

The Quest for a H1N1 Flu Vaccine

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On 24 April 2009, the World Health Organisation (WHO) put out an alert on an influenza-like illness in the United States (US) and Mexico. The United States Government reported 7 confirmed human cases of Swine Influenza A/H1N1 in the US. Six days later, following daily updates, the WHO pandemic alert was raised from Phase 4 to Phase 5. Phase 5 is characterised by human-to-human spread of the virus in at least 2 countries in one of the WHO regions. Just 3 days later, on 3 May 2009, 17 countries had officially reported 787 cases of confirmed infection.¹

Singapore reacted with characteristic speed to the pandemic threat. Preventive measures were put in place a broad scale with the aim to prevent or contain the outbreak and spread of the pandemic in Singapore.² The first confirmed H1N1 case was diagnosed in Singapore on 27 May 2009, and this number rose rapidly to 1217 confirmed cases by 7 July 2009, despite rigorous containment measures.³ The Ministry of Health (MOH) was able to secure vaccine supply from GlaxoSmithKline (GSK) on 17 September 2009, and on 26 November 2009 this vaccine was rolled out to the public.

Luckily, the H1N1 pandemic was mild. Nevertheless, the economic consequences were notable in terms of hotel occupancy rates and retail sales.⁴ A virus causing more serious pathology comparable to H5N1 or H7N9, with the transmission rate of H1N1 could have had devastating consequences.

This event, once again, as the SARS epidemics in 2003, underlined the vulnerability of Singapore regarding newly emerging viral diseases and prompted some of us to consider options to decrease the dependence of Singapore on overseas vaccine producers. Through a collaboration of Cytos Biotechnology AG with SIGN (A*STAR) in a different project area we were made aware of the vaccine platform of this company.^{5,6} This platform is based on virus-like particles (VLP) derived from Q β bacteriophages carrying chemically linked antigens of various sources. We hypothesized that a virus-derived protein could be linked to VLP and be tested for its immunogenicity in animals, for its protective efficacy against a homologous virus challenge. If

proven to be efficacious and safe, such a vaccine candidate could be developed for use in humans. Influenza viruses exhibit 2 major proteins at their surface: neuraminidase (N) which is the target of Tamiflu and Relenza, 2 anti-flu drugs) and haemagglutinin (H) as the major immunogenic protein that induces a strongly protective antibody response in humans. Both N as well as H are highly variable, and give rise to a bewildering variety of influenza viruses that are typed accordingly, e.g. H1N1, H5N1, H7N9, etc. Immune protection, as a rule, is linked to a given subtype requiring repeated vaccination against seasonally occurring variable subtypes.

The VLP platform would offer several major advantages:⁷

- (a) Haemagglutinins of influenza subtypes can be produced by recombinant techniques in innocuous bacteria such as *E.coli* thereby eliminating the need of working with live virus. This can be done as soon as the sequence of a newly emerging virus is known.
- (b) VLP particles are also easily manufactured in *E.coli* and therefore are safe; no replicating bacteriophage is ever present in the manufacturing process.
- (c) Manufacturing haemagglutinins and VLP in *E.coli* is a fast process and comparatively cheap.

These were the arguments that prompted us to create a project team, involving several Singapore research institutions. The fact that this could be done with a minimum of loss of time and bureaucratic hurdles is a clear manifestation of the collaborative spirit around goals that seem to be relevant to be pursued.

The participating institutions were:

- (a) Duke-NUS (Prof Ooi Eng Eong and Dr Veronika von Messling).
- (b) Defence Science Organisation, DSO (Dr Brendon Hanson).
- (c) Singapore Immunology Network, SIGN, A*STAR (Prof Paola Castagnoli, Dr John Connolly and Dr David Skibinski).
- (d) Cytos Biotechnology AG, Zurich (Prof Martin

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Bachmann and Dr Philippe Saudan) and subsequently, Biomeva in Heidelberg, Germany and a string of outsourced companies.

- (e) Singapore Clinical Research Institute (SCRI), National Medical Research Council, NMRC (Dr Sam Lim and Dr Helen Isaacs).
- (f) Experimental Therapeutics Centre, ETC/D3 (Dr Alex Matter, Sponsor and Project Leader, Dr Veronica Diermayr, Project Management, and Dr Kantharaj Ethirajulu, Technical Research and Development (R&D)).

This project team selected A/California/7/2009 (H1N1) and Q β bacteriophage as the basis of the VLP vaccine. Financial support was provided by grants from SIGN and Exploit Technologies Pte Ltd (ETPL) (both A*STAR) and direct in-kind contributions of ETC and SIGN. The project plan was upset several times. Unforeseen hurdles such as the sale of A-Bio, with which we had intended to manufacture the vaccine, the financial difficulties of Cytos as well as the lack of technical experience of some of our outsourced partners were seriously threatening the project. Nevertheless, all of these difficulties were overcome thanks to the perseverance and willingness of the project team to deliver high quality results.

We needed to demonstrate 3 things:

- (a) A stable and pure product that fulfilled the technical specifications, and was fit to be used in human clinical trials.
- (b) Evidence for the safety of this product in an animal species (rats).
- (c) Evidence for the efficacy of the product in 2 animal species (mouse and ferrets). These 2 species are recognised to have predictive quality for the outcome of clinical trials in humans.

This work laid the basis to develop a clinical trial plan that was aligned with classical Phase I clinical trial strategies in the influenza field. Healthy human volunteers were to be immunised day 1 and day 21, and tested for the appearance of protective antibody titres in the blood, 21 days and 42 days after the first immunisation. One group was to be treated without, a second group with an additional adjuvant (alum). The trial was designed as a double-blind study.

The technical data, the safety and efficacy data, Investigator's Brochure, informed consent form and the clinical trial protocol were submitted to the Centralised Institutional Review Board (CIRB) of SingHealth, and an approval was obtained on 29 November 2012. Subsequently, the Health Sciences Authority (HSA) gave their green light, and the clinical Phase I trial started successfully in April 2013 at the Investigational Medicine Units of SingHealth and the Changi General Hospital.

It is too early to speculate about the outcome of this trial. At the very minimum, we have shown that it is possible to form a coherent project team with members from several science institutions to pursue an ambitious scientific and clinical goal, and to jointly overcome the many technical, financial and managerial hurdles. This experience, in our view, augurs well for the expertise, the will and the stamina of Singapore R&D to tackle ambitious projects in the biomedical arena. In the case that the trial is positive, we feel that we have opened a door to a safe, comparatively fast and cheap technology which may usefully bolster the preparedness of this nation regarding newly emerging viral diseases. In the meantime, another group has shown that—using a slightly different technique—it is indeed possible to produce a safe and effective VLP-based H1N1 vaccine.⁸ We therefore are confident that our vaccine will also produce promising results.

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