Institution: University of Nottingham

Unit of Assessment: UOA3 (Pharmacy)

Title of case study: Commercialisation of fluorescent ligand technologies for advancing receptor pharmacology and drug screening

1. Summary of the impact

Research by the School of Pharmacy has underpinned the development of fluorescent ligand probes that have opened-up new pathways in drug discovery. These ligands have been commercialised through the formation of the spin-out company CellAura Technologies Ltd, and have been made globally available through a number of distributer agreements. Customers include pharmaceutical companies (e.g. Pfizer, AstraZeneca), drug discovery biotechs (e.g. Addex, Heptares) and drug discovery technology providers (e.g. CisBio). These ligands provide alternatives to the use of radio-ligands, giving more informative and safer solutions for industrial drug discovery. This has, for example, enabled: a new direction in G protein-coupled receptor research at Novartis Pharmaceuticals UK Ltd; validation of Promega Corporation's new drug-binding assay; and superior performance in the establishment of cell lines at inSCREENex GmbH.

2. Underpinning research

G protein-coupled receptors (GPCRs) are key drug targets in the pharmaceutical industry. They control or influence a diverse range of physiological functions and are the target for approximately 45% of all currently licensed therapeutic drugs. The ability to measure the binding of GPCR ligands and their resultant responses is therefore a key part of the drug discovery process. Conventional methods using radioactively labelled ligands (radio-ligand binding) are not suitable for the new fluorescence based high-throughput techniques being used for drug discovery. Radio-ligands also have significant health, safety and environmental implications. Fluorescent ligand technologies offer an updated approach with much improved detection limits compared to radio-ligands with the ability to determine receptor-ligand interactions at the single cell and single molecule level. This new approach encapsulates the synthesis of fluorescently tagged small molecule compounds that bind specifically and with high affinity to GPCRs, and other membrane receptors, to allow biological properties of the receptor-ligand interaction to be determined, and potential drug molecules to be identified.

Barrie Kellam (Professor of Pharmaceutical Medicinal Chemistry, University of Nottingham 1997-present) first synthesised fluorescently-tagged small molecule antagonists and agonists to the adenosine receptor in the School of Pharmacy from 2001-2006 (1,2). The first ligand synthesised was the antagonist XAC-BY630, based on the xanthine amine congener conjugated to a BODIPY630/650 fluorescent tag (fluorophore) (1). This was followed up with a series of agonists based on 5'-*N*-ethyl carboxamidoadenosine conjugated to BODIPY630/650 (2). Pharmacological validation of these ligands was carried out in collaboration with Prof Stephen Hill and Dr Stephen Briddon of the School of Biomedical Sciences. Hill and Briddon used functional assays and confocal imaging to confirm interaction of the ligands with the adenosine receptor and Fluorescence Correlation Spectroscopy (FCS) to quantify ligand binding in membrane microdomains of single living cells (1-3). Further research showed that the success of these fluorescently-tagged ligands depends greatly on the chemical linker used to conjugate the ligand to the fluorophore, and also the type of fluorophore used. Varying these parameters had a profound effect on the binding affinity and efficacy of the ligands. Factors influencing the binding affinity were evaluated between 2004 and 2006 and were shown to include the length and physicochemical properties of the linker, site of linkage, and size of attached fluorophore (3). Continuing research has led to additional GPCRs being targeted and more selective ligands for the adenosine receptor family being synthesised: ligands for the β-adrenoceptor family (therapeutic targets in cardiovascular and respiratory diseases) were synthesised and characterised in 2010 (4) and highly potent and selective ligands for the adenosine A1-receptor (involved in a broad range of signalling responses) and the adenosine A3-receptor (a therapeutic target for cancer, glaucoma and autoimmune inflammatory disorders) were synthesised and evaluated in 2011 (5,6).

Initial funding for this new research was provided by the University of Nottingham and the Wellcome Trust (7,8). The BBSRC funded work on adenosine receptor binding in 2005 (9), which was subsequently followed up with a major programme grant from the MRC in 2009 (10). Novartis

also funded a BBSRC CASE studentship in 2008 with Kellam to develop fluorescent muscarinic receptor ligands for their in house studies.

3. References to the research (School of Pharmacy researchers in bold):

Key Papers:

1. Briddon SJ, **Middleton RJ**, Cordeaux Y, Flavin FM, Weinstein JA, George MW, **Kellam B**, Hill SJ. 2004. Quantitative analysis of the formation and diffusion of A1-adenosine receptor-antagonist complexes in living cells. *Proceedings of the National Academy of Sciences (USA)* 101, 4673-4678. DOI: 10.1073/pnas.0400420101
2. **Middleton RJ**, Briddon SJ, Cordeaux Y, Yates AS, **Dale CL**, George, MW, Baker JG, Hill, SJ, **Kellam B**. 2007. New fluorescent adenosine A1-receptor agonists which allow quantification of ligand-receptor interactions in microdomains of single living cells. *Journal of Medicinal Chemistry* 50, 782-793. DOI: 10.1021/jm061279i
3. Baker JG, **Middleton R**, **Adams L**, May LT, Briddon SJ, **Kellam B**, Hill SJ. 2010. Influence of fluorophore and linker composition on the pharmacology of fluorescent adenosine A1 receptor ligands. *British Journal of Pharmacology* 159, 772–786. DOI: 10.1111/j.1476-5381.2009.00488.x
4. Baker JG, **Adams LA**, Salchow K, **Mistry SN**, **Middleton RJ**, Hill SJ, **Kellam B**. 2011. Synthesis and characterization of High-Affinity 4,4-Difluoro-4-bora-3a,4a-diaza-s-indacene-Labeled Fluorescent Ligands for Human β-Adrenoceptors. *Journal of Medicinal Chemistry* 54, 6874-6887. DOI: 10.1021/jm2008562
5. **Dale CL**, Hill SJ, **Kellam B**. 2012. New potent, short-linker BODIPY-630/650 (TM) labelled fluorescent adenosine receptor agonists. *MedChemComm* 3, 333–338. DOI: 10.1039/c2md00247g
6. **Vernall AJ**, Stoddart LA, Briddon SJ, Hill SJ, **Kellam B**. 2012. Highly Potent and Selective Fluorescent Antagonists of the Human Adenosine A3 Receptor Based on the 1,2,4-Triazolo[4,3-a]quinoxalin-1-one Scaffold. *Journal of Medicinal Chemistry* 55, 1771–1782. DOI: 10.1021/jm201722y

Grant Funding:

7. 1999-2002. Wellcome Trust Joint Infrastructure Fund: Fluorescent measurement of cell signalling and single molecular interactions in human cells in health and disease. Hill SJ, Williams P, **Bycroft B**, Kendall DA, Hall IP, **Kellam B**, **Chan W**. £881,988
8. 2002-2005. Wellcome Trust (066817/Z02/Z): The pharmacological characteristics of the human A1-receptor at the single molecular level. Hill SJ, **Kellam B**. £226,317
9. 2005-2008. BBSRC (BB/0521581): Adenosine A1-receptor binding and signalling in membrane microdomains of single living cells. Hill SJ, Briddon SJ, **Kellam B**. £295,119
10. 2009-2014. MRC programme grant (G0800006): Use of fluorescence correlation spectroscopy to study the adenosine A3-receptor in microdomains of single living cells. Hill SJ, Briddon SJ, **Kellam B**. £1,313,190

Key Patent Families:

11. **Kellam B**, **Middleton RJ**, George MW, Hill SJ. Library having several tagged non-peptide ligands or their salts, useful for assessing pharmacological properties of ligand, comprising ligand moieties linked to tag moieties through linker moieties. WO2004088312 (priority date 02/04/2003)
12. Hill SJ; Briddon SJ, **Kellam B**. Improvements in High Content Screening. WO2006032926 (priority date 24/09/04)

4. Details of the impact

Fluorescent ligand technologies developed at the University of Nottingham have been commercialised through the spin-out company CellAura. Distributer agreements have made these ligands globally available, enabling industry to gain new insights into receptor ligand interactions. In addition, the use of fluorescent ligands allows for faster, more cost-effective screening studies that negate the need for radio-labelling, making ligand binding studies safer and reducing the associated environmental impact.

The initial publication in the Proceedings of the National Academy of Sciences (1) described for the first time a pharmacologically-validated fluorescent ligand for a GPCR and the application of FCS to the study of non-peptide GPCR ligands in membrane micro-domains of single living cells. This work and the patents (11,12) that were filed before this publication formed the basis for the establishment of the spin-out company CellAura Technologies Ltd in 2004. CellAura, based at Nottingham BioCity, began trading in 2006 and has subsequently received investment funds of £1.8M (a). Since 2008, CellAura have maintained a workforce up to 7 FT and 4 PT staff (a).

CellAura currently market 51 products (17 validated ligands and 34 development ligands, www.cellaura.com). These products are underpinned by the novel ligand development process patented by the University of Nottingham (11,12), which has been exclusively licenced to CellAura since 2006. In some cases, ligands have been developed in collaboration with the University (4,6) via a pipeline agreement signed in 2008 (a). The unique selling point of these products is that they are pharmacologically validated and provide applications not previously available by conventional methods - they can be used on primary cells (including from diseased tissue), on single living cells, in real time and in the native environment – providing new information on binding kinetics and receptor distribution, internalisation and signalling. They can also be used to perform traditional receptor-ligand binding studies at comparable cost to using radio-ligands without the need for associated radioactive licences, infrastructure and safety monitoring, making these studies more amenable to smaller research companies for whom it is not practicable to have their own radiation facilities.

To facilitate access to CellAura's products, on-going regional distributor agreements were arranged with Fisher Scientific (UK and Scandinavia) and Funakoshi (Japan) in 2009, and worldwide agreements with Abcam and Sigma-Aldrich in 2011 (a). CellAura also make direct-to-customer sales in Europe, North America and Australasia, and provide custom ligand development contracts. Their customers include the major pharmaceutical companies AstraZeneca, Pfizer, Sanofi Aventis, Amgen and Takeda, and GPCR biotechnology companies such as Addex and Heptares (a). Novartis Pharmaceuticals UK Ltd has used CellAura ligands to understand the mechanism of 'tethered' drugs which has led to a new direction in their research into GPCR drugs. The Global Head of Respiratory at Novartis confirmed the benefits to the company "*Novartis' studies with fluorescent derivatives of therapeutic compounds have led to the ground-breaking concept of tethered drugs which Novartis has applied to better understand the pharmacological mechanism of drug compounds and to direct new research on longer acting drugs targeting GPCRs. It is therefore an enabling technology that brings benefits to the pharmaceutical industry by allowing the pursuit of approaches that were previously unavailable*". (b)

CellAura ligands are also utilised by drug discovery technology providers. Between 2009 and 2012, CellAura developed a number of custom fluorescent ligands for CisBio Bioassays, a French-based global drug discovery assay development company. CisBio adopted 18 'active' ligands (prepared exclusively for CisBio and not available via the CellAura catalogue) for use in their proprietary Tag-lite™ GPCR high throughput screening (HTS) platform (a,c). Promega Corporation, a US-based market leader in developing and commercialising novel reporter technologies and assay chemistries for interrogating GPCR signalling, has been using CellAura ligands since 2011 to successfully validate their innovative NanoLuc® Luciferase Technology. The combined use of this superior luciferase technology along with high affinity fluorescent ligands has also enabled GPCR-ligand interactions to be studied via Bioluminescence Resonance Energy Transfer (BRET; d). Promega state that "*The fluorescent ligands developed by CellAura have proven to be excellent tracers for measuring the affinities of various GPCR drugs in the competitive displacement format, and have helped to validate NanoLuc as an excellent reporter tag for ligand binding studies using BRET*. *Data presented to customers in the pharma industry in 2012 and 2013 have spurred considerable interest in NanoLuc/BRET for general target engagement assays*". (d)

Since 2008, CellAura have demonstrated that their technology works on a range of functional screening platforms used in the pharmaceutical industry, including high content analysis (HCA) imaging platforms. Demonstration results have been disseminated via poster presentations at various scientific meetings (e) and used in marketing materials. CellAura was voted 'best new

technology' at the European Laboratory Robotics Interest Group (ELRIG)/ SBS Drug Discovery meeting in 2008. Fluorescence techniques such as HCA rely on high resolution fluorescence image capture meaning that these techniques are not as fast as traditional methods for receptor ligand binding, and are in general being used to provide additional information alongside rather than replacing radio-ligands in HTS. In 2012 CellAura established a successful collaboration with BMG Labtech (a German-based global developer and manufacturer of microplate readers) that enables CellAura's ligands to be utilised on a conventional (non-imaging) PheraStar fluorescence plate reader. Ligand binding assays analogous to radio-ligand studies can be performed on the PheraStar with inherent safety advantages. The PheraStar also has time saving advantages over HCA (all inclusive 96 well plate assay time of <10 minutes, compared to approx. 1 hour) and gives comparable binding data, making this technique more amenable for HTS. Technical notes outlining this technique are made available via both the CellAura and BMG Labtech websites (f). In addition, a PheraStar plate reader costs much less than a HCA imaging platform (approximately £70K compared to £450K (i)). Together, these fluorescent ligand binding techniques provide improved methods for investigating ligand interactions with GPCRs (discussed by Comley in the industrial publication, *Drug Discovery World* Spring 2009, 32-50) and time and cost benefits (particularly when using specific screening platforms) for drug discovery, important factors when promoting new technology to a pharmaceutical industry that has undergone major realignment in recent years.

In addition to the use of fluorescent ligands in drug discovery, CellAura have diversified the impact of the technology to include live cell sorting. This new patented technology (g), filed by CellAura in 2007, describes the novel use of fluorescent ligands as alternatives to antibodies for fluorescence activated cell sorting. The method described can be used to easily sort cells expressing specific receptors when generating recombinant clonal cell lines. CellAura ligands have been used by the German SME inSCREENex GmbH since 2009 to establish cell lines for drug discovery. Their Managing Director confirmed the benefits of using CellAura ligands "*The performance [of the ligands] was great and superior to any of the antibodies we used in parallel. By using the ligands we were able to reduce the time to analyse our cell lines significantly. Further, the application of the ligands allowed us to screen a high number of novel cell lines for optimal expression. This is not possible with conventional methods like Western Blotting*." (h)

In summary, commercialisation of fluorescent ligands through CellAura has opened up new and improved methods for industrial drug discovery, and a number of distributer agreements have made these ligands globally available.

5. Sources to corroborate the impact

a. Supporting evidence provided by CEO, CellAura Technologies (on file)
b. Corroborative Statement from Global Head of Respiratory, Novartis (on file)
c. Zwier *et al*. 2010. *J. Biomol. Screen.* 15(10), 1248-1259. DOI: 10.1177/1087057110384611 (also on file)
d. Corroborative Statement from Senior Research Scientist, Promega Corporation (on file)
e. Technology Platform evaluation posters are available from the CellAura website, URL: www.cellaura.com/resources/index.html#posters (Accessed 20/08/13 – also on file).
f. Technical notes describing the use of CellAura ligands for GPCR binding studies on a PheraStar plate reader can be found on the CellAura website, URL: http://www.cellaura.com/resources/index.html#application-notes (Accessed 20/08/13) and the BMG Labtech website, URL: www.bmglabtech.com/application-notes/fluorescence-intensity/gpcr-cellaura-pherastar-fs-227.cfm (Accessed 20/08/13) (also on file).
g. Hill SJ, Kellam B, Middleton RJ. Method for generating a recombinant clonal cell line and novel reagents for use in the method. WO2009040555 (priority date28/09/07).
h. Corroborative Statement from Managing Director, InScreenEx (on file) and publication: Schucht *et al*. 2011. *J. Biomol. Screen*. 16(3), 323-331. DOI: 10.1177/1087057110396371 (also on file).
i. Equipment quotations for a fluorescence plate reader and a HCA Platform (on file)