Genetic Engineering for Hemophilia in Georgia

Hemophilia State Comprehensive Care Meeting
March 2, 2017
Chris Doering, PhD
Associate Professor
Department of Pediatrics
Aflac Cancer and Blood Disorders Center
Era of Gene Therapy?
Approved Gene Therapy Products

**Glybera®** *(alipogene tiparvovec)*
- Approved in 2012
- Indication – Lipoprotein lipase deficiency
- Price – $1,000,000, “world’s most expensive drug”
- Market – 100 new cases per year

**Strimvelis™**
- Approved in 2016
- Indication – Adenosine deaminase deficiency
- Price – $665,000
- Market – 250 new cases per year
Today’s Topics

1. Gene therapy background

2. History of clinical gene therapy for hemophilia

3. Gene therapy enlightenment

4. Liver-directed *in vivo* gene therapy

5. *Ex vivo* stem cell gene therapy
Human Gene Therapy: Basics

• Definition: the use of nucleic acids (DNA/RNA) as a pharmaceutical agent to induce therapeutic gene/protein expression in a patient

• A “vector” is a vehicle used to transfer genetic material to a cell or host (e.g. viruses).

• Vector can be administered *ex vivo* (outside the body) to extracted human cells prior to transplantation or *in vivo* (inside the body; e.g. intravenous delivery)

• Therapeutic effect can be acute or durable depending on the choice and design of vector as well as the type of cell targeted
Long Term Correction of Hemophilia Requires Gene Transfer

- Liver transplantation cures hemophilia
- However, no hepatocyte (Factor IX; FIX) or endothelial (Factor VIII; FVIII) stem cell clinical transplantation products exist
- Use of autologous cells will require gene editing/addition
- Functional FVIII biosynthesis occurs following gene addition into non-endothelial (heterologous) cells
## Initial Clinical Trial Disappointment 😞

<table>
<thead>
<tr>
<th>Vector/promoter (study sponsor)</th>
<th>Delivery/primary organ</th>
<th>Subjects (N)</th>
<th>Results</th>
<th>Complications</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Hemophilia A:</strong></td>
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</tbody>
</table>
| Non-viral/fibronectin with B domain-deleted factor VIII cDNA (Transkaryotic Therapies) | omental implantation after \textit{ex vivo} electroporation, selection & growth of autologous, cultured fibroblasts | 12 | • no sustained responses  
• possible transient, factor VIII levels in some but at level of detection  
• possible decreased infusion requirements in some | no significant adverse events; requires skin biopsy and laparoscopic surgery with factor VIII | Roth et al, 2001 |
| Retroviral/viral-LTR with B domain-deleted factor VIII cDNA (Chiron) | 3 daily intravenous doses/liver | 13 | • no sustained responses  
• possible prolonged survival of transfused factor VIII in some  
• possible decreased infusion requirements in some | none observed | Powell et al, 2003 |
| Adenoviral/albumin with full-length factor VIII cDNA (GenStar) | intravenous/liver | 1 | • no sustained response  
• possible transient factor VIII levels but at level of detection | transient liver dysfunction and thrombocytopenia | White, 2003 |


- “No sustained response” \textit{i.e.} plasma FVIII activity
- No complications observed in 2/3 trials
- 0/3 trials progressed to phase 2
Gene Therapy: HYPE or HOPE?

A. Original Gartner Hype-Hope Cycle

- Peak of Inflated Expectations
- Plateau of Productivity
- Slope of Enlightenment
- Trough of Disillusionment
- Technology Trigger
- Visibility
- Time

Standing on the shoulders of stem cell gene therapists

By JASON GARDNER

Enlightenment #1: Clinical Gene Therapy Vector Platforms

**Adeno-Associated Viral (AAV) Vector**
- Protein capsulated, ssDNA containing
- Family: *Paroviridae*
- 20 nm diameter
- 4.7 – 5.0 kb packaging limit
- Predominantly episomal (duration?)
- Wild-type virus non-pathogenic (?)
- Clinical safety and efficacy data exist
- Single approved product (Glybera)

**Retroviral Vector**
- Lipid enveloped, RNA virus
- Family: *Retroviridae*
- 100 nm diameter
- 7 – 10 kb packaging limit
- Predominantly integrating
- Wild-type HIV virus known cause of AIDS
- Clinical safety and efficacy data exist
- Single approved product (Strimvelis)
Enlightenment #2: Bioengineering
FVIII and FIX

• Increase specific activity
  (FIX Padua mutation)

• Codon optimization

• Increase sugar attachments

• Single chain design

• Decrease intracellular detection

• Adopt ancestral properties

• Spark Therapeutics

• University College London/ BioMarin;
  Sangamo, Dimension Therapeutics, Spark
  Therapeutics, Expression Therapeutics

• University of Michigan, University College
  London

• Children’s Hospital of Philadelphia

• University of Michigan, Emory University,
  Expression Therapeutics

• Emory University/Georgia Institute of
  Technology
Recombinant Porcine FVIII (OBIZUR): Product Development Timeline

- 1994: Patent Issued
- 1998: Company founded
- 2002: Lead molecule acceptance
- 2006: IND filing
- 2002: Phase 1 trial
- 2006: Phase 2 trial
- 2010: Phase 3 trial
- 2014: Bankruptcy
- 2018: Baxter purchase BLA
- 2018: Product approval/launch!

Pete Lollar, M.D.
OBI-1 (OBIZUR, Baxter) Inventor
ET3 inventor
Expression Therapeutics co-founder
Recombinant Porcine FVIII and the Discovery of High Expression FVIII

Bioengineering human FVIII for high expression
ET/Emory-FVIII (ET3) – 91% identical to BDD hFVIII

Doering CB et al. *J Biol Chem.*

Doering CB et al. *Mol Ther.*

Doering CB et al. *J Biol Chem.*
2002;277(41):38345-38349.
Mining ancient proteins for next-generation drugs

Robert A Lazarus & Friedrich Scheiflinger

Predicting the evolutionary ancestors of a protein drug provides a strategy for optimizing its pharmaceutical properties.

Robert A. Lazarus is in the Department of Early Discovery Biochemistry, Genentech, Inc., South San Francisco, California, USA, and Friedrich Scheiflinger is at Research EU, Shire, Vienna, Austria.

e-mail: lazarus.bob@gene.com or fritz.scheiflinger@shire.com
FVIII Bioengineering: A ‘biobetter’ FVIII variant with improved expression, activity, stability and reduced immune reactivity

Enhancing the pharmaceutical properties of protein drugs by ancestral sequence reconstruction

Philip M Zakas¹, Harrison C Brown¹, Kristopher Knight², Shannon L Meeks², H Trent Spencer¹,², Eric A Gaucher³ & Christopher B Doering¹,²

Dr. Phil Zakas

Dr. Eric Gaucher
Increased Production/Activity

**Recombinant Production**

**In Vivo Gene Therapy**

- Increased Production/Activity
- Recombinant Production In Vivo Gene Therapy
- AAV dose – $2 \times 10^{11}$ vg/kg
Decreased Immune Reactivity

- An-68
- An-53

**In vitro antigenicity**

**In silico immunogenicity**
Collaboration between Amit Nathwani (University College London) and Andrew Davidoff (St. Jude Children’s Research Hospital)
**In Vivo Liver-Directed AAV (or LV) Vector Gene Transfer**

Vector encoding a liver-directed FVIII expression cassette

Recipient (Affected individual)

Liver hepatocyte transduction by infused recombinant vector

Secretion of functional FVIII into the blood circulation
Results from First 10 AAV-FIX Patients

- AAV8, self complementary, liver-directed promoter with codon-optimized FIX transgene
- Phase 1 dose escalation, safety study
- No adverse events other than mild transaminitis that responds to corticosteroid treatment (high dose cohort only)
- All patients in high dose cohort are displaying 3–8% FIX activity levels
Other Hemophilia B Clinical Programs

- Shire (formerly Baxalta)
  - Trial halted and program terminated. Several patients developed anti-capsid immunity and simultaneous reduction in FIX activity

- Uniqure
  - Ongoing phase 1/2 trial. Several patients displaying FIX activity in the 2 – 5% normal range.

- Spark Therapeutics
  - Ongoing phase 1/2 trial. 6 of 7 patients displaying FIX activity in the 20 - 40% normal range with improved QoL.
  - Ongoing phase 1/2 trial. Data similar to uniqure.

- Dimension Therapeutics

- Christian Medical College (Vellore), Expression Therapeutics (USA), Emory University (USA) and University of Florida (USA)
  - More to come!
BMN-270 Phase 1/2 Study

Interim results of an open-label, Phase 1/2 study of BMN 270, an AAV5-FVIII gene transfer in severe hemophilia A

John Pasi
Professor of Haemostasis and Thrombosis
Barts and the London School of Medicine and Dentistry
The Royal London Hospital
UK

On behalf of
Savita Rangarajan, Glenn Pierce, David Perry, Jonathan Wilde, Bella Madan, Didier Rouy and Wing Yen Wong

July 27, 2016

- FVIII sequence in the vector encodes “SQ” B-domain deleted FVIII
- AAV5 capsid
- AAV2 ITRs flank expression cassette
- “HLP” promoter and synthetic polyadenylation site
- Vector genome is a single stranded DNA
- Manufactured in Baculovirus
Phase 1/2 Study: First-in-Human, Dose Escalation Study In Patients with Severe Hemophilia A

• Design
  • Subjects enrolled sequentially into one of up to three cohorts based on FVIII activity at 3 weeks
    • 1. 6 x 10^12 vg/kg given as a single intravenous dose
    • 2. 2 x 10^13 vg/kg, iv
    • 3. 6 x 10^13 vg/kg, iv
  • Dose escalation occurred if the resulting FVIII activity at Week 3 visit is < 5 IU/dL
  • Subjects on prophylactic FVIII therapy switched to an “on-demand” schedule
  • Out of abundance of caution to ensure patient safety and to protect against loss of FVIII activity expression, the protocol pre-specified:
    • Frequent monitoring of liver enzymes performed
    • ALT elevations 1.5 fold above baseline would trigger corticosteroid use in all subjects
    • ALT elevations 5 fold above baseline would lead to halting enrollment

Endpoints
• Safety of a single intravenous administration of a recombinant AAV human-coagulation FVIII vector
• Change from baseline FVIII expression level
• Impact on the frequency of FVIII replacement therapy and annualized bleeding rate

Kinetics of FVIII and ALT In 4 Weekly Intervals For Subjects Administered BMN 270 at 6E13 VG/KG Dose Level

[Graph showing Factor VIII Activity (IU/dL) and ALT (U/L) over weeks with number of subjects at each interval.]

Excludes FVIII values within 72 hours of exogenous FVIII administration.
Data as of 06 Jul 2016.

Clinical AAV-FVIII Conclusions

- *In vivo* AAV liver gene transfer PoC demonstrated
  - Dose 30 – 120 times higher than tested in hemophilia B trials
- Mild transaminitis
  - Prophylactic steroid treatment
- Dose response observed
  - 2% to >200% FVIII activity
- Maximum tolerated dose achieved
  - >150% FVIII activity levels
  - Elevated LFT
- No evidence of anti-FVIII immunity
AAV Serotype 3 Improves Liver-Directed Gene Delivery

Among the first AAV serotypes identified
Initially ignored as it does not transduce murine hepatocytes
Utilizes human hepatocyte growth factor receptor for cellular entry
Expert collaborators at University of Florida
Strategies to Improve Potency of AAV Vectors

Utilize improved viral capsid
• More transgenes successfully delivered per viral particle

Engineer stronger promoters
• More mRNA transcripts per transgene

Utilize novel codon optimization strategy
• More efficient FIX production

Bioengineer FVIII/IX for increased activity
• More hemostatic efficacy per FIX peptide

Multinational collaboration between Expression Therapeutics, Emory University, University of Florida, Powell Gene Therapy Center, Christian Medical College (Vellore, India)
Combined bioengineering results in 5X improvement over existing technology

![Graph showing plasma FVIII activity over time with 5X improvement]
Pre-clinical testing of a virus that does not work in mice requires creativity

**Primary human liver cells**
- Transduce cultured primary human hepatocytes
- Measure secreted FIX in media

**Hydrodynamic plasmid injection**
- High volume (10% body weight) injection mechanically delivers plasmid DNA to liver
- Gene expression remains durable for months

**Humanized mice**
- Up to 70% of mouse hepatocytes replaced with human hepatocytes
- Mouse becomes permissive to AAV3 infection
Proposed Hemophilia Clinical Trials Collaboration

**Hemophilia B Project Goal:** Initiate the first gene therapy clinical trial in India

**Lead candidate selection**
- *in vitro* testing of construct panel
- non-viral testing of top constructs *in vivo*
- Non-GMP viral packaging of lead candidate
- *in vitro* infection of cultured human hepatocytes with lead candidate
- **IND-enabling NHP study**

**Vector production**
- Process standardization
- Product biochemical characterization

**In vivo testing**
- Test lead candidate in humanized mice
- Murine tox studies

**Patient selection**
- No AAV3-neutralizing antibodies
- No history of immune reaction to fIX

**Vector administration and LTFU**
- Submit IND in 8-9 months to regulators
- Determine dose escalation
- Establish protocols for measuring fIX antigen levels

Chris Doering, Trent Spencer

Arun Srivastava, Barry Byme

Alok Srivastava
**Ex Vivo Blood Stem Cell Gene Therapy**

1. Stem cell harvest
2. Viral gene transfer
3. Pre-conditioning
4. Infusion of genetically-modified cells

*Drug Product*
SC-LV Gene Therapy: Pros, Cons and Mechanism of Action

Pros:
1) Potential for “cure”
2) Efficient gene transfer
3) Ex vivo process control
4) High potency due to cell expansion
5) Immune modulation – ‘tolerance’

Cons:
1) Conditioning side effects
2) Risk of insertional mutagenesis
3) Complexity of HSCT protocol
HSCT Gene Therapy of Hemophilia: Preclinical Small Animal PoC studies

General overview of the platform and experimental design

- Harvest BM
- Select HSCs
- Transplant into conditioned recipient
- Ex vivo Transduction

Monitor:
- Engraftment
- FVIII antigen levels
- Complete blood counts
- Plasma FVIII activity
- Transgene copy number
- Anti-FVIII inhibitors

HIV-1-based SIN – LV delivery
Non-Clinical Proof of Concept: CD68-ET3 LV

Efficacy testing in hemophilia A mice: CD68-ET3

Plasma FVIII Activity (IU/ml) vs Vector Copy Number (VCN)

- Normal
- Mild
- Moderate
- Severe
**CD68-ET3-LV CD34⁺ INTEGRATION ANALYSIS**

<table>
<thead>
<tr>
<th>Genomic Locations</th>
<th>CD68-ET3 LV (253 non-redundant IS)</th>
<th>Published Data* (1717 non-redundant IS)</th>
<th>Random Dataset (one million random genomic positions)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within Genes</td>
<td>All Genes</td>
<td>71.15%</td>
<td>76.50%</td>
</tr>
<tr>
<td>Cancer Genes</td>
<td>3.16%</td>
<td>5.10%</td>
<td>1.49%</td>
</tr>
<tr>
<td>All Genes</td>
<td>16.60%</td>
<td>12.70%</td>
<td>10.43%</td>
</tr>
<tr>
<td>&lt; 5kb from TSS</td>
<td>Cancer Genes</td>
<td>0.00%</td>
<td>0.30%</td>
</tr>
</tbody>
</table>

* Arens et al (Hum Gene Ther Meth 2012)
CD68-ET3 LV SCT: CLINICAL PROTOCOL SIMULATION

- Healthy volunteer mobilization and apheresis performed at CCHMC
- Mock shipping period then CD34+ selection and cell banking performed at CMO (Cincinnati Children’s Hospital Medical Center, CCHMC)
- 1/10th scale CD68-ET3 LV transduction of thawed and stimulated CD34+ cells
- Vector copy number (VCN) analysis performed on CFCs and cultured CD34+ cells at several time points post transduction
CD68-ET3-LV CD34+: Proposed Pilot Clinical Trial Design

Milestones and Timeline:

1. RAC review: Q2 2011
2. Pre-IND review: Q1 2012
3. Biosafety approved: Q4 2012
5. IRB submission, IND submission, Initiate clinical trial: Q4 2017

Emory University
Comprehensive Hemophilia Treatment Center
Winship Cancer Institute
Bone Marrow and Stem Cell Transplant Program
Aflac Cancer and Blood Disorders Center BMT Program
Lentigen Technologies (Miltenyi)
Opus Bio: Collaboration Partner
Cincinnati Children’s Hospital Medical Center

*** Funding from NIH, Children’s Healthcare of Atlanta and Hemophilia of Georgia
Future Expectations/Challenges

EXPECTATIONS:

1. Clinical gene therapy is demonstrating safety and efficacy
2. First FDA product approval(s) expected in Q4 2016/Q1 2017
3. Clinical gene therapy of hemophilia is progressing rapidly through phase 1/2 clinical trials

CHALLENGES:

1) Manufacturing – scale and cost
2) Potency – reduce required dose
3) Durability – unknown, limited clinical data
4) Side/adverse effects – liver function, genotoxicity, immunity
5) Profitability – yet to be demonstrated
Hemophilia Care in the 21st Century

- Curative Gene Therapy (LV-CD68-ET3)
- Extended Gene Therapy (AAV-ET3)
- Protein Replacement Therapy (ET3)

Institutions:
- Aflac Cancer & Blood Disorders Center
- Cincinnati Children’s
- Emory University School of Medicine
- UF University of Florida
- The Children’s Hospital of Philadelphia
- Center for Cellular & Molecular Therapeutics
Emory Gene Therapy Program and Our Collaborators

Emory University/Children’s Healthcare of Atlanta
H. Trent Spencer, PhD – Director of Gene and Cell Therapy
Pete Lollar, MD – Clotting Factor Guru
Christine Kempton, MD – Hemophilia Director
Edmund Waller, MD/PhD – Clinical BMT Director
Jordan Shields, MS
Andrew Fedanov, PhD
David McCarty, MS
Bagirath Gangadharan, PhD
Kristopher Knight
Stephan George
Lucienne Ide, MD/PhD
Kerry Dooriss, PhD
Jennifer Johnston, PhD
Philip Zakas, PhD
Harrison Brown
Allison Lytle
Robert Moot
Lauren Fleischer

Expression Therapeutics
Gabriela Denning, PhD – COO
Angel Rivera, PhD – R&D Director
Navdeep Jihta, MD – FVIII analytics
Eli Fine – Bioinformatics

Georgia Institute of Technology
Eric Gaucher, PhD
Wilbur Lam, MD, PhD
Reginald Tran

Lentigen Technologies/Miltenyi
Boro Dropulic, PhD

Cincinnati Children’s Hospital Medical Center
Punam Malik, MD
William Swaney, PhD
Diana Nordling

Funding Support:
NIH/NHLBI
Children’s Healthcare of Atlanta
Hemophilia of Georgia
Bayer
Georgia Research Alliance
Curing Kids Cancer

Christian Medical College, Vellore
Alok Srivastava

University of Florida
Arun Srivastava, PhD
Barry Byrne, MD/PhD
rET3i – a novel FVIII product for immune tolerance induction (ITI) and replacement therapy

*Leverage the high expression properties of ET3 + state of the art manufacturing technology + NIH small business resources to produce a more economical ITI product*

**Project tenets:**

- Pharmacological properties appear non-inferior to current products
- A serum-free media, suspension adapted research cell bank generated
- Upstream and downstream process development underway
- **rET3i manufacturing costs should be <1% of current rFVIII products**
- Primary safety concern to be addressed involves the potential immunogenicity of bioengineered FVIII sequences

**Clinical indications:**

- Primary indication – ITI
- Secondary indication – Post-ITI prophylaxis
- Tertiary indication – Standard replacement therapy in PUPs and/or PTPD
Available Project Support

**NIH/NHLBI and SMARTT Program:** To accelerate translation of research from bench to bedside by providing services that support pre-clinical studies and regulatory submissions.

1. **Manufacturing (ABL – SMARTT)**
   a. Tech transfer
      - Nov 2016  Dec 2016
   b. Process development
   c. Analytical method development
   d. Pilot Run (Non-GMP, 100L)
      - Jan 2017  Feb 2017  Mar 2017
      - Jan 2017  Feb 2017  Mar 2017
      - April 2017  May 2017

2. **Regulatory (RTI – SMARTT)**
   a. Approved 12/14/16
   b. First call 1/13/17

3. **Pharm/tox (SRI – SMARTT)**
   a. Waiting for ABL pilot run product to initiate testing
# ET3i Project Timeline (US-FDA path)

<table>
<thead>
<tr>
<th>Activity</th>
<th>Time Period</th>
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<tbody>
<tr>
<td>Technology Transfer</td>
<td>Q4 2016</td>
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<tr>
<td>Process Scale Up</td>
<td>Q4 2016</td>
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<tr>
<td>Pre-IND Meeting</td>
<td>Q2 2017</td>
</tr>
<tr>
<td>Manufacturing</td>
<td>Q3 2017</td>
</tr>
<tr>
<td>Preclinical Studies</td>
<td>Q1 2017</td>
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<tr>
<td>IND</td>
<td>Q4 2017</td>
</tr>
<tr>
<td>Phase 1 Study</td>
<td>Q1 2018</td>
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</table>
HYPE or HOPE Example: PDA

Leading Clinical Gene Therapy Vector Platforms

**Adeno-Associated Viral (AAV) Vector**
- Protein capsulated, ssDNA containing
- Family: *Paroviridae*
- 20 nm diameter
- 4.7 – 5.0 kb packaging limit
- Predominantly episomal (duration?)
- Wild-type virus non-pathogenic (?)
- Clinical safety and efficacy data exist
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**Retroviral Vector**
- Lipid enveloped, RNA virus
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- Predominantly integrating
- Wild-type HIV virus known cause of AIDS
- Clinical safety and efficacy data exist
- Single approved product (Strimvelis)
**In Vivo Liver-Directed AAV (or LV) Vector Gene Transfer**

Vector encoding a liver-directed FVIII expression cassette

Recipient (Affected individual)

Liver hepatocyte transduction by infused recombinant vector

Secretion of functional FVIII into the blood circulation
Current Activity in Gene Therapy of Hemophilia A

**Liver-Directed AAV**
- **Biomarin (AAV5)**
  - Codon-optimized BDD hFVIII transgene (omitting V3)
  - In phase 1/2 trial
- **Freeline Therapeutics (AAV?)**
  - University College London Start-up
- **Dimension Therapeutics/Bayer (AAV10)**
  - Hemophilia A and B programs
- **Uniqure (AAV5)**
  - Hemophilia B trial progressing into phase II (St. Jude construct)
  - Hemophilia A program slower to progress
- **Shire/Baxalta/Baxter/Chatham/UNC (AAV8)**
  - Hemophilia B trial terminated
  - Expect hemophilia A program to move forward
- **Spark/CHOP (AAV?)**
  - Hemophilia B trial ongoing
  - Hemophilia A program near IND
- **Sangamo (AAV6)**
  - Hemophilia A program near IND

**HSC and Liver-Directed Lentiviral vector**

**Expression Therapeutics/Emory/Cincinatti Children’s**
Projected to initiate clinical trial in 2016
Ex vivo transduction of autologous CD34+ cells
Bioengineered ET3 transgene/myeloid promoter

**Blood Center and Medical College of Wisconsin**
Ex vivo transduction of autologous CD34+ cells
Platelet-directed expression

**TIGET/Biogen**
Liver-directed in vivo lentiviral vector delivery
Hemophilia B and A programs

**Emory University/Expression Therapeutics/University of Florida**
- Bioengineered AAV3 platform
- Bioengineered liver optimized ET3 or An-FVIII transgene
- Engineered synthetic liver promoter
- Preclinical NHP studies in 2017

No open trials for inhibitor patients
# Bioengineering FVIII: Current Candidates

<table>
<thead>
<tr>
<th>Wild Type Human FVIII</th>
<th>A1</th>
<th>A2</th>
<th>B</th>
<th>A3</th>
<th>C1</th>
<th>C2</th>
</tr>
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<tbody>
<tr>
<td><strong>U of Michigan – S. Pipe</strong></td>
<td>A1</td>
<td>A2</td>
<td>B*</td>
<td>A3</td>
<td>C1</td>
<td>C2</td>
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<td><strong>Emory – C. Doering/H. Spencer/P. Lollar</strong></td>
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<td><strong>Emory/Georga Tech – C. Doering/E. Gaucher</strong></td>
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<td>A2</td>
<td>A3</td>
<td>C1</td>
<td>C2</td>
<td></td>
</tr>
</tbody>
</table>

Shortened B domain retaining 6 N-linked glycosylation sites

B domain deleted – 6 N-glycan sites

RH mutation in linker – Reduced Pace/Furin cleavage

Porcine A1/A3 sequences