Live Attenuated Influenza Vaccines with Altered NS1

Technology Overview

<table>
<thead>
<tr>
<th>Summary</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Transformative Technology:</strong> Live attenuated influenza vaccines (LAIVs) with precise, genetically stable truncations of the NS1 gene -- rationally modulated and optimized.</td>
</tr>
<tr>
<td><strong>Potent Immunogenicity, Self-adjuvanted:</strong> NS1-altered LAIVs elicit host interferon, activating T- and B-cell immune responses, and eliciting both mucosal and serum immunity.</td>
</tr>
<tr>
<td><strong>Proof of Concept:</strong> Preclinical studies in 4 different animal models demonstrate safety, immunogenicity and protection against matched and mismatched influenza strains.</td>
</tr>
<tr>
<td><strong>Seasonal and Pandemic Programs:</strong> Initial focus on LAIVs for seasonal influenza in adults age 50 and older, one of the most vulnerable groups to influenza and its complications; available vaccines have relatively poor efficacy in this age group.</td>
</tr>
<tr>
<td><strong>Advanced Product Concept:</strong> Single, low-dose immunization via intranasal administration (needle-free) can induce a potent immune response, with potential for long-lasting immunity and cross-protection.</td>
</tr>
</tbody>
</table>

Introduction

Vivaldi Biosciences Inc. is developing live attenuated influenza vaccines (LAIVs) for the prevention of seasonal and pandemic influenza. Vivaldi’s LAIVs are based on licensed technologies developed by Peter Palese, PhD and Adolfo García-Sastre, PhD at the Mount Sinai School of Medicine. Drs. Palese and García-Sastre devised methods to create specifically altered recombinant influenza virus genomes, and characterized the mode of action of nonstructural protein 1 (NS1), a key virulence factor of influenza and other negative-strand RNA viruses. In addition to influenza, Vivaldi’s proprietary technologies are applicable to development of vaccines and antiviral drugs for other human respiratory diseases, including respiratory syncytial virus and parainfluenza.

Vivaldi is developing LAIV candidates by altering the NS1 gene. By genetically engineering precise truncations in NS1, Vivaldi is developing LAIVs that replicate less well in the host than wild-type influenza and partially induce interferon, thus eliciting potent immune responses. Host interferon acts as an endogenous adjuvant, stimulating T and B cell responses. Vivaldi’s approach to LAIV development is unique in that: 1) the mechanism of attenuation is established, 2) the level of attenuation can be rationally modulated, 3) vaccine strains with multiple levels of attenuation can be evaluated simultaneously in the clinic to select a candidate with the proper balance of immunogenicity and safety (lack of reactogenicity), and 4) each NS1-altered vaccine strain has a single defined modification that is genetically stable.

Vivaldi’s initial focus is NS1-altered LAIVs for seasonal influenza for adults age 50 and older. This age group is the most vulnerable to influenza and its serious complications, yet available vaccines have relatively poor efficacy in individuals over age 50. Trivalent inactivated vaccines for seasonal influenza are only 30% to 40% protective for illness in the elderly, and 60% effective in the elderly, based on a death endpoint.
Vivaldi has established GMP production in egg substrate, and is developing methods to produce its NS1-altered LAIVs in standardized Vero cell culture. Cell substrate manufacturing is expected to enable rapid, scalable and efficient production, which is essential for timely distribution of seasonal vaccines, and especially crucial in the event of a pandemic.

**Live Attenuated Influenza Vaccines (LAIVs)**

Live attenuated vaccines are considered the “gold standard” for diseases such as polio, MMR, yellow fever and VZV; in particular, efficacy of live attenuated vaccines for VZV in the elderly is well-established. Live attenuated vaccines show similar promise and advantages for influenza. LAIVs are more rapidly and efficiently produced than inactivated vaccines, and because of in vivo expression and replication, they have the potential to be more effective. LAIVs are administered intranasally (needle-free). Unlike inactivated vaccines, LAIVs induce mucosal immunity, neutralizing the virus at the point of entry, as well as systemic humoral and cell-mediated immune responses. Host neutralizing antibodies mediate protection against re-infection; viral clearance is mediated by cellular immunity (Th1 response consisting of virus-specific IFN-γ-secreting CD4 T cells and cytotoxic CD8 T cells). The only available LAIV in the US is FluMist®, which was approved in 2003. FluMist® uses an influenza strain with multiple cold-adaptive and other function-modulating point mutations that result in attenuation. FluMist® is broadly protective in children, but is not approved for adults age 50 and older. In contrast, Vivaldi’s approach to viral attenuation is based on altering a virulence factor of influenza, NS1, rather than attenuation based on the virus’s own replication efficiency.

**Alteration of Nonstructural Protein 1 (NS1)**

NS1 is a multifunctional protein that is a key virulence factor of influenza due to its ability to block the host’s interferon response. The N-terminal domain of NS1 binds double-stranded RNA to block type 1 interferon induction, while the C-terminal effector domain modulates the strength of the anti-interferon activity by suppressing host post-transcriptional processing of mRNAs. NS1 also inhibits adaptive immunity by attenuating dendritic cell maturation and the capacity of these cells to induce T cell responses (Fernandez-Sesma, et al., 2006).
In wild-type influenza virus, NS1 blocks the host’s interferon-mediated response, allowing the virus to replicate and cause disease. Influenza virus in which the NS1 gene has been deleted completely cannot counter the host interferon response; infection of cells with virus lacking NS1 results in induction of host interferon and viral replication is blocked. When truncations are made in the NS1 gene, virus strains are generated with intermediate activity, able to replicate in the host and induce potent antibody and cellular immune responses without causing disease. Host interferon acts as an endogenous adjuvant by enhancing production of immunoglobulins and contributing to activation of dendritic cells required for antigen presentation and the cellular immune response. Thus, NS1-altered LAIVs are in effect “self-adjuvanted”. This adjuvant-like activity and potentially qualitatively different immune response with NS1-altered LAIVs may translate into lower doses of vaccine required to induce a robust, protective and possibly cross-protective immune response in man.

Attenuation by truncating the NS1 gene can be precisely manipulated by decreasing the length of the amino terminal portion of NS1 with a single defined alteration that is genetically stable. Thus, the levels of attenuation and immunogenicity can be optimized by the degree of truncation of the NS1 gene. Vivaldi is exploiting this property to develop LAIVs with different attenuation and replication characteristics, suitable for different patient groups, based on age or immune status. Vivaldi’s intellectual property includes domestic and international rights to over 25 issued patents relating to vaccines with alterations of the NS1 gene, including certain exclusive rights to the use of reverse genetics for viruses containing modifications of NS1.
Proof of concept for NS1-altered LAIVs has been demonstrated in 4 model species: mouse, pig, horse and macaque, and the data have been published (Talon, et al., 2000; Richt, et al., 2006; Vincent, et al., 2007, Quinlivan, et al., 2005). Preclinical studies with these animal models demonstrated viral attenuation based on NS1 partial truncations. Intermediate growth characteristics also were demonstrated in vitro. Safety was demonstrated in vivo, with self-limited local and systemic inflammatory responses, and non-productive respiratory infection. The NS1-altered LAIVs were shown to be immunogenic; the vaccine candidates induced both antibody and T-cell immunity, with robust CD4+ T-cell and hemagglutinin inhibition (HI) antibody responses. Moreover, protection and cross-protection were demonstrated. Studies of NS1-altered LAIVs in equine and porcine models demonstrated cross-protection upon challenge with mismatched strains. For example, an H3N2 strain with a specific truncation in the NS1 gene (amino acids 1-126 remaining) induced protective immunity in pigs when challenged with the wild-type strain, and also provided cross-protection when challenged with H1N1 (Richt, et al., 2006).

### Protection and Cross-Protection of Pigs Vaccinated with Tx98 H3N2 NS1 1-126 After Challenge with WT Tx98 H3N2 and Mn99 H1N1

<table>
<thead>
<tr>
<th>Immunization</th>
<th>PBS</th>
<th>Tx98 H3N2</th>
<th>Tx98 H3N2 1-126</th>
<th>Mn99 H1N1</th>
<th>Mn99 H1N1 1-126</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Titers, log pfu/ml</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nasal, day 2</td>
<td>2.4 ± 1.7</td>
<td>0.0 ± 0.0</td>
<td>1.7 ± 1.0</td>
<td>0.7 ± 1.2</td>
<td></td>
</tr>
<tr>
<td>Nasal, day 5</td>
<td>4.5 ± 0.0</td>
<td>0.0 ± 0.0</td>
<td>4.3 ± 0.4</td>
<td>1.4 ± 1.7</td>
<td></td>
</tr>
<tr>
<td>BAL, day 5</td>
<td>5.6 ± 0.5</td>
<td>0.0 ± 0.0</td>
<td>5.1 ± 0.4</td>
<td>0.4 ± 1.2</td>
<td></td>
</tr>
</tbody>
</table>

### NS1-Altered LAIV Strain Development and Production

Vivaldi’s reverse genetics advances include a plasmid-only rescue technology developed by Drs. Palese and Garcia-Sastre which makes it possible to engineer stable deletions in the influenza genome, and allows the preparation of recombinant vaccine seed strains much more rapidly than is possible with standard strain development techniques. The method takes as little as one week to generate appropriate seed viruses for vaccine manufacture, dramatically accelerating the timeframe for obtaining seed virus versus classical reassortment and passaging, which typically takes 6 to 8 weeks. Unlike conventional reassortment, this allows generation of standardized seed strains, and the NS1 mutations are genetically stable. The “backbones” of the recombinant viruses can be prepared, tested, and distributed in advance, providing a significant advantage over standard methods for preparing influenza vaccines. Vivaldi’s technologies thus provide for rapid, efficient and flexible development and production of seasonal vaccines for new circulating influenza strains, and pandemic vaccines to meet the urgent demands of an emergent pandemic strain.

Vivaldi has established GMP production of its NS1-altered LAIVs in egg substrate, and is developing methods for standardized production of NS1-altered LAIVs in interferon-deficient Vero cell lines. Vivaldi also is establishing feasibility of production in MDCK cells. Vivaldi’s proprietary Vero cell-based methods are expected to provide for rapid, scaleable and cost-effective production of NS1-altered LAIVs. Vero cell culture systems for human vaccines are well established, and have a solid regulatory track record. Vero cell culture provides several important advantages over conventional egg-based production of influenza.
vaccines, including an unlimited supply of substrate, the ability to control substrate formulation, rapid scale-up and greater capacity.

In summary, Vivaldi’s NS1-altered LAIVs are expected to provide the following important advantages:

- Superior efficacy in general and vulnerable populations, including individuals age 50 and over
- Cross-protection against variant (mismatched) strains via cell-mediated immunity and interferon response
- Vaccine attenuation / replication optimized for specific target populations (i.e., elderly)
- Protective immune response, conferring mucosal immunity, and cellular plus humoral systemic immunity
- Efficacy with a single dose, and low inoculation size in non-immune individuals and populations
- Intranasal (needle-free) administration
- Standardized egg substrate or cell culture production
- Faster development of relevant seed virus for seasonal and pandemic variants
- Stable, defined genetic sequence, with potential for streamlined release testing
- Highly feasible approach for production scale suitable for pandemic scenario

**Vivaldi Biosciences Scientific Advisory Board**

Peter Palese, PhD: Horace W. Goldsmith Professor and Chair, Department of Microbiology, Professor of Medicine, Mount Sinai School of Medicine; Member, National Academy of Sciences

Adolfo García-Sastre, PhD: Professor of Microbiology, Professor of Medicine, Director, Emerging Pathogens Institute, Mount Sinai School of Medicine

Elliott Kieff, MD, PhD: Harriet Ryan Albee Professor of Medicine, Professor of Microbiology & Molecular Genetics; Co-Director, Channing Laboratory, Department of Medicine, Brigham & Women’s Hospital / Harvard Medical School; Senior Physician, Brigham & Women’s Hospital; Member, National Academy of Sciences and Institute of Medicine

Robert Belshe, MD: Professor, and Director, Center for Vaccine Development, St. Louis University School of Medicine

Ann Arvin, MD: Lucile Salter Packard Professor of Pediatrics and Professor of Microbiology & Immunology, Stanford University School of Medicine, Vice Provost and Dean of Research, Stanford University

**Company Information**

Vivaldi Biosciences Inc.
Bellevue Hospital Center, 462 First Avenue, Building A, 9th Floor
New York, NY 10016-9196
Tel: 646-381-6680
www.vivaldibiosciences.com

Douglass B. Given, MD, PhD    David Liebowitz, MD, PhD
President & CEO    Chief Scientific Officer
Tel: 646-381-6681    Tel: 646-381-6682
doug.given@vivaldibiosciences.com    dave.liebowitz@vivaldibiosciences.com
Selected References


