PRODUCT R&D

ALTERMUNE'S ALPHAMERS

By Lauren Martz, Staff Writer

Altermune Technologies LLC is taking immunotherapy back to its day job of fighting infections with a new technology that coats bacteria with a sugar, and recruits circulating antibodies against the sugar to find and destroy the organisms. The strategy follows a trend in cancer immunotherapy of harnessing the immune system in new ways, and departs from standard antibacterials or immunotherapy approaches that depend on inducing or injecting antibodies against the pathogens themselves.

"It is really strange that we don't consider using the immune system in infectious disease therapy. We are seeing great results in cancer," said Victor Nizet, professor of pediatrics and pharmacy at the University of California San Diego, and a member of Altermune's scientific advisory board. "There are a lot of innovative ways in which this can be explored in infectious diseases, and we are starting to explore one of them now."

Altermune's platform — dubbed alphamers — is the latest brainchild of PCR inventor Kary Mullis, and involves conjugates containing the sugar α-Gal linked to an aptamer that targets a specific bacterial epitope and doesn't bind host cells. The idea is that once the sugar-aptamer conjugate coats the pathogens, it will attract antibodies that can opsonize the organisms and trigger their elimination.

Mullis is founder of Altermune and chief scientific advisor to the company.

Although humans don't express α-Gal naturally, the sugar is found in most other mammals and in many bacteria, and gives rise to anti-α-Gal antibodies when people are exposed to it from eating meat or coming into contact with the bacteria. According to Nizet, the anti-α-Gal antibodies constitute about 1% of the total number of antibodies in humans.

Instead of passive immunization, which involves delivering therapeutic mAbs directly into the circulation, Altermune's approach capitalizes on the existence of circulating anti-α-Gal antibodies, and avoids the short half-life of injected antibodies. By combining the specificity of aptamers with the abundance, longevity and heterogeneity of the anti-α-Gal antibodies, the company believes its compounds can provide a more effective and sustained response than traditional immunization.

"Our alphamers don't generate a monoclonal antibody response, they generate a polyclonal response, so we expect they will have increased potency," said CEO Mike Westby.

He added that the approach is differentiated from mAbs because the antibodies Altermune relies on "naturally pre-exist in everyone and are known to have good effector function. Many therapeutically used mAbs in oncology and infectious diseases are good at binding targets but are not optimized for killing."

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This month, Altermune and its collaborators at UCSD published the first data on alphamers showing the compounds specifically bind a conserved site on various Group A *Streptococci*, recruit the anti-sugar antibodies and induce antibody-mediated cell death. (See Distillery, page 19)

ANTIBACTERIAL SPREAD

Mullis and colleagues at Altermune joined with Nizet's team at UCSD to study the effects of bacteria-targeted alphamers in models of infection and explore the compounds' potential for drug development.

By screening a panel of DNA aptamers, the researchers identified an 80-nucleotide candidate that potently bound a region of a *Streptococcus* surface binding protein conserved across various strains, but did not recognize cells lacking the binding site.

The team conjugated the aptamer's 5' end to α-Gal to form an alphamer and showed there was no loss in bacterial binding.

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To determine whether alphamer binding could attract anti-α-Gal antibodies to the bacteria, the team cultured human or mouse anti-α-Gal antibodies with the alphamers and live Group A *Streptococci*, and measured antibody binding. Antibodies bound about 86% of Group A *Streptococcus* cells, but did not bind bacterial cells cultured with non-targeted alphamers or vehicle.

Next, the team tested whether antibody binding led to bacterial cell death. When human neutrophils or whole blood samples were added to the culture system, alphamer-bound bacteria were phagocytosed about three times more efficiently than the control alphamers or vehicle.

Results were published in the *Journal of Molecular Medicine*.

Nizet told BioCentury the team's next two priorities to support the preclinical program are developing and testing alphamers against multidrug resistant (MDR) bacteria and developing mouse models for *in vivo* evaluation.

"In this case, we took an important human pathogen, but it wasn't an antibiotic-resistant pathogen," he said. "Resistant Gram-negative bacteria are a big problem and may be a very good fit for this technology."

He noted that for Gram-negative bacteria, the cell walls can be ruptured and lysed by the complement system, which is effective at killing antibody-bound bacteria. That mechanism of cell death could complement the opsonization and phagocytic cell death seen in the Gram-positive bacteria, he said.

But Nizet added that the alphamer platform wasn't universally applicable, as it wouldn't work against intracellular pathogens such as those responsible for tuberculosis, typhoid fever or chlamydia.

Nizet is now focusing on developing new mouse models. First, the team will breed mice with a human mutation that will allow α-Gal to be recognized by antibodies, because mice naturally express α-Gal and don't normally develop antibodies. Next, the group will immunize the mice with the sugar to generate anti-α-Gal antibodies and create models of infection to use in testing alphamer candidate compounds.

The *in vivo* work will help the team sort out the mechanism of antibody-induced cell death, said Nizet. He noted that although it isn't yet clear whether endogenous antibodies bind the therapeutic alphamers first, or alphamers bind the target pathogen first, the binding order is unlikely to be important for efficacy.

ALPHAMER BENEFITS

The company believes its strategy has several advantages over antibiotics and other antibacterial approaches because of its versatility and the fact that it doesn't perturb the host bacterial population significantly.

"We think there is some real advantage to aptamer development because these are chemically synthesized molecules and we can rapidly explore structure-activity relationships and make changes to optimize the molecule at any position," said Westby. "In terms of discovery and optimization, there are a lot of advantages."

In addition, said Nizet, "Classical antibiotics wipe out the intestinal flora and normal bacteria, and this is a big problem. The targeted alphamers wouldn't have that problem."

Altermune believes the ability to fight Gram-negative bacterial infections could be one of the platform's biggest assets because of the high problem of resistance and lack of options for those organisms in particular.

"We are first going after the multidrug-resistant bacterial infections with high unmet need such as resistant forms of Gram-negative ESKAPE pathogens, including *Pseudomonas*

and *E. coli,*" said Westby. "But once a therapeutic is approved and shown to be safe and efficacious in patients who have an antibiotic-resistant pathogen, these therapeutics could possibly be extended to treat patients with antibiotic-susceptible infections."

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Westby said that although Altermune was founded in 2010 at a time when many companies were exiting the antibacterial space, the commercial incentives have changed since then because of the GAIN Act and because regulators are willing to view antimicrobial resistant infections as Orphan indications. In addition, technology developments in point-of-care diagnostics can improve both treatment and trials, and can benefit platforms such as alphamers.

Altermune also plans to expand the platform to cancer immunotherapy. According to Westby, that move wouldn't put Altermune in competition with another of his companies, Agalimmune Ltd., which is also exploiting natural immunity to α-Gal to treat cancer.

"We do not anticipate competition in cancer as the intratumoral route of administration for Agalimmune's technology versus the IV route for Altermune will lead to different indications being targeted," said Westby.

Agalimune's Alphaject platform involves directly injecting α-Gal-containing glycolipids into a solid tumor where the hydrophobic tail of the lipid-α-Gal conjugate inserts into the tumor cell surface, leaving α-Gal exposed to attract anti-α-Gal antibodies. Alphaject therapeutics have completed Phase I trials in melanoma.

Westby is CEO of Agalimmune Ltd., which was founded in 2013 with partial backing from Loxbridge Research LLP. Loxbridge was also a founding investor in Altermune.

The alphamer technology was patented by Mullis and the patent was assigned to Altermune. The company has rights to develop the technology for all indications including cancer. Agalimmune has a separate IP portfolio, and Mullis is not affiliated with the company.

Altermune hopes to file its first IND for an infectious disease indication in 2017.

COMPANIES AND INSTITUTIONS MENTIONED

Agalimmune Ltd., London, U.K.

Altermune Technologies LLC, Irvine, Calif.

University of California San Diego (UCSD), San Diego, Calif.

TARGETS AND COMPOUNDS

α-Gal - Galactose-α-1,3-galactosyl-β-1,4-N-acetyl-glucosamine

REFERENCES

Kristian, S., et al. "Retargeting pre-existing human antibodies to a bacterial pathogen with an alpha-Gal conjugated aptamer." *Journal of Molecular Medicine* (2015)