting of the enzyme by suggesting binding sites where small molecule inhibition should be possible; for example by inhibiting ATP or substrate binding to the kinase domain, or disrupting inositol lipid binding to the PH domain.

A patent (EP 0862622 B1) on methods for screening for PKB inhibitors was subsequently granted, citing the authors of the original GSK3 paper as co-inventors (1). The MRC and Novartis entered into an agreement to jointly exploit the IP in 2004.

These fundamental contributions that influenced multiple industrial drug discovery campaigns and led to several current clinical trials, were transmitted to members of the pharmaceutical industry prior to publication through the University of Dundee's unique Division of Transduction Therapy (DSTT) pre-competitive research consortium (as well as to the entire pharmaceutical industry following publication) – see Impact case study entitled "The impact of kinase profiling research at the University of Dundee on identifying areas of biology rich drug targets and accelerating product development in the pharmaceutical industry."

3. References to the research (indicative maximum of six references)
Publications:

1. Cross, D. A. E., **Alessi, D. R.**, **Cohen, P.**, Anjelkovic, M. & Hemmings, B. A. (1995) Inhibition of glycogen synthase kinase-3 by insulin mediated by protein kinase B. Nature 378,785-788. (doi:10.1038/378785a0) (Citations 2799, Scopus Nov 2013) - 3rd most highly cited basic research paper on PKB according to Scopus)

2. James, S.R., Downes, C.P., Gigg, R., Grove, S.J.A., Holmes, A.B., & **Alessi , D.R.** (1996) Specific binding of the Akt-1 protein kinase to phosphatidylinositol 3,4,5-trisphosphate without subsequent activation. Biochem. J, 315, 709–713. (ISSN: 02646021) (Citations 197, Scopus Nov 2013)

3. **Alessi, D. R.**, Andjelkovic, M., Caudwell, F. B., Cron, P., Morrice, N., **Cohen, P.** & Hemmings, B. A. (1996) Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J. 15, 6541-6551. (ISSN: 02614189) (Citations 1807, Scopus Nov 2013).

4. **Alessi, D. R.**, James, S. R., Downes, C. P., Holmes, A. B., Ga¡ney, P. R. J., Reese, C. B. & **Cohen, P.** (1997) Characterization of a 3-phosphoinositide-dependent protein kinase which phosphorylates and activates protein kinase Ba. Curr. Biol. 7, 261-269. (doi:10.1016/S0960-9822(06)00122-9) (Citations 1561, Scopus Nov 2013)

5. **Alessi, D. R.**, Deak, M., Casamayor, A., Caudwell, F.B., Morrice, N., Norman, D.G., Gaffney, P., Reese, C.B., Macdougall, C.N., Harnison, D., Ashworth, A. & Bownes, M. (1997) 3-phosphoinositide-dependent protein kinase-1 (PDK1): structural and functional homology with the Drosophila DSTPK61 kinase. Curr. Biol. 7, 776-789. (doi: 10.1016/S0960-9822(06)00336-8) (Citations 477, Scopus Nov 2013).

6. Thomas, C.C., Deak, M., **Alessi, D.R.**, & **Van Aalten, D.M.F.** (2002) High- resolution structure of the pleckstrin homology domain of protein kinase B/Akt bound to phosphatidylinositol (3,4,5) trisphosphate. Curr. Biol., 12, 1256 -1262. (doi:10.1016/S0960-9822(02)00972-7) (Citations 147, Scopus Nov 2013).

4. Details of the impact (indicative maximum 750 words)

The beneficiaries:

(a) The Pharmaceutical industry (particularly GlaxoSmithKline, Aeterna Zenartis and Merck & Co who benefited from the evidence that targeting PKB should be efficacious and from structural evidence for the druggability of PKB).

(b) Cancer patients (those who have already benefited in Phase II clinical trials and those likely to benefit from future drug combinations)

(c) Life sciences companies (through the production and sales of reagents used to probe the PKB/Akt signaling pathway in normal and diseased tissue).

Impacts:

Drug development of PKB/Akt inhibitors for the treatment of cancer
Dissection of the PKB signalling pathway and elucidation of the PKB crystal structure has been extremely influential, stimulating pharmaceutical companies to develop specific inhibitors of this pathway for therapy. PKB/Akt is associated with tumor cell survival, proliferation, and invasiveness. Activation of PKB/Akt is one of the most frequent alterations observed in human cancer, making PKB/Akt an attractive target for cancer therapy. The MRC non-exclusively licensed the IP from the PKB inhibitor screening methods patent (EP 0862622 B1) to a major pharmaceutical company (name withheld for confidentiality reasons) who has used the IP in their drug discovery efforts

resulting in clinical phase development. As part of this agreement, the MRC received a total of £150,000 (1).

A number of companies have initiated drug development campaigns in this area and several novel agents have been advanced into clinical trials including the three examples below:

GlaxoSmithKline (GSK)
In 2008, GSK developed GSK690693, a low-nanomolar pan-PKB kinase inhibitor (2). GSK690693 exhibited significant antitumor activity in ovarian, prostate and breast carcinoma xenograft models (3). Consequently, GSK690693 entered Phase I clinical trials for the treatment of hematologic malignancies. However, clinical development was suspended, perhaps due to potential side effects subsequently reported by GSK in rodents. GSK have since developed further PKB/Akt inhibitors that have now entered clinical trials. Currently 1 Phase II and 2 Phase 1 trials are underway testing the efficacy of the orally available ATP-competitive inhibitor GSK2110183 in multiple myeloma, haematologic malignancies and solid tumours (4) and 3 Phase I trials of the ATP-competitive inhibitor GSK2141795 are underway for the treatment of solid tumours, ovarian cancer and lymphoma (5). Promising results for GSK2141795 have been reported in gynecological, head and neck, prostrate, and colon cancers (6).

Aeterna Zentaris
Structural biology studies by Prof Daan Van Aalten at the University of Dundee showed that lipid binding to the PH domain activates PKB/Akt, thus revealing an alternative mechanism of PKB/Akt inhibition. Perifosine, an oral PKB/Akt inhibitor, was developed by Aeterna Zentaris using this strategy and this compound has been through 19 Phase I and 25 Phase II clinical trials and entered Phase 3 clinical trials for colon cancer and multiple myeloma in 2010 and 2009 respectively (7) (http://clinicaltrials.gov). While the Perifosine Phase 3 trials were not successful, the impact of the fundamental research on this drug development strategy was very significant.

Merck & Co
MK-2206 is an oral, non-ATP competitive allosteric PKB/Akt inhibitor with potential antineoplastic activity developed by Merck. There are currently 41 active or completed clinical trials testing the efficacy of this drug in a variety of cancers (http://clinicaltrials.gov). In 2009, Merck announced a partnership with AstraZeneca to develop a combination anti-cancer regimen composed of MK-2206 from Merck together with a mitogen-activated protein kinase 1 inhibitor AZD6244 from AstraZeneca (8).

Development of reagents by life science companies
The identification by the University of Dundee that phosphorylation of PKB/Akt at Thr308 and Ser473 is required for full activation of the kinase has led to the development of many new reagents by life science companies. The University of Dundee entered into an exclusive arrangement with Merck Millipore (formerly Upstate Biotechnology) to sell PKB reagents developed and produced by the MRC Protein Phosphorylation Unit and the Division of Signal Transduction Therapy. Subsequently, Merck Millipore designed a number of PKB-related catalogue items such as antibodies, assay kits, and fluorescently labelled proteins. Since 2008, total sales of PKB related products by Merck Millipore for which the University of Dundee has received royalties has amounted to $1,784,449 (9).

Phospho-specific antibodies specific to Thr308 and Ser473 residues to measure the activation of PKB/Akt are now sold by many life science companies including Cell Signaling Technology (http://www.cellsignal.com) (who currently sell 23 different phospho-specific antibodies to these sites, Invitrogen (http://www.invitrogen.com), Santa Cruz Biotech (www.scbt.com), BD Biosciences (www.bdbiosciences.com) and Sigma Aldrich (www.sigmaaldrich.com). Many of these companies also sell assay kits for detecting downstream physiological substrates of activated PKB such as GSK-3, identified as a target of PKB/Akt activity by the University of Dundee.

5. Sources to corroborate the impact (indicative maximum of 10 references)

1. Further information regarding the licensing of this patent can be obtained from the Senior Business Manager, MRC Technology, Edinburgh, EH4 2SP.

2. Heerding, D.A., Rhodes, N., Leber, J.D., Clark, T.J., Keenan, R.M., Lafrance, L.V., Li, M., Safonov, I.G., Takata, D.T., Venslavsky, J.W., Yamashita, D.S., Choudhry, A.E., Copeland, R.A., Lai, Z., Schaber, M.D., Tummino, P.J., Strum, S.L., Wood, E.R., Duckett, D.R., Eberwein, D., Knick, V.B., Lansing, T.J., McConnell, R.T., Zhang, S.Y., Minthorn, E.A., Concha, N.O., Warren, G.L., & Kumar. R. (2008) Identification of 4-(2-(4-Amino-1,2,5-oxadiazol-3-yl)-1-ethyl-7-{[(3S)-3-piperidinylmethyl]oxy}- 1H-imidazo[4,5-c]pyridin-4-yl)-2-methyl-3-butyn-2-ol (GSK690693), a Novel Inhibitor of AKT Kinase. J. Med. Chem. *51*, 5663–5679. (doi: 10.1021/jm8004527)

3. Rhodes, N., Heerding, D.A., Duckett, D.R., Eberwein, D.J., Knick, V.B., Lansing, T.J., McConnell, R.T., Gilmer, T.M., Zhang, S.Y., Robell, K., Kahana, J.A., Geske, R.S., Kleymenova, E.V., Choudry, A.E., Lai, Z., Leber, J.D., Minthorn, E.A., Strum, S.L., Wood, E.R., Huang, P.S., Copeland, R.A., & Humar, R. (2008) Characterization of an Akt Kinase Inhibitor with Potent Pharmacodynamic and Antitumor Activity. Cancer Res. *68*, 2366-2374. (doi: 10.1158/0008-5472.CAN-07-5783)

4. GSK Clinical study register http://www.gsk-clinicalstudyregister.com/quick-search-list.jsp?tab=protocols&phase=All&studyType=All&population=All&marketing=No&status=All&country=All&item=GSK2110183&letterrange=G-K&type=Compound

5. GSK Clinical study register http://www.gsk-clinicalstudyregister.com/protocol_comp_list.jsp?compound=GSK2141795&studyType=All&phase=All&population=All&marketing=All&status=All&country=All

6. Meeting Report in Pharmacy & Therapeutics: Alexander, W. (2011) Inhibiting the Akt Pathway in Cancer Treatment. P &T. *36*, 225-227. (ISSN: 10521372, http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3086120/pdf/ptj36_4p225.pdf)

7. Richardson, P.G., Eng, C., Kolesar, J., Hideshima, T., Anderson, K.C. (2012) Perifosine, an oral, anti-cancer agent and inhibitor of the Akt pathway: mechanistic actions, pharmacodynamics, pharmacokinetics, and clinical activity. *Expert. Opin. Drug. Metab. Toxicol. 8,* 623-633 (doi: 10.1517/17425255.2012.681376)

8. http://www.merck.com/licensing/our-partnership/astrazeneca-partnership.html

9. External corroboration can be obtained from the Research Collaboration Manager at Merck Millipore.