RSVing for site zero

By Tracey Baas, Senior Editor

Despite decades of research, respiratory syncytial virus (RSV) remains a highly prevalent childhood pathogen without an approved vaccine.¹ There is a marketed prophylactic—Synagis palivizumab—to prevent severe disease caused by RSV in at-risk infants, but the passive immunization provided by the antibody does not last from season to season, and its high cost precludes its use in other patient populations. Now, a team from the **NIH** has used structure-based design to generate RSV vaccines that showed strong neutralizing activity in both mice and macaques.²

The next step is picking a lead vaccine to advance into GMP production and clinical trials.

RSV is the most common cause of hospitalization in children under 5 years of age and results in more than 3 million hospital stays each year. RSV mortality in the elderly is comparable to that of influenza virus.^{3,4}

There are multiple barriers to developing RSV vaccines. These include the very young age of most patients, the lack of a good animal model that recapitulates human RSV infection, and the virus' ability to both evade innate and adaptive immunity and reinfect patients.

The most substantial barrier came to light decades ago when one of the first candidate vaccines—formalin-inactivated RSV (FI-RSV)—not only failed to protect children in early trials but also enhanced virus-induced respiratory disease and led to two deaths and a high hospitalization rate.

The vaccine-enhanced disease is thought to be caused by the induction of high levels of non-neutralizing antibodies.

Ideally, an effective vaccine would induce high levels of neutralizing antibodies.

Synagis is marketed by **AstraZeneca plc's MedImmune LLC** unit to prevent RSV in premature infants at high risk of infection. The humanized mAb targets RSV F, which is a trimeric glycoprotein the virus uses to enter host cells via membrane fusion. The mAb posted sales of \$1.04 billion in 2012.

Although RSV F is clearly a good starting point for vaccine development, the conformational diversity of the target makes engineering a good antigen difficult.

Before virus-cell interactions, RSV F exists in an unstable, lollipop-shaped conformation. After the merging of virus and cell membranes, RSV F exists in a stable, crutch-shaped conformation.

The stable, postfusion RSV F is currently being used to develop vaccine candidates⁵ (see Table 1, "RSV pipeline").

But in recent years, a team from the NIH and China identified 5C4 and another team from antibody company **AIMM Therapeutics B.V.** identified AM22 and D25—all three strongly neutralizing antibodies that target a site

dubbed antigenic site zero in prefusion RSV F.⁶ Antibodies targeting site zero were 10- to 100-fold more potent than Synagis.

Based on that work, the NIH team suspected that vaccines able to induce neutralizing antibodies against prefusion RSV F could be a better approach than using postfusion RSV F.

Now, the NIH team has bridged the gap between site zero-targeting antibodies and an actual vaccine. The group used structure-based design to engineer antigens based on soluble variants of the prefusion RSV F protein that have stably exposed antigenic site zero.

The researchers modified F protein to include cysteine pairs, cavity-filling hydrophobic substitutions and a C-terminal trimerization domain dubbed a foldon. They constructed more than 100 such variants and looked for those that bound the D25 antibody for at least a week.

Among the top four variants, X-ray crystallography showed that the stabilized proteins included some version of the antigenic site zero fixed in a D25-bound conformation.

The team dosed mice and rhesus macaques with the variants plus the adjuvant poly inosine:cytosine formulated in polylysine carboxymethylcellulose (poly-ICLC) and then tested the ability of the animals' sera to prevent RSV infection in human cells.

In mice, the top variant elicited neutralizing activity that was 8-fold better than that caused by stable, postfusion F protein and 40 times the threshold for protection. In macaques, this same variant led to neutralizing activity that was 70 times greater than that of the postfusion F protein.

Results were published in *Science*.

Vaccine reality

The next step for the team is GMP manufacturing of a lead variant in preparation for a Phase I trial.

"It's not clear if additional improvements to the antigen are needed," said Jason McLellan, the study's lead author, who is now an assistant professor of biochemistry at the **Geisel School of Medicine at Dartmouth**. "Additional modifications will be made and tested. Removal of the foldon domain may be required."

JoAnn Suzich, VP of research for infectious diseases and vaccines at MedImmune, agreed. "Ideally, a vaccine for humans would not contain extraneous protein sequence such as a foldon. At the very least, the similarity of foldon sequence to any human protein sequences would need to be explored, and the potential for antibodies or T cell responses directed against this domain to cross-react with human tissues should be addressed."

Gregory Glenn, SVP and CMO of vaccine company **Novavax Inc.**, said that it will be important to test the vaccine "with an adjuvant that is already approved for use in humans rather than the poly-ICLC adjuvant used in the study."

"In terms of challenge studies, there is unfortunately not an optimal animal study [or] challenge model for RSV. We will be moving forward toward clinical studies to evaluate safety."

**—Peter Kwong,
National Institutes of Health**

Table 1. RSV pipeline. Only one product is marketed to prevent serious lower respiratory tract disease caused by respiratory syncytial virus (RSV). At least 12 other compounds are being developed to prevent or treat RSV-induced respiratory disease.

Source: *BCIQ: BioCentury Online Intelligence*

Company	Product	Description	Status
Vaccines			
Novavax Inc. (NASDAQ:NVAX)	RSV F vaccine	RSV vaccine directed against RSV F protein and formulated in virus-like particles	Phase II
AstraZeneca plc's (LSE:AZN; NYSE:AZN) MedImmune LLC unit	MEDI-559	Live attenuated RSV vaccine	Phase I
GlaxoSmithKline plc (LSE:GSK; NYSE:GSK)	GSK3003891A	Immunostimulant vaccine	Phase I
	Pan-adenovirus type 3 (PanAd3)/modified vaccinia Ankara (MVA) virus RSV vaccine	Heterologous prime-boost regimen with vectors encoding a string of RSV F, N and M proteins bearing both B and T cell epitopes	Phase I
	RSV vaccine	Vaccine based on the company's PanAd3/MVA virus RSV vaccine	Phase I
Antibodies			
AstraZeneca/AbbVie Inc. (NYSE:ABBV)	Synagis palivizumab	Humanized mAb against RSV F	Marketed
MedImmune	Motavizumab (MEDI-524)	Humanized mAb against RSV F	Phase III
Ablynx N.V. (Euronext:ABLX)	ALX-0171	Single variable domain antibody fragment against RSV F	Phase I
Other modalities			
Alnylam Pharmaceuticals Inc. (NASDAQ:ALNY)/ Kyowa Hakko Kirin Co. Ltd. (Tokyo:4151)	ALN-RSV01	Small interfering RNA targeting RSV nucleocapsid gene	Phase II
Gilead Sciences Inc. (NASDAQ:GILD)	GS-5806	Undisclosed	Phase II
Arrow Therapeutics Ltd./Novartis AG (NYSE:NVS; SIX:NOVN)	RSV604	Nucleoprotein inhibitor	Phase II
MicroDose Therapeutx Inc.	MDT-637	Inhalable, small molecule, antiviral fusion inhibitor delivered using dry powder nebulizer technology	Phase I
Alios BioPharma Inc.	ALS-8176	Oral RSV nucleoside analog	Phase I

Glenn wanted to see the vaccine used in cotton rats challenged with RSV infection, but Peter Kwong said that such studies would yield little information. Kwong is chief of the Structural Biology Section at the NIH's Vaccine Research Center and one of the corresponding authors on the paper.

"In terms of challenge studies, there is unfortunately not an optimal

animal study [or] challenge model for RSV," he said. "We will be moving forward toward clinical studies to evaluate safety."

Barney Graham said "that while mice and cotton rats are both semipermissive for RSV infection, they are commonly used for preclinical evaluation of RSV vaccine safety. However, when vaccination results in such high neutralization titers, it is difficult to make an assessment of vaccine-enhanced disease because infection cannot be established."

Graham is chief of the Clinical Trials Core and Viral Pathogenesis Laboratory at the NIH's Vaccine Research Center and the other corresponding author on the paper.

On the safety front, McLellan said that the group's variants are unlikely to aggravate RSV infection. "Unlike FI-RSV, our prefusion-stabilized molecules elicit high titers of neutralizing antibodies," he said. "Also, our stabilized antigens are far less likely to elicit non-neutralizing antibodies since we're presenting the active form of the molecule to the immune system."

"These studies suggest that vaccines with the stabilized prefusion F have the best chance for efficacy of RSV vaccines tested to date," said Larry Anderson, a professor of pediatrics in the division of pediatric infectious diseases at the **Emory University School of Medicine**.

There also was consensus that the NIH's vaccine would not compete with Synagis.

"I believe a population different than the palivizumab target population would be best for this novel vaccine," said Suzich. "The immaturity of the newborn immune system makes vaccination in very young infants difficult."

According to Kwong, "The vaccine could target other populations affected by RSV such as the elderly. One could also target women of childbearing age or pregnant women." Vaccine-induced neutralizing antibodies from the mother would be actively transported to the newborn.

Graham said, "Eventually it may be possible to evaluate the vaccine in young children and perhaps, with an enlarged safety database, in infants. None of these vaccine approaches would really compete with Synagis."

The findings are patented and available for licensing from the Office of the Director at the NIH.

Baas, T. *SciBX* 6(44); doi:10.1038/scibx.2013.1247

Published online Nov. 14, 2013

REFERENCES

- Anderson, L.J. *et al. Vaccine* **31 Suppl. 2**, B209–B215 (2013)
- McLellan, J.S. *et al. Science*; published online Nov. 1, 2013; doi:10.1126/science.1243283
Contact: Peter D. Kwong, National Institutes of Health, Bethesda, Md.
e-mail: pdkwong@nih.gov
Contact: Barney S. Graham, same affiliation as above
e-mail: bgraham@nih.gov
- Graham, B.S. *Immunol. Rev.* **239**, 149–166 (2011)
- Lozano, R. *et al. Lancet* **380**, 2095–2128 (2012)

"These studies suggest that vaccines with the stabilized prefusion F have the best chance for efficacy of RSV vaccines tested to date."

—Larry Anderson,
Emory University School of Medicine

5. Swanson, K.A. *et al. Proc. Natl. Acad. Sci. USA* **108**, 9619–9624 (2011)
6. McLellan, J.S. *et al. Science* **340**, 1113–1117 (2013)

COMPANIES AND INSTITUTIONS MENTIONED

AIMM Therapeutics B.V., Amsterdam, the Netherlands

AstraZeneca plc (LSE:AZN; NYSE:AZN), London, U.K.
Emory University School of Medicine, Atlanta, Ga.
Geisel School of Medicine at Dartmouth, Hanover, N.H.
MedImmune LLC, Gaithersburg, Md.
National Institutes of Health, Bethesda, Md.
Novavax Inc. (NASDAQ:NVAX), Rockville, Md.