Subunit boost for TB vaccines

By Gaspar Taroncher-Oldenburg, Executive Editor

A long-term study in macaques by **GlaxoSmithKline plc** and collaborators bolsters the case for using a subunit tuberculosis vaccine to boost the effects of the only marketed TB prophylactic—bacille Calmette-Guérin (BCG).¹ Ongoing Phase I and Phase II trials of the subunit vaccine, dubbed M72, should pave the way for future trials of a combined BCG prime and M72 boost regimen in infants and adults.

The paper, published in the *Proceedings of the National Academy of Sciences*, is the first evidence of long-term protection against TB in adult nonhuman primates. Although BCG does a good job of protecting infants from pulmonary TB, its effects do not carry over to adults. Nor does the vaccine stop transmission or latent survival of *Mycobacterium tuberculosis*.

M72 is one of the most advanced TB boost vaccines in the clinic (see **Table 1, “Tuberculosis vaccines in development”**). It consists of a synthetic multiprotein antigen, Mtb72F, which incorporates fragments from two different *M. tuberculosis* antigens. The vaccine is formulated in GSK’s AS02A adjuvant.

The macaque data “demonstrate that protection from the standard BCG vaccine can be substantially enhanced by boosting with a defined vaccine,” said lead author Steven Reed, who is founder and CSO of the **Infectious Disease Research Institute (IDRI)** and cofounder and president of **Immune Design Corp.**

Long-term protection

There are two main strategies in TB vaccine development. The first is generating more potent prime vaccines, mainly better versions of BCG. The second is designing subunit boost vaccines consisting of defined sets of *M. tuberculosis* antigens that could be used to boost either the existing BCG vaccine or future, alternative prime TB vaccines.

“Since BCG is already a readily accepted component of pediatric vaccination worldwide, development of robust subunit vaccines that can boost an initial BCG vaccination is important for controlling TB,” said Michelle Larsen, assistant professor at the **Albert Einstein College of Medicine of Yeshiva University**. “The current vaccine, BCG, provides partial, but not long-term, protection against TB. New vaccines capable of boosting this partial immunity should be emphasized.”

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—Leander Grode,
Vakzine Projekt
Management GmbH

According to the **World Health Organization**, “it would be unethical and impractical to test new vaccine strategies that would not include BCG in early infancy. Therefore, most new TB vaccine development is being done in a BCG prime–vaccine boost strategy.” (http://www.who.int/vaccine_research/diseases/ari/en/index.html)

The BCG vaccine consists of an attenuated strain of *M. bovis*, a close relative of *M. tuberculosis*.

Because M72 consists of proteins that are present in both *M. tuberculosis* and BCG, “it provides a boost to the immune responses induced by BCG vaccination or *M. tuberculosis* infection in monkeys,” said Pascal Mettens, coauthor of the *PNAS* paper and a senior scientist for preclinical immunology at GSK’s GlaxoSmithKline Biologicals unit.

Previous work showed increased protection using the BCG prime and M72 boost approach in mouse, guinea pig and rabbit TB models.^{2–4}

The *PNAS* paper reports on a series of long-term experiments lasting about 100 weeks in which adult macaques received BCG followed 8–32 weeks later by M72, BCG or saline. All macaques were challenged with *M. tuberculosis* four weeks after the final immunization.

The combined BCG prime and M72 boost approach provided better protection than either vaccine alone, characterized by lower numbers or absence of lung infiltrates, low bacterial burdens in lung, almost a complete lack of necrotic lesions and substantially longer survival times.

According to Leander Grode, director of business development and project manager at **Vakzine Projekt Management GmbH**, “this

study represents state-of-the-art vaccine testing—in terms of the long-term protection model, it pushed the standard.”

Peter Andersen, director for infectious disease immunology and VP of vaccine R&D at the **State Serum Institute**, agreed. “The time span of 100 weeks is impressive,” he said. “The question remains whether the long-term protection reported in the paper also represents true long-term immunological memory, an important variable in assessing vaccine efficacy.”

To assess long-term immunological memory—the ability of an individual to fend off an *M. tuberculosis* infection long after immunization, “the animals should have ideally been challenged following a longer postimmunization period than the four weeks tested in the paper,” he added.

Mettens was optimistic that the results would be predictive of long-term protection in humans. “The long duration described in the *PNAS* study is unique and very encouraging since the nonhuman primate is one of the most relevant animal models for human TB,” he said.

M72 has been tested in Phase I and II trials in healthy adults in the U.S. and Europe. Phase II trials are ongoing in healthy adults in the Philippines and South Africa.

None of these trials has included infants, and although administering an M72 vaccination to an adult who had been given BCG decades before at birth is considered immunologically to be equivalent to a prime and

Table 1. Tuberculosis vaccines in development. Selected TB vaccines in clinical development.

Vaccine	Type	Description	Status	Companies/institutions
Crucell Ad35 (AERAS-402)	Boost	Adenovector 35 (Ad35)-based vaccine	Phase II	Crucell N.V. (Euronext:CRXL; NASDAQ:CRXL)/Aeras Global TB Vaccine Foundation
GSK TB010 (M72)	Boost	Fusion protein of two <i>Mycobacterium tuberculosis</i> antigens	Phase II	GlaxoSmithKline plc (LSE:GSK; NYSE:GSK)/Aeras
MVA85A	Boost	Recombinant modified vaccinia virus Ankara (rMVA) expressing antigen 85A	Phase II	University of Oxford/Emergent BioSolutions Inc. (NYSE:EBS)
SSI HyVac4 (AERAS-404)	Boost	Vaccine containing antigens Ag85B and TB10.4 combined with IC31 adjuvant	Phase I	Intercell AG (VSE:ICLL)/sanofi-aventis Group (Euronext:SAN; NYSE:SNY)/Aeras/State Serum Institute
VPM1002	Prime	Recombinant urease C-deficient listeriolysin expressing bacille Calmette-Guérin vaccine	Phase I	Vakzine Projekt Management GmbH/Max Planck Institute for Infection Biology

boost regimen, controlled BCG prime and M72 boost regimens have not yet been tested.

In addition to GlaxoSmithKline Biologicals, the team on the *PNAS* paper included scientists at the **Leonard Wood Memorial Center for Leprosy Research** and **Colorado State University**.

Vaccination markers

The *PNAS* paper has additional implications for TB vaccine developers because it provides a set of potential immunologic markers—interferon- γ (IFN- γ), tumor necrosis factor- α (TNF- α), IL-2 and IL-6—for assessing TB protection.

In the past, there has been no way to analyze the level of protection conferred by a TB vaccine. The lack of markers has thus hampered “preclinical development of novel TB vaccines as well as later-stage studies,” Yeshiva’s Larsen noted.

“The immunologic markers identified in the *PNAS* paper that may predict protection also lead us to believe that surrogate markers can be developed,” Jerald Sadoff, president and CEO of **Aeras Global TB Vaccine Foundation**, told *SciBX*.

The paper suggests that the main immune response in the macaque model is driven by CD4⁺ T cell-mediated mechanisms with a minor contribution by CD8⁺ T cells.

Immunization with M72 induced the production of IFN- γ , TNF- α and IL-2. Following *M. tuberculosis* challenge, a direct correlation emerged between the level of protection conferred by the different vaccination regimens and the ratio of IFN- γ to a fourth cytokine, IL-6.

If these markers “could be validated in terms of their ability to predict vaccine-induced protection in humans, they would become a critical tool for the development of new TB vaccines,” said Sadoff.

Reed noted that IFN- γ is a known component of the general immune response, and TNF- α has been correlated specifically with protection against TB in humans based on negative responses to drugs such as TNF- α inhibitors.

According to Andersen, Statens’ subunit TB booster vaccines are eliciting immune responses in humans that are similar to those seen for

M72 in macaques, although data haven’t been published.

Statens has two subunit vaccines in the clinic: H1, which consists of a synthetic multiprotein antigen construct incorporating the *M. tuberculosis* proteins Ag85B (fbpB) and early secretory antigenic target 6 (esxA; Esat6), and HyVac4, which contains Ag85B and the *M. tuberculosis* protein TB10.4. The two vaccines are given with IC31, an adjuvant from **Intercell AG**.

“Both vaccines have been evaluated in Europe and we have recently started testing in Africa. We have most immunogenicity data from H1 and it has given impressive levels of long-lived Th1 responses, up to 1.5 years post-vaccination,” said Andersen.

Such T helper type 1 cell (Th1)-mediated responses, which are characterized by increased levels of IFN- γ , TNF- α and IL-2, mirror the immune response seen with M72 in the *PNAS* study.

HyVac4 is now in Phase I testing in Africa with partner sanofi Pasteur, the vaccines division of **sanofi-aventis Group**, supported by Aeras. This month, Statens will start a Phase I African

study of H1 formulated in the institute’s CAF01 cationic liposomes.

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Aeras Global TB
Vaccine Foundation**

Broader responses

Although the primarily CD4⁺ T cell-mediated immune responses observed for naked subunit vaccines such as M72 and Statens Serum Institute’s H1 are encouraging, some researchers said a response that includes other arms of the immune system may be more desirable. This could potentially be achieved by developing better subunit vaccines or replacing the prime vaccine itself.

Several virus-based subunit vaccines in development are seeking to increase the CD8⁺ T cell response, including MVA85A and AERAS-402 (see **Table 1, “Tuberculosis vaccines in development”**).

According to Sadoff, virus-based subunit vaccines “are more capable of inducing CD8⁺ T cell responses” than naked subunit vaccines because the viral component of these vaccines stimulates a CD8⁺ T cell response.

University of Oxford’s MVA85A (AERAS-485) is being tested in collaboration with Aeras. Phase II trials in adults, children and infants

are underway in South Africa and a Phase IIb trial in infants is planned to begin there this year.

Crucell N.V.'s Crucell Ad35 (AERAS-402), also being tested in collaboration with Aeras, is in a Phase II trial in adults previously exposed to TB.

Other researchers are looking to replace BCG entirely in hopes of producing a stronger and more lasting immune response.

For example, Reed said "IDRI is developing next-generation vaccine candidates using novel antigens and adjuvants that can be used to prevent as well as to treat tuberculosis." He did not provide details.

Grode expects that it will take a combination of a new prime vaccine and a good booster subunit vaccine to successfully replace BCG.

A final consideration of TB vaccines is their safety and efficacy in humans already exposed to TB. According to WHO, about one-third of the world population is latently infected. As a result, said Andersen, "determining the effect of subunit vaccines such as M72 on this population and, more importantly, developing vaccines that may actually have a therapeutic effect, should be a high priority."

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COMPANIES AND INSTITUTIONS MENTIONED

Aeras Global TB Vaccine Foundation, Rockville, Md.

Albert Einstein College of Medicine of Yeshiva University, New York, N.Y.

Colorado State University, Fort Collins, Colo.

Crucell N.V. (Euronext:CRXL; NASDAQ:CRXL), Leiden, the Netherlands

GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), Rixensart, Belgium

Immune Design Corp., Seattle, Wash.

Infectious Disease Research Institute, Seattle, Wash.

Intercell AG (VSE:ICLL), Vienna, Austria

Leonard Wood Memorial Center for Leprosy Research, Cebu City, Philippines

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