Institution: Plymouth University

Unit of Assessment: 3

Title of case study:
Pre-Natal Screening and Diagnosis through Non-Invasive Methods and Molecular Blood Grouping

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

A long programme of research By Neil Avent has led to the development of powerful screening and diagnostic measures. It has enabled the implementation of molecular blood grouping and Non-invasive prenatal diagnosis (NIPD) into clinical use. The work began with research that took the lead in developing the commercially available products BLOODchip and MLPA, used extensively in the management of difficult to transfuse patients. This was developed into investigations of NIPD of fetal blood groups (particularly RhD), and through EC funding, drove workshops to establish non-invasive RhD typing as routine in the clinical management of haemolytic disease of the fetus and newborn. This work has shaped the standardisation of NIPT for fetal Rhesus D (RhD) and fetal sexing via External Quality Assessment (EQA) and the EC network Eurogentest.

2. Underpinning research (indicative maximum 500 words)

Avent's career has focussed over the past 25 years on establishing the molecular basis of blood group antigen expression, in particular the most complex of them, the Rh system (1,2). This work began at the University of the West of England and continued with Avent's move to Plymouth University in April 2009. The key foundational paper on the Rh system (1) has been in the top 30 read/downloaded articles of the journal Blood every year for the past decade. This research work directly led to the development of methods to prenatally define RhD status in the clinical management of HDFN (Haemolytic Disease of the Fetus and Newborn), launched as a service in the NHS Blood and Transplant. This directly led to the development of non-invasive methods using maternal plasma as a source of fetal material, and the first ever non-invasive pre-natal diagnosis (NIPD) clinical service in 2001 (4,5). Plymouth University has recently organised wet workshops in the UK for fetal sexing, and Internationally for RHD blood grouping as part of NIHR and EC FP7 funded initiatives. Concomitant with this research was the understanding of the complex genetics of the Rhesus and other blood group systems. Avent contributed significantly to this knowledge and translated this into practise by coordinating a highly successful EC FP5 demonstration project, Bloodgen, at UWE, Bristol between 2003-2006. This project developed the product in its entirety from the selection of blood group specific polymorphisms to be analysed a multiplex PCR to amplify all required genomic fragments and a protocol for screening a DNA chip by allele specific hybridisation. Following Avent's move to Plymouth in 2009, a CE-marked product, BLOODchip, was launched. The research at Plymouth has involved clinical trials of further patient samples 2007-2011) and has led to FDA approval of the product in 2013. Plymouth University continues to lead Molecular blood grouping methodology, and a method based on the Multiplex Ligation-dependent probe amplification technique (MLPA) was published in July 2013 and was developed jointly between Plymouth University and Sanquin, Netherlands (6), Two abstracts are in press of work led by Avent's Plymouth University group (BBTS meeting in Birmingham October 2013) relating to next-generation based sequencing of major blood groups (ABO, RH, KEL, FY, JK). This is, as far as we are aware the first description of the NGS-based analysis of these blood groups.

3. References to the research (indicative maximum of six references) Plymouth authors in bold

1. **Avent ND**, Reid ME (2000) The Rh blood group system: a review. *Blood* 95: 375-387. A highly cited review on Rh, among the top thirty most read Blood papers every year since publication. Blood is the leading journal in Haematology. Co-written by world famous blood group serologist, author of the factsbook series on blood groups. The review describes a decades worth of molecular workup (much pioneered by Avent) that led to the molecular characterisation of the Rh antigens.

2. **Avent ND** (2004) Blood groups: molecular genetic basis. In *Encyclopaedia of the human*

genome, Nature press 1: 333-343. A keynote chapter in the well renowned Nature series published to coincide with the completion of the human genome project.

3. **Avent ND** , Martin PG, Armstrong-Fisher SS, Liu, W, Finning, K., Maddocks, DG, Urbaniak SJ (1997) Evidence of genetic diversity underlying Rh D negative, weak D and partial D phenotypes as determined by multiplex PCR analysis of the RHD gene. *Blood* 89: 2568-2577.

4. Finning KM, Martin PG, Soothill PW, **Avent ND** (2002) Prediction of fetal D status from maternal plasma: introduction of a new noninvasive fetal RHD genotyping service. *Transfusion* 42: 1079-1085. Widely cited paper which describes the launch of the world's first non-invasive genotyping test.

5. **Avent ND, Madgett TE**, Maddocks DG, Soothill PW (2009) Cell free DNA in the maternal serum and plasma: current and evolving applications. *Curr Opin Obstet Gynecol* 21:175-179. A review of state of the art non-invasive prenatal diagnosis in major international journal.

6. Haer-Wigham, L., Veldhuisen, B., Jonkers, R., Loden, M., **Madgett, T.E., Avent, N.D.,** de Haas, M., van der Schoot., C.E. (2013) RHD and RHCE variant and zygosity genotyping via multiplex ligation-dependent probe amplification. *Transfusion* **53:** 1559-1574. Impact factor 3.526. First description of the application of the MLPA test to molecular blood grouping.

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

Non-Invasive Prenatal testing/diagnosis (NIPT/NIPD)
Mass elimination of risky amniocentesis and chorionic vilus sampling during the clinical management of HDFN has been achieved. There is an at least 1% spontaneous miscarriage rate during such procedures, and amniocentesis can exacerbate alloimmunisation in cases of HDFN. NIPD is required for Aneuploidy and when achieved is one of the greatest impacts in obstetric management over the past fifty years. Avent has contributed significantly to this goal, in particular the management of HDFN, and provided here is evidence supporting this contribution.

Work by Avent since the late 1990s has included the first ever introduction of a routine NIPD service for the RhD blood group, initially for the clinical management of RhD HDFN in 2001 (Finning et al 4 above). This work was then extended by the EC-FP6 network of excellence, Special Non-invasive Fetal and Neonatal Evaluation (SAFE), funded between 2004 – 2009 (1). Avent was the chair of the steering committee of this network which was funded in excess of €12M . Avent, through the work of the SAFE network, and more recently following moving to Plymouth University in April 2009 (funded by the NIHR and EC FP7) has standardised approaches to fetal RhD typing and fetal sexing and has held wet workshops (led by Plymouth) involving UK and EC-based laboratories. This approach to standardisation has resulted in the use of RhD non-invasive testing for all RhD negative pregnant mothers in the Netherlands (2009) and Denmark (2011) with others imminently launching such a service. This approach has been adopted in order to avoid exposure of pregnant mothers to a human product and to conserve stocks of prophylactic anti-D which is in short supply. Avent's lab, in collaboration with the RAPID project (2009-2014) (funded by the NIHR, www.rapid.nhs.net) and EuroGentest (www.eurogentest.org (2010-2013)), is driving the widespread implementation of next generation sequencing techniques for routine implementation of NIPD, especially for Down syndrome. The NGS-based approaches for Down syndrome typing is now in clinical use. Thus despite the major funding block for Avent's NIPD work (the SAFE network) concluding in 2009, just within the current REF period, the bulk of its impact has been felt clinically in the past 5 years.

Blood group genotyping (BGG)
BGG has now developed into routine use to support patients whom are transfusion dependent (2,6). These vulnerable individuals are prone to alloimmunisation and as a consequence can be life threatening unless they receive best match possible blood. Throughout the past fifteen years Avent and collaborators developed a commercially available product to manage such patients and is the most comprehensive system available until next generation sequencing (NGS) protocols are

available. Plymouth University is at the leading edge of implemention of NGS for Blood group determination – two abstracts relating to this work are currently in press (Halawani *et al*., 2013, Altayar *et al*., 2013 *Transfusion Medicine*). NGS will ultimately replace array based (i.e.BLOODchip below and MLPA) assays for BGG. Avent's group was among the first to publish this technique and therefore remains at the forefront of BGG. This work can readily be evidenced by references 2,4,6 and 7 below.

Bloodchip is used extensively worldwide to manage the difficult to transfuse patient, and in certain instances during pregnancy. The full impact of this development has only been made since 2008-2013 when the product was approved for diagnostic use by CE marking which allows its use as a diagnostic rather than solely research product. This product's major advantage over its commercial competitors is that it is able to comprehensively test for Rh variants and that can cause clinical complications during pregnancy. The major current use of blood group genotyping is the management of transfusion dependent individuals, for example sickle cell disease. Avent whilst at Plymouth has developed in collaboration with Sanquin (Amsterdam) to develop a rapid Multiplex Ligation Dependent probe amplification (MLPA) based assay for BGG, and has been published in July 2013 (see reference 6 section 3). Avent joined the Transfusion Medicine advisory board (TMAB) for Grifols AG (a large Spanish multinational pharmaceutical company) in 2011, Grifols acquired Progenika in 2012 primarily on the value of the BLOODchip product. The Grifols TMAB is advising the company on the full integration of blood group genotyping in Transfusion Medicine, and BLOODchip is now one of the world leading products for DNA based blood typing. Grifols also run educational courses to illustrate and guide the use of blood group genotyping - Avent participated in these in Japan (2012), Barcelona (x2) (2012 & 2013) and Birmingham (2013).

5. Sources to corroborate the impact (indicative maximum of 10 references)

1. Chitty LS, Hahn S, van der Schoot, Avent ND (2008) SAFE- The special non-invasive advances in fetal and neonatal evaluation network – aims and achievements. Prenatal Diagnosis 28: 83-88. A report on behalf of the 52-partner SAFE consortium describing its impact on the implementation of NIPD.

2. Avent ND (2009) Large scale blood group genotyping – clinical implications British Journal of Haematology 144 3-13. State of the art review, invited by prestigious Haematology journal, detailing the implementation of molecular blood grouping.

3. Anstee DJ (2009) Red cell genotyping and the future of pretransfusion testing. Blood 114: 248-256. A complementary review to (2) above indicating that Blood Group genotyping is making significant impact in Transfusion Medicine.

4. Director of Immunohematology, Sanquin, The Netherlands. Provides evidence of **Avent'**s role in the development of NIPD especially for RhD.

5. Bloodchip : http://www.progenika.com/eu/index.php?option=com_content&task=view&id=143&Itemid=187 Indication of the widespread use Internationally of BLOODchip which is distributed worldwide by Grifols AG. Avent is a member of the Transfusion Medicine Advisory Board of Grifols, which advises them on the mass-scale use of blood group genotyping.

6. Chief Scientific Officer and Intellectual property director, Progenika AG. Provides evidence of NDAs role in Bloodgen project and NIPD.

7. Avent ND et al., (2009) The Bloodgen project of the European Union 2003-2009. Transfusion Medicine and Haemostasis 36: 162-167. Description of the work of the Bloodgen consortium, culminating in the CE-marking of the BLOODchip product.

8. Quill E (2008) Blood grouping goes genetic. Science 319: 1478-1479. A Science report indicating that serological testing now has a viable alternative in molecular DNA-based testing.

9. Avent has been awarded the Kenneth Goldsmith award for 2013 from the British Blood Transfusion Society for “Outstanding contribution to Transfusion Medicine especially in molecular blood grouping and non-invasive prenatal diagnosis”.