Target on Ebola

By Tracey Baas, Senior Editor

Public Health Agency of Canada researchers have shown that a cocktail of three antibodies, dubbed ZMab, improved the survival of nonhuman primates infected by Ebola virus.¹ ZMab is licensed to **Defyrus Inc.**, which plans to establish commercial-scale manufacture of the therapy and carry out biodistribution and toxicology studies.

An ongoing challenge for Ebola therapies is the ability to treat the disease several days after infection, when patients typically present at the clinic. Ebola runs its course within 14–21 days, with patients first presenting nonspecific flu-like symptoms. Patients then progress and show impaired kidney and liver function, and in some cases internal and external bleeding.

There are no licensed antiviral therapies or vaccines for the virus, and treatments in development, including anticoagulants, antisense molecules and antibodies, are only effective when given within a few hours of infection.

For the past two years, the Public Health Agency of Canada (PHAC) researchers have been working to widen that treatment window using an antibody-based approach.

In prior work, they showed that each of three murine mAbs targeting the Ebola Zaire glycoproteins GP1 and GP2 and a precursor of GP1 and GP2, called GP, protected mice from Ebola challenge when given 48 hours after infection.² When combined into a cocktail, the 3 mAbs protected guinea pigs from Ebola challenge when given 48 hours after infection.³

Based on those data, the researchers decided to test the three mAbs as a cocktail in cynomolgus macaques.

At 24 hours after a lethal Ebola challenge, 3 doses of ZMab given 3 days apart protected all 4 animals from death. When the cocktail was started 48 hours after lethal challenge, 2 of 4 animals survived. In survivors, the treatment led to lower viral loads, mild symptoms, no shedding and normal blood biochemistry and hematology.

Virus isolated from one of the nonsurviving animals had developed two escape mutations, suggesting the cocktail could trigger resistance.

Results were published in *Science Translational Medicine*.

Mouse vs. human mAb

The PHAC researchers are collaborating with life sciences company Defyrus, which has exclusive rights to manufacture and commercialize ZMab.

Defyrus' lead compound is DEF201, a replication-defective adenovirus vector that encodes the gene for the antiviral human interferon- α (IFNA; IFN- α) protein. The broad-acting antiviral is in preclinical development for multiple disease indications.

Defyrus president and CEO Jeffrey Turner said DEF201 could be used in combination with ZMab. "With a suspected Ebola virus infection,

nasal delivery of DEF201 slows disease progression and buys time while diagnostics are performed to confirm an Ebola infection. Upon confirmation of Ebola infection, ZMab would then immediately be given," he said.

Defyrus plans to start clinical trials of murine ZMab within 24 months once GMP manufacturing is established and preclinical safety and toxicology are confirmed.

Gary Kobinger, PHAC team leader, said he is interested in having the murine mAbs humanized but added that "it's not a requirement to move into the clinic." Kobinger is chief of special pathogens, head of vector design and immunotherapy for the Special Pathogens Program at the National Microbiology Laboratory at PHAC and adjunct professor of medical microbiology and immunology at the **University of Manitoba**.

"We hypothesize that a humanized ZMab treatment might have a longer half-life, so we might be able to see protection with only two doses rather than three," he noted. "But our first priority is to move the murine ZMab treatment forward."

Patrick Iversen, SVP of research and innovation at **Sarepta Therapeutics Inc.**, told *SciBX* he would prioritize differently. "Humanizing the mAb is a critical next step because clinical studies using mouse mAbs for other indications showed that patients developed antibodies against mouse mAbs," he said.

Iversen also wanted to see "what type of immune responses the nonhuman primates showed toward the mouse ZMab cocktail. Further preclinical studies will need to include immunotoxicity testing in addition to standard safety pharmacology and repeat dose toxicology testing."

Sarepta's AVI-7537 antisense molecule is in Phase I testing for Ebola.

"I think his concern is legitimate because serum sickness is a real adverse effect when antibodies are administered in an individual with concomitant anti-antibodies," Kobinger said. "In our case, however, there are only three injections administered within nine days, which is not long enough for anti-antibodies to develop while the mouse antibodies are still being given."

He added, "That being said, we have already humanized the 3 monoclonals and hope to produce and test them within the next 12 months or so. In the meantime, the mouse monoclonals do work with no apparent side effect in nonhuman primates. Considering the probable 100% mortality of an accidental lab-acquired Ebola virus infection and what is available out there, I myself wouldn't worry too much about antimouse antibodies when deciding on the course of action would I be accidentally exposed to Ebola virus."

Also, deep sequencing methods will be needed to better understand potential mechanisms of emerging viral resistance, said Iversen. "The entire viral genome must be evaluated for resistance mutations in all infected animals, not just mutations in the GP gene and not just in euthanized nonsurvivors."

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—Patrick Iversen,
Sarepta Therapeutics Inc.

The group's ultimate goal "is a treatment or combination of treatments to push past the barrier of 72 hours after infection," said Kobinger. The team also wants to develop other antibody-based strategies to treat similar diseases, such as Lassa, Rift Valley and Crimean-Congo hemorrhagic fevers.

A patent application covering the paper's findings has been filed by PHAC and is exclusively licensed to Defyrus.

Baas, T. *SciBX* 5(29); doi:10.1038/scibx.2012.748
Published online July 26, 2012

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