BIOCENTURY
Innovations
FROM IDEA TO IND

REPRINT FROM AUGUST 6, 2015

PRODUCT R&D

IMPEDE A FEVER

By Stephen Parmley, Senior Writer

A team at the Massachusetts Institute of Technology has developed an antibody that could treat dengue infections caused by all four serotypes of the virus, and might be able to prevent the disease as well. While most companies are focusing on vaccines for dengue, Visterra Inc. has licensed the mAb and believes that an immunotherapy could produce a better and more lasting way to treat and prevent infection.

Researchers have struggled to generate treatments or vaccines that work broadly in dengue fever because antibodies against one dengue serotype rarely induce long-term protection against the others. On top of that, when patients recover from dengue infection, the antibodies they develop against the first serotype can exacerbate a second infection by enhancing viral uptake into host cells.

The most advanced products in the clinic are tetravalent vaccines that include a mixture of antigens from all four serotypes.

However, according to Visterra COO and CFO David Arkowitz, vaccine data so far have shown inconsistent protection across the different serotypes after three doses, and disease worsening has been observed in some of the younger vaccinated patients.

Although the field is dominated by strategies to prevent the disease, some of which use small molecule antivirals as alternatives to the tetravalent vaccines, some groups are trying to create cross-reactive antibodies that can treat active infections by any serotype. (See "Delving Into Dengue", page 6)

Now, the MIT team has described in *Cell* a strategy that involves targeting a conserved patch within a region of the dengue viral envelope not recognized as a dominant epitope by the immune system during the normal course of an infection. The mAb, dubbed VIS513, binds residues in domain III of the dengue surface protein DENV_gp1, neutralizes all four serotypes, and rapidly treated mice with severe dengue fever after a single dose.

"We believe that VIS513 can be a highly effective therapy to rapidly clear infection with a single administration," Arkowitz told BioCentury.

DOMAIN SEARCH

Because the first attempt to target the patch yielded a mAb that bound domain III but showed low affinity to serotypes 3 and 4, the group used structure-guided antibody engineering to improve and equalize the compound's affinity for all four serotypes.

The researchers introduced amino acid changes in the antibody's binding region that would create better contacts with matching residues of the bound DENV_gp1. For example, they reasoned a hydrophobic T33V mutation would improve the antibody's interaction with a hydrophobic valine 364 in domain III. In addition, the group introduced changes and amino acid deletions to alter the shape of the mAb's recognition site.

> "We believe that VIS513 can be a highly effective therapy to rapidly clear infection with a single administration."
>
> David Arkowitz, Visterra Inc.

"We predicted a deletion in the antibody that would increase the contact with the antigen such that it was able to provide a much broader specificity and that has never been done before," said Ram Sasisekharan, principal investigator on the study and a professor of biological engineering and health sciences and technology at MIT.

VIS513 bound domain III of dengue serotypes 1-4 with K_d values of 0.1-4.3 nM, and its affinity for serotypes 3 and 4 was about 10-fold higher than that of the parent mAb.

The team tested the mAb's ability to treat infection using mice engineered to produce human platelets. Treatment with VIS513 following infection with any of the four dengue serotypes increased platelet counts compared with a control antibody, which suggested the mAb could treat the infection-induced thrombocytopenia that is one of the hallmarks of severe dengue fever.

Next, the team tested *in vitro* the mAb's ability to block the phenomenon in which anti-dengue antibodies enhance infection, by comparing the activity of VIS513 with that of a different dengue mAb known to facilitate viral uptake into host cells expressing the antibody receptor FCGR. In human FCGR-

positive monocytes, VIS513 decreased infection by all four serotypes compared with the control antibody.

To demonstrate *in vivo* efficacy against exacerbated disease, the team tested whether VIS513 could treat dengue serotype 2 infections in newborn mice that had maternally transferred antibodies against serotype 1. In these mouse pups challenged with dengue serotype 2, VIS513 decreased clinical scores within a few days and increased survival compared with an unrelated isotype-matched mAb. VIS513 also decreased vascular leakage to levels similar to those seen in uninfected animals.

The authors concluded that the data support the idea that, unlike previous anti-dengue mAbs, VIS513 would not exacerbate infections, and could be used to treat active dengue serotype 2 infections even after a previous infection with a different serotype.

Finally, the team tested the antibody's efficacy in preventing infection in a mouse model of dengue serotype 2 infection, which is one of the more virulent serotypes. Pretreatment with VIS513 increased survival compared with a control antibody.

PROPHYLACTIC PRACTICALITIES

Visterra has partnered with the Agency for Science Technology and Research (A*STAR) and plans to take VIS513 into the clinic next year to treat acute dengue infections. But it's not clear how

DELVING INTO DENGUE

Dengue preventive vaccines and therapeutics in development. At least eight preventive vaccines, four therapeutic antivirals, one anti-infective nanoparticle and one therapeutic antibody against dengue virus are in development by biotech and pharma companies. The vaccines in development include components from dengue virus serotypes 1-4 (tetravalent vaccines). The four antivirals treat dengue fever by blocking viral replication. **NanoViricides Inc.**'s (NYSE-M:NNVC) nanoviricide micelles treat dengue fever by engulfing and dismantling the virus. **Visterra Inc.**'s mAb VIS513 treats dengue fever by blocking host cell infection by all dengue serotypes. *Sources: BCIQ: BioCentury Online Intelligence; www.clinicaltrials.gov; company websites*

COMPANY	PRODUCT	DESCRIPTION	STATUS
Sanofi (Euronext:SAN; NYSE: SNY)	ChimeriVax	Chimeric tetravalent dengue vaccine consisting of live attenuated yellow fever virus 17D strain with structural genes replaced with the corresponding dengue structural genes	Phase III
Takeda Pharmaceutical Co. Ltd. (Tokyo:4502)	TAK-003 (Dengue fever vaccine)	Recombinant tetravalent attenuated live dengue vaccine	Phase II
Globavir Biosciences Inc.	GBV006	Combination of two previously FDA-approved anti-infective drugs	Phase I
Merck Sharp and Dohme Corp. subsidary of **Merck & Co. Inc.** (NYSE:MRK)	V180	Tetravalent recombinant subunit vaccine against dengue fever	Phase I
United Therapeutics Corp. (NASDAQ:UTHR)	UV-4B	α-glucosidase inhibitor	Phase I
Vical Inc. (NASDAQ:VICL)	Vaxfectin-formulated tetravalent dengue DNA vaccine	Tetravalent vaccine containing genes encoding pre-membrane and envelope proteins for four dengue virus serotypes formulated with the Vaxfectin adjuvant	Phase I
Altravax Inc.	Dengue virus vaccine	Chimeric tetravalent vaccine against dengue virus envelope protein E (DENV_gp1)	Preclinical
NanoViricides Inc.	Anti-dengue nanoviricides (DengiCide, DengueCide)	Nanoviricides targeting certain regions of the dengue envelope protein structure	Preclinical
PaxVax Inc.	Dengue vaccine	Two-dose, whole-virus inactivated vaccine	Preclinical
Sarepta Therapeutics Inc. (NASDAQ:SRPT)	Viral PMO-X	RNA-based antiviral based on morpholino-modified phosphorodiamidate oligomers (PMO) antisense chemistry	Preclinical
Siga Technologies Inc. (Pink:SIGAQ)	Dengue antiviral	Orally bioavailable antiviral dengue fever compound	Preclinical
Themis Bioscience GmbH	Dengue fever vaccine	Tetravalent vaccine developed using Thermaxyn technology that uses a single measles viral vector expressing the conserved EDIII domain of DENV_gp1	Preclinical
Visterra Inc.	VIS513	Humanized mAb targeting a conserved region DENV_gp1	Preclinical

practical an antibody-based prophylactic therapy could be for a developing world disease.

"Prophylaxis with an antibody might not be very effective because the antibody has a limited half-life, you can't predict when you're going to get an infection, and it is not feasible to inject large amounts of the antibody on a weekly basis," said Sean Du, COO of infectious disease company Altravax Inc.

He noted that the mAb's prospects could be better if it works at low doses.

Arkowitz countered, "There is a long history of the use of monoclonal and polyclonal antibodies to protect against infection and we believe that passive prophylaxis could be an important use for VIS513."

He added: "We believe that the prophylactic dose will be meaningfully lower than a curative therapeutic, further reducing the cost from already affordable mAb ranges."

Although last week a group at Inovio Pharmaceuticals Inc. published a study in *Scientific Reports* about a DNA-based approach for reducing the cost of a prophylactic dengue immunotherapy, Arkowitz told BioCentury that Visterra plans to validate the therapeutic utility of VIS513 in the clinic before exploring other modalities.

Inovio's method uses a DNA vector encoding a neutralizing dengue antibody that generates the mAb when injected into mouse muscle. A DNA vector would be less expensive to manufacture than a protein and could require fewer doses if it elicits sustained expression of the anti-dengue mAb.

Arkowitz said, "Alternative vehicles and strategies, such as DNA vector electroporation for delivery, are welcome and needed, [but] we believe that the cost of goods for mAbs will continue to drop."

> "We predicted a deletion in the antibody that would increase the contact with the antigen such that it was able to provide a much broader specificity and that has never been done before."
>
> Ram Sasisekharan, MIT

The MIT team has a pending patent on the mAb for the treatment of dengue fever and has used a similar structure-guided engineering approach to develop neutralizing mAbs for additional undisclosed infectious agents.

COMPANIES AND INSTITUTIONS MENTIONED

Agency for Science Technology and Research (A*STAR), Singapore
Altravax Inc., Fargo, N.D.
Inovio Pharmaceuticals Inc. (NASDAQ:INO), Blue Bell, Pa.
Massachusetts Institute of Technology (MIT), Cambridge, Mass.
Visterra Inc., Cambridge, Mass.

TARGETS AND COMPOUNDS

DENV_gp1 - Dengue virus envelope protein E
FCGR - Fcγ receptor

REFERENCES

Flingai, S., et al. "Protection against dengue disease by synthetic nucleic acid antibody prophylaxis/immunotherapy." *Scientific Reports* **(2015)**

Robinson, L., et al. "Structure-guided design of an anti-dengue antibody directed to a non-immunodominant epitope." *Cell* **(2015)**

EDITORIAL & RESEARCH

NEWSROOM:
pressreleases@biocentury.com

SAN CARLOS, CA:
+1 650-595-5333; Fax: +1 650-595-5589

CHICAGO:
+1 312-755-0798; Fax: +1 650-595-5589

WASHINGTON, DC:
+1 202-462-9582; Fax: +1 202-667-2922

UNITED KINGDOM:
+44 (0)1865-512184; Fax: +1 650-595-5589

Editor-in-Chief: Karen Bernstein, Ph.D.
Editor: C. Simone Fishburn, Ph.D.
Associate Editor: Michael J. Haas
Senior Writer: Stephen Parmley, Ph.D.
Staff Writers: Selina Koch, Ph.D.; Lauren Martz; Mary Romeo; Karen Tkach, Ph.D.; Mark Zipkin
Director of Research: Walter Yang
Copy Editor: Claire Quang