Lab-Produced Antibody Prevents A Deadly Bacteria From Spreading Its Toxin

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Pseudomonas aeruginosa (Pa), a common bacterium found in soil and water, is called an opportunistic pathogen because it attacks individuals with weakened immune systems. In people whose lung function is already compromised — including critically ill patients who cannot breathe on their own and require assistance from a mechanical ventilator and those with cystic fibrosis (CF) — Pa infection destroys lung tissue, often leading to death.

Two scientists working across the country from one another joined forces — and laboratories — to develop a molecule called a monoclonal antibody capable of preventing the toxic effects of Pa. The antibody, now licensed to KaloBios Pharmaceuticals and humanized or converted for use in humans, is undergoing the clinical testing necessary for approval by the U.S. Food and Drug Administration."

About Pa

Because Pa thrives on moist surfaces, it can grow on ventilator tubes and move to a patient's lungs. As a result, Pa is

the most common cause of pneumonia in patients who are on mechanical ventilators. It is estimated that between 250,000-300,000 ventilator-associated pneumonia cases per year occur in the United States.

“Pa is one of the more dangerous organisms in the ICU,” says Geoffrey Yarranton, Ph.D., chief scientific officer and executive vice president of research at KaloBios. “Twenty to 30 percent of patients infected with Pa go on to die.”

Chronic Pa infection also contributes to the decline of lung function in children and young adults with CF, an inherited disease affecting about 70,000 individuals worldwide, according to the Cystic Fibrosis Foundation. In CF, a defective gene causes the body to produce unusually thick mucus that clogs the lungs, leading to life-threatening lung infections.

“By the time CF patients are in their teens, 80 percent have Pa,” says Jeanne Y. Jew, senior vice president of business development at KaloBios. “We hope this antibody will ultimately increase the lifespan and quality of life of CF patients.”

The Research Team

Dara W. Frank, Ph.D., director of the Center for Infectious Disease Research at the Medical College of Wisconsin (MCW), has been working to understand the interplay between Pa and its human host since 1989.

As a critical care physician, Jeanine Wiener-Kronish, M.D., sees many immunocompromised patients succumb to Pa infections, which are often resistant to antibiotics. She was studying the bacterium in her laboratory while at the University of California, San Francisco (UCSF) when she learned of Frank’s work and reached out to her to discuss testing various strains of Pa.

“They had common goals: both were looking for ways to neutralize the Pa bacteria,” explains Joseph O. Hill, managing director in the MCW Office of Technology Development.

Together, the scientists determined that a specific enzyme made certain strains of Pa more virulent — a discovery that led the two to engage in a more formal collaboration beginning in 1992 and lasting over a decade.

“Jeanine developed animal models that mirrored human infections, and I identified the genes required for Pa virulence in these models,” explains Frank, professor of microbiology and molecular genetics at MCW.

Attacking Pa

Initially, the scientists thought they’d go after the toxins secreted by Pa that kill off white cells in the lungs. Instead, they decided to target the toxins’ injection mechanism, a structure called the Type III Secretion System (TTSS). At the tip of the TTSS structure is a protein called PcrV that serves as a needle-like injector of the toxins.

Frank and Wiener-Kronish developed a mouse monoclonal antibody (called a murine antibody) that binds to the PcrV protein, essentially “capping” the injector and inhibiting it from injecting toxins into healthy cells. Monoclonal antibodies mimic the body’s natural antibodies that identify and neutralize foreign objects, or antigens. Part of the burgeoning field of medicine called biological therapy, these specially engineered molecules are increasingly being used to fight cancer and autoimmune disorders.

For Frank and Wiener-Kronish, the lengthy process of creating an anti-PcrV murine antibody involved immunizing mice with the Pa antigen (injecting mice with pure PcrV protein) and then harvesting antibody-producing B cells from the animals’ spleen. The B cells were then fused with fast-growing myeloma cells and cultured over a period of weeks. The

resulting cloned cells — called hybridomas — were then individually tested for their ability to produce antibodies that bound to the target antigen, the PcrV protein.

After years of work — funded in part by the MCW Department of Microbiology and Cancer Center, the Cystic Fibrosis Foundation, and the National Institutes of Health — testing revealed that one hybridoma, called monoclonal antibody (mAb) 166, was protective in animal models. A single dose of passively transferred antibody provided protection against Pa.

“This was the first mAb targeting a virulence system instead of a specific toxin,” says Frank.

Finding KaloBios

With the help of Joseph Hill and UCSF’s Office of Innovation, Technology & Alliances, the scientists filed their first patent on mAb 166 in 1998. To date, a total of 35 foreign and U.S. patents have issued covering a number of related discoveries, including the monoclonal antibody itself, its use in therapy, a Pa vaccine and a diagnostic test to determine whether the bacteria carries the PcrV protein.

The two tech transfer offices also divided their labor — MCW worked on the patents, while UCSF looked for potential licensees for the biological therapy. After a false start with a licensee that was unable to humanize the murine antibody, UCSF found KaloBios, which was just completing work on its humanizing technology and looking for animal antibodies to convert for human use.

“Jeanine was on the frontline, convincing KaloBios of the clinical market for a Pa antibody; Dara was able to provide the sophisticated technical and scientific basis for the antibody and toxin. Together they provided proof of concept in animal models of disease,” says Hill. “The market attracts the development partner, but it’s solid science that keeps them engaged.”

Humanizing a murine antibody is a difficult process that involves taking out the mouse genes and inserting human sequences within the antibody: The humanized antibody must mimic the mouse antibody while keeping all of its activity. Key to the process is creating an antibody that won’t be recognized as foreign by the human host, triggering an immune response in which the antibody is attacked by the body.

“We entered into a materials transfer agreement to see what we could do with it and agreed to talk about licensing later,” says Yarranton. “In 2004, we were able to engineer a high-affinity human antibody and entered into an exclusive license agreement with UCSF and MCW.”

The company also stabilized the human antibody by pegylating it to prevent degradation in the lung and improve the half-life of the drug. Named KB001-A, the anti-PcrV monoclonal antibody is actually a fragment of the laboratory-made protein, because it contains only the antigen-binding portion of the antibody, not the portion that triggers an inflammatory response, which is unwanted in these patients.

“Infection in the lung causes inflammation, which recruits immune cells,” says Yarranton. “This antibody works without stimulating more inflammation.”

Testing the New Antibody in Humans

KaloBios has conducted several successful trials in human patients to determine the safety and usefulness of the KB001-A for preventing and treating Pa infections in ventilator-assisted patients and individuals with CF.

The company has entered into its own collaboration by partnering with Sanofi Pasteur, a global healthcare company, to continue studying the antibody's ability to prevent ventilator-associated pneumonia. Meanwhile, KaloBios is continuing to test the antibody for its effectiveness in CF patients, working with 50 specialized CF centers across the country to recruit patients for a Phase II trial.

"We are recruiting up to 180 patients who will either be treated for four months with the antibody or will receive a placebo," says Jeanne Jew. "We hope to end recruitment by midyear and have results at the end of the year."

The study will measure the effect of multiple doses given to CF patients over a four-month period.

> *"What makes the antibody treatment unique and valuable is its selectivity and half-life," says Yarranton. "You don't have to treat multiple times a day. One injection covers 28 days."*

Industry–Academic Collaboration

Meanwhile, executives at KaloBios are keen on continuing to work with academic scientists like Frank and Wiener-Kronish.

"Three of our projects have come from academia," says Yarranton. "We want to work where there's the greatest scientific knowledge about a project, so we'll be successful. It's a two-way street because the academics are excited to have what they developed be tested in the clinic.

"There's a lot of knowledge and many good [technologies] out there to be licensed."