Institution: The Institute of Cancer Research

Unit of Assessment: UoA5

Title of case study: RAS/RAF/MEK/ERK signalling and identifying BRAF as a key target for the development of novel cancer therapeutics

1. Summary of the impact

Scientists at The Institute of Cancer Research (ICR) have played a central role in analysing the RAS/RAF/MEK/ERK cell signalling pathway and defining targets for novel cancer therapeutics. Their research work was key in stimulating an international effort to develop MEK inhibitors. Subsequently, ICR scientists predicted that the BRAF protein would be a key node in this pathway and they made the significant discovery that mutant *BRAF* is an oncogene. This prompted an international search for BRAF inhibitors, which was facilitated by the ICR's structural biology studies of BRAF. As a result, two novel drugs are now on the market.

2. Underpinning research

From 1993 onwards Professor Chris Marshall (ICR Faculty) was leading a team (which included Dr Richard Marais, a postdoc at the time) probing the RAS/RAF/MEK/ERK signalling pathway. This work showed that oncogenic RAS activates ERK through MEK and that the RAF proteins lie between RAS and MEK in the pathway sequence. The team cloned the *MEK1* gene, making the key discovery that constitutively activated MEK could oncogenically transform cells, thus demonstrating that MEK could be an important therapeutic target (Ref 1). These findings resulted in an international drug discovery effort to develop MEK inhibitors, for which the Marshall team provided many of the reagents.

In 1997, Marais (ICR Faculty, 1998-2012) and Marshall showed that oncogenic RAS maximally activates BRAF but not CRAF. This led to the hypothesis that the *BRAF* gene was the more likely candidate for possible oncogenic mutation in human cancer (Ref 2). Based on these studies, in 2001, Marais and Marshall began a collaboration on BRAF with Professor Michael Stratton (ICR Faculty) and Dr Richard Wooster (ICR Faculty).

This work resulted in the discovery of *BRAF* mutations in approximately 50% of melanomas, 10% of colorectal cancers and a smaller percentage of other cancers (Ref 3). The research teams of Marais and Marshall carried out further biological studies, including making the crucial observation that confirmed mutated *BRAF* as an oncogene (Ref 3). This discovery prompted a worldwide search for inhibitors of BRAF as potential cancer therapeutic agents. The development of effective inhibitors was facilitated by the research of Professor David Barford (ICR Faculty) and his team, who elucidated the crystal structure of mutant BRAF (Ref 4) enabling, for the first time, structure-based approaches to be used for therapeutic development.

Research into the function of BRAF continued at the ICR under the direction of Marais and Professor Caroline Springer (ICR Faculty). The team demonstrated that selective inhibition of BRAF in cells that have *NRAS* but not *BRAF* mutation results in a paradoxical activation of the MAPK pathway – through CRAF – which can lead to mutant RAS driven tumours (Ref 5). This discovery was based first on animal models and then on analysis of patients treated with selective BRAF inhibitors in which the development of squamous cell carcinomas was seen as a side effect (Ref 6). This research at ICR has contributed to the understanding of how to use specific BRAF inhibitors in anti-cancer therapy, and to the hypothesis that combination treatment with MEK inhibitors would be a more effective therapeutic approach.

3. References to the research

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

1. **Cowley S, Paterson H, Kemp P, Marshall CJ**. 1994, Activation of MAP kinase kinase is necessary and sufficient for PC12 differentiation and for transformation of NIH 3T3 cells, Cell. 77 (6), 841-852. (http://dx.doi.org/10.1016/0092-8674(94)90133-3)
2. **Marais R,** Light Y**,** Paterson HF**,** Mason CS**, Marshall CJ**. 1997, Differential regulation of Raf-1, A-Raf, and B-Raf by Oncogenic Ras and Tyrosine Kinases, J Biol Chem. 272, 4378-4383. (http://dx.doi.org/10.1074/jbc.272.7.4378)
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4. **Wan PTC, Garnett MJ, Roe SM, Lee S, Niculescu-Duvaz D, Good VM,** Cancer Genome Project**, Jones CM, Marshall CJ; Springer CJ; Barford D; Marais R**. 2004, Mechanism of Activation of the RAF-ERK Signaling Pathway by Oncogenic Mutations of B-RAF, Cell. 116 (6), 855-867. (http://dx.doi.org/10.1016/S0092-8674(04)00215-6)
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6. Su F, **Viros A, Milagre C**, Trunzer K, Bollag G, Spleiss O, **Reis-Filho JS**, Kong X, Koya RC, Flaherty KT, Chapman PB, Jung Kim M, **Hayward R, Martin M,** Yang H, Wang Q, Hilton H, Hang JS, Noe J, **Lambros M, Geyer F, Dhomen N, Niculescu-Duvaz I, Zambon A, Niculescu-Duvaz I, Preece N**, Robert L, Otte NJ, Mok S, Kee D, Ma Y, Zhang C, Habets G, Burton EA, Wong B, Nguyen H, Kockx M, Andries L, Lestini B, Nolop KB, Lee RJ, Joe AK, Troy JL, Gonzalez R, Hutson TE, Puzanov I, Chmielowski B, **Springer CJ,** McArthur PA, Sosman JA, Lo RS, Ribas A, **Marais R**. 2012, *RAS* Mutations in Cutaneous Squamous-Cell Carcinomas in Patients Treated with BRAF Inhibitors, N Engl J Med. 366, 207-215. (http://dx.doi.org/10.1056/NEJMoa1105358)

Selected research grant support

1. Marais – The role of C-RAF and MEK in B-RAF induced melanoma, 2007-2010, Cancer Research UK. £165,000.

4. Details of the impact

ICR research into the RAS signalling pathway has led to significant benefits for patients, especially for the approximately 50% of melanoma patients with tumours harbouring BRAF mutations. It has also had a major commercial impact in the pharmaceutical industry through the identification of new targets for therapeutic development. Many international pharmaceutical companies have added considerable shareholder value by adding MEK or BRAF inhibitor products to their cancer therapeutic pipeline; this commercial benefit has been enabled and facilitated by the ICR's ongoing underpinning research. Plexxikon, the biotech company which developed vemurafenib, the first BRAF inhibitor to reach the market, was acquired in 2011 by Daiichi Sankyo with an investment of over $800 million upfront. In this acquisition, vemurafenib was a major factor in the Plexxikon valuation [1].

BRAF Inhibitors

Malignant melanoma is the fifth most common cancer in the UK. Unlike many other malignancies, its incidence continues to rise on a yearly basis at a rate of 5-7%; this rising incidence is a global phenomenon, with the rate of increase being higher than for any other malignancy. Age demographics for the disease are also unusual, with over a quarter of new cases diagnosed in patients under 50.

Whilst early detection of localised disease allows complete surgical resection and a potential cure, the historical lack of effective treatment options for advanced (metastatic) disease means that melanoma accounts for a significant number of lives lost to cancer. Median survival with metastatic disease is approximately 6-9 months. Given the young patient population affected, this represents a significant burden of potential life years lost and thus melanoma is a malignancy of increasing epidemiological significance. The development of targeted therapies on the basis of ICR research (such as vemurafenib) have, for the first time in the history of metastatic malignant melanoma treatments, resulted in improved survival outcomes for patients.

In 2013, over 20 novel selective BRAF inhibitors are being developed commercially and two of these, vemurafenib (Roche) and dabrafenib (GSK), are now on the market. Vemurafenib received FDA marketing approval in 2011 [2] and Canadian and European [3] marketing approval in 2012; it is also NICE approved (NICE guidance TA269) [4]. Dabrafenib received FDA [5] and European [6] marketing approval in 2013 and is under review by NICE. It is estimated that globally over 12,000 patients were treated with vemurafenib in the period from launch to 31st July 2013 [7]. As evidenced by the approval by NICE of vemurafenib these new drugs represent a major improvement in treatment prospects for melanoma patients.

The ICR, in partnership with the Wellcome Trust, patented mutant BRAF as a target for drug screens and patient testing in 2001 [8]. The patent has five inventors of which four were ICR Faculty at that time (Marshall, Marais, Stratton, Wooster), which demonstrates the pivotal role that ICR scientists played in the discovery. The ICR and the Wellcome Trust made the strategic decision to file a patent early, well before the publication came out, so that they would have a dominant patent position. The patent has enabled us to out-license widely on a non-exclusive basis to facilitate worldwide drug discovery. Currently there are 12 licensees of this patent and, since 2008, £221,000 of licensing income has been received, indicating the investment of commercial resources into projects that relate directly to the ICR's underpinning research.

The discovery by the ICR that mutant BRAF is an oncogene has led to the clinical stratification of certain cancers such as melanoma. The development by pharmaceutical companies of inhibitors that specifically target mutant BRAF driven tumours means that patients have to be screened first before treatment to identify if they are suitable for these new therapies. A number of diagnostic companies have developed tests for mutant BRAF and have had these tests approved by the FDA and other regulatory authorities. The marketing of these tests has added commercial value to these companies and this is all a direct result of the ICR's fundamental research.

The publication of the molecular structure of mutant BRAF by ICR researchers was another key step in enabling companies worldwide to develop their own inhibitor design programmes based on the protein structural information. Over 70 commercial companies have cited the ICR structural biology work (Research Ref 4 above) (over 60 since 2008), including Plexxikon and GSK (Web of Science data).

The published ICR research on the paradoxical activation of MAPK through CRAF following BRAF inhibition demonstrated the mechanism by which selective BRAF inhibitors can induce squamous cell carcinomas (Research Refs 5 and 6 above). The elucidation of this mechanism has enabled companies developing selective inhibitors to invest in strategies to compensate for the side effects, for example by developing combination therapies with MEK inhibitors. Such a combination of dabrafenib (BRAF inhibitor) and trametinib (MEK inhibitor), developed by GSK, has shown significantly improved progression-free survival in patients [9].

MEK Inhibitors

ICR research on the oncogenic RAS signalling pathway and its key protein components has led to worldwide commercial impact through the development of novel cancer drugs. Following the publication of the pioneering work demonstrating activation of MEK and ERK by oncogenic RAS, and the finding that constitutively activated MEK leads to the neoplastic transformation of cells (Research Ref 1 above), over 20 international pharmaceutical companies have used and cited this research (Web of Science data). Although the impact began to be realised before 2008, it is only since that date that international pharmaceutical companies have commenced large-scale clinical trials of MEK inhibitors with the investment of major resources into their clinical development (ClinicalTrials.gov lists 112 clinical trials worldwide of MEK inhibitors, 104 of these commenced from 2008 onwards). Since 2008, over 12,000 patients have participated in MEK inhibitor clinical trials worldwide and 10 different products are in development (ClinicalTrials.gov). One product, trametinib, developed by GSK, was approved by the FDA in May 2013 for use in patients with unresectable or metastatic melanoma with BRAF V600E or V600K mutation [6] and is currently under review by the EMA's Committee for Medicinal Products for Human Use. It is now generating revenue in the international market with projected sales figures for 2013 of $26M (Thomson Reuters database).

The ICR, together with its partner the Royal Marsden NHS Foundation Trust (RM), has played a key role in the clinical studies of MEK inhibitors [10] (for example CinicalTrials.gov listed trials NCT01682083, NCT01584648, NCT00773526, NCT01245062 and NCT01449058) and has pioneered studies into combinations of MEK inhibitors with other drugs: as an example, being the first UK site to investigate the AKT/MEK combination, specifically MK2206 and AZD 6244. The combination studies point to improved patient outcomes over single therapy [11].

5. Sources to corroborate the impact

[1] http://www.plexxikon.com/view.cfm/88/press-releases

[2] http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm268241.htm

[3] http://www.ema.europa.eu/ema/index.jsp?curl=pages/medicines/human/medicines/002409/smops/Positive/human_smop_000318.jsp&mid=WC0b01ac058001d127&murl=menus/medicines/medicines.jsp

[4] NICE guideline TA269 – http://www.nice.org.uk/nicemedia/live/14005/61877/61877.pdf

[5] http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm354199.htm

[6] http://www.ema.europa.eu/ema/index.jsp?curl=pages/news_and_events/news/2013/06/news_detail_001836.jsp&mid=WC0b01ac058004d5c1

[7] Market Research Team, Genentech

[8] Patent publication number: WO/2003/56036. International application number: PCT/GB2002/005891. Inventors: Stratton, Futreal, Wooster, Marais, Marshall. Applicants: The Wellcome Trust, Stratton, Futreal, Wooster, Marais, Marshall. (http://patentscope.wipo.int/search/en/WO2003056036)

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