Institution: University of Dundee		
Unit of Assessment: UoA5 Biological Sciences		
Title of case study: Adoption by global Pharma of new therapeutics, tools and assays targeting LRRK2 to treat Parkinson's disease		
Period when the underpinning research was undertaken: 2007-present		
Details of staff conducting the underpinning research from the submitting unit:		
Name(s): Prof Dario Alessi	**Role(s) (e.g. job title):** Professor of Signal Transduction and Director of MRC Protein Phosphorylation and Ubiquitylation Unit	**Period(s) employed by submitting HEI:** 1991- present
Period when the claimed impact occurred: 2013-present		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

Parkinson's disease affects 6 million people worldwide**.** One genetic cause is mutation in the *LRRK2* gene. Research of Professor Dario Alessi and colleagues has: (1) underpinned over USD1billion investment in the development and clinical trial of LRRK2 inhibitors as therapeutics to treat Parkinson's; (2) provided 'gold standard' assays and tools to monitor LRRK2 activity that R&D in global pharma has relied on; (3) identified LRRK2-related biomarkers that are critical to evaluating the effectiveness of therapeutics in clinical trials; and (4) produced tools/reagents for LRRK2 preclinical research that are being marketed worldwide and used by industry.

2. Underpinning research

Parkinson's Disease is a debilitating progressive disorder of the nervous system. Genetic breakthroughs have uncovered genes linked to inherited forms of Parkinson's. One cause of familial Parkinson's is mutation within a protein kinase gene called *LRRK2* (for **l**eucine-**r**ich **r**epeat **k**inase **2**). When this became apparent in 2004, little was known of the physiological function of LRRK2. Research led by Alessi revealed robust quantitative evidence that the most common pathogenic mutation, located within the kinase domain of LRRK2, increases its kinase activity **[R1]**. This was an early strong indication that hyper-activity of LRRK2 could be the mechanism by which mutations in this protein cause disease and indicated that drug inhibitors of LRRK2 activity might offer a potential route to treatment.

To facilitate research on LRRK2, Alessi developed LRRKtide, a synthetic LRRK2 substrate for assessing LRRK2 kinase activity **[R1].** He also discovered key phosphorylation sites on LRRK2 that mediate its biological interactions and raised monoclonal antibodies against them **[R2]**. These have been universally used in academia and industry to assess *in vivo* efficacy of LRRK2 inhibitors. In 2011, in partnership with collaborator Nathanael Gray (Harvard), Alessi published the creation of the first potent and selective inhibitor of LRRK2 **[R3].**

Progress in the research field was stymied for a number of years by a lack of understanding of the implications of increased LRRK2 kinase activity on cell biology. Alessi devoted 12 years to identifying physiological substrates of LRRK2. In 2016 the breakthrough came from a collaboration between Alessi and Matthias Mann (Max Planck Institute), Merck, GlaxoSmithKline (GSK) and the Michael Fox Foundation for Parkinson's research (MJFF) with the seminal discovery that proteins known to be involved in trafficking between cell compartments (members of the Rab family of small GTPases) were the validated physiological phosphorylation targets of

LRRK2 **[R4].** All known Parkinson's-associated LRRK2 mutations significantly enhance Rab phosphorylation *in vivo* and this disrupts their normal trafficking within the cell **[R5].**

In order to analyse the signalling pathway, the Alessi lab developed a panel of sensitive, phospho-specific monoclonal antibodies to monitor the activity of the endogenous LRRK2 pathway *in vivo* **[R5]**. This significant undertaking resulted in a major step change in the biochemical research tools available to easily measure the activity of the LRRK2 pathway. Subsequently, Dr Esther Sammler (UoA1) together with Alessi used these reagents to develop the first biomarker assay measuring LRRK2 phosphorylation of pRab10 in human blood cells **[R6].** Alessi and Sammler also discovered a link between LRRK2 and how another Parkinson's mutation causes disease, indicating a value for LRRK2 inhibitors even in patients without LRRK2 mutation.

3. References to the research

[R1] Jaleel, M, **Nichols, RJ, Deak, M**, **Campbell, DG**, Gillardon, F, Knebel, A & **Alessi, DR** (2007) LRRK2 phosphorylates moesin at threonine-558: characterization of how Parkinson's disease mutants affect kinase activity, *Biochemical Journal*, vol. 405, no. 2, pp. 307-317. DOI: 10.1042/BJ20070209

[R2] Nichols, RJ, Dzamko, N, Morrice, NA, Campbell, DG, **Deak, M, Ordureau, A**, **Macartney, T,** Tong, Y, Shen, J, **Prescott, AR** & **Alessi, DR** (2010) 14-3-3 binding to LRRK2 is disrupted by multiple Parkinson's disease-associated mutations and regulates cytoplasmic localization, *Biochemical Journal*, vol. 430, no. 3, pp. 393-404. DOI: 10.1042/BJ20100483

[R3] Deng, X, **Dzamko, N**, **Prescott, A, Davies, P**, Liu, Q, Yang, Q, Lee, J-D, Patricelli, MP, Nomanbhoy, TK, **Alessi, DR** & Gray, NS (2011) Characterization of a selective inhibitor of the Parkinson's disease kinase LRRK2, *Nature Chemical Biology*, vol. 7, no. 4, pp. 203-205. DOI: 10.1038/NCHEMBIO.538

[R4] Steger, M, **Tonelli, F, Ito, G**, **Davies, P**, **Trost, M,** Vetter, M, Wachter, S, Lorentzen, E, Duddy, G, Wilson, S, Baptista, MAS, Fiske, BK, Fell, MJ, Morrow, JA, Reith, AD, **Alessi, DR** & Mann, M (2016) Phosphoproteomics reveals that Parkinson's disease kinase LRRK2 regulates a subset of Rab GTPases, *eLife*, vol. 5, e12813, pp. 1-28. DOI: 10.7554/eLife.12813

[R5] Lis, P, **Burel, S**, Steger, M, Mann, M, **Brown, F**, **Diez, F**, **Tonelli, F**, Holton, JL, Winglok Ho, P, Ho, S-L, Chou, M-Y, Polinski, NK, Martinez, TN, **Davies, P** & **Alessi, DR** (2018) Development of phospho-specific Rab protein antibodies to monitor in vivo activity of the LRRK2 Parkinson's disease kinase, *Biochemical Journal*, vol. 475, no. 1, pp. 1-22. DOI: 10.1042/BCJ20170802

[R6] Fan, Y, Howden, AJM, Sarhan, AR, Lis, P, Ito, G, Martinez, TN, Brockmann, K, Gasser, T, **Alessi, DR** & **Sammler, E** (2018) Interrogating Parkinson's disease LRRK2 kinase pathway activity by assessing Rab10 phosphorylation in human neutrophils, *Biochemical Journal*, vol. 475, no. 1, pp. 23-44. DOI: 10.1042/BCJ20170803

Key research grants relevant to this case study:

1. **Alessi, DR.** Understanding signalling pathways mutated in inherited disorders. Medical Research Council. (2013-present). Value: GBP6,415,000.
2. **Alessi, DR.** Regulation of the Parkinson's LRRK2 protein kinase by Rab29. Parkinson's UK. (2018-2021). Value: GBP91,389.
3. **Alessi, DR.** Towards a unifying theory of Parkinson's disease: Investigation of the biochemical and genetic role of Rab GTPases (2016-2018) Medical Research Council. Value: GBP240,758.
4. **Alessi, DR.** Characterisation of the LRRK2 protein kinase, mutated in inherited Parkinson's disease. Medical Research Council. (2008-2011). GBP254,088

5. **Alessi, DR.** The Michael J. Fox Foundation for Parkinson's Research. Cumulative grants from 2012- 2021. GBP 1,795,583
6. **Sammler, E.** Biomarkers in Parkinson's disease: A blood test to measure LRRK2. Parkinson's UK. 2017-2019. Value: GBP 49,270
7. **Sammler, E.** Bench-to-bedside Parkinson's disease research: Biomarkers in LRRK2 Research proposal. NHS Research Scotland (NRS) and Scottish universities. (2019-2023). Value: GBP1,046,335

4. Details of the impact

Beneficiaries:
(a) Pharmaceutical industry
(b) Patients
(c) Biotech reagent companies

Impacts:
(a) Focussing and enabling commercial drug discovery on LRRK2 inhibitors
The robust evidence that a common LRRK2 pathogenic mutation in Parkinson's increased its kinase activity, and the development of a synthetic LRRK2 substrate, had critical impact in directly stimulating the development of LRRK2 inhibitor therapeutics by industry **[E1]**. The Vice President of MJFF and former Merck LRRK2 Inhibitor Program Biology Lead confirms Alessi's contribution:

> "*During the early days of LRRK2 kinase inhibitor programs, all pharmaceutical companies relied on his published work to establish primary assays to screen for LRRK2 kinase inhibitors…*[and used his]*…synthetic LRRK2 substrate…to screen their compound libraries…These discoveries…had a major impact on the field since they stimulated the pharmaceutical industry to embark on LRRK2 kinase inhibitor drug discovery and development…*[His tools]*…directly facilitated the entry of the first LRRK2 kinase inhibitors into the clinic in 2017. People with Parkinson's are now testing these potential therapies thanks to Dr. Alessi's efforts"* **[E1]**.

(b) Underpinning investment in LRRK2 inhibitors and their entry into the clinic
As a result of the progress facilitated by Alessi's research, biopharma company Denali Therapeutics developed two brain-penetrant LRRK2 inhibitors that have generated positive results in Phase 1 and Phase 1b clinical trials in healthy volunteers and Parkinson's patients **[E2]**. One Denali inhibitor is now in preparation for late-phase clinical trials. This success stimulated significant investment in the company in 2020, with Biogen announcing a USD1billion deal to co-develop and co-commercialise Denali's LRRK2 inhibitors **[E3]**. Denali document many direct impacts of the research in enabling their trials **[E3]** which led to economic and patient benefits.

Global pharmaceutical company GlaxoSmithKline (GSK) has directly collaborated with Alessi on LRRK2 since 2008. GSK published nine World Intellectual Property Organization LRRK2 inhibitor patents 2015-2019 that cite Alessi's 2007 research **[R1]**; six also cite the 2016 paper **[R3]** which they had involvement with **[E4]**. In 2018, GSK announced a $300M partnership with DNA testing company 23andMe to expedite recruitment to their LRRK2 inhibitor clinical trials by targeting individuals with LRRK2 mutations **[E4]**. Worldwide, more than 43 patents were published 2014-2019 on small molecule LRRK2 inhibitors **[E5]**. Many belong to global pharmaceutical companies such as GSK, Pfizer, Merck, Roche and Genentech who all cite Alessi's work **[E5]**.

(c) Adoption of LRRK2 biomarkers with direct impact in enabling clinical trials
Alessi's research discoveries provided the two blood biomarkers that are essential to evaluating the effectiveness of treatments for LRRK2-mutation Parkinson's in clinical trials. Monitoring, via Alessi's UDD2 antibody, phosphorylation at a key site in LRRK2 became "*a gold standard measure of target engagement for LRRK2 kinase inhibitors*" according to MJFF **[E1]**. MJFF's

precompetitive alliance with Denali, Pfizer, Merck and Biogen, the 'LRRK2 Detection in PBMC Consortium', depended on UDD2 to develop a biomarker for their therapeutic trials **[E6]**. Similarly, referring to Alessi's identification of Rab GTPases as LRRK2 substrates, Denali say they "*immediately recognized the importance of this published insight and applied this to*…[develop] *high throughput clinical grade assays to measure Rabs as a pathway engagement biomarker*" **[E3]**. Both types of biomarker were measured in participant/patient blood during the Denali clinical trials to evaluate LRRK2 inhibitor effectiveness **[E2]**. Alessi's research also enabled Denali to find the correct inhibitor dose for the trials **[E3]**. MJFF and others are sponsoring clinical studies in the USA and Europe measuring the same blood biomarkers to see if they can identify people with enhanced LRRK2 pathway activity whether or not they have LRRK2 mutations or Parkinson's symptoms **[E7]**. The Vice President MJFF states:

> "*All of the target engagement work being conducted by pharmaceutical companies to measure LRRK2 and Rabs in*…[blood] *cells is due to Dr. Alessi's efforts and collaboration with MJFF to generate and make available at cost, crucial monoclonal antibodies*" **[E1]**.

(d) Marketing of LRRK2 products by laboratory reagent supply companies

LRRK2-related reagents from the Alessi lab are marketed to industry and academia by Abcam, NeuroMab, and the Dundee MRC-PPU Reagents and Services facility **[E8]**. Revenue from sales to industry by MRC-PPU was approximately GBP200,000 over the REF period **[E8]** and is likely to have been higher for the commercial vendors. These reagents are used extensively by Pharma, including Pfizer, Boehringer Ingelheim and Merck, for Parkinson's preclinical research **[E9]**.

(e) Bringing hope and encouraging people with Parkinson's to participate in research

In 2017, Parkinson's UK established the Dundee Parkinson's Research Interest Group (DRIG). Researchers from the University of Dundee regularly interact with DRIG, enabling people affected by Parkinson's to input to the research. The Chairperson of DRIG comments: "*DRIG has benefitted greatly from our association with the MRC PPU*…[We contribute]*…through occasionally commenting on research proposals…as well as by funding some small research projects."* **[E10]**. The group keeps up-to-date with Sammler's genetic testing for early-onset or inherited Parkinson's patients diagnosed in Scotland. People identified as having LRRK2 mutations become eligible to join clinical studies she leads for Dundee/UK such as the industry-led Rostock International Parkinson's Disease Study enrolling participants worldwide **[E7]**, and the LRRK2 International Parkinson's Disease Study **[E7]**. Informed involvement in such research benefits people with Parkinson's:

> *"…working with the MRC-PPU gives us not just hope, but also knowledge and a little bit of control over our future, which strengthens our determination to carry on and ultimately beat Parkinson's*" – Chair of DRIG **[E10]**.

5. Sources to corroborate the impact

[E1] Testimonial from the Vice President, The Michael J Fox Foundation for Parkinson's Research.

[E2] Phase 1/1b Clinical trials of LRRK2 inhibitors DNL201 and DNL15 using measurement of biomarkers identified by Alessi, pS935 and pRab10, as secondary outcome measures: (NCT04551534) 16th September 2020; (NCT03710707) 18th October 2018; (NCT04557800) 22nd September 2020; (NCT04056689) 14th August 2019.

[E3] Testimonial from Chief Medical Officer and Head of Development, Denali Therapeutics, and related press release.

[E4] GSK World Intellectual Property Organisation LRRK2 patent list citing R1 and R4 and GSK Press Release.

[E5] Xiao Ding & Feng Ren (2020) 'Leucine-rich repeat kinase 2 inhibitors: a patent review (2014-present)', *Expert Opinion on Therapeutic Patents,* vol. 30, no.4, pp275-286. DOI: 10.1080/13543776.2020.1729354 and example patents from GSK (WO2019012093A1), Pfizer (US9695171B2), Roche/Genentech (WO2013139882A1) and Merck (US9493440B2).

[E6] Publication by MJFF of results of pre-competitive collaboration with industry partners with the goal of optimizing the measurement of pLRRK2 in human PBMCs. Alessi is named as advisor and use of his UDD2 antibody supplied by Abcam is noted in Table 1.

[E7] Clinical Observational studies to assess LRRK2 activity (including phosphorylated LRRK2 and LRRK2 phosphorylated Rabs, detected using Alessi's antibodies) in blood cells as biomarkers in Parkinson's Disease: (NCT03545425) 4th June 2018; (NCT03866603) 7th March 2019; (NCT04214509) 2nd Jan 2020; (NCT04436848) 18th June 2020.

[E8] Panel of LRRK2 associated reagents developed by Alessi marketed by (a) Abcam (Anti-LRRK2 antibodies ab133450, ab133518) (b) anti-LRK2 clone 8G10 from NeuroMAb (75-308) and (c) LRRK2 reagents available to purchase from the UoD MRC PPU Reagents and Services website, and statement corroborating sales of reagents.

[E9] Biopharma publications corroborating use of Dundee UDD2 antibody (Abcam ab133450) in industry LRRK2 inhibitor programmes.

(i) Pfizer LRRK2 assay: Galatsis, P *et al*. (2014), 'Kinase domain inhibition of leucine rich repeat kinase 2 (LRRK2) using a [1,2,4]triazolo[4,3-b]pyridazine scaffold', *Bioorganic & Medicinal Chemistry Letters* vol. 24, no. 17, pp 4132-40. DOI: 10.1016/j.bmcl.2014.07.052

(ii) Merck LRRK2 assay along with LRRKtide and antibodies against it replicated and marketed by ThermoFisher: Fell, MJ *et al*. (2015) 'MLi-2, a Potent, Selective, and Centrally Active Compound for Exploring the Therapeutic Potential and Safety of LRRK2 Kinase Inhibition', *Journal of Pharmacology and Experimental Therapeutics.* vol. 355, no. 3, pp.397-409. DOI: 10.1124/jpet.115.227587

(iii) Boehringer Ingelheim: Speidel, A *et al* (2016) 'Leucine-Rich Repeat Kinase 2 Influences Fate Decision of Human Monocytes Differentiated from Induced Pluripotent Stem Cells', *PloS One* vol. 11,11 e0165949. DOI: 10.1371/journal.pone.0165949

[E10] Testimonial from the Chair of the Dundee Parkinson's Research Interest Group.