Institution: University of Oxford

Unit of Assessment: UOA5

Title of case study:

Revolution in influenza vaccine production

1. Summary of the impact

Research from the University of Oxford has played a major role in the development of effective vaccines to combat the urgent worldwide problem of influenza. This methodology, licensed to AstraZeneca, has been used to prepare the currently licensed live attenuated influenza vaccine FluMist. Since its introduction in 2006 it is estimated that FluMist or other vaccines produced using reverse genetics have saved the lives of thousands of people worldwide who would otherwise have died from flu and its complications. FluMist has generated close to $1 billion income for the manufacturers (MedImmune, owned by AstraZeneca).

2. Underpinning research

The World Health Organization estimates that 250,000 to 500,000 deaths occur worldwide every year from flu-related illnesses. There is therefore an urgent need for improved production of effective vaccines. Classical 'reassortant' techniques used for virus design and production are inefficient, time consuming (taking several months) and frequently unsuccessful.

In 1998, Professor Ervin Fodor joined the research group of Professor George Brownlee at the University of Oxford and, working in collaboration with Drs Palese and Garcia-Sastre (Mount Sinai School of Medicine, New York), devised a method for producing a wide range of influenza viruses within the laboratory. The breakthrough was the successful generation of recombinant influenza viruses after plasmid transfection using reverse genetics[1, 2]. It surprised many people in 1999, and still surprises scientists now, that it was possible to transfect twelve independent plasmids into a single cell.

Fodor and colleagues' new method resulted in the release of recombinant influenza viruses by the transfected cells after only a few days. By virtue of this method, and because manipulation of DNA plasmids is now routine, any desired mutation can be introduced into any of the eight individual RNA segments comprising the influenza RNA genome. This approach has made the development of new vaccine strains rapid and straightforward.

In 2003, collaborations between the University of Oxford and Dr Subbarao at the Centre for Disease Control, Atlanta, resulted in a pivotal paper showing the use of plasmid-based viral rescue in the first successful attempt to prepare a vaccine candidate against H5N1 flu virus[3]. There is widespread fear that this highly pathogenic avian flu virus could adapt to permit human-to-human spread, resulting in a new global flu pandemic. Such a pandemic has not so far emerged, but work by the University of Oxford researchers and their collaborators has repeatedly shown that vaccines which are effective in protecting chickens and mice against H5N1 bird flu virus[4, 5] can be produced using this method.

The key research achievement was the ability to prepare recombinant influenza virus easily within the laboratory. In addition to the practical aims of improving vaccines, the new methodology has accelerated basic research into influenza by providing a better understanding of (i) the influenza virus RNA polymerase promoter and its regulation, (ii) the proteins and domains involved in viral replication, and (iii) the determinants of virulence. These insights have opened up new avenues for drug development by the pharmaceutical industry.

3. References to the research

1. Fodor E, Devenish L, Engelhardt OG, Palese P, Brownlee GG, García-Sastre A. (1999) Rescue of influenza A virus from recombinant DNA. Journal of Virology 73: 9679-9682. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC113010/. ***The original study describing the development of the reverse genetics technology to generate recombinant influenza virus by transfecting 12 plasmids into Vero cells (derived from green monkey kidney), a cell line licensed for the production of vaccines for use in humans.***

2. García-Sastre A, Palese P, Brownlee GG, Fodor E. (2003) Helper-free rescue of negative strand RNA virus. United States Patent 6649372. http://www.google.com/patents/US6649372 ***This patent described the invention and its potential applications.***

3. Subbarao K, Chen H, Swayne D, Mingay L, Fodor E, Brownlee G, Xu X, Lu X, Katz J, Cox N, Matsuoka Y. (2003) Evaluation of a genetically modified reassortant H5N1 influenza A virus vaccine candidate generated by plasmid-based reverse genetics. Virology 305: 192-200. doi: 10.1006/viro.2002.1742 ***This study applies reverse genetics to generate a candidate vaccine against highly pathogenic H5N1 avian influenza as proof of principle. It also reports that the method allows the genetic modification of the virus to eliminate determinants of high pathogenicity, an important safety consideration during vaccine production.***

4. Nicolson C, Major D, Wood JM, Robertson JS. (2005) Generation of influenza vaccine viruses on Vero cells by reverse genetics: an H5N1 candidate vaccine strain produced under a quality system. Vaccine 23: 2943-2952. doi: 10.1016/j.vaccine.2004.08.054 ***This study describes the generation of a candidate vaccine against the highly pathogenic H5N1 influenza virus in Vero cells licensed for human vaccine production under quality controlled conditions. It also demonstrates the short time frame in which a seed vaccine can be produced using the novel reverse genetics technology.***

5. Chen Z, Wang W, Zhou H, Suguitan AL Jr, Shambaugh C, Kim L, Zhao J, Kemble G, Jin H. (2010) Generation of live attenuated novel influenza virus A/California/7/09 (H1N1) vaccines with high yield in embryonated chicken eggs. Journal of Virology 84: 44-51. doi: 10.1128/JVI.02106-09 ***This study demonstrates that the method can be used to introduce mutations into the genome of the vaccine virus leading to improved yields in subsequent viral production.***

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4. Details of the impact

Research and development pioneered within the University of Oxford revolutionised the laboratory production of viruses, enhancing the speed, specificity, reliability and safety of vaccine production. The innovative reverse genetics technology resulted in the rapid production (within a few days) of quantities of virus suitable for use as a vaccine[6] and was invaluable for the development of the FluMist vaccine formulation for the 2008 flu season[7].

A further crucial advantage of the reverse genetics technology pioneered by Oxford is the ability to mutate any nucleotide in the plasmids used for the expression of the viral RNA genome segments. This enables the design of new, ever more effective viruses. The advantage of producing 'custom' viruses was highlighted when insertion of a mutation into the H1N1 vaccine strain, proved to be critical for increasing the virus yield in the commercial production of the successful vaccine used in the 2009 bird flu outbreak[6]. These achievements - optimising the yield of the FluMist vaccine and genetically engineering the bird flu multibasic sequence - would have been highly problematical using the old 'reassortant' technology. Oxford's technology provides an essential tool to combat future influenza pandemics.

The reverse genetics technology developed by the University of Oxford and collaborators was licensed to AstraZeneca and MedImmune Inc. (later part of AstraZeneca) for use in the preparation of FluMist vaccine[8]. MedImmune later also non-exclusively licensed the technology to other companies such as Novartis and Sanofi Pasteur. FluMist was the first intranasal vaccine, containing the highly attenuated live flu virus (A/Ann Arbor/ 6/60), able to infect and successfully immunise people against influenza A and B viruses without causing disease. Later vaccine developments using the Oxford technology included the cold-adapted LAIV (tri-valent vaccine currently marketed as FluMist) and FluMist Quadrivalent vaccines.

FluMist as an intra-nasal rather than an injectable vaccine has been shown to be highly successful [9,10] and is marketed worldwide. Already administered to immunocompetent children from the age of two in the USA, FluMist (licensed under the name Fluenz in the EU) is to be offered to all such children from the age of two to 17 in Europe in 2012/3[11]. Results from a clinical trial have shown the further promise of FluMist as a vaccine for children from the ages of 5 to 17 years who are immunocompromised with cancer[12]. In 2012 a new vaccine, FluMist Quadrivalent, prepared using reverse genetics technology, was licensed for use in children, adolescents and adults (two to 49 years of age) in the USA[13]. This vaccine, which contains an additional attenuated virus, should offer even broader protection against flu.

Over 5 years, several million people worldwide have been vaccinated with the FluMist vaccine that uses the reverse genetics technology. In the UK alone, annual outbreaks of seasonal flu affect 5 to 20% of the population, and flu-related deaths vary from 3,300 to 48,860 per annum. Thus, extrapolating from the UK figure of 562 people dying from flu in 2009 in a UK population of 60 million, it is estimated that vaccination would probably have saved the lives of hundreds of people. Flu also has particularly serious consequences for the elderly. In excess of £22M is spent on hospital care for the elderly every winter and influenza also results in more than 400,000 GP appointments annually in England and Wales. In addition to healthcare costs, flu also places a heavy burden on productivity and the economy. More than 6 million working days are estimated to be lost in the UK due to seasonal influenza every year. This is even before the potential problems of global flu epidemics with the attendant problems of increased virulence are taken into account. The "economic savings" made from vaccination are therefore significant, although very difficult to estimate with any accuracy.

MedImmune announced FluMist sales of $104M in 2008, $145M in 2009 and $174M in 2010, plus $389M in 2009 and $39M in 2010 of pandemic vaccine against H1N1. Total sales revenue is thus close to $1 billion since 2008[14].

Royalties arising from the University of Oxford patents, which were jointly filed with the US collaborators (licensed by Isis, the Technology Transfer Office of the University of Oxford, to Mount Sinai School of Medicine, New York, who licenced it to MedImmune) have already provided income of over £3M to the University of Oxford.

5. Sources to corroborate the impact

6. Broadbent AJ, Subbarao K. (2011) Influenza virus vaccines: lessons from the 2009 H1N1 pandemic. Current Opinion in Virology 1: 245-262. doi:10.1016/j.coviro.2011.08.002 ***This review article summarises the benefits of the reverse genetics technology and its impact for the production of vaccines against the 2009 swine-origin pandemic influenza virus.***

7. Roumeliotis G. MedImmune cleared to offer Flumist the reserve genetics treatment. in-PharmaTechnologist.com. 13 Jul 2006. Available from: http://www.in-pharmatechnologist.com/Processing/MedImmune-cleared-to-offer-Flumist-the-reverse-genetics-treatment ***Link between reverse genetics and the future production of FluMist.***

8. MedImmune. MedImmune announces FDA approval of first four-strain flu vaccine, FluMist Quadrivalent (influenza vaccine live, intranasal) [internet)]. Gaithersburg (MD): U.S. MedImmune; 29 Feb 2012. Available from: http://www.medimmune.com/media/press-

releases/2012/02/29/medimmune-announces-fda-approval-of-first-four-strain-flu-vaccine-flumist-sup-sup-quadrivalent-%28influenza-vaccine-live-intranasal%29 ***Website reporting FDA approval for intranasal FluMist.***

9. Centers for Disease Control and Prevention. The nasal-spray flu vaccine (live attenuated influenza vaccine [LAIV]) [Internet]. Atlanta: Centers for Disease Control and Prevention. Available from: http://www.cdc.gov/flu/about/qa/nasalspray.htm ***Website of the CDC describing the efficacy of FluMist.***

10. Carter NJ, Curran MP. (2011) Live attenuated influenza vaccine (FluMist; Fluenz): a review of its use in the prevention of seasonal influenza in children and adults. Drugs 71: 1591-622. doi: 10.2165/11206860-000000000-00000 ***Paper describing the effectiveness of FluMist/Fluenz compared with an injectable flu vaccine.***

11. AstraZeneca Global. European Commission approves nasal spray vaccine FLUENZ for the prevention of seasonal influenza in children [Internet]. U.S. AstraZeneca Global; 1 Feb 2011. Available from: http://www.astrazeneca.com/Media/Press-releases/Article/AstraZeneca-approves-nasal-spray-vaccine-Fluenz-for-flu ***Press release describing the use of Fluenz in children in Europe.***

12. Halasa N, Englund JA, Nachman S, Weinberg GA, Huber VC, Allison K, Dubovsky F, McCullers JA, Flynn PM. (2011) Safety of live attenuated influenza vaccine in mild to moderately immunocompromised children with cancer. Vaccine 29: 4110-4115. doi: 10.1016/j.vaccine.2011.03.097 ***Paper describing the safety the FluMist vaccine in immunocompromised children with cancer.***

13. AstraZeneca Global. FDA approves first four-strain flu vaccine [Internet]. U.S. AstraZeneca Global; 1 Mar 2012. Available from: http://www.astrazeneca.com/Media/Press-releases/Article/20120301--fda-approves-first-fourstrain-flu-vaccine ***Website reporting Food and Drugs Administration approval for intranasal FluMist.***

14. AstraZeneca. Therapy area review. Infection AstraZeneca; 2011. AstraZeneca annual report and form 20-F Information. Available from: http://www.astrazeneca-annualreports.com/2011/documents/pdfs/infection.pdf ***Financial reports from AstraZeneca describing sales of FluMist.***