NIH PROGRAMS For the PREVENTION of INHIBITORS

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Toward the Rational Design of Optimally Functional Non-Immunogenic Factor VIII Therapeutics

- *NHLBI-convened Working Group*
  - June 8-9, 2015
  - Planned with FDA ; CDC participation
- *Invited:*
  - factor VIII and glycan biochemists; immunologists; physician-scientists in hematology; computational biologists in the field of protein immunogenicity
- *Goals:*
  - discuss current/ applicable knowledge in this area
  - map out a research pathway toward an actionable understanding of the mechanisms underlying FVIII immunogenicity and the development of anti- FVIII antibodies in severe HA.

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NHLBI WG : Topics of Discussion

- *Emerging insights into FVIII biochemistry*
- *Critical aspects of FVIII immunogenicity and tolerance*
- *Exploring new tools /models for protein immunogenicity*
  - *In silico* design of functional non-immunogenic proteins
  - Therapeutic protein immunogenicity: animal models
- *Understanding the interrelationship between FVIII biology and immunogenicity : Critical scientific gaps and opportunities*

https://wcms.nhlbi.nih.gov/research/reports/national-heart-lung-and-blood-institute-working-group-toward-rational-design-optimally-functional

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1.Address *critical knowledge gaps in FVIII biochemistry* key to an actionable understanding of FVIII immunogenicity

.FVIII Biosynthesis:

- *Endothelial co-expression of FVIII and VWF*
- Native/bioengineered post-translational *FVIII modificatio*n e.g. glycosylation
- Cellular regulation of *misfolded FVIII & FVIII fragment/aggregate secretion:* **ubiquitination**

FVIII Structure/Function

- Relative roles *of FVIII C1 and C2 epitopes in both VWF and PL binding*
- Comparative biochemistry/immunogenicity of ***FV and FVIII***
- Role of *differential FVIII glycan signatures*

FVIII Protein and Cellular Interactions: Activation and Clearance

- Physiologic cellular/ membrane requirements for *Xase assembly/ function*
- *Role of VWF in FVIII clearance*
- Role of FVIII-fibrin complex *binding to platelets*
- Higher resolution *FVIII structural data* (VWF-bound and Xase-complexed) for *in silico* protein engineering and/or FVIII-mimetic scaffold design;

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2. Encourage targeted investigation *of critical gaps in current knowledge of human host immune response and tolerance* to *endogenous /exogenous FVI*II

Role of FVIII Protein

- *Intrinsic immunogenicity of* FVIII zymogen and activated protein
- *FVIII intrinsic immune co-stimulatory and pro-inflammatory* functions
- Nature/circumstances of first *FVIII encounter with host immune system*

B and T Cell Immunity

- Characterize *pre- and post-treatment FVIII-specific T / B cells* in infants/ children
- Further delineate *B and T cell FVIII epitopes*; pathogenic/non-pathogenic abs
- Characterize *T cell bias and naïve T cell counts in sHA;* requirement for *in silico modelling* of protein immunogenicity
- *Thymic education of T cells: ? Role of maternal-fetal mixing in utero*

Development and Restoration of Tolerance

- *Potential for modified/expanded Tregs: single-chain chimeric antigen receptors (CARs), B-cell activating receptors (BARs) to establish/restore FVIII tolerance;*
- *? Autologous TReg ex vivo production/transfusion;*
- *Oral FVIII antigen tolerance induction in neonates and children: Role of T reg LAP+*
- *Collaborations with the NIH Immune Tolerance Network*

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2. Encourage targeted investigation of *critical gaps in current knowledge of human host immune response and tolerance* to endogenous /exogenous FVIII (cont'd)

Genome/Epigenome/Transcriptome/Microbiome

- *Whole Genome Sequencing (WGS*) approaches to understanding immunogenicity; GWAS a challenge in small sample size populations. Consider:
  - ❑ Family trios, inclusion of extreme phenotypes to mitigate need for large sample size traditionally required for association studies
  - ❑ Combined cohorts (e.g., RBC immunogenicity) to increase sample size
  - ❑ N of 1 WGS in rare disease with extreme phenotype
- *Transcriptomics* to discriminate individuals who will develop pathogenic and non-pathogenic antibodies
- *Epigenomic characterization of the host immune response to FVIII*
- Role of the *microbiome in FVIII immunogenicity*

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3. Facilitate *the development/refinement/validation of and access to next generation technology* required to investigate critical knowledge gaps in FVIII biochemistry and immunogenicity.

Physicochemical technology

- Higher resolution *X-Ray crystallography*; small angle X-Ray scattering
- *Cryo EM* for near-atomic resolution of protein molecules and complexes
- Techniques to capture *glycan structure on the FVIII protein scaffold*
- Hydrogen-deuterium exchange *(HDE) mass spectrometry*
- Surface plasmon resonance *(SPR) spectroscopy*
- *Real time imaging of hemostasis*

Bioassays

- *Immune signature biomarkers/assays* for 80-95% prediction of inhibitor risk /early FVIII neutralizing and non-neutralizing FVIII antibody profiles
- Assays with increased sensitivity and specificity for *FVIII detection in cells/tissues*
- *Micro-assays* for studies in pediatric target populations
- *Endothelial cell factor expression* systems; *Protein conformation-specific antibodies*

Animal models

- Refinement /optimization of *humanized mouse model (*hematopoietic BLT-SCID)

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4. Encourage/facilitate the development *of national longitudinal hemophilia A cohorts* (PUPs, trios) generating robust datasets required to an actionable understanding of FVIII immunogenicity

Prospective longitudinal studies

- Robust clinical and laboratory genomic /phenotype characterization (including HLA typing)
- National biospecimen repository for multi-parameter studies
- Prioritized data collection during high risk periods for antibody development
- Long term assessment of the immunogenicity of novel engineered FVIII products
- Harmonized with European efforts

Prospective tolerance therapy trials

- Eventual antigen-specific (e.g., TRegs , orally administered FVIII)
- Interim antigen-agnostic (e.g., immunomodulatory drugs)

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5. Facilitate the attraction of scientists in diverse fields to the *collaborative multidisciplinary study of FVIII immunogenicity.*

Critical scientific disciplines include:

- ❑ FVIII biochemistry
- ❑ Structural biology
- ❑ Glycan biochemistry
- ❑ Immunology (B and T cell tolerance)
- ❑ Bioengineering
- ❑ Genomics/ Systems biology
- ❑ Computer modelling
- ❑ Data science
- ❑ Clinical/translational hematological science
- ❑ Novel technology development (including public-private partnerships)

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NHLBI/DBDR Next Steps

- ➢ Encourage and facilitate basic and translational science in the mechanisms of FVIII immunogenicity and the application of new discoveries to novel diagnostics and therapeutics
- ➢ Multilevel approach
  - ➢ Facilitate scientific innovation
    - ➢ Genomics- ***TOPMed***
  - ➢ Facilitate training in basic, translational and clinical science relevant to rare diseases
  - ➢ Stimulate novel technologies development
  - ➢ Explore partnerships for national hemophilia cohort development
    - ➢ *Request of Informatio (RFI)* anticipated

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TOPMed Goals

- *Build a WGS Resource*
  - Diversity
  - Depth and breadth of information
  - Large sample size
  - Ability to integrate/analyze data
- *Early Discovery*
  - Focus on specific diseases
  - Focus on specific patient groups
  - Focus on underrepresented groups

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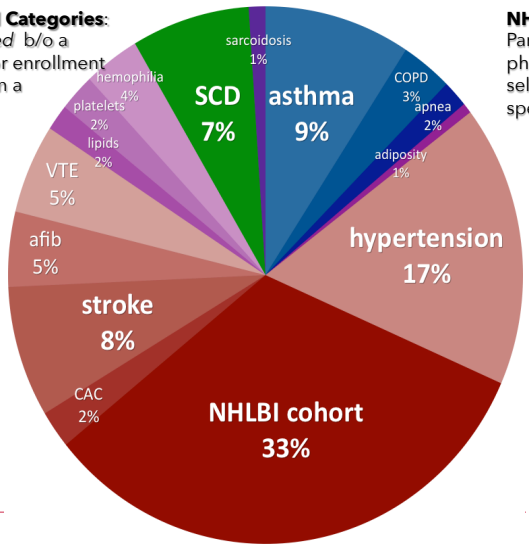
Phenotype Focus in TOPMed

Phase 1 and 2: 62,045 subjects

Phenotype Focused Categories: Selected into *TOPMed* b/o a specific phenotype or enrollment in a study focused on a specific phenotype

NHLBI Cohort Category: Participants with broad phenotypes that were not selected into *TOPMed* for a specific phenotype.



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Blood Disease Cohorts

- ***Hemophilia Blood Disease Cohort (4%)***
  (*Genetic Modulation of Inhibitor Risk in Hemophilia)*
  - Source**: MY Life/Our Future** biospecimens / ATHN phenotype data
  - Cohort sample size: 2,188 expanding to >5,000
  - PI: Barbara Konkle (University of Washington/Blood Works)
- ***GeneStar Cohort (2%)***
  (Family-based Exome Sequencing to Identify Platelet Aggregation Genes)
  - Cohort sample size: 1,400
  - PI: Rasika Mathias
- ***ARIC Cohort (5%)***
  (Risk factor identification in a VTE sub study of DVT/PE subjects)
  - Cohort sample size: 6,000
  - PI: Eric Boerwinkle

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Discussion

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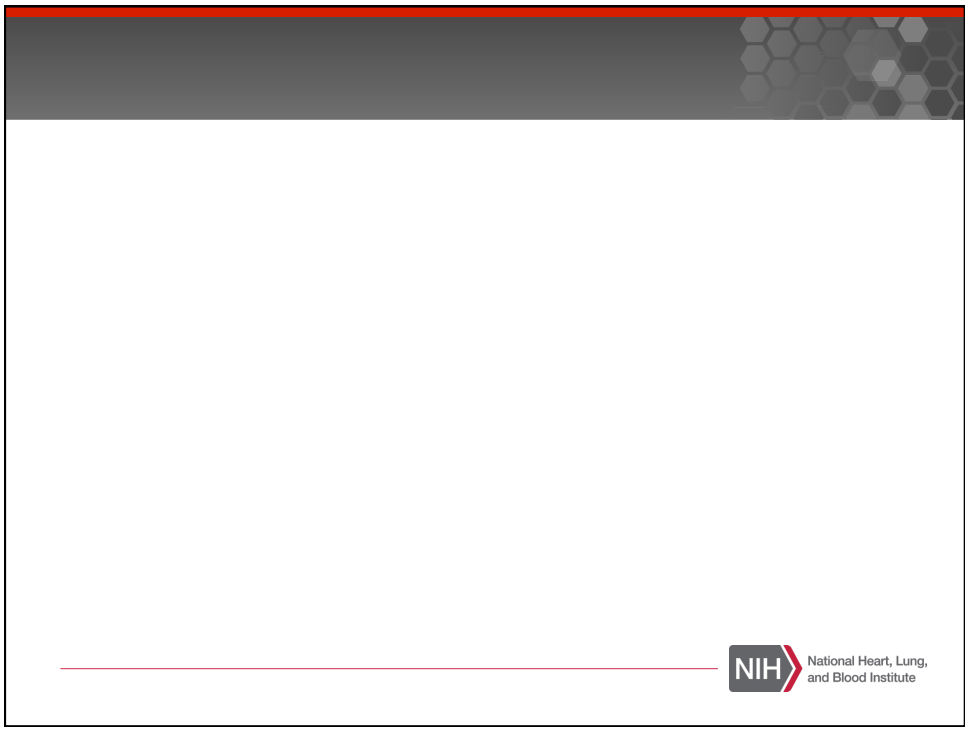

SCD Blood Disease Cohorts

SIT	***James Casella***	***1,074***
WALK PHASST	***Mark Gladwin***	***720***
REDS III Brazilian Cohort	***Busch***	***2,809***

Combined: 7 % of the TOPMed Cohort
Other Prospective SCD cohorts in the pipeline

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