Institution: King's College London

Unit of Assessment: UoA5

Title of case study: Developing drugs targeting neuroregeneration in stroke

1. Summary of the impact

Neurons in the central nervous system do not normally regenerate following injury, due in part to the presence of 'inhibitory' molecules that actively prevent the growth and/or collateral sprouting of axons. King's College London scientists identified myelin associated glycoprotein (MAG) as the first myelin inhibitory molecule and demonstrated that inhibition of MAG function with a monoclonal antibody promotes axonal regeneration. They have gone on to promote MAG and its receptor (called the NgR1) as druggable therapeutic targets. Their discovery has led the UK's largest pharmaceutical company – GlaxoSmithKline – to develop monoclonal antibodies to MAG and a second myelin inhibitor as clinical drug candidates. The anti-MAG therapeutic successfully completed Phase I and II clinical trials in humans for stroke during 2008-2013.

2. Underpinning research

Stroke is a leading cause of disability with around 90% of patients living with major loss of neurological function and decreased quality of life. Current front-line treatment involves the use of thrombolytic drugs; however they are only effective if administered within an hour or two following the stroke and the effects are not compelling. Research has been focussed on traditional small molecule drug programs, largely based on a neuroprotective strategy targeted at the acute phase of stroke, however, despite considerable effort and expenditure all programs to date have failed despite significant effort and funding resulting in most major companies reducing their efforts in this area. A 'holy-grail' for stroke patients would be the development of restorative therapies that can be administered days, if not weeks, after the insult but as yet no such therapy has been approved for clinical use.

Work on therapies that can promote axonal growth and sprouting has been carried out at King's College London (KCL) by Prof Frank Walsh (1990-present, Staff scientist then Visiting Professor, 1997-present), Prof Patrick Doherty (1990-present, Head of the Wolfson Centre for Age-Related Diseases), Dr Emma Williams (1994-present, Senior Research Fellow) and Dr Gareth Williams (1997-present, Wolfson Bioinformatics Lead).

Neurons in the central nervous system (CNS) do not normally regenerate following injury such as that seen after a stroke. This is due in part to the presence of 'inhibitory' molecules that actively prevent the growth and/or collateral sprouting of axons. At least some of these inhibitory molecules were thought to arise from CNS myelin, produced by oligodendrocytes, however the nature and action of such molecules was not known. KCL scientists developed assays to investigate the neurite outgrowth response from postnatal rat cerebellar neurons cultured on monolayers of immortalised fibroblast cells that they genetically engineered to express a number of different adhesion molecules (Williams EJ, et al. Neuron, 1994). In collaboration with colleagues from Hunter College in New York, USA, KCL scientists used these assays to test the function of the oligodendrocyte cell surface protein myelin-associated glycoprotein (MAG). They demonstrated that MAG strongly inhibits neurite regeneration from the postnatal cerebellar neurons and extended this to show MAG also inhibited neurite outgrowth responses from adult dorsal root ganglion neurons. They also showed that an anti-MAG antibody could promote a robust regenerative response of neurites, based on the antibody's ability to inhibit MAG function (Mukhopadhyay G, et al. Neuron, 1994).

KCL scientists and Hunter College colleagues went on to reinforce the view that MAG is an important therapeutic target with the demonstration that soluble MAG extracted from an *in vivo* source also inhibits *in vitro* regeneration of neurites from postnatal cerebellar neurons (Tang S, et al. Mol Cell Neurosci, 1997). Importantly, work done at KCL demonstrated that soluble MAG could prevent neurons from regenerating their axons on a wide variety of cellular substrates as well as over surfaces coated with a number of different matrix molecules. They also confirmed that an antibody to MAG inhibits the activity of soluble MAG.

Recognition of the contribution of the KCL scientists made to the identification of MAG as a therapeutic target is reflected in a long-standing KCL/industry collaboration that has resulted in a number of additional key findings. For example, in work sponsored by Wyeth Pharma, KCL scientists developed a small peptide agonist to the TrkB neurotrophin receptor and showed that this peptide can overcome MAG inhibition by activating the TrkB receptor (Williams G et al. J Biol Chem, 2005). In addition, KCL scientists used a structural bioinformatics approach to identify the first functional binding loop sequence on the MAG receptor (NgR1). Based on this, they designed a series of constrained cyclic peptides that can act as MAG antagonists by mimicking this NgR1 sequence and demonstrated that they promote a regenerative response by inhibiting MAG function (Williams G, et al. J Biol Chem 2008). Patent protection, with the KCL scientists named as the sole inventors, was granted for the TrkB agonist peptides (WO 2005/025514 A2) and as joint inventors for the NgR1 antagonist peptides (WO2008006103). Based on the NgR1 peptide work, KCL scientists also identified a "druggable" pocket on the NgR1 and, in collaboration with Wyeth, tested ~130 compounds identified in a Wyeth-led, KCL-run virtual screen against the pocket. This has led to the identification and patent protection for several small molecules that act as direct NgR1 antagonists (WO2008006103).

3. References to the research

Mukhopadhyay G, Doherty P, Walsh S, Crocker PR, Filbin MT. A novel role for myelin-associated glycoprotein as an inhibitor of axonal regeneration. Neuron 1994;13:757-67. Doi:10.1016/0896-6273(94)90042-6 (674 Scopus citations)

Tang S, Woodhall RW, Shen YJ, DeBellard ME, Saffell JL, Doherty P, Walsh FS, Filbin MT. Soluble myelin-associated glycoprotein (MAG) found in vivo inhibits axonal regeneration. Mol Cell Neurosci 1997;9:333-46. Doi: http://dx.doi.org/10.1006/mcne.1997.0633 (83 Scopus citations)

Williams EJ, Furness J, Walsh FS, Doherty P. Activation of the FGF receptor underlies neurite outgrowth stimulated by L1, NCAM and N-cadherin. Neuron 1994;13:583-94. Doi:10.1016/0896-6273(94)90027-2 (429 Scopus citations)

Williams G, Williams E-J, Maison P, Pangalos MN, Walsh FS, Doherty P. Overcoming the inhibitors of myelin with a novel neurotrophin strategy. J Biol Chem 2005;280:5862-69. Doi: 10.1074/jbc.M411121200 (21 Scopus citations)

Williams G, Wood A, Williams EJ, Gao Y, Mercado ML, Katz A, Joseph-McCarthy D, Bates B, Ling HP, Aulabaugh A, Zaccardi J, Xie Y, Pangalos MN, Walsh FS, Doherty P. Ganglioside inhibition of neurite outgrowth requires Nogo receptor function: identification of interaction sites and development of novel antagonists. J Biol Chem 2008;283:16641-52. Doi: 10.1074/jbc.M802067200 (20 Scopus citations)

Examples of Grant support

- 2001-2003. Design of novel agonists and antagonists for stimulation of neuronal repair. GlaxoSmithKline, £350,000. Principle Investigator: P Doherty.
- 2003-2005. TrkB agonist and antagonist collaboration. Wyeth Research, £176,471. Principle applicant: P Doherty
- 2004- 2007. Overcoming the inhibitors of myelin. Wyeth Research, £466,176. Principle applicant: Doherty

Patent (Applicants Wyeth and KCL)

- WO2005/025514 (A2). Compounds that modulate neuronal growth cones and their uses (inventors Doherty P and Williams G). Publication date: 25.3.2005: http://www.google.com/patents/WO2005025514A2
- WO2008006103 (A2). Nogo receptor functional motifs, peptide mimetics, and mutated functional motifs related thereto, and methods of using the same (inventors Wood, Katz, Gao, Bates Doherty and Williams). Publication date: 20.3.2008: https://www.google.com/patents/WO2008006103A3?cl=en&dq=%E2%80%A2%09WO2008006103&hl=en&sa=X&ei=szRxUuHvGI_g7QaD_oCQAQ&ved=0CDkQ6AEwAA

4. Details of the impact

Research by Profs Walsh and Doherty at King's College London (KCL), in collaboration with Hunter College, New York, USA, provided identification of the first inhibitory molecule on myelin and a proof-of-principle that an anti-MAG antibody might have therapeutic potential as a biopharmaceutical based on its ability to promote a regenerative response. Walsh moved from KCL in 1997 (while maintaining a visiting Professorship) to become head of Neuroscience drug discovery at SmithKline Beecham, now GlaxoSmithKline (GSK), then went on to Wyeth (2002-2007). Doherty remained at KCL, but served on the GSK Neurology-CEDD (1997-2002) and then the Wyeth scientific advisory boards to advise and collaborate on the MAG and related programs (2002-2007).

The above discovery has led to a long-standing KCL/industry partnership that has resulted in over £2.5M in grants being awarded to Prof Doherty from GSK and Wyeth to pursue MAG and related molecules (e.g. the NgR1) as therapeutic targets for regenerative medicine. This has generated a pipeline of compounds that range from anti-MAG monoclonal antibodies, biologically active peptides derived from NgR1 and small molecules that bind to a functional pocket on the NgR1 receptor. KCL scientists have identified and developed most of these and shown that they can all function as MAG antagonists and thereby promote axonal growth in an inhibitory environment.

As a direct consequence of the research carried out by KCL, Walsh initiated a clinical development program to "humanise" a MAG antibody, as a novel first-in-class biopharmaceutical for stroke, and an anti-Nogo antibody, as a first-in-class biopharmaceutical for amyotrophic lateral sclerosis (ALS) when he took up his position at GSK. This was very significant as it was the first time that a biopharmaceutical program had been undertaken within the Neurology group at GSK and very much reflected a "sea-change" in their approach to drug discovery that until that point had been based on the traditional small molecule approach. The KCL pre-clinical work assisted greatly in staff scientists in GSK being able to make the case for a paradigm shift and move to biological approaches in areas where small molecule approaches predominated and as such could be regarded as transformational.

To date GSK has invested at least $33M in the pre-clinical and clinical work. The furthest forward program for the clinic is the development of a humanised monoclonal antibody (GSK249320) against MAG aimed at the enhancement of recovery of function poststroke. A 'first in man' Phase I study involving 48 healthy subjects was conducted between 2007 and 2009 and results showed the biopharmaceutical to be safe to use in man and the justification for the clinical study was directly linked to the KCL work (1a-d). A Phase IIa study between July 2009 and Jan 2011 in patients with stroke was then conducted building on the positive Phase I data (2a,b). The study reported that "while not powered to demonstrate efficacy, gait velocity data from MAG111539 suggest a trend toward benefit with GSK249320 treatment which warrants further exploration" (2c). This project is currently in the GSK portfolio of clinical drugs and should proceed further very soon. It should be noted that the vast majority of drugs that are tested for efficacy in animals models do not make it into human clinical trials as they need to pass a very rigorous evaluation process that scrutinises all aspects of the drug including the logic underpinning it's development, such as the results obtained in animal models of disease, the manufacturing conditions and the design of the clinical trial. It is estimated that only around one in a thousand compounds discovered in the pre-clinical stage finally gain FDA approval (3).

A program to develop humanised antibodies to block the function of a second myelin inhibitory molecule called Nogo-A was also initiated by GSK based on the KCL work on MAG. This has led to the development of an antibody to NOGO A called Ozanezumab/GSK1223249. Three Phase I trials in patients with multiple sclerosis and ALS have been successfully completed between 2009-2011 (4a-c). Phase II trials are currently being undertaken with the Motor Neurone Disease Association and the ALS Therapy Institute, a nonprofit biotechnology organization dedicated to developing effective treatments for ALS. They have highlighted Ozanezumab in a news report and podcast interview of one of the clinicians involved in the study (4d). Importantly, the anti-MAG and anti-Nogo-A monoclonal antibodies are listed as assets in the current GSK pipeline at Phase II with

indications of stroke and ALS respectively. There are no other NOGO compounds listed as being developed for these devastating conditions. To date GSK has invested up to $28M on this project in pre clinical and clinical studies based on the KCL work (5).

5. Sources to corroborate the impact

1. First clinical trial on anti-MAG therapy

a. Anti-MAG First Administration to Human: http://clinicaltrials.gov/ct2/show/NCT00622609?term=GSK249320&rank=1
b. Study Report: http://www.gsk-clinicalstudyregister.com/result_detail.jsp?protocolId=MAG103114&studyId=E9C77BA1-C903-4996-879E-4C99172A1FE6&compound=GSK249320
c. Abila B, Cunningham E, Simeoni M (2013). First-time-in-human study with GSK249320, a myelin-associated glycoprotein inhibitor, in healthy volunteers. Clin Pharmacol Ther 2013;93:163-69. Doi: 10.1038/clpt.2012.227
d. Thompson HJ, Marklund N, LeBold DG, Morales DM, Keck CA, Vinson M, Royo NC, Grundy R, McIntosh TK. Tissue sparing and functional recovery following experimental traumatic brain injury is provided by treatment with an anti-myelin-associated glycoprotein antibody. Eur J Neurosci 2006;24(11):3063-72. Doi: 10.1111/j.1460-9568.2006.05197.x

2. Phase IIa trial on anti-MAG therapy

a. Safety Escalating Repeat IV, in Stroke Patients (MAG111539): http://clinicaltrials.gov/ct2/show/NCT00833989?term=GSK249320&rank=3
b. Study Report: http://www.gsk-clinicalstudyregister.com/result_detail.jsp?protocolId=111539&studyId=C17374B6-C24E-413E-8568-59858CF9BE3D&compound=GSK249320
c. Cramer SC, Abila B, Scott NE, Simeoni M, Enney LA; on behalf of the MAG111539 Study Investigators. Safety, pharmacokinetics, and pharmacodynamics of escalating repeat doses of GSK249320 in patients with stroke. Stroke 2013;44(5):1337-42. http://stroke.ahajournals.org/content/early/2013/03/07/STROKEAHA.111.674366

3. Fierce Biotech report on drug development:
http://www.fiercebiotech.com/topics/fda_approval_process.asp

4. Phase I anti-Nogo studies

a. First Time in Human Study of GSK1223249 in Amyotrophic Lateral Sclerosis:
   - Trial: http://clinicaltrials.gov/ct2/show/NCT00875446?term=GSK1223249&rank=1
   - Study Report: http://www.gsk-clinicalstudyregister.com/result_detail.jsp?protocolId=111330&studyId=6B53306A-9CFF-42EE-987C-B5D9F45BA40B&compound=GSK1223249
b. NOGO-A in Multiple Sclerosis FTIH
   - Trial: http://clinicaltrials.gov/ct2/show/NCT01424423?term=GSK1223249&rank=2
   - Study Report 1: http://www.gsk-clinicalstudyregister.com/result_detail.jsp?protocolId=112988&studyId=02EEC5BE-1F88-4808-BEB8-B4E7730088D9&compound=GSK1223249
   - Study Report 2: http://www.gsk-clinicalstudyregister.com/result_detail.jsp?protocolId=114840&studyId=008EEB15-83CB-491B-90FC-BDF2755BB100&compound=GSK1223249
c. Study of Ozanezumab (GSK1223249) Versus Placebo in the Treatment of Amyotrophic Lateral Sclerosis: http://clinicaltrials.gov/ct2/show/NCT01753076?term=GSK1223249&rank=4
d. ALS Therapy Institute: http://blogs.als.net/post/A-Go-For-anti-NOGO-A.aspx

5. Current GSK pipeline: http://www.gsk.com/research/our-product-pipeline.html