Institution: The University of Oxford

Unit of Assessment: 1

Title of case study:

SAFE, ACCURATE AND NON-INVASIVE PRENATAL DIAGNOSIS

Summary of the impact:

University of Oxford researchers have developed the first safe, accurate and non-invasive prenatal diagnostic tests. After confirming that fragments of fetal DNA circulate in maternal blood, University of Oxford scientists used the polymerase chain reaction technique to accurately identify fetal DNA in maternal serum and plasma. This technique, known as cell-free fetal DNA testing, has enabled the first non-invasive prenatal genetic tests for the determination of fetal gender and the diagnosis of genetic disorders. Patented in 2001 and commercially released in 2011, cell-free fetal DNA testing is now recommended by the UK National Health Service as a safe and accurate alternative to invasive prenatal tests.

Underpinning research:

Prenatal screening for genetic diseases in a fetus or embryo was first introduced in the 1960s. Although traditional diagnostic techniques such as amniocentesis and chorionic villus sampling continue to be used clinically for prenatal diagnosis, these invasive tests have been known to cause damage to the fetus and miscarriage in approximately 1% of cases.

Using a polymerase chain reaction (PCR) to analyse fetal DNA in the maternal bloodstream, Professor James Wainscoat and Professor Dennis Lo, at the University of Oxford's Nuffield Department of Clinical Laboratory Sciences, saw great potential for this technique in prenatal diagnosis[1]. In 1994 they reported the detection of fetal cells in maternal circulation as early as six weeks into pregnancy[2]. The Oxford researchers began researching the potential for prenatal diagnosis and in 1996 used PCR to detect male cells in the peripheral blood of pregnant women bearing male fetuses; however, this technique proved difficult to make reliable enough for clinical use[3].

The breakthrough came in 1997 when their seminal paper was published in the Lancet describing the presence of fetal DNA circulating in the plasma of pregnant women. Although the absolute amount of fetal DNA was small, the relative concentration of fetal to maternal DNA circulating was much higher than in the cellular part of blood[4]. This finding gave rise to the field of non-invasive prenatal diagnosis.

The University of Oxford's Professor Wainscoat continued to collaborate with Professor Lo following the latter's relocation to the Department of Chemical Pathology at the Chinese University of Hong Kong. In 1998 they showed that fetal DNA can be readily detected in maternal plasma or serum, confirming that maternal blood samples may be a valuable source of fetal DNA for non-invasive diagnosis[5]. In a subsequent paper published that same year, they conclusively showed that rapid and reliable non-invasive fetal genotyping for Rhesus Disease could be performed in the second trimester of pregnancy using maternal plasma[6].

References to the research:

1. Lo, Y. M. *et al.* Prenatal sex determination by DNA amplification from maternal peripheral blood. *Lancet* **2**, 1363–1365 (1989). ***Paper showing the potential for the use PCR technique in the analysis of fetal DNA in the bloodstream.***

2. Lo, Y. M., Fleming, K. A. & Wainscoat, J. S. Strategies for the detection of autosomal fetal DNA sequence from maternal peripheral blood. *Ann. N. Y. Acad. Sci.* **731**, 204–213 (1994) doi: 10.1111/j.1749-6632.1994.tb55772.x ***Paper showing that fetal cells appear in maternal circulation as early as six weeks into pregnancy.***

3. Lo, Y. M. *et al.* Two-way cell traffic between mother and fetus: biologic and clinical implications. *Blood* **88**, 4390–4395 (1996). ***Paper showing the use of PCR to detect male cells in the peripheral blood of pregnant women bearing male fetuses.***

4. Lo, Y. M. *et al.* Presence of fetal DNA in maternal plasma and serum. *Lancet* **350**, 9076, 485–487 (1997) doi.org/10.1016/S0140-6736(97)02174-0. ***A landmark paper describing the presence of fetal DNA circulating in the plasma of pregnant women, giving rise to the field of non-invasive prenatal diagnosis.***

5. Lo, Y. M. *et al.* Quantitative analysis of fetal DNA in maternal plasma and serum: implications for noninvasive prenatal diagnosis. *Am. J. Hum. Genet.* **62**, 768–775 (1998) doi.org/10.1086/301800. ***A paper showing that fetal DNA can be readily detected in maternal plasma or serum.***

6. Lo, Y. M. *et al.* Prenatal diagnosis of fetal RhD status by molecular analysis of maternal plasma. *N. Engl. J. Med.* **339**, 1734–1738 (1998) doi: 10.1056/NEJM199812103392402. ***A paper showing that non-invasive fetal genotyping for Rhesus Disease can be performed in the second trimester of pregnancy through maternal plasma.***

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Details of the impact:

University of Oxford research has led to the first non-invasive prenatal genetic diagnostic tests. This non-invasive alternative to traditional tests has significantly improved the quality of care offered to pregnant women at a high risk of carrying a genetic mutation. This research has also significantly reduced fetal risk, as traditional early diagnostic tests, such as chorionic villus sampling, carry a risk of miscarriage of around 1%.

Fetal Sex Determination

Prenatal fetal sex determination is recommended to women at high risk of serious genetic disorders affecting a specific sex, such as congenital adrenal hyperplasia, haemophilia or Duchenne muscular dystrophy. The identification of cell-free fetal DNA in maternal circulation has allowed the development of non-invasive prenatal diagnostic tests, which are capable of determining fetal sex early and without risk. An independent paper published in 2008, showed that early determination of fetal sex is feasible and reliable using cell-free fetal DNA tests from just seven weeks, and should be made available to all women at risk of X-linked disorders as well as metabolic conditions[7]. The report also stated that use of this technology could reduce the need for invasive procedures by up to 50%[7]. A national audit assessing the effectiveness and clinical utility of non-invasive prenatal genetic diagnosis for fetal sex determination in the UK concluded that non-invasive prenatal diagnosis through cell-free fetal DNA testing is highly accurate in determining fetal sex when performed in NHS laboratories[8]. In addition to fetal sex determination, non-invasive cell-free fetal DNA tests are now available in the UK to test the rhesus blood group in a fetus within 12 weeks of pregnancy without any risk[9], enabling fast effective prenatal care for mother and child.

Diagnosis of Down Syndrome

The first non-invasive maternal blood test for Down syndrome, the MaterniT21 assay, became available to physicians on request in 20 regions within the United States in October 2011. The MaterniT21 assay, developed by Sequenom Ltd, is capable of accurately testing maternal blood as early as 10 weeks into gestation, and has been made available to pregnant women at a high risk of carrying a fetus with Down syndrome. With an estimated 750,000 high-risk pregnancies in the

United States each year, the test offers a safer, more specific and sensitive alternative to invasive tests such as amniocentesis and chorionic villus sampling. The accuracy of the MaterniT21 assay was confirmed in a 2011 study, which showed the Down syndrome detection rate for the MaterniT21 test was 98.6%[10]. The MaterniT21 assay is not yet available for clinical use outside of the US.

Clinical Practice Guidelines

In the March 2011 NHS UK Genetic Testing Network's "best practice guidelines for non-invasive prenatal diagnosis to determine fetal sex for known carriers of congenital adrenal hyperplasia", recommended the use of cell-free fetal DNA testing as an alternative for prenatal sex determination[11].

The NHS UK Genetic Testing Network guidelines also report that there are no cost implications for service providers, due to testing being cost-neutral, and that the cell-free fetal DNA technique is a highly sensitive and specific diagnostic test[11].

In November 2011 the NHS released additional guidance for commissioners and public health officials, supporting the use of cell-free fetal DNA for fetal sex determination in serious genetic disorders[12].

Commercialisation

The patent for "*non-invasive prenatal diagnosis using cell-free fetal DNA"* was filed in the United Kingdom in March 1998, application number: PCT/GB98/00690[13]. Yuk-Ming Dennis Lo and James Stephen Wainscoat are listed as co-inventors, while Isis Innovation Limited is the named patent holder. Under this patent family a US patent was filed in July 2001, US Patent number 6258540[13], and since 2005 Sequenom Inc. have held an exclusive licence to the invention.

The Sequenom Inc. 2010 Annual Report states that in October 2005 Sequenom Inc. acquired exclusive rights in certain countries, including the United States, United Kingdom and other countries in Europe and elsewhere, to non-invasive prenatal diagnostic intellectual property from Isis Innovation Ltd. After receiving upfront royalties totaling $0.8 million, an amendment to the agreement in November 2009 led to a second one-time royalty payment of $1,000,000. During the years 2010, 2009 and 2008, the amount of royalties paid to ISIS in connection to product sales was $0.1 million annually[14].

The total number of MaterniT21 PLUS tests run since the initial product launch in 2011 increased from 20,000 in the first year, to 65,000 by mid 2012. Due to the rapid growth in adoption the Sequenom Inc. now predicts they will bill 50,000 MaterniT21 PLUS tests in 2012[15].

Sources to corroborate the impact:

7. Finning, K. M. & Chitty, L. S. Non-invasive fetal sex determination: impact on clinical practice. *Semin Fetal Neonatal Med* **13**, 69–75 (2008) doi.org/10.1016/j.siny.2007.12.007. ***An independent paper reporting that early determination of fetal sex is feasible and reliable using cell-free fetal DNA tests from just seven weeks, reducing the need for invasive procedures by up to 50%.***

8. Hill, M. *et al.* Non-invasive prenatal determination of fetal sex: translating research into clinical practice. *Clin. Genet.* **80**, 68–75 (2011) doi: 10.1111/j.1399-0004.2010.01533.x. . ***A national (UK) audit reporting that cell-free fetal DNA testing is highly accurate in determining fetal sex.***

9. NHS Choices, Rhesus disease - Diagnosis (Accessed 2013) Available from http://www.nhs.uk/Conditions/Rhesus-disease/Pages/Diagnosis.aspx ***NHS Choices webpage showing that non-invasive cell-free fetal DNA tests are now available in the UK to test the rhesus blood group in a fetus within 12 weeks of pregnancy.***

10. Palomaki, G. E. *et al.* DNA sequencing of maternal plasma to detect Down syndrome: an international clinical validation study. *Genet. Med.* **13**, 913–920 (2011) doi: 10.1097/GIM.0b013e3182368a0e. ***A 2011 study confirming the Down syndrome detection rate for the MaterniT21 test is 98.6%.***

11. NHS UK Genetic Testing Network. Best practice guidelines for non -invasive prenatal diagnosis to determine fetal sex for known carriers of congenital adrenal hyperplasia (CAH) (undated) (Accessed 2013) <Available from http://www.ukgtn.nhs.uk/gtn/digitalAssets/1/1050_BPCAREPATWAYSNIPDCAHFINAL.pdf> ***NHS UK Genetic Testing Network's "best practice guidelines" recommending the use of cell-free fetal DNA testing as an alternative source of fetal DNA for prenatal sex determination.***

12. Burton, D. H., Farndon P., Westwood, J., Chitty, L. Cell-free fetal DNA for fetal sex determination in serious genetic disorders (2011) (Accessed 2013) . <Available from http://www.rapid.nhs.uk/wp-content/uploads/2011/11/Commisioning-Guide.pdf> ***Additional guidance from the NHS for commissioners and public health officials, supporting the use of cell-free fetal DNA for fetal sex determination in serious genetic disorders.***

13. Lo, Y-MD, and Wainscoat, J.S.Inventors; 2001 Jul 10 Non-invasive prenatal diagnosis, United States patent US6,258,540 (Accessed 2013) . <Available from http://www.google.com/patents?id=0eUHAAAAEBAJ&printsec=abstract&zoom=4#v=onepage&q&f=false>***Patent information for non-invasive prenatal diagnosis using cell-free fetal DNA.***

14. Sequenom Inc Financial Reports (Accessed 2013) <Available from http://www.sequenom.com/home/investors/financial-reports-ir> ***Sequenom, Inc. 2010 Annual Financial Report.***

15. Sequenom, Inc. Reports Financial Results For The Second Quarter Of 2012 And Increases Full-Year MaterniT21™ PLUS Test Volume Goal To 50,000 - Jul 26, 2012. *sequenom.investorroom.com* (2012) (Accessed 2013) <Available from http://sequenom.investorroom.com/2012-07-26-Sequenom-Inc.-Reports-Financial-Results-For-The-Second-Quarter-Of-2012-And-Increases-Full-Year-MaterniT21-PLUS-Test-Volume-Goal-To-50-000> ***Press release from Sequenom, Inc. reporting financial results between 2011 and 2012.***