Institution: University of Glasgow (UofG)

Unit of Assessment: UoA4 (Psychology, Psychiatry and Neuroscience)

Title of case study: Imaging-based diagnostics for patient selection and treatment of acute ischaemic stroke

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:
(1) Prof Keith Muir	**(1)** SINAPSE Chair of Clinical Imaging	**(1)** 2001–present
(2) Prof Ian Ford	**(2)** Professor of Biostatistics; Senior Research Fellow	**(2)** 1992–2018; 2018–present
(3) Dr Celestine Santosh	**(3)** Honorary Clinical Associate Professor	**(3)** 2016–present
(4) Prof I Mhairi Macrae	**(4)** Professor of Neuroscience; Honorary Senior Research Fellow	**(4)** 1985–2017; 2017–2020
(5) Dr William Holmes	**(5)** Clinical Research Fellow; Senior Research Fellow	**(5)** 2005–2014; 2014–present
(6) Dr Graeme Deuchar	**(6)** Research Associate; Research Fellow; Honorary Research Fellow	**(6)** 2010–2011; 2011–2015; 2015–present

Period when the claimed impact occurred: 2015–present

Is this case study continued from a case study submitted in 2014? No

1. Summary of the impact

Therapeutic options for acute ischaemic stroke (AIS) are constrained by the limited insights into brain pathophysiology offered by current imaging techniques. Only 10% of patients receive thrombolytic treatment, with many excluded owing to clinical uncertainty around the time of onset (wake-up stroke) or severity of brain injury. UofG research has improved selection of these patients for treatment through two imaging approaches. First, findings from the WAKE-UP study of magnetic resonance imaging (MRI)-guided thrombolysis informed European, US and Australian clinical guideline recommendations on eligibility for thrombolysis, providing increased diagnostic certainty and access to treatment. Second, working in collaboration with NHS Scotland, UofG developed a novel MRI diagnostic technique that led to a spin-out, Aurum Biosciences Ltd, which has attracted GBP3.67 million in investment and received UK regulatory approval to commence clinical trials.

2. Underpinning research

UofG researchers have a long-standing international reputation in the field of stroke research and patient care, pioneering the use of thrombolysis since the early 1990s. For example, in 2011, data from the ECASS III study supported extension of the approved timeframe for treating AIS with the thrombolytic (clot-busting) drug alteplase from 3.0 hours up to 4.5 hours. This work was the subject of a REF2014 impact case study.

Nonetheless, fewer than 10% of all AIS patients receive reperfusion therapy with alteplase or endovascular thrombectomy. This situation reflects the fact that many of the remaining 90% of affected individuals arrive too late at the hospital to benefit from such treatment (>4.5 hours after AIS onset) or else the time of onset is unknown due to waking with symptoms or an inability to communicate. To address this unmet clinical need, UofG researchers developed, assessed and validated imaging techniques to enable individualised treatment of AIS. Decisions are based on brain pathophysiology rather than time alone, thereby widening eligibility for thrombolytic therapy and aiding clinical assessment of the balance between benefit and risk.

Imaging to guide treatment for stroke of unknown onset: the WAKE-UP trial

Wake-up stroke accounts for approximately one in five AIS cases. Use of MRI among AIS patients with known time of onset detects a visible ischaemic lesion on diffusion-weighted imaging (DWI). When combined with a lack of visible hyperintense signal in the same region on fluid-attenuated inversion recovery (FLAIR) sequences, this lesion correlates with symptom onset within 4.5 hours before MRI. Therefore, MRI offers a potential tool for the selection of patients with unknown onset of AIS who might benefit from thrombolytic therapy.

Between 2012 and 2017, an EU-funded multicentre trial (WAKE-UP; NCT01525290) evaluated

the safety and efficacy of MRI to direct thrombolysis among 503 patients with mild stroke of unknown onset [3.1]. WAKE-UP was led by Dr Götz Thomalla (Universitätsklinikum Hamburg-Eppendorf, Germany), with **Prof Keith Muir** as WAKE-UP Co-Investigator and UK Chief Investigator. In addition, **Muir** and **Prof Ian Ford** were members of the WAKE-UP Steering Committee. Their specific contributions included design and management of the trial (**Muir**, **Ford**); leading UK recruitment and training (**Muir**); leading dissemination activities (**Muir**); and data analysis (**Ford**). Published in 2018, WAKE-UP demonstrated that thrombolytic treatment with alteplase substantially improved the outcome of patients with the MRI marker used as a surrogate for stroke onset within the previous 4.5 hours [3.1]. Based on the modified Rankin Scale score for neurologic disability, patients in the alteplase group reported better functional outcomes than those receiving placebo. The proportion of patients with a score of 0 (no symptoms) or 1 (no significant disability) at 90 days was 11.5 percentage points higher in the alteplase group versus the placebo group (a similar magnitude of absolute benefit to that seen in the <3 hours group treated in other clinical trials). A 2020 systematic review and meta-analysis that pooled the WAKE-UP data with similar studies from Europe, Japan and Australia confirmed the utility of this approach for identifying patients with stroke of unknown onset who could benefit from thrombolysis (**Muir**) [3.2].

A diagnostic and therapeutic (theranostic) approach to identify patients suitable for thrombolytic therapy

Since 2007, UofG researchers have worked in partnership with colleagues at NHS Scotland Greater Glasgow and Clyde Health Board (NHS GGC) to develop and validate Glasgow Oxygen Level Dependent (GOLD) technology, a novel method to determine metabolic function as an indicator of viable brain tissue among AIS patients. GOLD was developed by **Muir** and **Dr Celestine Santosh** (Southern General Hospital, Glasgow), and validated in preclinical rat models and human imaging studies by **Muir**, **Dr William Holmes**, **Prof Mhairi Macrae** and **Santosh** [3.3–3.6]. This method comprises non-invasive contrast T2* signal-weighted MRI under oxygen challenge (5 minutes, 100% O_2). Used together with an injectable perfluorocarbon-based small-molecule oxygen carrier (Oxycyte) as a signal contrast enhancer, GOLD measures oxyhaemoglobin formation characteristic of metabolically active (i.e. salvageable) brain tissue. Furthermore, Oxycyte limits the time penalty associated with additional imaging as it provides a therapeutic supply of oxygen that can reach ischaemic tissue, protecting the brain from further damage—a finding first confirmed in animal models (**Dr Graeme Deuchar**, **Holmes**, **Muir**, **Santosh**, **Macrae**) [3.7, 3.8].

This work led to patents being filed for the GOLD technology, with **Holmes**, **Deuchar** and/or **Santosh** listed as inventors for patents WO2008023176AI (USA, EU), WO2011027165A1 (USA, EU) and WO2014083331AI (USA).

2. References to the research

1. Thomalla G *et al*. (2018) MRI-guided thrombolysis for stroke with unknown time of onset. N Engl J Med;379(7):611–622 (doi:10.1056/NEJMoa1804355). WAKE-UP study (**Ford** and **Muir** listed as co-authors).
2. Thomalla G *et al*. (2020) Intravenous alteplase for stroke with unknown time of onset guided by advanced imaging: systematic review and meta-analysis of individual patient data. Lancet;396(10260):1574–1584 (doi:10.1016/S0140-6736(20)32163-2). WAKE-UP study (**Muir** listed as a co-author).
3. **Santosh C**, Brennan D, McCabe C, **Macrae IM**, **Holmes WM**, Graham DI, Gallagher L, Condon B, Hadley DM, **Muir KW**, Gsell W (2008) Potential use of oxygen as a metabolic biosensor in combination with T2*-weighted MRI to define the ischemic penumbra. J Cereb Blood Flow Metab;28(10):1742–1753 (doi:10.1038/jcbfm.2008.56). Proof-of-principle of the image contrast approach to identify ischaemic penumbra (rat study).
4. Dani KA, **Santosh C**, Brennan D, McCabe C, **Holmes WM**, Condon B, Hadley DM, **Macrae IM**, Shaw M, **Muir KW** (2010) T2*-weighted magnetic resonance imaging with hyperoxia in acute ischemic stroke. Ann Neurol;68(1):37–47 (doi:10.1002/ana.22032). Imaging study among patients with AIS.
5. Robertson CA, McCabe C, Gallagher L, Lopez-Gonzalez Mdel R, **Holmes WM**, Condon B, **Muir KW**, **Santosh C**, **Macrae IM** (2011) Stroke penumbra defined by an MRI-based oxygen

challenge technique: 1. Validation using [^{14}C]2-deoxyglucose autoradiography. J Cereb Blood Flow Metab:31(8):1778–1787 (doi:10.1038/jcbfm.2011.66). Validation study in rats.
6. Robertson CA, McCabe C, Gallagher L, Lopez-Gonzalez Mdel R, **Holmes WM**, Condon B, **Muir KW**, **Santosh C**, **Macrae IM** (2011) Stroke penumbra defined by an MRI-based oxygen challenge technique: 2. Validation based on the consequences of reperfusion. J Cereb Blood Flow Metab;31(8):1788–1798 (doi:10.1038/jcbfm.2011.67). Data processing for T2* oxygen challenge assessment (rat study).
7. Robertson CA, McCabe C, Lopez-Gonzalez MR, **Deuchar GA**, Dani K, **Holmes WM**, **Muir KW**, **Santosh C**, **Macrae IM** (2014) Detection of ischemic penumbra using combined perfusion and T2* oxygen challenge imaging. Int J Stroke;10(1):42–50 (doi:10.1111/ijs.12327). T2* oxygen challenge combined with perfusion imaging has advantages over other MRI techniques for penumbra imaging (rat study).
8. **Deuchar GA**, Brennan D, **Holmes WM**, Shaw M, **Macrae IM**, **Santosh C** (2018) Perfluorocarbon enhanced Glasgow oxygen level dependent (GOLD) magnetic resonance metabolic imaging identifies the penumbra following acute ischemic stroke. Theranostics;8(6):1706–1722 (doi:10.7150/thno.21685). The first use of both lactate change and oxygen challenge techniques (rat study).

Grants

Muir (Co-Investigator and UK Chief Investigator): WAKE-UP study [3.1, 3.2], European Union Seventh Framework Program grant 278276 (2011–2016); EUR15,899,832, with UofG receiving EUR1,171,858.

All research to support development of the theranostic [3.3–3.8] resulted from grant funding to UofG. For example:

- **Macrae (Principal Investigator):** Medical Research Council '*Imaging the ischaemic penumbra using BOLD MRI with oxygen challenge as a biotracer*' grant G0700439 (2008–2011); GBP224,167.
- **Muir (Co-Investigator):** Wellcome Trust Health Innovation Challenge Fund '*GOLD imaging in acute stroke: further technology development incorporating a perfluorocarbon oxygen carrier (Oxycyte)*' grant 102567 (2015–2020); GBP1,813,034.

4. Details of the impact

The benefit of thrombolytic therapy for AIS is well established. Nonetheless, its use was previously limited to a window of less than 4.5 hours from symptom onset, during which timeframe only 20%–40% of all stroke patients present to hospital. Treatment choices are particularly difficult for patients with unknown time of AIS onset; for example, individuals who go to bed well but wake up with symptoms. UofG researchers pioneered the use of imaging to help to personalise therapeutic options for those patients who will benefit the most in terms of post-treatment outcomes. The impacts of this research include **(1)** changes to clinical practice through evidence-based guideline recommendations, and **(2)** product development through creation of a spin-out company.

Impact 1: Increasing patient access to thrombolytic therapy through imaging

Of the 1.5 million patients experiencing AIS each year in the EU, 20% wake up with symptoms and so have been excluded from thrombolytic therapy. Thus, this group is disadvantaged by a substantial barrier to post-stroke recovery.

Release of the WAKE-UP data in 2018 [3.1] demonstrated that tissue appearance by MRI can be used to direct appropriate thrombolytic treatment among patients with mild stroke of unknown onset, offering substantial clinical benefit when compared with treatment as usual. Approximately 33%–50% of patients with wake-up stroke qualify for these criteria. The WAKE-UP findings [3.1] were reflected in updates to international clinical guidelines (2018–2019):

- The 2019 European Stroke Organisation guidelines [5.A] recommend the use of intravenous alteplase among patients with AIS of unknown onset time in the presence of a DWI–FLAIR mismatch on acute MRI on the basis of WAKE-UP [3.1] (grade B evidence; support from one randomized controlled trial or one statistical review).
- The 2019 joint American Heart Association–American Stroke Association (AHA/ASA) guidelines for the early management of AIS [5.B] included a new recommendation regarding

eligibility for intravenous alteplase (Section 2.2.2, Recommendation 3). WAKE-UP [3.1] provided the sole evidence to support this recommendation: *"In patients with AIS who awake with stroke symptoms or have unclear time of onset >4.5 hours from last known well or at baseline state, MRI to identify diffusion-positive FLAIR-negative lesions can be useful for selecting those who can benefit from IV alteplase administration within 4.5 hours of stroke symptom recognition."* WAKE-UP [3.1] also supported a new recommendation on the time window for alteplase administration (Section 3.5.2, Recommendation 3): *"IV alteplase (0.9 mg/kg, maximum dose 90 mg over 60 minutes with initial 10% of dose given as bolus over 1 minute) administered within 4.5 hours of stroke symptom recognition can be beneficial in patients with AIS who awake with stroke symptoms or have unclear time of onset >4.5 hours from last known well or at baseline state and who have a DW-MRI lesion smaller than one-third of the MCA territory and no visible signal change on FLAIR."*

- The Australian Stroke Foundation guidelines for stroke management were first published in 2017 but have since been superseded by online 'living guidelines' that are updated as substantial evidence changes become available [5.C]. In 2019, a new recommendation solely based on WAKE-UP [3.1] was added to Chapter 3 of these guidelines: *"For patients with potentially disabling ischaemic stroke of unknown onset time who meet MRI FLAIR-diffusion mismatch criteria in addition to standard clinical criteria, intravenous alteplase (dose of 0.9 mg/kg, maximum of 90 mg) may be administered."* The strength of this recommendation reflects the Working Party opinion that "*benefits outweigh harms for the majority, but not for everyone. The majority of patients would likely want this option.*"
- The European Stroke Action Plan 2018–2030 covers seven domains of stroke, including management of AIS, and sets consensus targets for the long-term development of stroke care across the region [5.D]. This document highlights the use of MRI as a state-of-the-art option for treatment stratification, citing WAKE-UP [3.1] as the evidence source. The action plan includes the following research and development priorities: (1) identifying which reperfusion options should be used "*based on patient-, service- and cost-specific factors*" and (2) determining how the *"speed, safety and effectiveness of reperfusion approaches (drugs or devices) [can] be optimised in Europe."* Achieving intravenous thrombolysis rates above 15% in all European countries by 2030 is a key target.

Together, the WAKE-UP findings [3.1, 3.2] and clinical guidelines [5.A–5.D] are driving increased uptake of MRI as a tool to select AIS patients for thrombolysis. The AHA/ASA recommendations [5.B] have been adopted in US clinical practice, with the national quality registry (Get with the Guidelines-Stroke) capturing data on imaging-based selection for late therapy with alteplase in over 1,700 hospitals [5.E]. The WAKE-UP approach is also being used in European countries whose healthcare systems have wide application of MRI in stroke units. For example, many of the 90 stroke units offering alteplase in the Netherlands have implemented this approach, with the Dutch Neurological Association developing a draft module that translates the WAKE-UP data [3.1] to clinical practice [5.E]. MRI-based stratification has been widely implemented in France [5.E], with hyperacute stroke centres in the UK using the WAKE-UP protocol to guide treatment decisions [5.E]. Such uptake is likely to contribute to the goal of increasing European thrombolysis rates by 2030 [5.D, 5.E].

Impact 2: Creation of a spin-out company to develop the stroke theranostic

Research on GOLD technology and Oxycyte [3.3–3.8] and the associated patents provided sufficient intellectual capital to support the creation of a spin-out company from UofG and NHS GGC. Co-founded by **Santosh** and based at the UofG Imaging Centre of Excellence (Queen Elizabeth University Hospital, Glasgow), Aurum Biosciences Ltd (ABL) is developing the stroke theranostic for use among patients with AIS [5.F], an indication forecast to be worth USD31 billion by 2021. In November 2015, ABL announced GBP3 million in funding from UK and US investors, including Tricapital (an angel investment group that supports innovative businesses) and the Scottish Investment Bank (part of Scottish Enterprise) [5.G]. These funds enabled ABL to employ several full-time staff, including a Chief Executive Officer; a Research and Development Director; a Director of Project Management; and a Director of Quality and Operations. **Santosh** currently serves on the Scientific Advisory Board.

Investment in ABL also drove development of the GOLD technology and Oxycyte as the company's lead product (known as 'ABL-101'). ABL owns or has licenced patents for use of ABL-101 as a method of imaging metabolic function in the EU and USA (granted 2015–2019) [5.H]. In November 2015, ABL signed a commercial licensing agreement with the developer of Oxycyte (Tenax Therapeutics, Morrisville, NC, USA), which gave ABL full rights to develop and commercialise ABL-101 in AIS [5.G, 5.H].

The November 2015 round of investment in ABL included a GBP1.8 million grant from the Wellcome Trust Health Innovation Challenge Fund (see section 3) to initiate phase 2 clinical trials of ABL-101. The clinical trials programme is sponsored by UofG and NHS GGC, with **Muir** as the Chief Investigator. In December 2017, ABL received approval from the UK Medicines and Healthcare Products Regulatory Agency to conduct the first phase 2 placebo-controlled trial of ABL-101 [5.I]. The POST-IT trial will evaluate the safety and tolerability of three dose levels of ABL-101 and supplemental oxygen among 18 AIS patients [5.I]. In October 2020, ABL secured new investments totalling GBP670,000 from Infinion Biopharma, Scottish Health Innovations Ltd, Tricapital and the Scottish Investment Bank [5.I]. These funds will allow ABL to manufacture ABL-101 locally, with POST-IT set to commence in May 2021 once supply of this investigational medicinal product is assured.

Beyond AIS, the ABL-101 theranostic is in development for other indications requiring oxygenation such as myocardial infarction [5.J]. Furthermore, data released in November 2019 indicated that ABL-101 can be used to track cells and monitor inflammation (e.g. macrophage accumulation) when detected by 19-fluorine MRI [5.J].

5. Sources to corroborate the impact

PDFs uploaded for all listed items.

A. The consensus statements and recommendations from the European Stroke Organisation Karolinska Stroke Update Conference (2019). See summary version, p.316. WAKE-UP [3.1] is cited as ref. 12 in Session 10 (Talk 3) of the full version (p.82–p.83).
B. AHA/ASA guidelines for the early management of patients with AIS (2019). See sections 2.2.2 (Recommendation 3) and 3.5.2 (Recommendation 3); WAKE-UP [3.1] is cited as ref. 88. WAKE-UP is also cited in online Data Supplement 1 (Evidence Table XIX).
C. Australian Stroke Foundation clinical guidelines for stroke management (2017; online update 2019). See chapter 3 (Acute medical and surgical management) for recommendation on thrombolysis (see p.6 and p.73–p.76). WAKE-UP [3.1] is cited as ref. 59.
D. European Stroke Action Plan 2018–2030 (doi:10.1177/2396987318808719). See section on management of AIS (p.315–p.316). WAKE-UP [3.1] is cited as ref. 51.
E. Testimonials from key opinion leaders regards uptake of clinical guideline recommendations.
F. ABL website and promotional video.
G. ABL press release announcing key investments and the agreement with Tenax Therapeutics (November 2015).
H. Patents: (1) ABL patent list; (2) EU patents 2309849 (2015; Oxycyte licensed to ABL by Tenax Therapeutics), 2053967 (2018) and 2464286 (2019); (3) US patents 9144392 (2015), 9322895 (2016) and 10278642 (2019).
I. POST-IT phase 2 clinical trial: (1) ABL press release announcing UK Medicines and Healthcare Products Regulatory Agency approval (December 2017); (2) Clinicaltrials.gov registration NCT03463551; (3) ABL press release announcing new funding (October 2020).
J. Other indications for ABL-101: (1) ABL pipeline; (2) ABL press release on inflammation imaging (November 2019); (3) Darçot *et al*. NMR in Biomedicine 2020;33:e4212 (doi:10.1002/nbm.4212; online November 2019).