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TITLE: A Factor H-Fc Fusion Therapy for Methicillin-Resistant Staphylococcus aureus Infection

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CONTRACTING ORGANIZATION: Planet Biotechnology Inc.

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14. ABSTRACT

The purpose of this project is to develop a new drug for methicillin-resistant *S. aureus* (MRSA), engineered from two human proteins, that will undermine the mechanism that *S. aureus* uses to evade detection and destruction by the human immune system. We have produced FH(18-20)/Fc, an engineered protein that combines the *S. aureus*-binding CCPs from human Factor H with immunoglobulin Fc, an antibody fragment that activates complement on surfaces to which it is bound. In a preliminary experiment, we showed that FH(18-20)/Fc binds to *S. aureus*.

In this reporting period we completed our first aim, which was to use our plant expression system to produce FH(18-20)/Fc along with two control FH/Fc fusions: one with a mutation that eliminates Fc's ability to activate complement and one with modified Fcs designed to dramatically enhance complement-activating activity. We made significant progress on our second aim, showing that FH(18-20)/Fc blocks binding of human FH to *S. aureus*. We also began experiments to measure how FH(18-20)/Fc bound to *S. aureus* affects complement activation. In the remaining grant period we will measure the efficacy of FH/Fc fusions in boosting killing of MRSA by human polymorphonuclear cells.

15. SUBJECT TERMS

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Introduction

The purpose of this research is to develop a novel therapy for methicillin-resistant *Staphylococcus aureus* (MRSA) infections. *S. aureus* has developed immune-evasion strategies to promote survival within the host. One of the ways it does this is by recruiting to its surface the soluble human complement regulator Factor H (FH). Specifically, *S. aureus* binds to FH C-terminal domains 19-20 *via* the surface protein serine-aspartate repeat protein E (SdrE). In a preliminary experiment, we showed that a fusion of human FH domains 18-20 and the Fc of human IgG1, FH(18-20)/Fc, binds to the surface of *S. aureus*. We hypothesize that FH(18-20)/Fc will displace intact FH from the surface of *S. aureus*, increasing complement-mediated opsonization and targeting the bacterium for destruction by phagocytes. Ultimately, we believe that FH/Fc could become a novel class of potent anti-infectives that can contribute to treating disease caused by MRSA. Using a plant expression system we will produce FH(18-20)/Fc along with three control FH/Fc fusions. We will first test the extent to which FH/Fc competes for binding to *S. aureus* in the presence of intact serum FH. Next, we will measure the extent to which *S. aureus*-bound FH/Fc affects complement activation by examining its impact on factor-I mediated cleavage of C3b, the level of C3-fragment deposition, and its effect on C5a generation. Finally, we will measure the efficacy of FH/Fc fusions in boosting opsonophagocytosis of MRSA by human polymorphonuclear cells.

Key words

Methicillin-resistant *Staphylococcus aureus* (MRSA)
Complement Factor H (FH)
Immunoglobulin
Fc fusion
Anti-infective
Opsonophagocytosis

ACCOMPLISHMENTS

Major goals of the project:

Goal (Major Task) 1: Produce FH/Fc transiently in *N. benthamiana*

We proposed producing three variants of FH(18-20)/Fc:

- Human FH(18-20) fused to Fc of human IgG1
- Human FH(18-20) fused to Fc of human IgG1with two mutations (D270A/K322A): lacks ability activate complement
- Human FH(18-20) fused to Fc of human IgG3: enhanced ability to activate complement

Milestone: Purify at least 10 mg of each of four variants of FH(18-20)/Fc. Demonstrate purity of >95%. Quantify success of labeling.

This aim was modified from the proposed methods due to steric hindrance considerations not previously realized. Further, the labeling was performed by the Sharp Lab using the Thermo Fisher SiteClick Antibody Azido Modification Kit and Biotium CF Dye BCN 405M.

Click chemistry was used to specifically modify terminal galactose residues and label with a fluorescent dye. Targeting the CH2 region of the Fc portion of the fusion proteins with a low molecular weight fluorescent dye is not expected to interfere with either FH(18-20) or Fc interaction with SdrE or spA (protein A), respectively.

Due to the propensity of FH(18-20)-Fc to aggregate, the first modification attempt yielded no protein. We substituted spin filters with dialysis to circumvent this difficulty. Labeling of FH(18-20)-Fc was performed twice to yield enough labeled FH(18-20)-Fc for Aim 2.1 (competition assay detecting FH(18-20)-Fc bound to intact cells). For a negative control protein, DAF-Fc (decay accelerating factor-Fc) was chosen due to similar N-glycosylation sites to FH(18-20)-Fc. Another negative control Fc fusion, DPP4-Fc (dipeptidyl peptidase 4), has significantly more terminal galactose sites compared to FH(18-20)-Fc, making it an unsuitable protein for labeling.

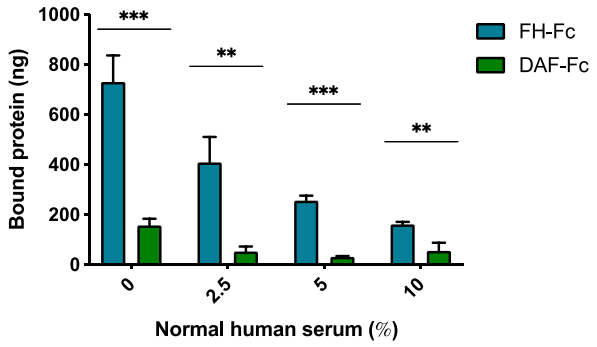
Target date for completion: End of month 4

Actual completion date: End of month 4

Goal (Major Task) 2: Examine potency of FH/Fc by measuring its effect on the binding of serum FH by *S. aureus*

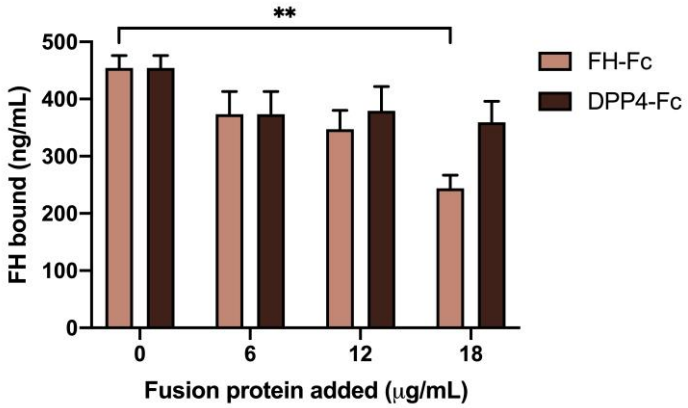
Subtasks 1 and 2: Completed the HRPO review/approval process and isolated serum from healthy volunteers for use in subsequent experiments involving complement.

Subtask 3: Assess the level of FH(18-20)-Fc binding to MRSA in the presence of serum FH (NHS, normal human serum). *S. aureus* strain R7 was incubated with 3 µg fusion protein with various amounts of NHS. After washing, bound fusion protein was measured using a fluorescent plate reader. A standard curve of purified labeled fusion protein was used for quantitation purposes. Binding of FH(18-20)-Fc was compared to DAF-Fc.

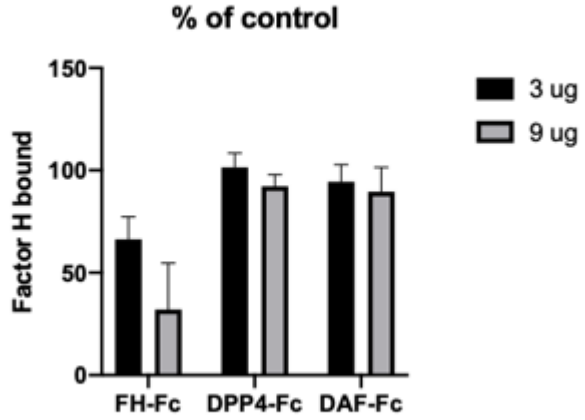


Using this data (Figure 1), we extrapolated that NHS inhibits the binding of 3 µg of FH(18-20)-Fc by 50% at a concentration of 3.269% serum.

The next experiment was a competition assay: assessing serum FH bound in the presence of increasing amounts of non-labeled fusion protein (2.5% Normal Human Serum). ELISA probing with anti-CCP1 mAb (Quidel); this mAb binds to an epitope found on serum FH but not on FH(18-20)-Fc.



The reduction of serum FH binding in the presence of 18 μg/mL FH(18-20)-Fc compared to control (no fusion protein) was significant, indicating that FH(18-20)-Fc competes with serum FH for SdrE binding under these conditions (Figure 2).



Extracts were also evaluated via anti-FH western blot (WB), with FH bands compared to % of control to determine effect. Due the semi-quantitative nature of a WB, these results are not as tight (Figure 3); however, they confirm the ability of FH(18-20)-Fc to compete with serum FH for R7 recruitment in a specific manner (DPP4-Fc and DAF-Fc did not compete).

Milestone Achieved: Optimize concentration of FH(18-20)-Fc that competitively reduces serum FH binding by MRSA.

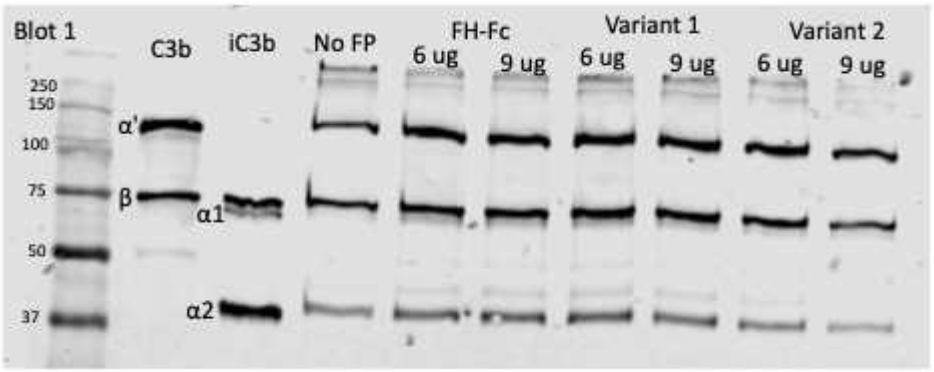
Target date for completion: End of month 8

Actual completion date: End of month 8

Goal (Major Task) 3: Examine potency of FH(18-20)-Fc by measuring its effect on complement-mediated opsonization of *S. aureus*

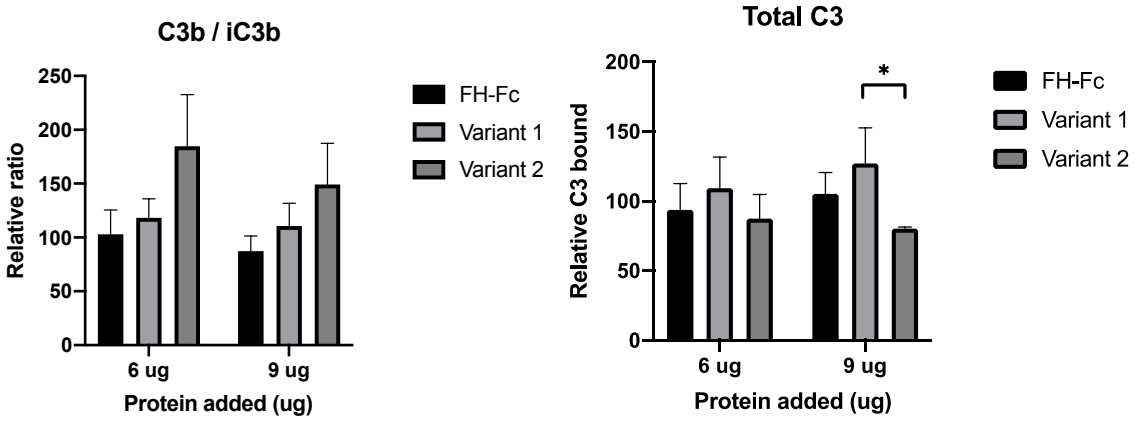
Subtask 1: Investigate effect of *S. aureus*-bound FH(18-20)-Fc on C3-fragment deposition

S. aureus strain R7 was incubated in 2.5% NHS and various amounts of fusion protein. To assess the level of C3-fragment deposition, bound C3 was stripped with 25 mM methylamine. We tested FH(18-20)-Fc as well as DAF-Fc; however, since DAF-Fc retains functionality, it was found to be an unsuitable control when measuring effect of FP on C3-fragment deposition (complement activation).



Anti-C3 WB verified C3-fragment presence and identity (iC3b (α1 + α2), C3b (α'), total C3 (β)). Figure 4 (left) is data from the WB using 6 μg vs 9 μg of FH(18-20)-Fc.

Optical densitometry of the WB resulted in Figure 5:



Interestingly, Variant 2 leads to less overall C3-fragment deposition, but with a higher proportion of C3b vs iC3b. In Variant 2, the CH2 and CH3 domains of IgG1 have been replaced with the corresponding domains of IgG3. IgG3 activates complement- and FcγR-mediated functions more effectively than other subclasses, so this was a surprising result.

Based on this result, we varied the method by doubling the reaction volume and using 30 µg of FH(18-20)-Fc in 1.25% NHS vs 45 µg of FH(18-20)-Fc and 2.5% NHS, keeping the amount of R7 constant. We saw a trend with this change (n=1); however, the experiment needs to be repeated then quantified with a C3 ELISA.

Target date for completion of Subtask 1: End of month 1

Actual completion date: End of month 12

- **What opportunities for training and professional development has the project provided?**
  - *Nothing to Report*
- **How were the results disseminated to communities of interest?**
  - *Nothing to Report*
- **What do you plan to do during the next reporting period to accomplish the goals?**

During the remaining 6 months of the grant we will:

- Determine how the three Variants of FH(18-20)-Fc affect the generation of anaphylatoxin C5a. This will complete Major Task 3, optimizing FH(18-20)-Fc effects on complement-mediated opsonization of MRSA.
- Measure the potency of the three FH(18-20)-Fc Variants in boosting opsonophagocytosis of MRSA.

IMPACT:

- **What was the impact on the development of the principal discipline(s) of the project?**
  - *Nothing to Report*
- **What was the impact on other disciplines?**

- *Nothing to Report*

- **What was the impact on technology transfer?**
  - *Nothing to Report*
- **What was the impact on society beyond science and technology?**
  - *Nothing to Report*

CHANGES/PROBLEMS:

There was a minor change to the protein labeling protocol, as described under Accomplishments. In addition, Dr Sharp's research laboratory was closed down for ___ months due to the COVID-19 pandemic.

PRODUCTS:

Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

Name:	*Keith Wycoff*
Project Role:	*Principal Investigator*
Researcher Identifier (e.g. ORCID ID):	0000-0002-5343-5329
Nearest person month worked:	1
Contribution to Project:	*Dr Wycoff was the Principal Investigator of this project.*
Funding Support:	

Name:	*Julia A. Sharp, Ph.D.*
Project Role:	*Co-PI (PI at EVMS)*
Researcher Identifier (e.g. ORCID ID):	*0000-0001-7473-1058*
Nearest person month worked:	*3*
Contribution to Project:	*Dr. Sharp oversees the grant at EVMS including experimental design and performance, data analysis, reporting, and training staff.*
Funding Support:	

Name:	*James Maclean*
Project Role:	*Molecular biology*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	0.6
Contribution to Project:	*Dr Maclean*
Funding Support:	

Name:	*Y Tran*
Project Role:	*Protein purification*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	*Mr Tran*
Funding Support:	

Name:	*Megan Golliher*
Project Role:	*Graduate Student*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*3*
Contribution to Project:	*Ms. Golliher performed experiments associated with Aim 2 of the grant, specifically factor H competition assays and data analysis. She also fluorescently labeled fusion proteins (Aim 1) for use in Aim 2.*
Funding Support:	*Effort not monetarily compensated*

Name:	*Michele Semeraro, M.S.*
Project Role:	*Research Assistant*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*5*
Contribution to Project:	*Ms. Semeraro performed experiments associated with Aim 2 of the grant, specifically fusion protein binding comparisons (including optimization). She also contributed to data analysis, protocol troubleshooting, and assisted with technical issues.*
Funding Support:	

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

- **Changes to active other support since the grant started is provided below**

Updated Previous/Current/Pending Support (PCPS)

Pending support

Project title	**FH-Fc as a Pre-Exposure Prophylactic for Tickborne Disease**	
Funding agency		NIH
Goals	The overall goal of this project is to demonstrate the efficacy of SCR6,7/Fc and SCR(18-20)/Fc in preventing Lyme borreliae infection in a mouse model of LD and determine a minimal effective dose.	
Aims/Tasks	Aim 1. Modify the Fc of SCR6,7/Fc to improve complement activating activity Aim 2. Determine the relative potency of FH-Fc variants in killing TBPs *in vitro* Aim 3. Determine the efficacy of FH-Fc to prevent TBP infections *in vivo*	
Start and end dates		07/01/2020 – 06/30/2022
Level (%) of effort	Keith Wycoff	10%
	James Maclean	12.5%
	Y Tran	12.5%
Grants officer	Alford, Trevor T.	trevor.alford@nih.gov
Level of overlap		No overlap

New support

Project title	**An immunotherapeutic to prevent gonorrhea**	
Funding agency		NIAID
Goals	Planet Biotechnology leads a collaborative project to evaluate 5 new variants of FH(18-20)/Fc for improved ability to kill *Neisseria gonorrhoeae in vitro* and *in vivo*. The best variant will be formulated into pod-IVRS and tested for PK and safety in non-human primates. A formal 6 month stability study will be conducted and a techno-economic analysis of manufacturing will be performed.	
Aims/Tasks	Aim 1: Evaluate the *in vivo* efficacy of FH*/GS-hFc variants Aim 2: Test for bactericidal activity against an expanded repertoire of clinical isolates Aim 3: Purification scale-up Aim 4: Evaluate PK and safety of intravaginal ring administration in NHP Aim 5: Perform 6 month drug product stability study Aim 6: Initiate technology transfer to CMO and consult with FDA	
Start and end dates		07/01/2019 – 01/31/2022
Level (%) of effort	Keith Wycoff	16.7%
	James Maclean	16.7%
	Y Tran	41.7%
Grants Officer	Lundgren, Jason A.	lundgrenj@mail.nih.gov
Level of overlap		No overlap

Project title	**Recombinant Fc fusions for treatment of uropathogenic *E. coli***
Funding agency	NIDDKD
Goals	Urinary tract infections are among the most common bacterial infections, of which up to 80% are caused by *E. coli*. At increasing frequency, these pathogens are resistant to most or even all last line antibiotics. We have devised a hybrid protein combining a protein fragment that can block bacterial cell attachment with an immune defense-activating portion of human antibody. We will demonstrate the ability of this hybrid to facilitate killing multi-drug resistant uropathogenic *E. coli*, *Enterobacter* and *Klebsiella pneumoniae*.
Aims/Tasks	Aim 1: Produce DPP4-Fc with varying N- glycosylation and compare potency in *vitro* Aim 2: Modify the Fc of DPP4-Fc to improve complement activating activity
Start and end dates	04/01/2020 – 03/31/2021

Level (%) of effort	Keith Wycoff	16.7%
	James Maclean	16.7%
	Y Tran	16.7%
Grants Officer	Keane-Myers, Andrea	andrea.keane-myers@nih.gov
Level of overlap		No overlap

Project title	**Improving gene expression via Massively Parallel Synonymous Codon Variant Screening**	
Funding agency		NIGMS
Goals	A novel method for identifying synonymous codon variants that result in optimal protein expression will be developed.	
Aims/Tasks	Aim 1: Generate diverse synonymous codon libraries to express human FIX Aim 2: Express libraries in tobacco plants and mice Aim 3: Next generation sequencing to "count" transcripts of each codon variant Aim 4: Test RNA and protein expression of the "best" variants individually	
Start and end dates		03/01/2020 – 02/28/2021
Level (%) of effort	Keith Wycoff	12.5%
	James Maclean	12.5%
	Y Tran	16.7%
Grants Officer	Lynch, Jennifer M.	jennifer.lynch@nih.gov
Level of overlap		No overlap

Recently ended support

Project title	**An immunoadhesin therapy for glioblastoma targeting CD97**	
Funding agency		NCI
Goals	Develop a recombinant therapeutic protein for glioblastoma	
Aims/Tasks	Aim 1: Produce multiple forms of human DAF-Fc using a plant expression system Aim 2: Evaluate the ability of DAF-Fc to target glioblastoma cells in culture Aim 3: Evaluate DAF-Fc in a human xenograft model of glioblastoma in mice	
Start and end dates		07/04/2018 – 06/30/2019
Level (%) of effort	Keith Wycoff	9.2%
	James Maclean	12.5%
	Y Tran	30%
Grants Officer	Meininger, Jennifer S.	jennifer.meininger@nih.gov
Level of overlap		No overlap

What other organizations were involved as partners?

- **Organization Name: Eastern Virginia Medical School (EVMS)**
- **Location of Organization:** Norfolk, VA
- **Partner's contribution to the project**
  - **Collaboration**