



## A pandemic influenza vaccine in India: From strain to sale within 12 months

Rajeev Dhere\*, Leena Yeolekar, Prasad Kulkarni, Ravi Menon, Vivek Vaidya, Milan Ganguly, Parikshit Tyagi, Prajakt Barde, Suresh Jadhav

Serum Institute of India Limited, 212/2 Off Soli Poonawalla Road, Hadapsar, Pune 411028, Maharashtra, India

### ARTICLE INFO

#### Keywords:

LAIV  
H1N1  
Influenza vaccine  
Pandemic  
Production  
Developing countries

### ABSTRACT

In the event of a highly pathogenic influenza pandemic, the Indian subcontinent would need 1.2 billion doses of vaccine to immunize its entire population, double if two doses were required to assure immunity. Serum Institute of India Limited (SII) thus became one of six initial grantees of the World Health Organization (WHO) technology transfer initiative to create capacity in developing countries to manufacture H5N1 pandemic influenza vaccine. At the outbreak of the A(H1N1) 2009 influenza pandemic, experience gained from the H5N1 project was used to develop a live attenuated influenza vaccine (LAIV), since this was the only option for the level of surge capacity required for a large-scale immunization campaign in India. SII took <12 months to develop and market its LAIV intranasal vaccine from receipt of the seed strain from WHO. As of November 2010, over 2.5 million persons have been vaccinated with Nasovac® with no serious adverse reactions or vaccine failure after 3 months' post-marketing surveillance. The product has been submitted for prequalification by WHO for purchase by United Nations agencies. In parallel, SII also developed an inactivated influenza vaccine, and is currently looking to ensure the sustainability of its influenza vaccine manufacturing capacity.

© 2011 Elsevier Ltd. Open access under [CC BY-NC-ND license](http://creativecommons.org/licenses/by-nc-nd/3.0/).

### 1. Introduction

The Serum Institute of India (SII) is the world's fifth largest producer of vaccines, with an installed capacity of over 1 billion doses. SII's core competence in mass production of cell-culture derived products makes it a major supplier of measles, mumps and rubella, as well as diphtheria, pertussis and tetanus vaccines through the United Nations Children's Fund. Given this experience and capacity, SII was selected in 2006 to participate in the World Health Organization (WHO) technology transfer initiative to strengthen the capacity of developing countries to produce pandemic influenza vaccine [1].

Countries such as India, with very large populations but no demand for seasonal influenza vaccine, face additional technological and financial challenges in ensuring an adequate supply of influenza vaccine. Although inactivated influenza vaccines (IIV) are generally preferred for high-risk populations such as immunocompromised individuals or pregnant women, the sheer number of doses required during a pandemic to immunize over a billion people means that among currently licensed vaccines only live attenuated influenza vaccines (LAIV) could meet the challenge of protecting the whole Indian population. In addition to the less complex downstream manufacturing process and higher yields, the

intranasal route of administration of LAIV imitates natural infection and presents a lower risk to the recipient compared to the injectable administration of IIV, making it the most appropriate candidate for mass immunization during a pandemic.

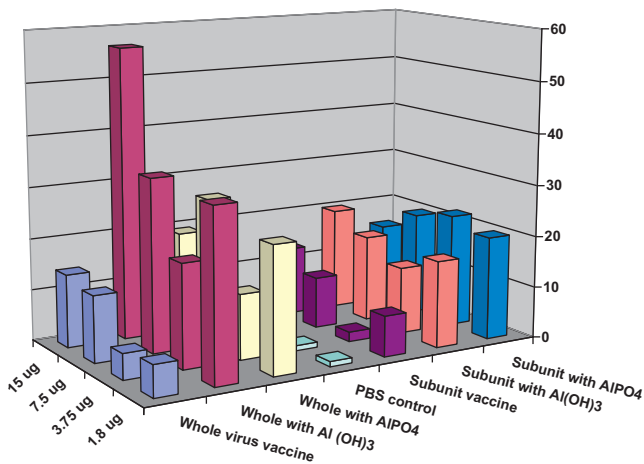
With these considerations, SII initiated the development of IIV and also approached WHO to obtain a sub-licence for the Russian LAIV technology. We present here our activities, as one of the WHO grantees, to develop, manufacture and license both IIV and LAIV for use in the event of an influenza pandemic.

### 2. Experimental studies with inactivated influenza vaccine

Our initial objective was to gain experience and generate technical and preclinical experimental data on influenza vaccines in order to decide on the best options for large-scale vaccine manufacture. Most influenza vaccines are produced in embryonated eggs. However, due to our extensive experience with production of cell-culture derived vaccines, we started by exploring the development of cell-based IIV to compare yields with those of egg-based vaccines.

We undertook extensive work between June 2007 and June 2009 on upstream and downstream processing of egg- and tissue culture-based IIV. A developmental and an analytical laboratory were set up to establish protocols for vaccine production and analytical testing, respectively. A/PR/8/34 (H1N1) prototype strain and seasonal influenza vaccine strains A/Solomon Islands/3/2006 (H1N1), A/Wisconsin/67/2005(H3N2) and B/Malaysia/2506/2004

\* Corresponding author. Tel.: +91 20 26602393; fax: +91 20 26602211.  
E-mail address: [rajeev.dhere@seruminstitute.com](mailto:rajeev.dhere@seruminstitute.com) (R. Dhere).



**Fig. 1.** Comparative immune response to H5N1-NIBRG-14 whole virus and subunit vaccine. HAI titres of adult Swiss albino mice, inoculated with two doses of 1.0 ml vaccine three weeks apart through intraperitoneal route containing 15 µg, 7.5 µg, 3.75 µg and 1.8 µg of HA antigen of H5N1 virus in the absence or presence of either aluminium phosphate or aluminium hydroxide.

were used to establish virus growth and purification methods, compare yields in eggs and tissue culture, generate trivalent seasonal influenza vaccine and carry out immunogenicity studies. The vaccine prepared using seasonal influenza strains induced an immune response in mice comparable to that in commercially available vaccine using the same strains.

The H5N1 NIBRG-14 strain was used to generate prototype whole and subunit pandemic vaccine and immunogenicity studies were conducted with and without adjuvants. The H5N1 whole virion inactivated vaccine induced considerable immune response using aluminium adjuvant (Fig. 1). Adequate exposure and successful handling of the NIBRG-14 strain along with promising immunogenicity data in mice provided confidence to advance the project to clinical development and large-scale manufacture of a H5N1 pandemic influenza vaccine at the beginning of 2009 [2].

The sudden outbreak of novel H1N1 pandemic influenza virus in May 2009 shifted our focus away from our comparative studies to develop a vaccine against the novel pandemic strain in the quickest possible time. We adopted the egg-based system because our studies indicated that egg-based technology showed slightly superior yields and was easier to retrofit into our existing production facility. Now that the H1N1 pandemic is under control, we will resume our studies to compare yields from egg- and cell-based technologies, but we will continue to use eggs for the manufacture of IIV as well as LAIV for the foreseeable future.

### 3. H1N1 pandemic vaccine development

In May 2009, SII signed an agreement with WHO to secure a sub-licence for the development, manufacture and sale of a LAIV using the backbone of attenuated strain A/Leningrad/134/17/57 from the Institute of Experimental Medicine (IEM), Russian Federation. This was fortuitous as it enabled us to shift the focus of vaccine manufacturing from IIV to LAIV in view of the certainty of higher yield of vaccine doses per egg. The development of IIV was maintained given the lack of data in administering LAIV to pregnant and lactating women, seriously immunocompromised recipients and recipients with known respiratory–pulmonary related ailments. This made it necessary to ensure that stocks of IIV were also available.

#### 3.1. Live attenuated influenza vaccine

The experience gained in growing and testing different influenza strains proved useful in designing the manufacturing process of LAIV. However, two main issues had to be tackled within the limited time available. The first challenge was to ensure stability of the vaccine, and the second was to develop a delivery system that ensured the use of the vaccine through intranasal route and not through the injectable route due to inadequate training of health-care workers. Once these challenges were overcome, proving clinical safety and immunogenicity was the final step. Scientific groups subdivided into independent virological, analytical, formulation and intranasal delivery device development, and clinical activities were put into action with clearly defined goals.

#### 3.2. The first challenge: stability of the vaccine

Today, LAIV is marketed in the United States of America (USA) as a liquid and in the Russian Federation as a freeze-dried product. Since the liquid version did not meet SII's shelf life (9 months stored at 2–8 °C) or cold chain (compatible with –20 °C) requirements for a pandemic vaccine, we opted for the freeze-dried route. SII has a lyophilization capacity of 30 million doses per year, which can be increased to 40 million doses in the existing plant in an emergency situation. The need for the process to be compatible with existing equipment was a prerequisite for rapid scale-up of operational capacity to meet the pandemic requirement.

The freeze-drying cycle development activity involves the creation and study of multiple formulations and narrowing these down to the most suitable. To reduce time, we adopted a novel approach of 'plugging' the attenuated influenza virus into a formulation containing excipients proven to be safe and effective in stabilizing an established (measles) attenuated virus vaccine. In addition to accelerating the development process, this allowed the seamless adaptation of personnel and equipment to the manufacturing process, and use of the existing supply chain and safety data for the materials. We therefore developed a LAIV formulation, the physicochemical properties of which were known. Estimates for methods and temperatures of filtration, expected losses in processing, procedures for setting titres and use of a diluting medium were based on experience with the measles vaccine. Results of subsequent studies on this 'plug in' approach matched scientifically predicted expectations.

Being a pandemic vaccine, there was a need for it to be available in multi-dose vials for mass campaigns as well as in single doses for the commercial market.

#### 3.3. The second challenge: the intranasal delivery system

The vaccine was to be reconstituted with water and administered using a system that ensures accurate measurement of dose, maximum reusable parts and for multi-dose vials, no shared contact of the device among recipients. However, certain hurdles were encountered such as producing water for inhalation for the single-dose diluent as the interaction of water for inhalation in such small volumes with type 1 glass vials resulted in conductivity shifts. While it is possible to overcome this issue with more expensive type 1 vials treated with ammonium sulphate, regulatory agencies need to review if this increase in cost is justified, as conductivity is not as relevant a parameter for intranasal administration as it is for parenteral administration.

An intranasal spray, rather than drops, was developed in order to maximize the coverage area and reduce the potential of pulmonary entrainment in recipients in the upright position. The development of the device presented major challenges since it had to be inexpensive and have a dead volume <100 µL (a loss of vaccine easily



Fig. 2. Intranasal spray device for administration of H1N1 pandemic vaccine.

compensated by increasing the titre). Existing snap-on metered dose sprays did not fit SII's 13 mm vials and would not guarantee that a consistent dose could be safely administered to multiple recipients. Therefore, a spray device fitted to the tip of a syringe was employed (Fig. 2). The syringe measured the dose accurately, and the spray device, in conjunction with the syringe, generated a spray that maximized coverage and ensured sufficient positive displacement. This eliminated the need for the recipient to lie down during administration.

Regarding packaging, there was a concern that vaccinators might mistake the vaccine as an injection if a needle is provided, especially since training in the field is not always optimum. The package was made needle free by developing a “needle-free transfer device” that cannot be used to inject the vaccine accidentally. This device is attached to a syringe to draw water from the vial, add it to the vaccine container and to withdraw the reconstituted vaccine. Similarly, the diluent was called “sterile water for inhalation” (SWFI<sub>nh</sub>) instead of “water for injection” to avoid errors. Sterile water for inhalation is covered in the US pharmacopoeia.

#### 3.4. Pandemic vaccine manufacture and clinical studies

A/17/California/2009/38 LAIV reassortant strain generated through reassortment of the A/Leningrad/134/17/57 master donor attenuated strain (IEM, Russian Federation) with H1N1 pandemic A/California/07/2009 was received in August 2009. Seed lots were prepared and characterized and a trial lot prepared to optimize processes including inoculation, harvesting clarification, purification and concentration. The same lot was used to assess the formulation and freeze-drying procedures, as well as to validate quality control tests.

A second lot was prepared for toxicity studies in mice and rats in October 2009. These studies revealed no toxic effects at doses higher than the intended human dose. The vaccine was tested in mice challenge studies (National Institute of Virology, Pune, India) and was found to induce protective immunity against the wild type strain. Ferret challenge studies were conducted with a single dose of LAIV with significant induction of haemagglutination inhibition (HAI) and microneutralization (MN) antibodies and complete protection against virus challenge (Fig. 3 and Table 1). This study was conducted in collaboration with WHO at Viroclin, The Netherlands. A third lot was prepared and released for clinical trial purposes by the SII quality control laboratory and the Indian National Control Authority (NCA) in January 2010.

A Phase I, double-blind randomized study in 50 healthy adults aged 18–49 years compared a placebo and a single dose of the study vaccine [ $10^7$  of the 50% egg infectious dose (EID<sub>50</sub>)] to assess safety over 42 days (CTRI/2010/091/000008). No serious adverse events (SAEs) or unsolicited events were reported. All solicited reactions were mild in intensity and all were resolved without sequelae within 2–3 days.

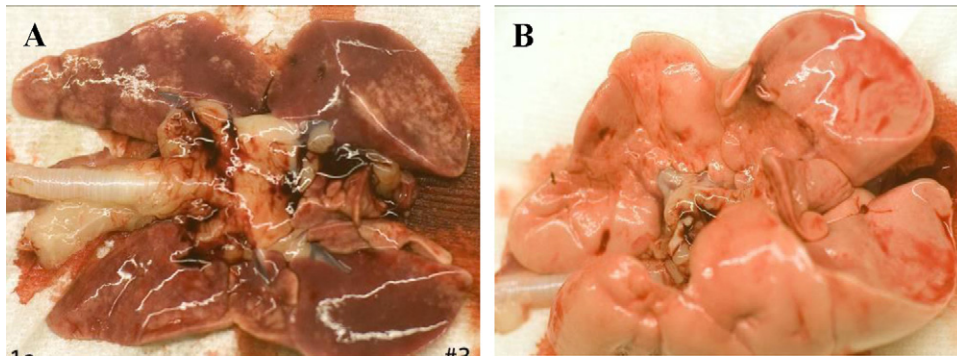
The Phase II/III double-blind randomized trial involved 330 individuals (110 adults, 110 elderly and 110 adolescents and children  $\geq 3$  years) at five sites in India (CTRI/2010/091/000092). Subjects received either a placebo or  $10^7$  EID<sub>50</sub> dose of the study vaccine. The vaccine was found safe in all age groups. No SAEs were reported and none of the unsolicited events in either group was causally related to the study products. The solicited reactions were similar in both groups, all of which were mild and all resolved without sequelae.

Although LAIV has been proved to be highly efficacious in preventing influenza virus infection, the serological correlates of protection are not well established. From studies characterizing the immune response following intranasal administration of LAIVs, cell-mediated immunity (CMI) is considered to have a role in protection in adults and children that cannot be entirely explained by mucosal or serum antibody responses. So far, the role of CMI in protection against clinical influenza has not been established in the field, due to the technical difficulties of using these complex assays. WHO recommended that an appropriate approach to evaluate the immunogenicity of LAIVs in clinical trials would be to show significant uptake (e.g., that a majority of vaccinees respond), measured by combining results from a panel of tests.

In our study, immunogenicity was assessed on Day 0 and 21 by HAI, MN, and IgG from serum samples. An in-house IgA detection assay from nasal wash/swab samples was developed, validated and used to test mucosal IgA response. The immune response induced by the vaccine was in line with published studies on LAIV [3–5].

The above studies were conducted in accordance with the International Conference on Harmonization–Good Clinical Practice (ICH–GCP) guidelines; the Declaration of Helsinki (Seoul 2008); Guidelines for Clinical Trials on Pharmaceutical Products in India – GCP Guidelines issued by the Central Drugs Standard Control Organization (CDSCO), 2001; Requirements and Guidelines for Permission to Import and/or Manufacture of New Drugs for Sale or to undertake Clinical Trials (Schedule Y, 2005); and Ethical Guidelines for Biomedical Research on Human Subjects issued by the Indian Council of Medical Research (ICMR), 2006.

Once the production process was optimized for bulk LAIV vaccine lots, process validation studies were completed on three



**Fig. 3.** Protective efficacy studies of pandemic LAIV in ferrets. Lungs from ferrets challenged with wild type H1N1 were examined macroscopically. (A) Severely affected dark hyperaemic and consolidated lung from placebo-treated control group. (B) Lungs from a vaccinated animal without discernible lesions. Normal aerated lung tissue is bright pink in colour.

**Table 1**

Immunogenicity of LAIV A/17/CALIFORNIA/2009/38 (H1N1) in ferrets after a single-dose immunization.

Haemagglutination inhibition assay Group/testing viruses	Average haemagglutinin titre (6 ferrets)		
	Serum Day 0 A/California/EURRG4/09	Serum Day 20 A/California/EURRG4/09	Serum Day 20 A/Netherlands/EURRG02/09
Negative control	5	5	5
SII clinical trial grade PLAIV	5	433	487

Source: ViroClinics, The Netherlands.

consecutive lots for licensing. The results of these studies met all critical process parameters for the manufacturing process. Following review by the Drug Controller General of India (DCG(I)) and the NCA, the final licence was issued on 3 July 2010. The vaccine was launched in India on 14 July 2010 under the brand name Nasovac® and as at November 2010, more than 2.5 million doses have been distributed.

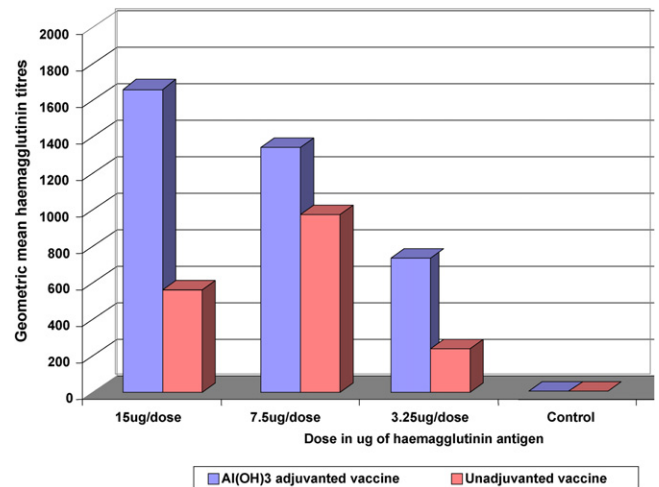
### 3.5. Inactivated influenza vaccine

In order to be able to provide vaccine for pregnant and lactating women, seriously immunocompromised recipients and recipients with known respiratory–pulmonary related ailments, the IIV development programme was undertaken in parallel to the LAIV programme.

A seed lot was prepared using the NYMC X-179A vaccine strain (similar to the A/California/07/2009 (H1N1) strain) obtained from the National Institute for Biological Standards and Control (NIBSC), United Kingdom in July 2009. A trial lot of inactivated H1N1 pandemic vaccine was prepared based on the knowledge acquired during the development of the H5N1 candidate vaccine. This trial lot adjuvanted with aluminium hydroxide gel was filled in single dose vials and used for in-house immunogenicity testing in mice. The data from these tests were very encouraging as two doses given 21 days apart at a concentration as low as 3.75 µg per dose produced ≥1:40 haemagglutination inhibition (HAI) titres in all immunized mice (Fig. 4).

A second lot was filled, quality tested and released, and used for toxicology studies: two single-dose and two repeated-dose studies in mice and rats were successfully completed by an external accredited laboratory. Upon approval from the DCG(I), an Independent Review Board and the Institutional Ethics Committee for the Phase I trial, a production scale IIV bulk lot was prepared for clinical studies in a renovated and Good Manufacturing Practice (GMP)-compliant facility.

The Phase I, double-blind, randomized study in 50 healthy adults aged 18–49 years (CTRI/2010/091/000082) compared the safety of two Al(OH)<sub>3</sub> adjuvanted whole virion formulations (10 µg and 15 µg haemagglutinin (HA) per dose). Satisfactory



**Fig. 4.** Inactivated H1N1 influenza vaccine immunogenicity studies in mice.

42-day follow-up data led to authorization for the Phase II/III double-blind, randomized study, carried out in 330 individuals (110 adults, 110 elderly and 110 adolescents and children ≥3 years) at five sites in India (CTRI/2010/091/000093). Following single dose of either 10 µg or 15 µg HA of the study vaccine given intramuscularly at 21 days apart, safety and immunogenicity were assessed and the vaccine found safe in all age groups. After 42 days of follow-up, no SAEs were reported and none of the few unsolicited events detected in each group was causally related to the study products. All solicited reactions reported in the groups were similar, mild in intensity and resolved without sequelae. Immunogenicity was assessed on Day 0 and 21 by HAI assay. The vaccine-induced immune responses of both formulations were in line with published studies [6–8]. Seroprotection and seroprotection (HI titres ≥1:40) rates met the requirements of the European Medicines Agency (EMA) and the US Food and Drug Administration (FDA) for licensure in all three age groups.

The DCG(I) granted the licence to market the 15 µg adjuvanted vaccine on 6 August 2010. As soon as six months of stability data are available, the 10 µg formulation will be registered and launched under the brand name Enzavac® in India.

#### 4. Regulatory support

All the clinical studies were approved by the DCG(I), the Independent Review Board and the Institutional Ethics Committee. Additionally, all data were periodically reviewed and approved by an independent Data Safety Monitoring Board. Among other controls, an on-site regulatory audit for the manufacturing processes and quality control testing was carried out by an inspection team from WHO, the CDSCO/DCG(I), and local FDA in April 2010.

During the entire clinical development and licensing of the IIV and LAIV, SII was actively supported by the government agencies since the need for a pandemic vaccine in India was clear. As a result, they approved importation of the H1N1 vaccine strain, clinical trial protocols and finally licensure on a fast-track basis.

In parallel, SII proactively apprised these agencies of developments at each stage of the project. For instance, while requirements for the production and use of IIV are long established, the WHO guidelines for the manufacture and evaluation of LAIV were being updated. Policy-makers and the scientific community were also apprehensive over issues such as potential reversion of attenuation to virulent phenotype, emergence of more pathogenic viruses from reassortant between vaccine strain and wild type strain, and limited safety data. Extensive brainstorming activities took place with these groups under the auspices of the ICMR and the Ministry of Health and Family Welfare to clarify any uncertainties. WHO's position on the use of LAIV during an influenza pandemic, and data on its use for routine immunization in the Russian Federation for the last 30 years and in the USA since 2003 were also presented. This approach was invaluable in developing an objective understanding of the safety and efficacy of LAIV, and aided in obtaining marketing authorization.

#### 5. Post-marketing surveillance

Exhaustive post-marketing surveillance in a large population has been completed and has shown the vaccine to be safe. No SAE caused by Nasovac®, or vaccine failure, have been reported after widespread use. Periodic Safety Update Reports were submitted every two weeks for the first 3 months and these will continue to be submitted on a monthly basis for a further year. The same post-marketing surveillance activities will be followed for IIV (Enzavac®).

#### 6. Discussion

SII is the only private manufacturer among the initial six grantees of the WHO influenza technology transfer project. Important advantages of this have been our flexibility in making decisions both on financial and technical issues, which is critical in handling an emergency situation. At the onset of the H1N1 outbreak, for example, we immediately converted a renovated measles vaccine production block for influenza vaccine and dedicated a complete facility to fill and freeze-dry the vaccine. In addition, we could rapidly reposition a pool of experts to oversee influenza vaccine manufacture along with the necessary budgetary and management support to address technical, scientific and regulatory issues. On the other hand, a disadvantage observed during interactions with policy-makers was the notion that the intentions of a commercial enterprise are automatically biased. Significant effort had to be invested to prove this assumption wrong.

Regarding production prospects, we plan to produce at least 3–5 million doses of live attenuated seasonal trivalent vaccine and examine the potential market for the combined North–South hemisphere vaccine production with a view to manufacturing seasonal influenza vaccine for the following year. Our installed capacity is currently around 15 million doses of trivalent vaccine with the potential for scale-up to nearly 30 million doses in 2011. We have enormous freeze-drying capacity, which means that we need to focus only on considerations of bulk production.

However, in order to sustain the production of influenza vaccine and to be able to address a pandemic situation, we need to maintain a pool of qualified human resources who are up-to-date on the latest developments in the field of influenza, along with a small R&D capacity to undertake virological experiments. The ability to handle a pandemic threat also depends to some degree on the existence of a routine influenza vaccination programme because this would create the demand needed to make influenza vaccine manufacturing financially feasible. We cannot rely on the Government of India as a prospective buyer for this purpose since there are many health priorities that precede the adoption of a seasonal influenza vaccination policy. On the other hand, with WHO prequalification, a user-friendly delivery system and an affordable vaccine, we expect to be able to offer LAIV to United Nations agencies for inclusion by developing countries in their national immunization programme (the WHO technology transfer grant stipulates that at least 10% of our pandemic influenza production must be made available to this channel). In this way, we hope to be able to sell sufficient vaccine to sustain our manufacturing activity.

Given that LAIV will be new to most countries, we also expect the need for awareness-building over at least a year before the vaccine will be taken up. To this end, SII proposes to undertake further studies on LAIV, for example to elucidate immunological correlates of protection. To understand better the mechanisms of LAIV protection with homologous as well as drifted strains, SII would like to explore a human challenge trial using LAIV and carry out well controlled experiments to collect more data on cell-mediated immunity and other immunological parameters. However, this research would require additional financial and scientific support.

The opportunity to work on influenza vaccine has opened up a new era of South–South cooperation. For example, SII and the Government Pharmaceutical Organization (GPO) in Thailand have been collaborating on the development of LAIV ever since seed strains became available from IEM. Among other initiatives, the GPO team visited SII to acquire the techniques and skills for their own development of LAIV. In a health crisis such as an influenza pandemic, science should override commerce and SII is committed to such collaborations.

#### 7. Conclusion

The WHO project to build capacity in developing countries to manufacture pandemic influenza vaccine has provided India with the critical skills needed to help protect its 1.2 billion population from a deadly influenza pandemic. The technical inputs and excellent coordination by the WHO team were of immense help in resolving several technical issues and enabling swift and pivotal decision-making. Our ability to develop and market a pandemic LAIV in such record time was partly due to our long-standing experience in vaccine manufacture, our qualified staff, and this WHO collaboration. However, with hindsight, this would not have happened without the exceptional ingenuity and commitment of the SII team, who subdivided into independent virological, formulation, analytical methods and clinical development groups, and achieved their defined goals in the face of stringent time constraints.

In the future, LAIV and tissue culture may be the way forward, and SII will continue its research and development efforts to remain at the forefront of providers of solutions to major public health priorities.

SII also intends to complete the work on development of candidate H5N1 vaccine which we were compelled to discontinue in the wake of H1N1 outbreak.

### Acknowledgements

The authors are particularly grateful to the following individuals and their colleagues for their invaluable contribution to the success of this project: Dr Marie-Paule Kieny, WHO, Switzerland; Dr John Wood, NIBSC, United Kingdom; Professor Larisa Rudenko, IEM, Russian Federation; the Centers for Disease Control and Prevention, USA; Dr A.C. Mishra, Dr V.A. Arankalle, Dr S.D. Pawar, and Dr J. Mullick, National Institute of Virology, India; Dr Albert Osterhaus, ViroClinics, Erasmus University, The Netherlands.

*Conflict of Interest Statement:* Funding for this study was partially provided by The World Health Organization. Rajeev Dhere, Leena Yeolekar, Prasad Kulkarni, Ravi Menon, Vivek Vaidya, Milan

Ganguly, Parikshit Tyagi, Prajakt Barde and Suresh Jadhav are employees of Serum Institute of India, Pune, India.

### References

- [1] Meeting with international partners on influenza vaccine technology transfer to developing country vaccine manufacturers, 29–30 October 2007. World Health Organization, WHO/IVB/08.09.
- [2] Jadhav S, Dhere R, Yeolekar L, Gautam M. Influenza vaccine production capacity building in developing countries: example of the serum institute of India. *Procedia Vaccinol* 2010;2:166–71.
- [3] Rudenko LG, Lonskaya NI, Klimov AI, Vasilieva RI, Ramirez A. Clinical and epidemiological evaluation of a live, cold-adapted influenza vaccine for 3–14-year-olds. *Bull World Health Organ* 1996;74(1):77–84.
- [4] Powers DC, Sears SD, Murphy BR, Thumar B, Clements ML. Systemic and local antibody responses in elderly subjects given live or inactivated influenza A virus vaccines. *J Clin Microbiol* 1989;27(12):2666–71.
- [5] Karron RA, Callahan K, Luke C, Thumar B, McAuliffe J, Schappell E, et al. A live attenuated H9N2 influenza vaccine is well tolerated and immunogenic in healthy adults. *J Infect Dis* 2009;199(March (5)):711–6.
- [6] Zhu FC, Wang H, Fang HH, Yang JG, Lin XJ, Liang XF, et al. A novel influenza A (H1N1) vaccine in various age groups. *N Engl J Med* 2009;361(25):2414–23.
- [7] Clark TW, Pareek M, Hoschler K, Dillon H, Nicholson KG, Groth N, et al. Trial of 2009 influenza A (H1N1) monovalent MF59-adjuvanted vaccine. *N Engl J Med* 2009;361(25):2424–35.
- [8] Greenberg ME, Lai MH, Hartel GF, Wichems CH, Dawson G, Hu W, et al. Response to a monovalent 2009 influenza A (H1N1) vaccine. *N Engl J Med* 2009;361(25):2405–13.