Overview of
Hemostasis Branch

Meeting of the Blood Products Advisory Committee
22 June 2018

Hemostasis Branch

Researcher-Reviewers

- Chava Kimchi-Sarfaty, PhD, Research Chemist
  - Nobuko Katagiri, PhD, Research Biologist
  - Aikaterini Alexaki, PhD, Staff Fellow
- Mikhail Ovanesov, PhD, Research Biologist
  - Yideng Liang, PhD, Staff Fellow
  - Mark Verdecia, PhD, Staff Fellow
- Andrey Sarafanov, PhD, Chemist
  - Svetlana Shestopal, PhD, Staff Fellow
  - Haarin Chun, PhD, Visiting Associate
- Zuben Sauna, PhD, Research Biologist
  - Vijaya Simhadri, PhD, Visiting Associate
  - Wojciech Jankowski, PhD, Staff Fellow

Hemostasis Branch

Full-time Regulatory Reviewers

- Tim Lee, PhD, Branch Chief
- Natalya Ananyeva, PhD, Chemist / Team Lead
- Alexey Khrenov, PhD, Senior Staff Fellow
- Coty Huang, MS, Biologist
- Ze Peng, PhD, Biologist
- FTE

Licensed products regulated by HB

- **Coagulation Factors**
  - Factors VIII and IX (Human plasma-derived & Recombinant)
  - Factor VIII/von Willebrand Factor Complex
  - Fibrinogen Concentrate
  - Factor X
  - Factor XIII
- **“Bypassing” Agents**
  - AICC (e.g., FEIBA)
  - Recombinant activated Factor VII
- **Hemostatic Agents**
  - Thrombin (Bovine, Human & Recombinant)
  - Fibrin Sealant
  - CryoSeal FS System
  - Fibrin Sealant Patch
- **Anti-coagulants**
  - Protein C
  - Antithrombin III (Human plasma-derived & Recombinant)
- **Reversal Agents for Anticoagulants**
  - Prothrombin Complex Concentrate
  - Recombinant Factor Xa Variant, Inactivated

Hemostasis Branch
Regulatory Responsibilities

- Review applications for:
  - Investigational products
  - Marketing of new products
  - Changes in manufacturing of, or indications for licensed products
- Serve as product specialist at Inspection of manufacturing facilities
- Test product batches

Hemostasis Branch
Regulatory Responsibilities (Cont.)

- Review of Biological Product Deviation Reports
  - Assessment of risk and response
- Develop policy and guidance
  - Product safety and efficacy
  - Current Good Manufacturing Practices
- Pre-submittal Support
  - Review of briefing material
  - Meeting with sponsors
  - Preparation of summaries

Original BLAs Approved between 2013 & 2017

- Tretten
- IXinity
- Alprolix
- Eloctate
- Coagadex
- Raplixa
- Obizur
- Nuwiq
- Adynovate
- Kovaltry
- Vonvendi
- Idelvion
- Afstyla
- Rebinyn
- Fibryga

Regulatory Activities between 2013 & 2017

Completed

- Over 650 BLA Supplements, Annual Reports and other miscellaneous submissions
- 250 original INDs & their associated amendments

Participated in

- Facility inspections on-site and by phone
- International calibration studies for reference standards

Emerging Products

- Improve pharmacological properties
  - New recombinant coagulation factor variants
  - New fusion proteins of coagulation factors, e.g., with XTEN or CTP
  - New PEGylated coagulation factors

- Recombinant variants for the treatment of arterial thrombosis and thromboembolism

- Recombinant ADAMTS13 for TTP

- Plasma-derived proteins to treat respective congenital deficiencies

Hemostasis Branch Research Activities

Towards more effective treatment of blood clotting disorders: Pharmacogenomic Studies of ADAMTS13, Factor VIII and Factor IX

Chava Kimchi-Sarfaty, PhD

Mission relevance

1. Develop scientific expertise to understand the biology and physiology of biological products, specifically the outcome of mutations/variations in therapeutic proteins
2. Facilitate the development of safe and effective biological products by providing the public with prediction tools to estimate the consequence of changes in coding sequence of therapeutic proteins during product design
3. Characterize the biology and functionality of ADAMTS13 and von Willebrand Factor in the population and in specific disease states to further recognition of efficacy and safety implications of these therapeutic proteins in patient-specific contexts (e.g., pediatric congenital heart disease, sickle cell disease)

Main goals of Kimchi Laboratory (Project # 1)

When and how do synonymous variants impact protein biogenesis?

- Examine the effect of *F9* non-synonymous and synonymous mutations on splicing (*in silico* and *in vitro*)
- Improve the tools to identify which mRNA and protein domains are more or less favorable to manipulation (*in silico* and *in vitro*)
- Identify correlations between experimental ribosomal profiling data and *in silico* predictions, through statistical analysis

Progress since October 2013 (Project # 1/a)

- Established single gene copy cell lines with a defined integration site to study the effects of codon optimization under a controlled genetic environment (*in vitro*)
- Designed new, bi-codon usage tables (*in silico*)
- Developing a hemophilia-specific prediction tool to estimate the consequence of synonymous and non-synonymous mutations and validate this tool (*in silico*)
- Studying the effect of synonymous polymorphisms in *ADAMTS13* on protein expression, conformation and function (*in vitro*)
- Examining the effect of *F9, ADAMTS13* bi-codon optimization on protein expression, conformation and function (*in silico* and *in vitro*)

Progress since October 2013 (Project # 1/b)

- Examining various codon optimized *F9* altered antigen processing / presentation (*in silico* and *in vitro*)
- Testing codon optimized *F8* using ribosome profiling to develop better algorithms for the optimization of *F8* sequence (*in silico* and *in vitro*)
- Developing codon usage tables (CUTs) for a variety of healthy and cancer tissues and primary cells in order to better understand tissue-specific codon usage bias (*in silico*)

Main goals of Kimchi Laboratory (Project # 2)

Investigate the role of ADAMTS13 in different hematologic conditions

- Develop *in vitro* assays to measure ADAMTS13 expression and function
- Understand the biology and impact of ADAMTS13 & vWF in sickle cell disease (SCD) population (*in vitro*)
- Examine ADAMTS13 expression and function in various non-activated and activated primary cells (*in vitro*)

U.S. FOOD & DRUG ADMINISTRATION

Study of Regulation of Blood Coagulation by Coagulation Factors VIIa, IXa and XIa

Mikhail V. Ovanesov, PhD

Mission Relevance

- **Standardization**
  - Biological reference standards for coagulation factor activity and antigen support accurate potency assignment, detection of harmful procoagulant impurities, and diagnosis of bleeding disorders
- **Harmonization**
  - Ensure analytical procedures give comparable results in different laboratories
- **Mechanisms of action**
  - The mechanisms of action of hemostasis and thrombogenicity of some coagulation factors are poorly understood, which limits our ability to monitor & control their potency and level as a therapeutic protein or impurity.

Collaborative Studies for Standards

- Assigned potency and antigen values to new and replacement WHO International Standard (IS) for Coagulation Factors IX, IXa, XI, XIa and XII and the Working Standard for FEIBA
- Investigated commutability of reference standards by applying assay kits sourced from different vendors and assays based on different technology
- Currently, participating in Global Working Group on standardization of IG procoagulant assays, and leading its sub-group on harmonization of TG assays
- Future studies for the WHO 2nd IS for FV, the 5th ISTH SSC working plasma standard for Factors II, V, IX, X, VII, VIII and XI, the WHO 1st IS for Thrombin-Activatable Fibrinolysis Inhibitor (TAFI), and the WHO 1st IS for human FXa

Discrepancies between different Coagulation Factor Assay Methodologies

- Effective dosing and monitoring of coagulation factor products require reconciliation of the clotting factor potency assigned on the product label with the activity recovered in post-infusion patient plasma samples.
- Discrepancies in activity values between potency and PK assays for genetically and chemically modified long-acting coagulation factors can lead to the potential of over- or under-dosing of patients.
- We investigate assay conditions to understand discrepancies, and support development of assay harmonization approaches for long-acting products.

Mechanisms of Action of Factors VIIa and XIa

- **Factor VIIa (FVIIa)**
  - Over a dozen genetically and chemically modified FVIIa variants entered the product development pipeline in the last decade, with promises of extended intervals between doses, and faster or safer hemostatic responses.
  - We use a hemophilia A mouse model to study the efficacy of FVIIa variants *in vivo,* and compare several assays of FVIIa antigen, FVIIa activity, and thrombin generation for their ability to predict the duration of FVIIa action.

- **Factor XIa (FXIa)**
  - In 2010, FDA found that FXIa was a root-cause for thrombogenicity in immune globulin products, which were later withdrawn from distribution due to increased rates of adverse events.
  - We are now investigating the molecular mechanisms that block FXIa inactivation by plasma inhibitors, allowing FXIa activity to remain in blood for 24 hours and longer.

U.S. FOOD & DRUG ADMINISTRATION

Towards Longer Acting Factor VIII Products with Better Purity

Andrey Sarafanov, PhD

Mission Relevance

- Understanding the mechanisms of interactions between FVIII and FVIII/vWF complex and their clearance receptors facilitates regulation of long-acting FVIII and vWF products
- Development of method(s) to control impurities in FVIII products will improve product quality
- Taken together, this will improve the safety and efficacy of products for treatment of Hemophilia A

Research Goals

- **Investigation of mechanisms of Factor VIII (FVIII) clearance in two pathways:**
  - when FVIII is in complex with vWF
  - when FVIII is not bound to vWF

- **Characterization of inactive protein(s) in FVIII products and development of method(s) to control this impurity(ies)**

Progress since October 2013 (I)

- **Further characterized interactive sites of FVIII and its clearance receptors:**
  - LDLR: low-density lipoprotein receptor;
  - LRP: LDLR-related protein 1[1,2].

- **Proposed a "dynamic bivalent" model of interaction of FVIII with LDLR[3]. This could be a new mechanism of action for biomolecular interaction, particularly relevant to receptors from the LDLR family and their ligands.**

- **Performed initial mapping of LRP sites for binding vWF[4].**

Progress since October 2013 (II)

- **Expressed and characterized a codon-optimized B-domain deleted Factor VIII[1]:**
  - demonstrated an approach to characterize new products based on codon-optimized FVIII, which are expected to be on the market
  - proposed an explanation on the root cause of the atypical assay discrepancy with transgene FVIII in the ongoing clinical study for gene therapy for hemophilia A
- **Advanced the methodology of analyzing impurities in Factor VIII products[2]:**
  - optimized conditions of affinity chromatography
  - determined limitations of the hydrophobic interaction chromatography

Immunogenicity of Protein-based Therapeutics

Zuben E. Sauna, PhD

Mission Relevance

- Immunogenicity compromises the safety and/or efficacy of protein therapeutics and is a priority for regulatory agencies
- The human and economic costs of immunogenicity to patients, their caregivers and the healthcare system are considerable
- Immunogenicity adds to the risk and cost associated with drug development; the lack of predictive tools may discourage industry from developing products to treat rare diseases

Research Goals

- Identify the pharmacogenetic determinants of immunogenicity
- Develop *in silico*, *in vitro* and *ex vivo* tools for non-clinical predictions of immunogenicity
- Develop tools to assess neo-sequences in bioengineered protein therapeutics for immunogenicity risk
- Develop strategies to de-immunize protein therapeutics

Logical Progression of Research Program

1. Used computational methods on existing clinical data to ***propose*** that sequence mismatch between endogenous and infused proteins and affinity of the foreign peptides for an individual patient's HLA molecule could predict immunogenicity risk

2. Used a genotyped cohort of hemophilia A patients to ***demonstrate*** that the paradigm in (1) can be used to assign personalized immunogenicity risk

3. Based on (2) ***proposed*** that neo-sequence-HLA affinity in engineered therapeutic-proteins could be risk factors for immunogenicity

4. **D*emonstrated*** that the paradigm described in (3) is valid

Progress since October 2013

- Developed algorithms and experimental methods for non-clinical immunogenicity risk assessment
- Developed a mouse model to study the immune consequences of Fc-engagement with the numerous Fc-receptors found in mammals
- Demonstrated by using a post-hoc immunogenicity assessment of a recombinant Factor VIIa analog that the sequences introduced were strong T-cell epitopes
- Used an emerging technology, MHC Associated Peptide Proteomics (MAPPs) to identify Factor VIII-derived peptide on patient cells, which can be used to test hypotheses from clinical studies showing differing prevalence of inhibitors among Factor VIII product classes
- Developed assays to identify pre-existing antibodies to Cas9 which is used for genome editing, and established prevalence of anti-Cas9 antibodies in the human population.

General Conclusions of 2017 Site Visit

- The Site-visit Committee was satisfied with the progress made in each of the research programs.
- The Site-visit Committee was in broad agreement with the direction of the research programs in HB.
- The Site-visit Committee supports the recommendations for personnel actions put forth by the Division.

Thank You