Pettit: Just Biotherapeutics Support for HIV bNAbs

OVERVIEW

During the past few years the Bill & Melinda Gates Foundation has engaged with universities developing broadly neutralizing monoclonal antibodies (bnAbs) against HIV. Molecules under investigation by CAVD grantees include bnAbs 3BNC117, 10-1074, PGT121, PGDM 1400, bi-specific antibody constructs, and the HIV-1 entry inhibitor eCD4-Ig. While the molecules mentioned here show good *in vitro* neutralization activity and are being tested in clinical trials they have not been sequence optimized for commercialization, which may lead to significant late stage commercialization problems during scale-up, formulation and fill/finish operations. Just Biotherapeutics, Inc., has developed a proprietary set of *in silico* tools for sequence optimization of antibodies and related proteins to significantly increase success during commercialization.

The focus of the current grant proposal from Just is 3-fold: 1) delivery of 2 commercially optimized HIV bnAbs from the set 3BNC117, 10-1074, PGT121 and PGDM 1400 for First-In-Human (FIH) clinical trials, which, if successful could be taken through to commercialization; 2) process economic modeling of the drug substance manufacturing; and 3) sequence optimization for non-traditional bnAbs and other proteins. The HIV bnAbs, from the set including 3BNC117, 10-1074, PGT 121 and PGDM 1400 will be sequence optimized using Just's Abacus *in silico* design, experimentally validated *in vitro*, and tested by academic PI's in non-human primate PK studies. In concert with the optimization, it is important to know if and how cost targets for manufacturing of the drug substance can be met. This will be accomplished through modeling, focusing only on the most cost effective manufacturing option. In addition to optimization of the parental HIV bnAbs, the bi-specific bnAbs will be sequence optimized through *in silico* modeling only, and technical expertise provided for the anti-HIV Fc-fusion eCD4-Ig, with additional scale-up directed through the development laboratories. Specific sequence improvements for the molecules include addition of mutations for increasing half-life, increasing expression, decreasing self-interaction, and limiting the effects of multiple potential degradative modifications. Together, the sequence optimization of the antibodies will significantly improve our ability to enhance expression and intensify the purification process (important for reducing manufacturing costs), while limiting phase separation, aggregation, and high viscosity associated with poor sequences. The sequence optimization should allow for improved properties leading to lower cost biotherapeutics.

This grant is led by Dean Pettit, PhD and Bruce Kerwin, PhD (Just Biotheraputics Inc.) with the collaborative engagement of Dan Barouch, MD, PhD (Beth Israel Deaconess Medical Center) and Michel Nussenzweig MD, PhD (The Rockefeller University) for *in vivo* evaluations of bnAbs in non-human primate models, David Ho (Aaron Diamond AIDS Research Center) for *in vitro* characterization of bi-specific bnAbs, Michael Farzan (The Scripps Research Institute) for scientific issues concerning eDC4-Ig, and David Volkin (University of Kansas) for structural analytics on purified constructs. In addition, the grant will leverage the following Central Services Facilities: The Vaccine Product Development Center for bnAb toxicology studies in rodents and non-human primates; and the Comprehensive Antibody Vaccine Immune Monitoring Consortium for *in vitro* neutralization measurements. The award was made in October 2015 with an original agreement length of 50 months.

Grant at a Glance

Principal Investigators
Dean Petttit, PhD
Bruce Kerwin, PhD

Grantee Institution
Just Biotherapeutics
Seattle, USA

Project Title
Just Biotherapeutics Support for HIV bNAbs

OPPID
1138851

Grant Award
Up to $9.5 million, awarded in October, 2015

Collaborating Institutions
The following institutions receive support under separate grants:

- Beth Israel Deaconess Medical Center, Boston, USA
- The Rockefeller University, New York, USA
- Aaron Diamond AIDS Research Center, New York, USA
- The Scripps Research Institute, Jupiter, USA
- University of Kansas, Lawrence, USA

RESEARCH OBJECTIVES

1.) Delivery of 2 commercially optimized HIV bnAbs from the set 3BNC117, 10-1074, PGT121, and PGDM 1400

   a.) Develop optimized bnAb cell lines that allow for maximizing specific productivity for efficient utilization within a J.POD facility, and

   b.) Increase bnAb stability allowing for time outside of the cold chain for greater distribution within Sub-Saharan Africa

2.) Process economic modeling for manufacturing the bNAbs

3.) Sequence optimization for bi-specific bnAbs and the eCD4-Ig entry inhibitor

PROGRESS

Significant progress has been made toward optimization of 3BNC117, 10-1074, PGT121, and PGDM1400 with optimization sites identified by *in-silico* modeling and variants produced and tested for retention of neutralization and increases in desired biophysical parameters. For 3BNC117, the data showed that sites which provided significant biophysical optimization interfered with neutralization activity leading to continued use of the parental molecule. In contrast, 10-1074 could be significantly modified with no loss of activity allowing for optimization resulting in increased thermodynamic stabilization which manifested as increased low pH stability and severely reduced aggregation during 40°C storage. Mouse PK studies also indicated that the sequence optimized 10-1074 bnAb had longer elimination half-live as compared to the parental sequence, and the LS modified parental sequence. Cell line development has been completed for both 3BNC117 and 10-1074 and scale-up for GMP production has been initiated to produce material for clinical trials. Optimization of PGT121 and PGDM1400 has also been completed. For PGT121 optimization resulted in increased thermal stability and resistance to chemical unfolding. Additionally, residues were identified that were responsible for low pH instability such that the bnAb can now be incubated at acidic pH with little to no aggregation while the parent shows up to 90% aggregation. Importantly, for room temperature stability the accelerated stability at 40°C demonstrated negligible aggregation during a 2-month incubation while the parental molecules show approximately 2% over the same timeframe. For PGDM1400 significant increases in thermal stability and resistance to chemical unfolding were achieved with retention of neutralization activity against a select set of viruses. Mouse PK studies in Tg276 human FcRn knock-in mice have been completed for the optimized PGT121 variants. The results showed that the optimized variants which include the LS mutation showed a 2.2-fold increase over the parental PGT121-LS molecule.

In addition to the optimization programs, process economic modeling has been conducted to identify processes that significantly affect the manufactured cost of goods. Based on the analysis, specific productivity provides the greatest impact on initially lowering the cost. Modeling of the bnAbs shows that costs in the $60 - $80 per gram range can be achieved based on volumetric productivity with further reductions achieved through facility scheduling, bioreactor size, chemically defined media and purification procedures.