Institution: University of Northumbria at Newcastle

Unit of Assessment: 3 (Allied Health Professions, Dentistry, Nursing and Pharmacy)

Title of case study: Rapid and robust clinical diagnosis of childhood brain tumours for improved patient outcomes

Period when the underpinning research was undertaken: October 2013 – 2020

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:
Edward Schwalbe	Associate Professor	October 2013 – present

Period when the claimed impact occurred: 2015 – 2020

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

1. Summary of the impact (indicative maximum 100 words)

Medulloblastoma is the most common malignant childhood brain tumour, affecting approximately 70 patients each year in the UK. Current treatment methods are aggressive and often cause long-term, life-limiting side effects. Research by Associate Professor Edward Schwalbe at Northumbria University has led to the development of a novel diagnostic tool that quickly and robustly identifies four recognised molecular subgroups of medulloblastoma. This rapid assay, called MIMIC (**Mi**nimal **M**ethylation **I**n **C**ancer), enables identification of the important WNT subgroup of patients in as little as three-to-four days, compared with three-to-four weeks for established diagnostics, making it applicable for decision making in treatment. The MIMIC assay has been used in the European PNET5 trial that investigates whether WNT patients can be treated with lower doses of radiotherapy. Adoption through the PNET5 trial means that it may reach all of the medulloblastoma patients in Europe. Of these, 20 children from 11 UK centres were found to be suitable to receive a greatly reduced dose of radiotherapy. Lower doses of radiotherapy reduce side effects and lead to better quality of life after treatment. Economic benefits from this research include income for the company providing the test, NewGene, and clinical cost decreased by half.

2. Underpinning research (indicative maximum 500 words)

Schwalbe's research is focused on improving outcomes for patients with medulloblastoma, the most common malignant childhood brain tumour, affecting approximately 70 patients each year in the UK and approximately 350 patients across Europe. Although cure rates have improved from 2% in the 1970s to approximately 75% in 2018, survivors face life-long side effects of receiving radiotherapy to their brains, including poor general functional outcomes such as speech and hearing, a sustained drop in IQ, as well as difficulties forming relationships, completing schooling, and getting work. By improving tumour diagnostics, patients can receive treatments that are optimised for them, helping to reduce treatment side-effects as far as possible while maintaining cure.

Four distinct molecular subgroups are well established: WNT, SHH, Grp3, and Grp4. Northumbria's Schwalbe, in collaboration with colleagues from the Newcastle University research group led by Professor Steven Clifford, was the first to show how medulloblastoma could be classified into these four types through the application of DNA methylation microarrays [**R1**-**R2**]. Each group has distinct molecular, clinical, and pathological features, and patient stratification is an important step in determining the most appropriate course of treatment and follow-up for each patient. For example, patients classified as belonging to the WNT subgroup have the most favourable outcomes and survival rates of approximately 95% [**R1**], while patients with SHH subgroup tumours may benefit from specific inhibition of the SHH pathway as part of their treatment.

Unfortunately, the microarray route to disease profiling is difficult to implement clinically, particularly for diseases such as medulloblastoma. This is because the method requires batched assessment using multiple samples, has high sample input quality and quantity requirements, and is also expensive. To tackle these challenges, Schwalbe conceived a novel method through the characterisation of minimal DNA methylation signatures using mass-spectrometry. The technique is called MIMIC (**Mi**nimal **M**ethylation **I**n **C**ancer) [**R1**]. This assay is suitable for assessment of scant and/or poor quality pathological specimens, such as those acquired during surgery for routine histology, and has clear applications both to routine molecular subgrouping in patients and for unlocking previously-inaccessible historical cohorts for contemporary molecular analysis. The technique was validated on 120 pre-existing samples [**R1**].

The utility of the new MIMIC assay for informing treatment protocols via subgrouping was assessed by retrospectively applying it to samples acquired during the PNET4 medulloblastoma clinical trial [**R2**]. The PNET4 trial ran from 2001-2006 and was designed before the molecular subgroups of medulloblastoma were identified. Unfortunately, no provision was made in the trial design to collect tumour material for this type of analysis. Only formalin-fixed, paraffin-embedded sections and cytosine nuclear preparations on glass slides remained, more suitable for microscopy. The DNA extracted from these specimens was insufficient for conventional subgrouping but was, however, successfully analysed using MIMIC.

Subsequently, Schwalbe and colleagues analysed the PNET4 trial findings, taking the resulting four subgroups and using these to identify a group of tumours with distinct patterns of chromosomal gain and loss that were associated with excellent (100%) survival rates [**R3**]. Substantial biological heterogeneity and differences in survival were apparent within each of the four subgroups. Schwalbe and Clifford investigated whether additional molecular subtypes existed within Grp3 and Grp4, and whether these could be used to improve disease subclassification and prognosis predictions [**R4**]. The patient group from the PNET4 trial was analysed in the light of these novel medulloblastoma subtypes [**R3**].

The new molecular subtypes described in 2017 [**R4**] were refined in a 2019 study that was carried out in collaboration with German Children's Cancer Centre in Heidelberg, Germany; St Jude Children's Research Hospital in Memphis, Tennessee; and the Broad Institute in Boston, Massachusetts [**R5**]. The work confirmed eight subtypes from the two subgroups (Grp3 and Grp4), which provides a foundation for future treatment risk stratification and clinical trial designs.

Schwalbe is now working to identify new subgroups by developing a DNA sequencing-based successor to the MIMIC assay. This new assay will not only give information on molecular subgroup but will also assign novel subtypes of the four subgroups of medulloblastoma. Moreover, it will provide information on accompanying patterns of chromosomal gain and loss and, crucially, will be compatible with the upcoming reorganisation of National Health Service (NHS) genomic testing that is a consequence of the UK-wide 100,000 genomes project.

3. References to the research (indicative maximum of six references)

R1. Edward Schwalbe, Hicks*, D., Rafiee*, G. *et al.* (**2017**) 'Minimal methylation classifier (MIMIC): A novel method for derivation and rapid diagnostic detection of disease-associated DNA methylation signatures' *Scientific Reports* **7**: 13421 https://doi.org/10.1038/s41598-017-13644-1 (Please note: **E. Schwalbe**, D. Hicks, and G. Rafiee contributed equally to this work)

R2. Clifford*, S. C., Lannering**, B., **Edward Schwalbe**, *et al.* (**2015**) 'Biomarker-driven stratification of disease-risk in non-metastatic medulloblastoma: Results from the multi-center HIT-SIOP-PNET4 clinical trial' *Oncotarget* **6** (36): 38827–38839 https://doi.org/10.18632/oncotarget.5149

R3. Goschzik**, T., **Edward Schwalbe**, Hicks*, D., *et al.* (**2018**) 'Prognostic effect of whole chromosomal aberration signatures in standard-risk, non-WNT/non-SHH medulloblastoma: a

retrospective, molecular analysis of the HIT-SIOP PNET 4 trial' *The Lancet Oncology* **19** (12): 1602–1616 https://doi.org/10.1016/S1470-2045(18)30532-1

R4. Edward Schwalbe, Lindsey**, J. C., Nakjang**, S., *et al.* (**2017**) 'Novel molecular subgroups for clinical classification and outcome prediction in childhood medulloblastoma: a cohort study' *The Lancet Oncology* **18** (7): 958–971 https://doi.org/10.1016/S1470-2045(17)30243-7

R5. Sharma**, T., **Edward Schwalbe**, Williamson*, D. *et al.* (**2019**) 'Second-generation molecular subgrouping of medulloblastoma: an international meta-analysis of Group 3 and Group 4 subtypes' *Acta Neuropathologica* **138**: 309–326 https://doi.org/10.1007/s00401-019-02020-0 (Please note: T. Sharma and **E. Schwalbe** contributed equally to this research)

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**Co-authors not based in the UK

4. Details of the impact (indicative maximum 750 words)

Schwalbe's research has made it more straightforward to identify medulloblastoma and classify the disease into its four major subgroups (WNT, SHH, Grp3 and Grp4). The assay developed for this classification can be used to support clinical practice, leading to improved treatment and better patient outcomes, ultimately reducing the burden on the NHS.

The four subgroups were only recognised by the World Health Organisation in 2016, which means that progress towards treatments relying on this classification is very recent. The MIMIC assay, underpinned by Northumbria's research, enables clinically appropriate, rapid, robust, and economically viable identification of these four molecular subgroups by deploying Agena Bioscience's Mass Array genetic testing system [**E1** DNA laboratory report confirms the use of MIMIC to classify Mass Array medulloblastoma data and refers to two publications by Schwalbe *et al.*], a technique previously used for assessing mutations in cancer samples.

The development of the MIMIC assay has changed practice by being adopted as a subgrouping assay for the Europe-wide PNET5 clinical trial (trial ID: NCT02066220) running from 2014 to 2024. This trial of standard-risk medulloblastoma is investigating whether WNT patients can be treated with lower-intensity therapies, aimed at minimising the side effects of treatment whilst maintaining the rate of cure. WNT patients enrolled in the trial receive a tailored and much-reduced dose of radiotherapy, at 18Gy instead of the current standard dose of 23Gy [**E2**, p2]. Simon Bailey, Chair Elect of the International Paediatric Oncology group (SIOPE), which designed and implemented PNET5, has confirmed the MIMIC assay *'has enabled the routine and rapid subgrouping of tumour samples'* [**E3**]. This is because of its efficacy in analysis of small sample sizes and improved turnaround times, enabling real-time diagnosis [**E3**].

Adoption through the PNET5 trial means that all of the medulloblastoma patients in Europe are reached. The Royal Victoria Infirmary Hospital at Newcastle coordinates samples from 11 centres in the UK. Since 2016, it has commissioned the MIMIC assay for 56 patient samples [**E4**]. Since the assay is rapid, it is compatible with clinical decision-making. After surgical removal of the tumour, patients recover for 30 days before receiving chemo- and/or radiotherapy, and any molecular analysis needs to be completed within this time. The Great North Children's Hospital in Newcastle upon Tyne is one of the major centres treating children with paediatric brain tumours in the UK. Simon Bailey, a Consultant in Paediatric Oncology at the hospital (who also serves as the chair of the SIOPE trial), stated:

> *'These tailored therapies can only be achieved with real time molecular diagnostics. From a clinical perspective, the turnaround time of MIMIC is much more rapid than alternative assays (median 8 days) [and can be as little as three-to-four dates, compared to three-to-four weeks using previous methods] and allows real-time rather than retrospective decision*

making. The MIMIC assay has allowed clinically useful molecular subgrouping to be done robustly and rapidly, to aid treatment stratification and prognostication' [**E3**].

The implementation of the assay has enabled patients with WNT subgroup medulloblastoma – a group associated with a particularly good prognosis and survival rate – to be identified and stratified into the appropriate treatment arm of the PNET5 trial. This lessens the intensity of radiotherapy. In the UK, 20 patients have been classified with WNT tumours since 2016 and were deemed to be suitable candidates to receive reduced doses of radiotherapy [**E3**]. Simon Bailey confirmed:

'Lowering the dose of radiotherapy can lead to better patient quality of life post-treatment, so only giving a dosage that is required and no more can have huge benefits for the patient in terms of quality of life. These tailored therapies can only be achieved with real time molecular diagnostics … The PNET5 trial is still ongoing; however, it is important to mention that no excess relapse has so far been shown in patients with lower treatment doses, with lower incidences of neurocognitive deficits being seen. In short, the use of the assay has led, so far, to more appropriate treatment doses and better outcomes for patients' [**E3**].

The MIMIC assay is delivered using Agena Bioscience's Mass Array genetic testing system [**E5**]. Since 2016, NewGene has used this system for subgrouping medulloblastoma in the UK [**E6**]. NewGene is an NHS-accredited molecular diagnostics company. The company runs the assay to Good Clinical Laboratory Practice compliant standards for clinical samples and to a defined standard operating procedure. It also provides a clinical report in a standardised format which makes use of a classification web server developed for this project. This takes the test out of the lab and enables its use in clinical practice. Angela Silmon, The Operational Director of the Yorkshire and North East Genomic Laboratory Hub (GLH), said:

'Dr Schwalbe wanted to develop and optimise an assay using this platform [Agena Bioscience's Mass Array genetic testing system] for the routine classification of medulloblastoma molecular subgroups using characteristic DNA methylation signatures … Dr Schwalbe developed the algorithm to classify medulloblastoma samples into molecular sub-groups. We worked with Dr Schwalbe to optimise the assay and determine how to implement it clinically. As a result of this, it enabled NewGene to develop an expertise in a new method of detecting DNA methylation signatures without additional capital investment' [**E6**].

Since 2016, NewGene has performed [text removed for publication] tests at a price of [text removed for publication] per test, giving the company new income of [text removed for publication] [**E6**]. The economic benefits of this price extend to the user too. For example, in Spain the previous test cost nearly twice as much as MIMIC (approximately [text removed for publication]) [**E7**].

As a result of its use by the Yorkshire and North East Genomic Laboratory Hub (GLH) – an NHS service which has provided genetic analysis for inherited and acquired diseases since 2018 – the NewGene assay has become an NHS provision and elevated the profile of the GLH. Angela Silmon said: *'This development provided the company with a competitive edge in the market and excellent international reputation and raises the profile of the Yorkshire and North East GLH. This would not have been possible without Northumbria University and Dr Ed Schwalbe'* [**E6**].

The wider applicability of the assay to any disease characterised by differential DNA methylation patterns has resulted in training and knowledge exchange activities for European groups. These include the Karolinska Institute (Stockholm, Sweden) and the Leiden University Medical Centre (Netherlands), working in partnership on the development of equivalent assays.

5. Sources to corroborate the impact (indicative maximum of 10 references)		
Ref.	**Source of corroboration**	**Link to claimed impact**
E1	Medulloblastoma DNA Laboratory Report	Corroborates that MIMIC assay is used to classify medulloblastoma molecular subgroups, and that Dr Schwalbe's research underpins the development of MIMIC
E2	Information about PNET5 trial	Corroborates that patients of certain subgroups enrolled on the PNET5 trial receive lower doses of radiotherapy
E3	Testimonial - Simon Bailey, Consultant in Paediatric Oncology at the Great North Children's Hospital in Newcastle upon Tyne	Corroborates the number of samples that were analysed using MIMIC assay in the UK since 2016, confirms the number of WNT patients who received a lower dose of radiotherapy
E4	Email from Stephen Crosier, Neuropathology Department, Royal Victoria Infirmary, Newcastle Hospitals NHS Foundation Trusts	Confirms that the Royal Victoria Infirmary Hospital at Newcastle commissioned the MIMIC assay for 56 patient samples
E5	NewGene marketing materials	Corroborates transfer of the MIMIC method to a private company and economic benefits of research
E6	Testimonial - Angela Silmon, Operational Director of the Yorkshire and North East Genomic Laboratory Hub	Corroborates economic impact for NewGene, adopting of the existing mass spectrometry platform to perform MIMIC assay, leading to new branch of business and competitive edge on the market
E7	Testimonial - Idoia Martin, Paediatric Oncology Group at the Biocruces Health Research Institute	Corroborates use of MIMIC in Spain, median time of diagnostics, and savings associated with adoption of MIMIC