Institution: The Institute of Cancer Research

Unit of Assessment: Biological Sciences

Title of case study: Enabling the discovery and development of AKT inhibitors as novel cancer therapeutics

Period when the underpinning research was undertaken: 2002 to 2007

Details of staff conducting the underpinning research from the submitting unit:

Name(s):	Role(s) (e.g. job title):	Period(s) employed by submitting HEI:
Professor Udai Banerji	ICR Team Leader	01/04/2000–30/11/2005; 01/04/07–Present
Professor David Barford	ICR Team Leader	01/05/1999–01/11/2013
Professor Ian Collins	ICR Team Leader	10/11/2003–Present
Dr Michelle Garrett	ICR Team Leader	15/03/1999–31/07/2014
Professor Nicholas Turner	ICR Team Leader	01/09/2008–Present
Professor Paul Workman	ICR Team Leader	18/08/1997–Present

Period when the claimed impact occurred: 2013 onwards

Is this case study continued from a case study submitted in 2014? Yes. The underpinning research is the same as submitted to REF 2014. This research enabled the discovery and clinical development of AKT inhibitors. Since the REF 2014 submission, there has been additional significant impact with the clinical development of capivasertib—which is based on a chemical series discovered jointly by the ICR and Astex—including the evaluation of capivasertib in three phase 3 trials in breast and prostate cancers.

1. Summary of the impact

Professor David Barford's team at The Institute of Cancer Research (ICR) solved the crystal structure of AKT (also known as protein kinase B, PKB) and helped directly to develop two innovative AKT inhibitor drug discovery approaches, leading to the following impacts:

- **Commercial interest.** There are currently at least 12 AKT inhibitors in clinical development, including drugs from GSK, AstraZeneca, Genentech, and Merck. One of the AKT inhibitors in clinical development, capivasertib, is from a joint ICR and Astex AKT drug discovery programme.
- **Patient benefit.** There have been over 80 trials of AKT inhibitors in a variety of cancers. Capivasertib is being evaluated in three phase 3 trials in endocrine-resistant advanced breast cancer, triple-negative breast cancer, and PTEN-deficient prostate cancer.

2. Underpinning research

Background. Proliferation, angiogenesis and programmed cell death are three cellular activities often found deregulated in cancer. The serine/threonine kinase AKT, also known as protein kinase B (PKB), regulates all three of these processes and is itself regulated through the PI3 kinase signalling pathway. This pathway is one of the most frequently activated pathogenic signalling routes in human cancer—affecting 30–50% of malignancies. In addition, the three closely related isoforms of AKT (AKT1, 2, and 3) have been found mutated, amplified, overexpressed, or inappropriately activated in a number of tumour types. As such, AKT is a potential target for anti-cancer drugs—but development of inhibitors of AKT had been hindered by the lack of protein structural information.

Elucidating the crystal structure of AKT. By the early 2000s, it had been established that AKT 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. There was therefore no procedure for generating activated AKT protein for structural studies. In 2002, Professor David Barford (ICR Team Leader) and his team described a method whereby they introduced a peptide containing a particular hydrophobic motif (known as PIFtide) to mimic a phosphorylated Ser474 residue, which circumvented the need to

phosphorylate Ser474. This enabled Professor Barford's team to produce activated AKT 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. Following this major technical advance, Professor Barford's team, in collaboration with a team at the Friedrich Miescher Institute, published the crystal structure of AKT, and provided a molecular explanation for the regulation of AKT by Ser474 phosphorylation (**Ref. 1** and **2**). The ICR teams made the expression systems encoding the engineered protein widely available to commercial companies and academic researchers. The ICR also filed several patents relating to the crystal coordinates and methods for crystallizing the protein (GB0119860.5, filed 14 August 2001; GB0209985.1, filed 1 May 2002; and GB0216215.4, filed 12 July 2002).

Facilitating the discovery of potential AKT inhibitors. The Barford team collaborated with ICR drug discovery teams led by Dr Michelle Garrett (ICR Team Leader 1999 to 2014) and Professor Paul Workman (current ICR Team Leader, UOA1), with Professor Ian Collins (current ICR Team Leader, UOA1) as lead chemist. They began an in-house drug discovery research programme in 2002, with an aim to find ATP competitive inhibitors of AKT. In 2003, the ICR team began a collaboration with the UK company Astex on this research programme. The collaboration saw the use of a number of innovative approaches, including fragment-based lead discovery, which facilitated the rapid identification of novel, low-molecular weight AKT inhibitors (**Ref. 3**). Another innovative approach was a 'back-soaking' method for obtaining AKT-ligand crystal structures, which allowed the teams to fully exploit AKT structural information within the drug discovery process (**Ref. 4**).

The collaborative ICR-Astex drug discovery research programme elaborated two fragment hits from the AKT screen, using structure-based design and medicinal chemistry based on the protein structure information from Professor's Barford team (**Ref. 1** and **2**). Four chemical series were found by this approach—two were prioritised—which also helped validate AKT as a potential oncology target. A lead series that was discovered by the ICR and Astex was licensed in 2005 to AstraZeneca who then selected AZD5363 (capivasertib) for clinical development. Capivasertib has been evaluated in various clinical trials, including ones led by ICR researchers Professors Nicholas Turner and Udai Banerji (*see Section 4*).

3. References to the research

Key: **ICR employed staff** at the time of publication, **ICR Team Leaders** at the time of publication.

(Ref. 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. (https://doi.org/10.1016/S1097-2765(02)00550-6). *Times Cited: 337 (WOS).*

(Ref. 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). *Times Cited: 389 (WOS).*

(Ref. 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). *Times Cited: 113 (WOS).*

(Ref. 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). *Times Cited: 72 (WOS).*

Quality Indicators.

Selected peer reviewed research grant support:

- Workman | "Cancer Research Campaign Centre for Cancer Therapeutics", 2001–2006, GBP22,500,000, programme grant (which included the AKT project)
- Workman | "Cancer Research UK Centre for Cancer Therapeutics", 2006–2011, GBP29,156,418 programme grant (which included the AKT project)

Prizes:

- American Association of Cancer Research Team Science Award 2012 for the impact in preclinical and clinical studies relating to cancer therapeutics, which included the highly promising inhibitors of AKT: https://www.aacr.org/professionals/research/scientific-achievement-awards-and-lecturships/scientific-award-recipients/aacr-team-science-award-recipients/
- Professor Workman was elected as a Fellow of the Royal Society (FRS) in 2016.

4. Details of the impact

Following the ICR-led publication of the AKT crystal structure, there has been widespread activity in developing AKT inhibitors across the pharmaceutical industry with at least 12 AKT inhibitors in clinical development and over 80 AKT inhibitors in clinical trials—either alone or in combination with other therapies **[A]**. Importantly, the AKT inhibitor capivasertib, which is based on a chemical series discovered jointly by the ICR and Astex, has now entered phase III trials in three therapeutic indications and is therefore benefiting cancer patients directly by offering new treatment opportunities. A second lead series (AT13148) was also identified, which was retained by Astex. However, based on the narrow therapeutic index and the pharmacokinetic profile seen in the first-in-human study, the decision was taken not to develop this compound further.

Clinical development of capivasertib and patient benefit. The first-in-human trial of capivasertib was carried out by Professor Udai Banerji (ICR Team Leader, UOA1) with The Royal Marsden NHS Foundation Trust (RM) and the Christie Hospital NHS Trust, both in the UK and in the Netherlands. This study recruited 90 patients with advanced solid tumours to determine the safety, tolerability, and preliminary anti-cancer activity of capivasertib. The study identified a recommended phase 2 dose using an intermittent dosing schedule and demonstrated that capivasertib was well-tolerated and achieved robust target modulation. The dosing schedules were optimised using pharmacodynamic biomarker assays, which were developed and run at the ICR. The study also explored single-agent activity of capivasertib in populations of patients who had metastatic breast and gynaecologic cancers with PIK3CA mutations. Results suggest that future efforts in developing this class of drugs for the treatment of solid tumours, including PIK3CA-mutated breast and gynaecologic cancers, could benefit from combination studies with other anti-cancer drugs **[B]**. Professor Nicholas Turner led the BEECH study, which investigated capivasertib combined with paclitaxel in patients with estrogen receptor-positive advanced or metastatic breast cancer, and in a PIK3CA mutant sub-population.

Following identification of a recommended phase 2 dose, the safety and efficacy of capivasertib was further explored in multiple molecularly and histologically defined phase 1 expansion cohorts. Through an expansion cohort, involving Professor Banerji, proof of concept was demonstrated for both single-agent activity, and a combination of capivasertib with fulvestrant for the treatment of breast cancer with AKT mutations **[C]**.

As of December 2020, there were 27 clinical trials of capivasertib registered on clinicaltrials.gov, providing patients with experimental treatment options **[D]**. In 2019, the phase 2 FAKTION trial of 140 patients (NCT01992952) reported that addition of capivasertib to fulvestrant for patients with endocrine-resistant advanced breast cancer resulted in significantly longer median progression-free survival (10.3 months compared to 4.8 months for placebo) and an improvement in overall survival (26.0 months compared to 20.0 months for placebo) **[E]**. On the basis of these results, the CAPItello-291 trial (NCT04305496) was initiated, which will enrol 830 patients across 20 countries to evaluate capivasertib combined with fulvestrant.

Data from the phase 2 trial of capivasertib with paclitaxel, PAKT (which involved Professor Turner), demonstrated the impact of this combination on progression-free survival and overall

survival in previously untreated, metastatic triple-negative breast cancer. This trial involved 140 patients and showed a median overall survival of 19.1 months for capivasertib compared to 12.6 months for placebo **[F]**. The results from PAKT formed the basis of a phase 3 trial of capivasertib with paclitaxel in triple-negative breast cancer (NCT03997123). This trial opened to recruitment in 2019 and expects to enrol 800 patients in the United States, United Kingdom, Argentina, Brazil, Canada, China, Colombia, Czechia, France, Greece, Hungary, Japan, India, Korea, Mexico, Philippines, Poland, Portugal, Russia, Saudi Arabia, Spain, South Africa, Sweden, Thailand, Taiwan, Turkey, and Vietnam.

The ProCAID phase 2 study (NCT02121639) showed that the combination of capivasertib with docetaxel improved overall survival in patients with metastatic castration resistant prostate cancer **[G]**. Further, a phase 3 study of capivasertib in combination with abiraterone in PTEN-deficient prostate cancer is ongoing (NCT04493853), and is expected to enrol 1,000 patients.

Other ICR trials of capivasertib. In an ICR trial, antitumor activity of capivasertib combined with olaparib, a PARP inhibitor, was observed in patients harbouring advanced solid tumours with germline *BRCA1/2* mutations and *BRCA1/2* wild-type cancers with or without somatic DNA damage response and/or PI3K–AKT pathway alterations. These results support the development of the combination of olaparib and capivasertib as a promising strategy that warrants further exploration in future clinical trials **[H]**. Professor Turner is investigating capivasertib in plasmaMATCH (NCT03182634), a phase 2 trial looking at circulating tumour DNA testing in advanced breast cancer. In this trial, capivasertib had high activity in patients with ctDNA-identified AKT mutations, both in hormone receptor-positive cancer with fulvestrant and in hormone receptor-negative cancer as a single agent.

Other AKT inhibitors in clinical development. Several other AKT inhibitors have entered clinical studies including the ATP-competitive AKT inhibitors GDC-0068 (ipatesertib) and GSK2110183 (afuresertib), as well as Merck's allosteric AKT inhibitor MK2206 **[A]**. The ICR's Professor Johann de Bono led a phase 3 trial of ipatesertib in combination with abiraterone in metastatic castration-resistant prostate with PTEN loss and initial results have shown improved progression free survival **[I]**. There are AKT inhibitors whose use with bortezomib, rituximab, and ofatumumab is being explored for the treatment of haematological malignancies. Finally, there are ongoing clinical studies exploring combinations of AKT and MEK inhibitors in gastric and colorectal cancers. This large number of trials benefits patients with a variety of cancers by providing them access to new experimental treatments when no other options are available.

The international AKT drug discovery effort has been facilitated by ICR's work. The ICR has made a major impact on the international search for AKT and its inhibitors. This research has enabled a number of pharmaceutical companies to advance their research programmes for the development and commercialisation of novel drugs. The ICR patents on crystal coordinates and crystalisation methods (GB0119860.5, GB0209985.1, and GB0216215.4) have been licensed to six commercial companies (not named for confidentially reasons), alongside experimental material (e.g. plasmid vectors and baculovirus encoding AKT). This demonstrates the interest from the commercial sector in AKT as a therapeutic target.

A lead series that was discovered by the ICR and Astex was licensed in 2005 to AstraZeneca who then selected AZD5363 (capivasertib) for clinical development. In addition to this discovery, ICR researchers have worked with AstraZeneca to develop the pharmacodynamic biomarkers that supported the early clinical development of capivasertib. The ICR team analysed these biomarkers in platelet-rich plasma and hair follicles, providing evidence of target modulation in the first-in-human trial—which was pivotal in determining a recommended dose and schedule of capivasertib **[B]**—and also on six additional capivasertib trials, including two ICR/RM academic investigator-initiated studies of capivasertib in combination with enzalutamide (RE-AKT, NCT02525068) or the PARP inhibitor olaparib (ComPAKT, NCT02338622)**.**

ICR-led research on the AKT crystal structure was described in two publications (**Ref. 1** and **2**). 27 different international commercial companies have cited Reference 1 (54 citations) and 40 different international commercial companies have cited Reference 2 (>90 citations) (data from

Web of Science, 2019)—evidence that ICR work played a vitally important role in the research and investment of commercial companies.

Japanese company Otsuka Holdings Co. acquired Astex Pharmaceuticals Inc. for about USD886,000,000 **[J].** Their drug discovery/development pipeline, which included the AKT project, was a key attraction. At the time James Manuso, PhD, chairman and chief executive officer of Astex Pharmaceuticals stated:

> "*We believe that Otsuka's financial resources and development expertise will enhance Astex's ability to build further its oncology portfolio, pipeline, and discovery prowess.*" **[J]**

5. Sources to corroborate the impact

A. Clinical development of AKT inhibitors: Brown JS, Banerji U. 2017. Maximising the potential of AKT inhibitors as anti-cancer treatments. Pharmacol Ther. Apr;172:101-115. (https://doi.org/10.1016/j.pharmthera.2016.12.001)

B. The first in human trial of capivasertib: Banerji U et al., A Phase I Open-Label Study to Identify a Dosing Regimen of the Pan-AKT Inhibitor AZD5363 for Evaluation in Solid Tumors and in PIK3CA-Mutated Breast and Gynecologic Cancers. *Clin Cancer Res.* 2018 May 1;24(9). (https://doi.org/10.1158/1078-0432.CCR-17-2260)

C. Phase 1 trial of capivasertib with fulvestrant: Smyth LM, Tamura K, Oliveira M, Ciruelos EM, Mayer IA, Sablin MP, Biganzoli L, Ambrose HJ, Ashton J, Barnicle A, Cashell DD, Corcoran C, de Bruin EC, Foxley A, Hauser J, Lindemann JPO, Maudsley R, McEwen R, Moschetta M, Pass M, Rowlands V, Schiavon G, Banerji U, Scaltriti M, Taylor BS, Chandarlapaty S, Baselga J, Hyman DM. Capivasertib, an AKT Kinase Inhibitor, as Monotherapy or in Combination with Fulvestrant in Patients with *AKT1*^{E17K}-Mutant, ER-Positive Metastatic Breast Cancer. *Clin Cancer Res.* 2020 Aug 1;26(15). (https://doi.org/10.1158/1078-0432.CCR-19-3953)

D. Clinical trials of capivasertib: https://clinicaltrials.gov/ct2/results?cond=&term=AZD5363&cntry=&state=&city=&dist=

E. FAKTION trial results: Jones RH, Casbard A, Carucci M, Cox C, Butler R, Alchami F, Madden TA, Bale C, Bezecny P, Joffe J, Moon S, Twelves C, Venkitaraman R, Waters S, Foxley A, Howell SJ. Fulvestrant plus capivasertib versus placebo after relapse or progression on an aromatase inhibitor in metastatic, oestrogen receptor-positive breast cancer (FAKTION): a multicentre, randomised, controlled, phase 2 trial. *Lancet Oncol*. 2020 Mar;21(3):345-357. (https://doi.org/10.1016/S1470-2045(19)30817-4)

F. PAKT trial results: Schmid P, Abraham J, Chan S, Wheatley D, Brunt AM, Nemsadze G, Baird RD, Park YH, Hall PS, Perren T, Stein RC, Mangel L, Ferrero JM, Phillips M, Conibear J, Cortes J, Foxley A, de Bruin EC, McEwen R, Stetson D, Dougherty B, Sarker SJ, Prendergast A, McLaughlin-Callan M, Burgess M, Lawrence C, Cartwright H, Mousa K, Turner NC. Capivasertib Plus Paclitaxel Versus Placebo Plus Paclitaxel As First-Line Therapy for Metastatic Triple-Negative Breast Cancer: The PAKT Trial. J Clin Oncol. 2020 Feb 10;38(5):423-433. (https://doi.org/10.1200/JCO.19.00368)

G. ProCAID trial: https://ascopubs.org/doi/abs/10.1200/JCO.2020.38.15_suppl.5520

H. Phase 1 Trial of the Olaparib and Capivasertib: Yap TA, Kristeleit R, Michalarea V, Pettitt SJ, Lim JSJ, Carreira S, Roda D, Miller R, Riisnaes R, Miranda S, Figueiredo I, Rodrigues DN, Ward S, Matthews R, Parmar M, Turner A, Tunariu N, Chopra N, Gevensleben H, Turner NC, Ruddle R, Raynaud FI, Decordova S, Swales KE, Finneran L, Hall E, Rugman P, Lindemann JPO, Foxley A, Lord CJ, Banerji U, Plummer R, Basu B, Lopez JS, Drew Y, de Bono JS. Phase I Trial of the PARP Inhibitor Olaparib and AKT Inhibitor Capivasertib in Patients with *BRCA1/2*- and Non-*BRCA1/2*-Mutant Cancers. *Cancer Discov*. 2020 Oct;10(10):1528-1543. (https://doi.org/10.1158/2159-8290.CD-20-0163)

I. de Bono JS, Bracarda S, Sternberg CN, et al., IPATential150: Phase III study of ipatasertib plus abiraterone vs placebo plus abiraterone in metastatic castration-resistant prostate cancer. ESMO Virtual Congress 2020. (https://doi.org/10.1016/j.annonc.2020.08.2250)

J. Astex press release: https://astx.com/wp-content/uploads/2016/11/ASTX_News_2013_9_5_General_Releases.pdf