REPRINT FROM APRIL 1, 2013

BioCentury

THE BERNSTEIN REPORT ON BIOBUSINESS™

Product Discovery & Development

Producing provocative proteins

By Emily Cukier-Meisner
Senior Writer

Tetragenetics Inc. has been keeping a low profile with partnerships for its protein expression and manufacturing platform, but this week was set to unveil deals with **Amgen Inc.** and **Pfizer Inc.** to produce proteins for antibody screens that would be difficult to make in standard expression systems.

The company also is releasing further details of its work under a grant from the **Bill & Melinda Gates Foundation**'s Grand Challenges Explorations initiative to use discoveries from the same platform to produce antigens for vaccines.

Under its deal with Amgen, Tetragenetics is using its TetraExpress *Tetrahymena thermophila*-based technology to express an undisclosed surface antigen selected by Amgen. The partners then will jointly develop antibodies against the target.

In its deal with Pfizer, Tetragenetics will use TetraExpress and its SionX technologies to express an undisclosed ion channel selected by Pfizer for antibody development. The pharma has an option for an exclusive license to the resulting immunogen preparations.

Details of the deals are not disclosed.

The TetraExpress platform of promoters, vectors and cell lines can achieve up to 18,000 foreign gene copies per cell. SionX is used specifically for ion channel expression.

"People are turning to us to generate the proteins they have not been able to produce in other systems," said Tetragenetics Chairman Doug Kahn.

Immunogenic membrane proteins are difficult to produce in bacterial expression hosts such as *Escherichia coli* because the bacteria do not consistently fold proteins accurately or make post-translational modifications necessary for function. In addition, such hosts have a cell wall that can impede downstream protein purification.

In mammalian cells, membrane proteins do not readily traffic to the plasma membrane and may have only short extracellular loops, making it difficult to identify or raise antibodies that recognize them.

"The more protein you can get on the surface of the membrane, the better the chances are that you're going be able to raise an antibody against it, or screen for existing antibodies that might be in libraries created in phage or yeast," said Tetragenetics founder and CSO Ted Clark.

> ***"People are turning to us to generate the proteins they have not been able to produce in other systems."***
>
> **Doug Kahn, Tetragenetics**

The eukaryotic microbe *T. thermophila* provides several advantages for expressing large quantities of correctly folded protein and targeting it to the plasma membrane.

T. thermophila lacks a cell wall, makes mammalian-like post-translational modifications to proteins, grows rapidly and creates high copy numbers of foreign genes through ribosomal DNA amplification.

According to Clark, *T. thermophila* looked promising for membrane protein and ion channel expression because it devotes much of its metabolism to membrane protein production and has a natural propensity to produce potassium channels.

He said *T. thermophila* can obtain thirtyfold higher expression per cell of certain ion channels compared to mammalian cells.

See next page

BioCentury®
THE BERNSTEIN REPORT ON BIOBUSINESS

PO Box 1246
San Carlos CA 94070-1246
Voice: 650-595-5333
Fax: 650-595-5589
www.biocentury.com

DAVID FLORES
President & CEO

KAREN BERNSTEIN, Ph.D.
Chairman & Editor-in-Chief

Product Discovery & Development, *from previous page*

"It can fold eukaryotic proteins including human proteins properly, it can post-translationally modify those proteins, and unlike some of the other unicellular eukaryotes like yeast, it doesn't have a cell wall, so it's very easy to get to the proteins that you put on the cell surface by lysing the cells with detergents," said Clark.

G-SOME challenge

The other focus of Tetragenetics' platform is producing vaccine antigens using *T. thermophila* and its associated proteins.

Under a Grand Challenges Phase II grant received last November, Tetragenetics is developing new expression systems for a transmission blocking vaccine against malaria. It is based on nanoparticles the company has developed, called G-SOMES, that incorporate the Pfs48/45 antigen.

G-SOMES are vaccines composed of fusion proteins of an antigen of interest and an undisclosed scaffold protein from *T. thermophila.* "These scaffold proteins have the ability to self-assemble. That assembly gives rise to a particle, and the G-SOME is what we call those purified particles," said Clark.

Tetragenetics believes G-SOMES have advantages over synthetic particle vaccines because they form under physiological conditions and do not require synthetic chemistry that could denature the protein during particle assembly.

Compared to virus-like particles, G-SOMES are more stable and can be formed from a much larger range of proteins.

The G-SOME for malaria is an "altruistic vaccine" that is intended to block transmission of the parasite from an infected person by generating antibodies that are ingested by the mosquito along with the parasite that prevent completion of the parasite sexual phase in the mosquito.

So-called altruistic vaccines provide no benefit to the individual, but are intended to block transmission.

"There are a number of antigens that have been proposed as candidates for that kind of a vaccine," said Clark, "but the challenge for many of these vaccines is being able to raise antibodies against them in the human, and this is where we think the G-SOME technology is going to be good."

Clark said the size and repetitive arrangement of antigens on the surface of G-SOMES makes them particularly effective for B cell stimulation and antibody production.

The initial Phase I grant investigations used *T. thermophila* to produce vaccine nanoparticles in secretory granules. Tetragenetics later determined that fusion proteins containing the *T. thermophila* scaffold protein could form particles (G-SOMES) *in vitro*, freeing Tetragenetics to produce vaccines in cell lines other than *T. thermophila*.

For Phase II, the Gates foundation encouraged Tetragenetics to move into traditional expression systems such as *E. coli* that have already been used to produce FDA-approved vaccines, thus providing a smoother regulatory pathway, according to Clark.

Clark believes these proteins will not be difficult to express in other systems.

"We're finding that the scaffold proteins that we're using to create these nanoparticles seem to have benefits for protein expression in *E. coli* that we didn't anticipate," he said. "There are benefits in terms of not just yield, but also functionality of the protein."

Tetragenetics will use the particles formed in *E. coli* to vaccinate animals and test the ability of the resulting antibodies to block development of malaria parasites in the mosquito gut.

Moving forward

In addition to the Amgen and Pfizer deals, Kahn said Tetragenetics has about half a dozen additional undisclosed partnerships.

Tetragenetics does not intend to bring a product to the clinic without a partner, Clark said, but said the company's first product could be in human trials in months.

Tetragenetics, which was founded in 2004, has raised $4.6 million from angel investors including **New England Biolabs Inc.** and received $4 million in grants, including the Phase II Grand Challenges grant of $824,000 and the Phase I grant of $100,000 it received in 2009.

At least one other company, **Cilian AG**, is exploring *T. thermophila* to produce complex proteins and vaccines. In 2009, **Lonza Group Ltd.** divested IP covering *T. thermophila* to Cilian in exchange for right of first refusal to market products based on the technology and a 5% equity stake.

Clark declined to comment on how the approaches differ, saying Cilian has not disclosed enough information to allow comparison.

COMPANIES AND INSTITUTIONS MENTIONED

Amgen Inc. (NASDAQ:AMGN), Thousand Oaks, Calif.
Bill & Melinda Gates Foundation, Seattle, Wash.
Cilian AG, Munster, Germany
Lonza Group Ltd. (SIX:LONN), Basel, Switzerland
New England Biolabs Inc., Ipswich, Mass.
Pfizer Inc. (NYSE:PFE), New York, N.Y.
Tetragenetics Inc., Cambridge, Mass.