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Hemostasis Research Program

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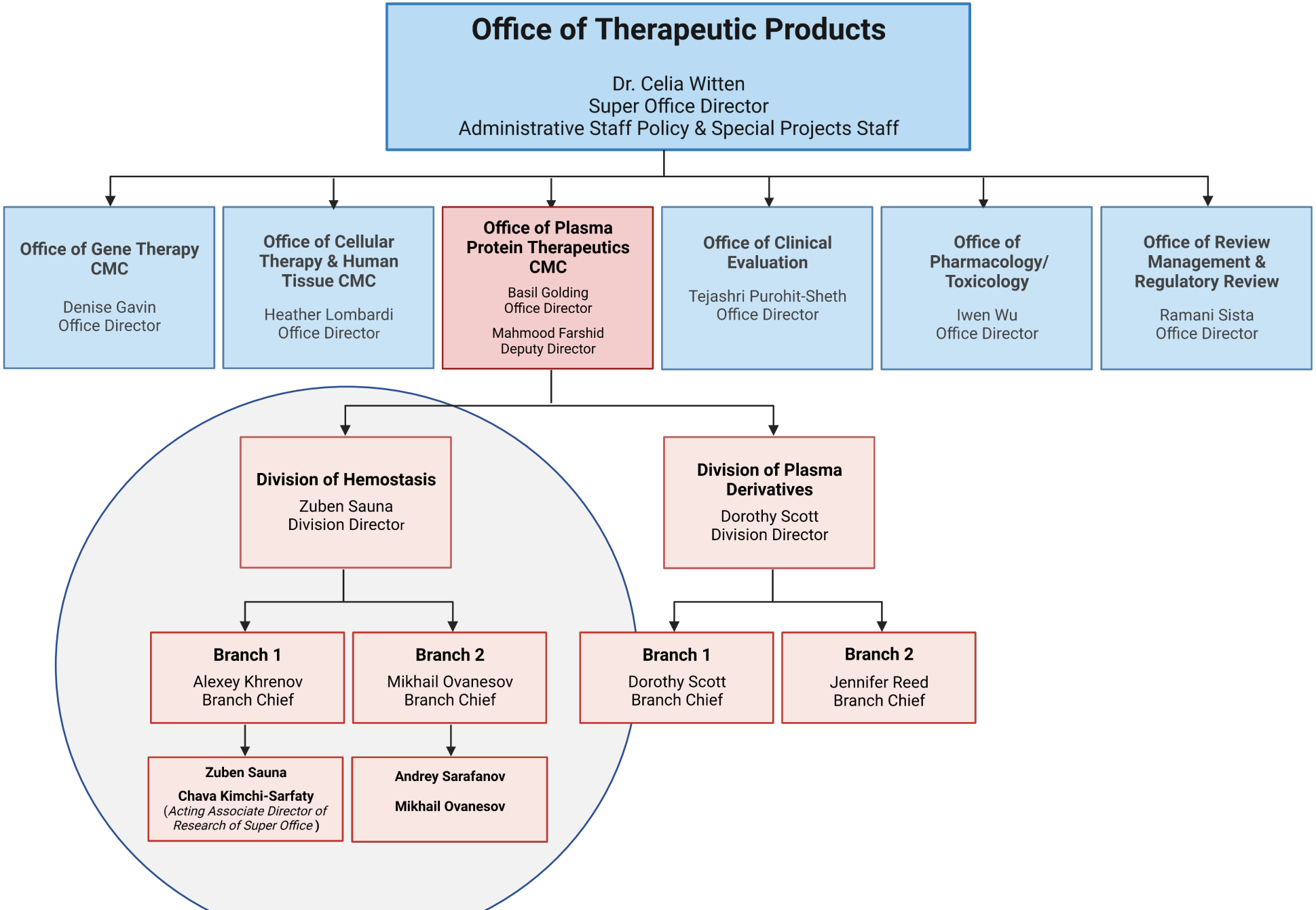
Office of Plasma Protein Therapeutics (OPPT)
Office of Therapeutic Products (OTP)
Center for Biologics Evaluation and Research (CBER)
U.S. Food and Drug Administration (FDA)

BPAC Meeting
April 26th, 2023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Administrative Staff Policy & Special Projects Staff
Office of Gene Therapy CMC
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Heather Lombardi
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Office of Pharmacology/ Toxicology
Iwen Wu
Office Director
Office of Review Management & Regulatory Review
Ramani Sista
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Division of Hemostasis
Zuben Sauna
Division Director
Division of Plasma Derivatives
Dorothy Scott
Division Director
Branch 1
Alexey Khrenov
Branch Chief
Branch 2
Mikhail Ovanesov
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Branch 1
Dorothy Scott
Branch Chief
Branch 2
Jennifer Reed
Branch Chief
Zuben Sauna
Chava Kimchi-Sarfaty
(Acting Associate Director of Research of Super Office)
Andrey Sarafanov
Mikhail Ovanesov

Outline

- **Introduction**
- **Immunogenicity of Protein-based Therapeutics:**
  - Zuben E. Sauna, Ph.D.
- **Study of the Regulation of Blood Coagulation by Coagulation Factors VIIa, IXa and XIa:**
  - Mikhail V. Ovanesov, Ph.D.
- **Towards Longer-acting Factor VIII Products with Higher Purity:**
  - Andrey G. Sarafanov, Ph.D.
- **Synonymous Variations in Health and Disease:**
  - Chava Kimchi-Sarfaty, Ph.D.
- **Concluding Remarks**

Research Program #1: Immunogenicity of Protein-Based Therapeutics

Zuben E. Sauna, Ph.D.

Goals of the research program

1: THE IMMUNOGENICITY OF PROTEINS USED IN THERAPEUTIC APPLICATIONS

Identify the genetic and phenotypic determinants of immune responses to therapeutic proteins and elucidate the molecular mechanisms of immune responses

2: ENGINEERING PROTEINS FOR IMPROVED THERAPEUTIC OUTCOMES

Engineer proteins that have desirable therapeutic outcomes and/or minimal immunogenicity

3: COMPUTATIONAL AND BIOPHYSICAL STUDIES RELATED TO THE SARS-COV-2 VIRUS

Use bioinformatics tools to understand the diverse clinical symptoms and pathologies reported in COVID-19 patients and design novel antibody and non-antibody-based SARS-CoV-2 binding reagents

Representative published findings since 2017

1. Used statistical methods and an Artificial Intelligence based tool to identify variables associated with risk of developing anti-drug antibodies to Factor VIII used to treat hemophilia A.
2. Developed approaches for assessing the immunogenicity risk of Cas proteins used in gene editing.
3. Designed and evaluated Factor Xa variants that could successfully reverse uncontrolled bleeding following use of the Direct Oral Anti-Coagulant (DOAC) apixaban.
4. Developed a workflow to design antibodies that bind specifically to multiple SARS-COV-2 variants with nanomolar binding affinity.

Research from this research program resulted in 32 publications since 2017; of these 4 papers were published after the Site Visit in November 2022

Scientific accomplishments since site visit in Nov 2022

1. Initiated work on addressing the question: How do different populations cluster with respect to HLA and could this impact immunogenicity between populations?
2. Initiated work on comparing immunogenicity of Cas from different organisms
3. Initiated studies on safety (with respect to thrombogenicity) of reversal agents for DOAC bleeding
4. Design of less immunogenic variants of Cas9 with respect to engagement of HLA Class I molecules is ongoing
5. Initiated studies on evaluation of neutralization by antibodies that target the SARS-CoV-2 virus
6. Initiated studies on evaluating next generation SARS-CoV-2 binding agents (Ankyrons)
7. Six manuscripts were submitted for publication

2-7 were proposed future studies during site visit

Research Program #2:
Study of the Regulation of Blood Coagulation by Coagulation Factors VIIa, IXa, and XIa

Mikhail V. Ovanesov, Ph.D.

Overall approach

Drug development & control of drug quality require
better, more predictive and accurate hemostasis assays

The Ovanesov laboratory:

Collaborates on assay harmonization and reference standards

Refines new and existing hemostasis assays by:

- evaluating assay limitations
- comparing assay results with evidence of safety and efficacy in animal models

Goals of the research program

1. DEVELOP ANALYTICAL METHODS THAT CAN MITIGATE THE RISKS OF BLEEDING AND THROMBOSIS IN PATIENTS TREATED WITH PLASMA PROTEIN PRODUCTS:

Develop method modifications to measure hemostatic and thrombotic activity more accurately or with better sensitivity.

Connect evidence of assays' predictive value with the mechanisms of drug action and thrombogenicity.

2. DEVELOPMENT OF INTERNATIONAL STANDARDS FOR COAGULATION FACTORS

The laboratory participates in collaborative studies to develop harmonized methods and international standards for coagulation factors.

Major scientific accomplishments since 2017

- Thrombin Generation assay variability & standardization
- Factor XIa thrombogenicity
- Mouse pharmacokinetics and tail bleeding studies

16+2* peer-reviewed publications

Public meetings and workshops regrading hemophilia therapeutics, such as, WHO hearing on the development of product-specific reference materials for FVIII and FIX products (2019)

International reference standards and assay studies, such as, WHO 3rd IS for von Willebrand Factor, plasma (three methods) and the WHO 3rd IS for thrombin (three methods)

10+2* collaborative lab studies

* Since Site Visit in Nov 2022

Research Program #3: Towards Longer-acting Factor VIII Products with Higher Purity

Andrey G. Sarafanov, Ph.D.

Overall approach

The Sarafanov laboratory seeks to:

- Facilitate development of longer-acting (extended plasma half-life) therapeutical FVIII products

- Understand structurally compromised FVIII protein in approved products as an impurity and facilitate the control of these impurities

The overarching approach is to introduce modifications in recombinant proteins and test the proteins in:

1. A purified system
2. Cell culture
3. Animal models
4. In-silico modeling

Goals of the research program

1. ELUCIDATE THE MECHANISMS OF FVIII CLEARANCE FROM THE CIRCULATION

- Characterize of mechanisms of FVIII interactions with plasma clearance receptors
- Generate model FVIII fusion proteins having extended plasma half-life
- Elucidate the reasons for FVIII activity assay discrepancy in Hemophilia A patients subjected to gene therapy

2. Characterize structurally compromised protein in FVIII products

- Characterize FVIII subpopulations unable to bind von Willebrand Factor (VWF) in recombinant FVIII (rFVIII) products
- Characterize FVIII tyrosine sulfation as a factor affecting safety and efficacy of rFVIII products
- Development of analytical methods to address these questions

Major scientific accomplishments since 2017

- Elucidated the mechanism of FVIII interaction with its major clearance receptor in plasma, hepatic LRP.
- Developed a method to analyze the VWF-nonbinding protein in rFVIII products, characterized all U.S. rFVIII products for the content of this impurity, and characterized the structural properties of this protein.
- Characterized the degree of tyrosine sulfation in all U.S. rFVIII products.*
- Published 4 papers, and presented data in 19 research conferences (including oral presentations in ISTH conferences).

* Since Site Visit in Nov 2022

Research Program #4: Synonymous Variations in Health and Disease

Chava Kimchi-Sarfaty, Ph.D.

Goals of the Research Program

1. Major Project: THE IMPACT OF GENETIC VARIANTS ON THERAPEUTIC PROTEINS

Investigate the impact of synonymous variants on the biological and chemical properties of therapeutic proteins and establish artificial intelligence (AI) tools to predict the performance of genetically engineered therapeutics.

2. ADAMTS13 IN HEALTH AND DISEASE

Explore methods for assessing ADAMTS13 activity and investigate the role of ADAMTS13 and VWF in unique patient populations and in health and disease (e.g., Sickle Cell Disease).

3. SARS-COV-2: GENETIC CHARACTERISTICS AND INTERACTIONS WITH COAGULATION PROTEINS

Evaluate the nucleotide composition, codon usage, and mutational properties of SARS-COV-2 and identify host-viral protein complexes between coagulation-related and SARS-COV-2 proteins.

Major Scientific Accomplishments since 2017

1. Major Project: Impact of Genetic Variants on Therapeutic Proteins

Identified novel examples of how synonymous variants and codon optimization can impact mRNA and protein properties in multiple different models (e.g., *ADAMTS13, F9*) and established codon usage resources for their evaluation.

Published **18** peer-reviewed publications and a book (co-editor)*

2. ADAMTS13 in Health and Disease

Elucidated the role of the VWF-ADAMTS13 in many clinical conditions, including its role in preoperative thrombosis, COVID-19 associated bleeding disorders, sickle-cell disease and in patients with severe congenital heart disease.

Published **8** peer-reviewed articles and established a provisional patent application # 63/106,699 (mrADAMTS13)*

3. SARS-COV-2: Genetics and Interactions with Coagulation Proteins

Identified promising sites within the nucleocapsid and spike proteins for deoptimization and vaccine development and established pipeline for identifying coagulation-related host-viral protein complexes

Published **5** peer-reviewed articles*

*We chaired sessions and participated in internal and external numerous conferences

Scientific Accomplishments since site visit in Nov 2022

1. Initiated pharmacological and immunogenicity studies on codon and codon-pair optimized *ADAMTS13* and *F8* and the implications of lentiviral and AAV delivery.

2. Developing a tRNA bioinformatics pipeline and synonymous variant prediction tool.

3. Ongoing projects on investigating ADAMTS13-independent VWF regulation in other disease models.

4. Performing a meta-analysis on the relationships between endothelial dysfunction, coagulation, and SARS-CoV-2 infection.

5. Two manuscripts accepted (methods for evaluating synonymous variants and comprehensive analysis of SARS-CoV-2 mutational profiles); Two manuscripts are under review.

Concluding Remarks

- OPPT have established 4 productive programs in the Hemostasis Research Program with a strong track record of publications.
- Research from this program has been presented at many conferences (e.g., ISTH).
- Our programs have initiated numerous emergency SARS-CoV-2 projects in response to the public health crisis.
- Our programs have characterized numerous biological products and developed novel assays for their assessment to enhance FDA regulation.

Thank you!