Institution: University of Oxford		
Unit of Assessment: 4 - Psychology Psychiatry and Neuroscience		
Title of case study: Optimising diagnosis of treatable autoimmune disorders of the central nervous system		
Period when the underpinning research was undertaken: 2008-2019		
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:**
Sarosh R Irani	Associate Professor & Head of Oxford Autoimmune Neurology Group (OANG)	11/2014 - present
Patrick Waters	Co-Director of OANG	04/2002 - present
Angela Vincent	Professor of Neuroimmunology	03/1998 - 03/2016
Bethan Lang	Associate Professor	08/1988 - 01/2017
Period when the claimed impact occurred: 1/08/2013 - 31/07/2020		
Is this case study continued from a case study submitted in 2014? N		

1. Summary of the impact

Specific autoantibodies cause severe central nervous system (CNS) diseases including neuromyelitis optica and encephalitis. Although rare, these conditions can lead to permanent disability but are treatable with immunotherapies after an accurate diagnosis of the autoantibodies. Hence, these are widely considered “not-to-miss" neurological and psychiatric conditions. Research by the University of Oxford Autoimmune Neurology Group (OANG) has identified four new autoantibody targets and developed and refined diagnostic tests which have become the gold standard. The OANG now performs about 20,000 tests annually for UK patient care. Many of these have been evaluated in large multinational studies, leading to changes in laboratory practices across multiple international testing centres. This greater diagnostic accuracy has improved the worldwide clinical identification of patients who respond to immunotherapies and prevented others from receiving inappropriate, potentially harmful, immunotherapies. Research by OANG also identified clinical features that facilitate early diagnosis and treatment, enabling prompt deployment of immunotherapies that lead to improved outcomes, and major industry investments in clinical trials.

2. Underpinning research

Autoantibodies produced by the immune system can erroneously target host cells, leading to autoimmune diseases. Autoantibodies targeting the nervous system can cause conditions such as neuromyelitis optica (NMO) and encephalitis. Patients with these conditions can respond well to immunotherapies, but only after accurate diagnosis. Researchers in the OANG have identified new autoantibody targets and pioneered the use of live cell-based assays in order to develop highly specific diagnostics for these conditions. These diagnostic autoantibody tests, and OANG’s discovery of clinical features by which these patients can be identified, have facilitated early recognition and diagnosis, prevented inappropriate use of potentially toxic immunotherapies, and enabled targeted recruitment of patients to clinical trials.

Discovery of clinically valuable autoantibodies: Between 2008 - 2015, the Oxford team identified new autoantibody targets (LGI1, CASPR2, and the glycine receptor) which showed clinical utility in diagnosing patients with immunotherapy-responsive CNS diseases in which patients develop memory loss, seizures and psychiatric illness. As an example of this work, in 2010 OANG discovered that autoantibodies in patients with neuromyotonia, Morvan’s syndrome or encephalitis, previously thought to bind the voltage gated potassium channel (VGKC), often bind two other proteins: LGI1 or CASPR2 [1]. The research showed that the autoantibodies against LGI1 and CASPR2 are associated with distinctive, well-defined syndromes, with diagnostic and prognostic value, and these patients almost universally responded to immunotherapies. In contrast, they also showed that patients with autoantibodies against the VGKC complex itself showed no distinct phenotypes and showed no clear response to

immunotherapies [2]. Separately, OANG used a candidate antigen approach to identify the α1 subunit of the glycine receptor as a new autoantibody target in patients with progressive encephalomyelitis with rigidity and myoclonus and stiff person/limb syndrome [3].

Development of accurate diagnostics: Conventional laboratory tests use denatured or chemically modified autoantibody targets, or present intracellular components of autoantibody targets that are inaccessible to circulating autoantibodies *in vivo*, that may lead to poor test accuracy. To exclusively detect binding to the native extracellular domains of proteins, the OANG researchers developed live-cell-based systems which present native surface targets to patient antibodies, to mimic what the circulating patient antibodies 'see' *in vivo*.

Waters developed a highly specific live cell-based assay for aquaporin-4 antibodies, which cause neuromyelitis optica [4]. This test greatly improved accuracy by presenting the native, surface-accessible aspects of aquaporin-4 to patient autoantibodies. In direct comparisons with assays in routine use at the Mayo Clinic, USA, the OANG live cell-based assay improved sensitivity of aquaporin-4 autoantibody detection by 50%. The system proved to be superior to any available commercial tests, including a newly developed commercial ELISA technique [4].

Autoantibodies to myelin oligodendrocyte glycoprotein (MOG) have traditionally shown limited syndrome specificity and were described in patients with multiple sclerosis, neuromyelitis optica and other conditions, all of which respond to different treatments. The OANG researchers developed and refined a live-cell-based assay for MOG antibodies to specifically detect IgG1 autoantibodies targeting the extracellular domain of natively-expressed MOG. This optimised test was proven to detect individuals at disease onset with an inflammatory demyelinating disease that is distinct from multiple sclerosis. In international multicentre studies led by the Oxford team, the test demonstrated diagnostic superiority to commercial tests and those routinely performed at the Mayo Clinic [5]. The research found that a positive result in the optimised assay rules out a diagnosis of multiple sclerosis, an observation subsequently supported by numerous publications.

OANG also advanced these concepts with LGI1 and VGKC antibodies [1, 2, 6]. As LGI1 is a secreted protein, OANG researchers developed the novel concept of tethering LGI1 to a membrane domain, for display on the live cell surface [1]. Further, development of live cell-based assays demonstrated that the patients with VGKC antibodies, but without reactivity to native LGI1 or CASPR2, neither benefited from treatment with immunotherapies nor had an immune mediated illness. These findings encouraged a widespread international move away from VGKC testing to avoid exposure of patients to unnecessary immunotherapies.

Recognition of disease specific clinical features which themselves support early treatment: Accurate diagnostic tests can correctly identify patients with a single disease that benefits from a specific treatment. Using OANG's LGI1-autoantibody assay, Irani and colleagues identified a new type of seizure, faciobrachial dystonic seizures' (FBDS), that is pathognomonic for the presence of LGI1-antibodies. Irani directed an international multicentre study of 103 patients with FBDS and LGI1-antibodies, showing that frequently used anti-seizure medications have limited clinical efficacy, whereas 90% of patients respond well and rapidly to immunotherapies [6]. In addition, early treatment of FBDS with immunotherapy was found to prevent development of cognitive impairment. OANG's LGI1 test validated FBDS as a pathognomonic clinical feature that permits early diagnosis and treatment.

3. References to the research (bold denotes Oxford researcher at the time of the study; citations from Google Scholar on 16/12/2020)

1. Irani SR, Alexander S, **Waters P**, Kleopa KA, Pettingill P, Zuliani L, Peles E, **Buckley C**, **Lang B**, **Vincent A**. (2010). Antibodies to Kv1 potassium channel-complex proteins leucine-rich, glioma inactivated 1 protein and contactin-associated protein-2 in limbic encephalitis, Morvan's syndrome and acquired neuromyotonia. *Brain* 133(9):2734-48. DOI: 10.1093/brain/awq213 (1096 citations)

2. **Lang B**, **Makuch M**, **Moloney T**, Dettmann I, Mindorf S, Probst C, Stoecker W, **Buckley C**, Newton CR, **Leite MI**, Maddison P, Komorowski L, Adcock J, **Vincent A**, **Waters P**, **Irani SR**.

(2017). Intracellular and non-neuronal targets of voltage-gated potassium channel complex antibodies. J *Neurol Neurosurg Psychiatry*. 88(4):353-361. DOI: 10.1136/jnnp-2016-314758 (76 citations)

3. Hutchinson M, **Waters P**, McHugh J, Gorman G, O' Riordan S, Connolly S, Hager H, Yu P, Becker CM, **Vincent A**. (2008). Progressive encephalomyelitis, rigidity, and myoclonus: a novel glycine receptor antibody. *Neurology*. 71(16):1291-2. DOI: 10.1212/WNL.0b013e318227b176 (283 citations)

4. **Waters PJ**, McKeon A, **Leite MI**, Rajasekharan S, Lennon VA, Villalobos A, Palace J, Mandrekar JN, **Vincent A**, Bar-Or A, Pittock SJ. (2012). Serologic diagnosis of NMO: a multicenter comparison of aquaporin-4-IgG assays. *Neurology* 78(9):665-71. DOI: 10.1212/WNL.0b013e318248dec1 (419 citations)

5. **Waters P**, **Woodhall M**, O'Connor KC, Reindl M, **Lang B**, Sato DK, Juryńczyk M, Tackley G, Rocha J, Takahashi T, Misu T, Nakashima I, Palace J, Fujihara K, **Leite MI, Vincent A**. (2015). MOG cell-based assay detects non-MS patients with inflammatory neurologic disease. *Neurol Neuroimmunol Neuroinflamm*. 2(3):e89. DOI: 10.1212/NXI.0000000000000089 (277 citations)

6. Thompson J, Bi M, Murchison AG, **Makuch M,** Bien CG, Chu K, Farooque P, Gelfand JM, Geschwind MD, Hirsch LJ, Somerville E, **Lang B, Vincent A, Leite MI, Waters P, Irani SR;** Faciobrachial Dystonic Seizures Study Group. (2018). The importance of early immunotherapy in patients with faciobrachial dystonic seizures. *Brain*. 141(2):348-356. DOI: 10.1093/brain/awx323 (108 citations)

Funding included a Wellcome Trust Intermediate Clinical Fellowship to Irani at the University of Oxford, 'The immunobiology of autoantibody-mediated diseases of the central nervous system', GBP884,681 (104079/Z/14/Z, Nov 2014 – Feb 2021).

4. Details of the impact

1. Patient Benefit

a. Widespread adoption of OANG tests with improved diagnostic accuracy:

In 2014, OANG tested 8,944 samples for autoantibodies against 8 targets. Of these, more than 95% were UK patients. By 2019, the OANG testing had expanded to 24,552 samples on 12 targets, including approximately 10,000 for the Nationally Commissioned Service for patients with neuromyelitis optica (NMO) which focuses on aquaporin-4 antibodies [A], and is the only UK provider of this optimised live-cell based assay. OANG's test identifies 80 new UK patients/year with aquaporin-4 antibodies. Given that aquaporin-4 antibodies have an overall incidence of 1.3/million person-years (equivalent of 85 new cases/year in the UK), these accurate tests are capturing the whole of the relevant UK patient population. In addition, this aquaporin-4 antibody test is run on 30,000 samples/year at the Mayo Clinic (USA), covering about 60% of USA test requests [B].

OANG's assay to detect MOG antibodies is the most specific worldwide in comparison to four international testing centres with an interest in MOG antibody testing [5]. OANG is the only provider of MOG-antibody IgG1 testing in the UK. UK test requests for MOG autoantibodies have increased by 61%/year over the last 5 years and this test received statutory approval in the USA in 2018. It is now run on 25,000 samples/year, approximately 55% of USA requests [B].

"*The Oxford Autoimmune Neurology Diagnostic Laboratory has had a significant impact, not only on the field of autoimmune neurology but also on the development and optimization of platforms for neural antibody testing, many of which have been incorporated into the current Mayo Clinic Neuroimmunology Laboratory testing platforms*."

Director of the Neuroimmunology Laboratory, Mayo Clinic. [B]

Since the OANG's original report in 2008, a 2018 literature review identified descriptions of 186 patients with glycine receptor antibodies who were almost universally responsive to immunotherapies. Although rare, the consistent clinical findings and response to immunotherapy in patients from multiple countries has led to the establishment of glycine receptor antibody testing in major testing centres in Germany (Jan 2019, [Ci]), and the USA (Aug 2020; [A], [Cii]).

b. More accurate diagnosis to improve treatments and avoid harm:
These autoantibody findings dramatically alter patient care. Three clinically similar diseases: multiple sclerosis, aquaporin-4 antibody and MOG antibody disorders all respond to different therapies and require different durations of administration. Patients with MOG-antibodies typically require short-term immunotherapies. Patients with aquaporin-4 antibodies have lifelong disease relapses which respond to long-term immunotherapies but worsen with treatments that are effective in multiple sclerosis (MS). In particular, misdiagnoses of NMO patients as MS means many patients suffer preventable disability or death and can respond to costly MS treatments such as interferons with catastrophic worsening [D]. OANG's improved live cell-based assay for aquaporin-4 antibody detection has led to 50% more patients identified with NMO in the USA each year - this equates to 1,658 extra patients captured between 2014 and 2020 using live cell-based assays [4; E].

Importantly for patient benefit, the time to diagnosis was reduced (1 versus 18 months). In 2019, class I evidence led to FDA approval for three effective immunotherapies which treat NMO, highlighting the impact of the new diagnostic criteria. A neurology clinician at the Massachusetts General Hospital summarised the benefits to patients being that, "*without the MOG assay, these patients would be left without a diagnosis or treatment, and would have a poor prognosis*", including hundreds of his patients alone [F(i)].

OANG's work has revealed that of all patients with VGKC antibodies, only those with either LGI1- or CASPR2-antibodies benefit from immunotherapies [1, 3, 6], and that treating the remaining 80-95% with VGKC-antibodies is harmful, or at least non-beneficial [G]. Consequently, VGKC testing was ceased by the Mayo Clinic in 2019 [H] and replaced by the OANG tests for LGI1 and CASPR2 antibodies, now performed on approximately 50,000 samples per year [B]. Many other centres worldwide have followed suit, including the Oxford NHS Trust immunology laboratory in 2020 [H]. Hence, the approximately 30,000 and approximately 100,000 VGKC-antibody tests performed in the UK and USA, respectively, are now preferentially tested for OANG's discovery of LGI1/CASPR2 antibodies. The new tests help avoid unnecessary immunotherapies in approximately 20,000 individuals/year in the USA, Europe and the UK [G].

2. Changes to international guidelines and professional practice
The OANG's development and evaluation of the most accurate aquaporin-4 and MOG autoantibody assays [4, 5] led to their uptake during the current REF Impact period in South Korea [Fiii], USA (Mayo Clinic, Minnesota [B], Harvard, Boston [Fi]) and Canada [Fii]. In South Korea, this has led to an increase in identification of patients with NMO (by 18.5%) and a 10% increase in the disease diagnosis, patients who would previously been left undiagnosed and untreated. The OANG's aquaporin-4-antibody test has been established at the British Columbia Neuroimmunology laboratory in Vancouver, Canada, now providing 100 tests/month. Cell-based assays are now recommended as gold standard in the 2015 international guidelines for diagnosis of NMO spectrum disorders [I, 2,090 citations]. These highly cited criteria have improved the diagnosis of NMO globally, with validation studies from every continent demonstrating improved patient identification: e.g. France (97% accuracy vs. 82%), in a single centre in one year in India (91 vs. 30 patients), and in four countries in Latin America (104 vs. 64 patients).

3. Economic benefits to industry
a. Commercial clinical trials: Identification of LGI1 and CASPR2 antibodies [1, 6] has been used as an inclusion criterion for an industry-funded clinical trial in the field [G]. Patients benefited from a reduced frequency of seizures on the test immunotherapy (intravenous immunoglobulins), which confirmed the previous OANG data [6]. This result now permits approximately 250 US patients per year access to immunotherapies otherwise denied by insurers [B].

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b. New technologies to support drug discovery: OANG's LGI1- and CASPR2-antibody tests have been commercialised and sold in 57 countries worldwide [K]. The OANG-pioneered membrane-tethering technology, used in the development of the LGI1-antibody assay [1], has been taken

up by Retrogenix Ltd, to detect binding partners of soluble molecules and off-target binding of biotherapeutics [K]. Since 2017 this technology has been integrated into nearly every client project run by Retrogenix including screening 1,000 drug molecules in more than 400 projects with more than 200 clients worldwide. The data generated supported 75 successful commercial clinical trial applications in the last 2 years. Retrogenix describe the benefit to their clients who "*have been able to make more informed decisions as to which molecules to progress within their pipelines, and into the clinic. This reduces their failure rates, and allows safer drugs to enter clinical trials, meaning an economic benefit, and even more importantly, less likelihood that patients will encounter unexpected toxicities."* [L].

4. Benefits to the NHS

Correct patient diagnosis and treatment leads to better patient outcomes, often with a return to activities of normal daily living. Accurate assays have also provided savings to the NHS since 2019 by treating patients who are CASPR2 or LGI1 antibody positive, and not treating (80-95%) those with VGKC antibodies (without CASPR2 or LGI1 reactivities) where there is no evidence of benefit on immunotherapy.

5. Sources to corroborate the impact

A. 2013/14 NHS Standard Contract for Neuromyelitis Optica Service, citing AQP4-Ab seropositive status, provided by the OANG testing service, as part of the diagnostic criteria.

B. Letter from Director, Neuroimmunology Laboratory, Mayo Clinic, on AQP4 and MOG assay set-up and the use of LGI1/CASPR2 versus VGKC.

C. Glycine receptor assay (i) in specification list at the Clinical Immunological Laboratory, Lubeck; (ii) description of new test at the Mayo Clinic laboratories introduced August 2020.

D. Studies showing harm of treating MNO patients as multiple sclerosis: (i) Williams J et al. *Lancet Neurol.* (2020) Jan;19(1):31-33. DOI 10.1016/S1474-4422(19)30445-4. (ii) Shimizu J et al. *Neurology* 2010;75;1423-1427 DOI https://pubmed.ncbi.nlm.nih.gov/20826711/. (iii) Stellmann JP et al. *J Neurol Neurosurg Psychiatry* 2017;88:639–647. DOI 10.1136/jnnp-2017-315603

E. Calculation of AQP4 antibody positive patients captured using live cell-based assays that are missed by indirect immunofluorescence based on reference [4].

F. Letters to corroborate the utility and establishment of AQP4 and MOG antibody assays from (i) Associate Professor of Neurology, Harvard Medical School and Massachusetts General Hospital; (ii) CEO and Medical Director; BC Neuroimmunology Lab Inc, Canada; (iii) Associate Professor, Seoul National University Hospital, Korea.

G. VGKC calculation of patients receiving inappropriate immunotherapy when the diagnosis is based on VGKC antibody positive result (based on Gadoth-A et al. Ann Neurol. 2017 Jul;82(1):79-92).

H. Data sheet from Mayo Clinic Laboratories, and letter from Head of Clinical Immunology laboratories at Oxford University Hospitals NHS Trust, stating that the VGKC Assay are replaced as first line tests by the LGI1 and CASPR2 assays.

I. Practice guideline: 'International consensus diagnostic criteria for neuromyelitis optica spectrum disorders', highlighting the importance of cell-based assays. Wingerchuk DM et al., *Neurology* (2015) Jul 14;85(2):177-89 DOI: 10.1212/WNL.0000000000001729.

J. [Text removed for publication]

K. Extract from Oxford University Innovation royalty report for use of LGI1 and CASPR2 tests during the REF period.

L. Letter from Director, Retrogenix, corroborating benefits to the company and to its clients.