Institution: University of Durham

Unit of Assessment: UoA 8-CHEMISTRY

Title of case study: Fluorescent Retinoids

Period when the underpinning research was undertaken: 2007 - present

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:
Prof. Andrew Whiting (AW)	Professor	2001- present
Prof. Ehmke Pohl (EP)	Professor	2007- present

Period when the claimed impact occurred: 2015 - present

Is this case study continued from a case study submitted in 2014? N

1. Summary of the impact

Durham University spin-out, LightOx Ltd (Est: Aug-2016) is underpinned by interdisciplinary patented research spanning the fields of spectroscopy (fluorescence, emissive), organic and medicinal chemistry and biology. LightOx has raised over GBP2m in funding, currently employs 11 scientists, and is commercialising Durham research, to provide innovative technologies focussed on retinoid signalling pathways. They sell a range of functional probes, diagnostic imaging kits and new phototherapeutic drugs. LightOx was winner of the Bionow 'Start-up of the Year' and 'Product of the Year' (Nov-2018) categories. Recently established Nevrargenics Ltd (Est: Dec-2019), a sister company also based upon Durham retinoid chemistry, aims to develop drugs for neurodegenerative diseases. The value of LightOx is GBP9m following substantial third-party investment rounds, and Nevrargenics has a pre-investment valuation of GBP15m.

2. Underpinning research

The relationship between structure and function of synthetic retinoids has been extensively researched in Durham by Profs Andy Whiting and Ehmke Pohl [R1-4]. This research has led to the development of fluorescent retinoids, [R2] which have promise to change biological imaging, drug screening and subsequent drug design [R4]. Retinoids such as all-trans-retinoic acid (ATRA) are natural signalling molecules (mediating transcription events) that control diverse biological functions such as vision, cell proliferation/differentiation, growth of skin and bone tissue and immune function; retinoids have recently been identified as activators of tumour suppressor genes [R1,2]. Ongoing research at Durham [R1-6] is helping to map the complex and intricate retinoid signalling pathways and in so doing uncover new treatments for a range of conditions including cancers and neurodegenerative conditions [R6].

ATRA and its endogenous isomers are susceptible to photoisomerization and degradation because of their polyene structure, making their use as routine diagnostic reagents or future use as therapeutics problematic [R1]. In collaboration with LightOx, Durham University has designed and synthesised a range of synthetic (>300 fluorescent compounds, each offering unique physiological and photophysical properties), light stable ATRA analogues, such as EC23 [R1] (Fig.1) designed to exhibit strong, intrinsic, solvatochromic fluorescence [R5] whilst retaining the biological activity of the parent molecule, for example, maintaining reproducible stem cell differentiation [R2,3].

Other species, e.g., DC271 (Fig.1) have been synthetically evolved to act as functional probes allowing unprecedented imaging of cellular localisation using confocal fluorescence microscopy, enabling correlation between cellular target and biological activity [R4]. This has enabled Durham

researchers to develop a highly reproducible in vitro fluorometric binding assay to characterise and quantify specific cellular binding targets and thereby unravel the intricate network of gene regulation controlled by retinoids [R5]. The small molecular size of the probes and high fidelity to the original ATRA structures is of particular importance and means that they avoid traditional problems associated with larger fluorescent tagged molecules (e.g. requiring tagging to a carrier molecule) [R1,2]. As direct retinoid mimics there is facile delivery into the cell (high cell permeability and diffusion rate), minimal disruption of key cell activity, and minimal off-target tissue accumulation [R4,5]. The retinoid mimics have been tested on a range of different cellular systems including keratinocytes, neuroblastoma and teratoma cells, all of which have successfully indicated localisation of the fluorescent retinoids. This has also been observed in living zebra fish [R5].

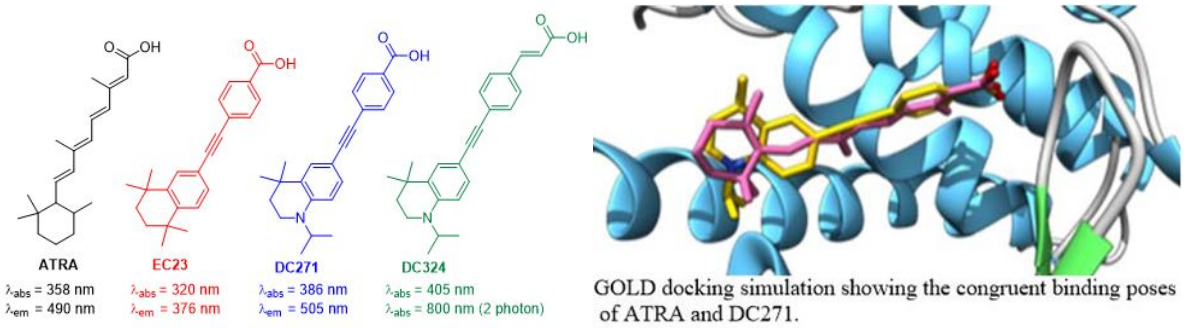

Figure 1. Synthesised fluorescent retinoids and docking simulation in GOLD showing the binding poses of ATRA (natural retinoid) and DC271 synthetic analogue.

Recent Durham research has shown that further modified structures, e.g. DC324 can be used as photosensitisers to elicit the production of reactive oxygen species (direct photoactivation by UV-A or two photon absorption by near-IR 800 nm light) resulting in powerful cytotoxic activity [R5]. This offers many options for photodynamic therapy of which microbial infections, neoplasias and tumour treatment are currently being investigated by LightOx supported by Durham researchers.

Concurrent research is also targeting neurodegenerative disease drug development for conditions such as Alzheimer's [R6]. The principal challenge has been to generate dual-acting retinoic acid receptor modulators (RAR-M) as the basis for a series of new rationally-designed and effective treatments [R6]. The research has identified a sub-nanomolar potency lead drug with high blood brain barrier penetration and ultra-low dose potential. The compound is currently being examined in relevant disease efficacy models.

3. References to the research

Citations according to Web of Science:

[R1] G. Clemens, K. R. Flower, A. P. Henderson, A. Whiting, S. A. Przyborski, M. Jimenez-Hernandez, F. Ball, P. Bassan, G. Cinque, P. Gardner, "The action of all-trans-retinoic acid (ATRA) and synthetic retinoid analogues (EC19 and EC23) on human pluripotent stem cells differentiation investigated using single cell infrared microspectroscopy" *Mol. BioSyst.*, **2013**, *9*, 677-692. DOI: 10.1039/C3MB25505K. **[19 citations]**

[R2] G. Clemens, K. R. Flower, P. Gardner, A. P. Henderson, J. P. Knowles, T. B. Marder, A. Whiting, S. A. Przyborski, "Design and biological evaluation of synthetic retinoids: Probing length vs stability vs activity" *Mol. BioSyst*., **2013**, 3124-3134. DOI: 10.1039/C3MB70273A **[21]**

[R3] V. B. Christie, D. J. Maltman, A. Whiting, T. B. Marder, S. A. Przyborski, "Retinoid supplementation of differentiating human neural progenitors and embryonic stem cells leads to enhanced neurogenesis in vitro" *J. Neurosci. Meth.*, **2010**, *193*, 239-245. DOI: 10.1016/j.jneumeth.2010.08.022. **[19]**

[R4] D. R. Chisholm, C. Tomlinson, G.-L. Zhou, C. Holden, V. Affleck, R. Lamb, K. Newling, P. Ashton, R. Valentine, C. Redfern, J. Erostyak, G. Makkai, C. A. Ambler, A. Whiting, E. Pohl, "Fluorescent retinoic acid analogues as probes for biochemical and intracellular characterization

of retinoid signalling pathways" *ACS Chem. Biol.*, **2019**, *14*(3), 369-377. DOI: 10.1021/acschembio.8b00916. **[10]**

[R5] D. R. Chisholm, R. Lamb, T. Pallett, V. Affleck, C. Holden, J. Marrison, P. O'Toole, P. D. Ashton, K. Newling, A. Steffen, A. K. Nelson, C. Mahler, R. Valentine, T. S. Blacker, A. J. Bain, J. Girkin, T. B. Marder, A. Whiting, C. A. Ambler, "Photoactivated cell-killing involving a low molecular weight, donor–acceptor diphenylacetylene" *Chem. Sci.,* **2019**, *10*, 4673–4683. DOI: 10.1039/c9sc00199a. **[4]**

[R6] T. Khatib, D. R. Chisholm, A. Whiting, B. Platt, P. McCaffery, "Decay in retinoic acid signalling in varied models of Alzheimer disease and restoration of gene expression with novel receptor acid receptor ligands (RAR-Ms) to Regulate Protective Genes" *Alzheimers Res. Ther*., **2020**, *73*, 935-954; DOI: 10.3233/JAD-190931. **[2]**

4. Details of the impact

The impact of Durham's synthetic retinoids research is being commercially developed via two Durham Chemistry spin-out companies: LightOx Ltd (www.lightox.co.uk) which focuses on diagnostic tools and imaging technologies, with its major emphasis upon photodynamic therapy for treating surface, especially oral cancers; and Nevrargenics Ltd (www.nevrargenics.com) a pharmaceutical start-up devising new medicines for neurodegenerative debilitating diseases that do not have current treatments such as Alzheimer's and Parkinson's.

LightOx (Est: Aug-2016) based upon patented Durham research [Patents: WO2008025965A2, WO2016055800, WO2017174999A1], markets synthetic retinoids developed by Durham Chemistry as tools for imaging, and assay development in the life science industries [R1-6, E1]. Its retinoid analogues enable cell imaging and bioactive molecule tracking using simple fluorescence and Raman spectroscopy techniques. LightOx has created a small molecule-receptor binding assay kit (see Fig.2). The kits have been purchased by >40 organisations worldwide spanning Brazil, USA, and Europe (sales of >GBP18k). Kits are now distributed globally by Merck Life sciences (www.sigmaaldrich.com LightOx™-range) along with an extended range of propriety retinoids through an exclusive 5 year deal (signed Dec-2018), current sales USD13.7k. Dr Wilke, Senior Product Manager for Emerging Chemical Synthesis at Sigma Millipore has worked closely with LightOx to develop the range and has indicated 'excellent sales and repeat interest' [E2].

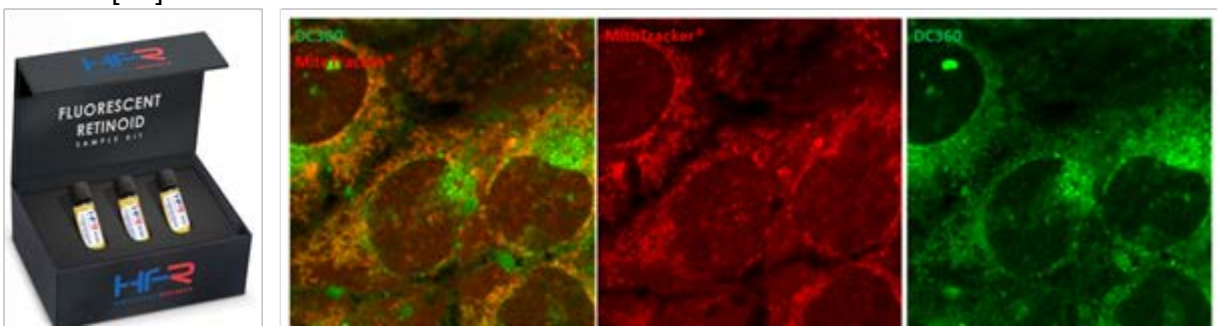

Figure 2. Small molecule receptor binding assay kit, DE360 co-imaged with MitoTracker® mitochondria stain. Cells fixed after 72 hours in 1µM DC360

Durham researchers have also developed a programmable light-box (Fig. 3) specifically tailored for use with these probes [R1-4] facilitating rapid diagnostic analysis. This is now also marketed on behalf of LightOx through Merck (released Aug-2019; 60-units commissioned, unit price GBP3,790). Following a successful RSC-run webinar the first production run of units has sold out [E2]. The unit is constructed to permit flexible configuration and provide defined and reproducible wavelength irradiation. The PhotoReact-365 nm version has been used in

Figure 3. The LightOx light-box

investigations of new anti-cancer treatments [R6]. A joint venture between LightOx and the Centre for Process Innovation (CPI) (National Healthcare Photonics Centre) [E3] led to the identification of several new lead candidates obtained by conjugating known HDAC inhibitors to a LightOx photosensitiser (Patent App:2019:GB1910239.1) [E1] creating a light activated therapeutic molecule for the treatment of pre-cancerous and early-stage oral cancer.

LightOx history: [E4]: Following creation and spin-out of the company from Durham University in Aug-2016, in Oct-2016 High Force Research Ltd (HFR) [E5] took an initial investment position of GBP180k and a follow-up tranche of GBP250k (Jan-2018 over 24 months) for a 30% shareholding in the Durham spin-out generating an initial company valuation of GBP1.4m. LightOx relocated from Biohub (Alderly Park, Cheshire) to its new business premises (Newcastle upon Tyne) in April-2019. The company has grown rapidly and currently employs 7 full-time scientists (biology/chemistry) and 4 part time (50-80%-FTE) managers as CEO/CTO/CSO. In addition, recruitment of other key personnel includes Steve Emery (Jun-2018) as non-executive director (former head of diligence AstraZeneca) to focus on business strategy, Tristan Sillars as CFO (Oct-2018), Amy Wright as Business Development Manager and Administrator (Jun-2019) and Nicola Emmett as Quality Control Manager (Jan-2020). The company has a salary outlay of GBP468k pa which would also equate to a further Gross Value Added (GVA) of ~GBP455k pa to the local economy [based upon Office of National Statistics data for the North East of England].

LightOx development: LightOx raised an additional GBP1.93m (July 2018-Jan 2020) from HFR, Meneldor & private investors [E6]. This has allowed LightOx to expand marketing of its biological probes, create prototype and commission the building of its light-box units and importantly for the long-term aspirations of the company to aggressively pursue its phototherapies for cancer [R5]. To date LightOx has invested over GBP1.51m in its technology and product development.

LightOx and Durham University has been widely recognised for its pioneering research and commercial development through the award of several business prizes and grants and has been highlighted in the national press (Daily Mail, Daily Express) [E7]. LightOx was twice silver medallist in the Medilink and Northern Powerhouse Alliance Business awards ‘Start-Up category’ (Feb/March-2018), a finalist in the Innovation Showcase at VentureFest (Nov-2018) and winner of the Bionow ‘Start-up of the Year’ and ‘Product of the Year’ (Nov-2018) categories. In addition, LightOx has been supporting its staff to become future scientific leaders and entrepreneurs; for example, Dr David Chisholm (a Durham Graduate), LightOx's Chemistry Team Leader was recently awarded "Promising technologist of the year" at the Bionow awards [E8].

Nevrargenics: [E9]: was founded in Dec-2019 (originally as RAR-M Therapeutics Ltd) to exploit specific neurodegenerative disease knowledge accumulated within Durham Chemistry [R1-3], LightOx, and Aberdeen University but outside LightOx’s primary business sector. Nevrargenics’ focus is primarily the development of neurodegenerative treatments based upon intellectual property described in two patents based on Durham research (WO2017174999 & WO2018029473) [E1]. The key lead candidate NVG0645, has already passed early-stage preclinical testing showing excellent PK/ADMET properties and is currently being taken through key aspects of: 1) target validation in advanced Alzheimer's cellular and animal models; 2) GMP scale-up and 3) preclinical toxicity testing in two animal models. The research has been presented under NDA to several major pharma companies who have acknowledged the significant commercial impact of the research, see support letter from Dr Fiona Marshall, VP Neuroscience and Head of UK Discovery Research at MSD [E10].

Durham University Research Impact Fund provided GBP10k for testing Nevrargenics lead compound DC645 in rats for bioavailability (Nov-2020). The results indicated exceptional efficacy even at exceptionally low dose levels, i.e., 0.02mg/kg. Further dosing studies are currently underway and additionally support this data. In Dec-2019 the Northern Accelerator for business support awarded a grant of GBP30k to Nevrargenics to recruit Dimitri Dimitriou (>30 years' experience in the pharmaceutical and biotech industry, https://ownyourventure.com/equitySim.html) to provide support raising ~GBP4-5m to pursue preclinical and Phase I/IIa work. A provisional valuation of GBP15m (ING Biotec Biotech valuation, Pharmaceuticals Western Europe) had been

assigned to Nevrargenics pre-investment raise based upon the value proposition and 3rd party interests that would have seen the raise occur in March/April 2020. Unfortunately, the Covid-19 pandemic has significantly impacted progression of the fund raise and, in negotiation with core investors, it was agreed to postpone until the financial situation becomes more stable. Research and business development has, however, been on-going with a Chairman having been appointed; namely, James Bromhead (https://www.linkedin.com/in/jabromhead/). A revised funding call based upon additional positive data is planned for Q1 2021. Following the several high-profile closures and numerous failures of many large pharma Alzheimer's and dementia programmes Nevrargenics aims to bring the first successful disease reversing drug for Alzheimer's to the market by 2025.

5. Sources to corroborate the impact

[E1] Patent filing history including patents.

[E2] Merck – (Sigma Millipore Europe) collaboration and engagement. Retinoid Screen Kits and components. See: https://www.sigmaaldrich.com (LightOx range) and https://www.merckmillipore.com/ (LightOx range). RSC webinar on PhotoReact-365: https://www.chemistryworld.com/webinars/illuminating-research-with-the-lightox-photoreact-365-benchtop-photoreactor/4012217.article.

[E3] Announcement of the joint venture between LightOx and the Centre for Process Innovation, the National Healthcare Photonics Centre. The identification of several new lead candidates as light activated therapeutic molecule for the treatment of pre-cancerous and early-stage oral cancer. See: https://optics.org/news/10/6/7).

[E4] LightOx Ltd: www.lightox.co.uk UK registered company 10308130, formed 2nd Aug 2016. Company House Reports provided. Company based on IPR from several DU patent families. Notch signalling for preparing a population of cells in autologous cell replacement therapy (WO2012035309-A1), Synthetic retinoids in cell killing (GB1613712.7), Fluorescent synthetic retinoids (WO2016055800).

[E5] https://www.highforceresearch.com/ UK registered company 02248615, formed 27 April 1988. Registered office address: Bowburn North Industrial Estate, Bowburn, Durham, DH6 5PF. Company reports. News of collaboration (https://contactmagazine.co.uk/hotbed-for-growth-puts-businesses-on-fast-track-to-global-success/).

[E6] Meneldor (https://meneldor.nl/) specifically managed by the founding partners Paul Lelieveld and Frans van Dalen.

[E7] Example press releases: https://www.insidermedia.com/insider/national/medilink-healthcare-business-awards-shortlist-unveiled?utm_source=southyorkshire_newsletter&utm_campaign=southyorkshire_news_tracker&utm_medium=business_article Alzheimer drug screening featured in many press releases such as 1) http://www.dailymail.co.uk/health/article-4063076/Do-Brussels-sprouts-hold-key-Alzheimer-s-pill-Festive-food-contains-nutrient-combats-dementia.html and http://www.express.co.uk/life-style/health/746739/Alzheimers-disease-dementia-brussels-sprouts-cure (All information is included in the article pack on awards and news).

[E8] November 2019 Dr David R. Chisholm "Promising technologist of the year" award at the Bionow annual awards https://bionow.co.uk/news/b5ddfadcd2cb62/diagnosis-companies-triumph-at-the-bionow-awards.

[E9] RAR-M Therapeutics Limited. Incorporated 18 December 2019. Name changed to Nevrargenics (trademarked from August 2020). UK registered company 12369627. Company House Reports provided.

[E10] MSD letter of support from Dr Fiona Marshall FMedSci, FRSC, FBPharm. VP Neuroscience and Head of UK Discovery Research for Merck Sharp & Dohme