What’s New in Inhibitor Research?

Michael Callaghan, MD
Assistant Professor of Pediatrics
Wayne State University
Children’s Hospital of Michigan Hemophilia Treatment Center
Disclosures

• Paid Consultant or service on Advisory Boards:
  • Baxter Healthcare
  • CSL Behring
  • Biogen Idec
  • Pfizer Hemophilia
  • Green Cross

• I will talk about many approaches that are not standard of care and are not FDA approved

• Most of these approaches are experimental and their use outside of a study is not advisable
Research in Inhibitors

• Very Difficult to answer research questions
  • Many fantastic Investigators/Ideas
  • Heterogenous population
  • Rare disease
    • 1/10,000 people in population with hemophilia
    • 20-30% severe A, 2-4% hemophilia B
      • Transient
      • Low titer
      • Easily tolerated
      • About 1-4% of people with hemophilia have long standing difficult inhibitors

• Little consensus about best approach
• Funding is tenuous
• High profile international studies fail to enrol enough patients
• We need your help!
Outline

• Treatment
  • Prophylaxis
  • Monitoring with Global Coaguation Assays
  • TFPI inhibitors
  • Anti-thrombin III siRNA
  • Porcine Factor VIII

• Eradication
  • RESIST Trial – plasma derived factor in ITI
  • Immunosuppressive Regimen
  • Targeting the Unfolded Protein Response

• Prevention (Dr. Tom Howard)
  • Haplotype directed therapy
  • Danger Theory
  • New approaches to stratifying inhibitor Risk
Identifying Inhibitors

- FDA requires Pharmaceuticals to report inhibitor development
- Most Physicians don’t routinely report development of inhibitors
- Pharma companies have pharmacovigilance departments that read publications about their products
- New products entering the market may increase or decrease inhibitor risk – it will be important to closely track
Treatment- Prophylaxis

• Prophylaxis
  • Novoseven
    • 90 ug/kg/d or 270 ug/kg/d
  • Feiba
    • 85 units/kg 3 times per week

• High Risk Inhibitors
  • High titer > 5 Bethesda Units (> 2 BU – novo)
  • Not on ITI
  • More than 6 bleeds in the past 6 months, 12 in 12 months or 4 in 1 month
  • The average patient in these studies had >28 bleeds per year

• 3 patients died out of 89 in studies lasting about a year
  • These were not because of the medications
  • In this high risk group ?5% Annual mortality
Anti-Inhibitor Coagulant Complex Prophylaxis in Hemophilia with Inhibitors

Cindy Leissinger, M.D., Alessandro Gringeri, M.D., Bülent Antmen, M.D., Erik Berntorp, M.D., Chiara Biasoli, M.D., Shannon Carpenter, M.D., Paolo Cortesi, M.Sc., Hyejin Jo, M.S., Kaan Kavakli, M.D., Riitta Lassila, M.D., Massimo Morfini, M.D., Claude Négrier, M.D., Angiola Rocino, M.D., Wolfgang Schramm, M.D., Margit Serban, M.D., Marusia Valentina Uscatescu, M.D., Jerzy Windyga, M.D., Bülent ZülfiKar, M.D., and Lorenzo Mantovani, D.Sc.
Figure 1. Randomization and Follow-up of the Study Participants.

34 Patients underwent randomization

17 Were assigned to prophylaxis (mo 1–6)
17 Were assigned to on-demand therapy (mo 1–6)

1 Withdrew before start of study
1 Withdrew at 2 mo
1 Was lost to follow-up at 3 mo
1 Withdrew at 4 mo
1 Withdrew at 6 mo
1 Died at 8 mo

Washout (mo 7–9)

14 Were assigned to on-demand therapy (mo 10–15)
14 Were assigned to prophylaxis (mo 10–15)

1 Withdrew because of allergic reaction at 10 mo
1 Died at 13 mo

14 Could be evaluated per protocol
12 Could be evaluated per protocol
Figure 2. Bleeding Episodes during the Two Treatment Periods.
Panel A shows the mean number of total patient-reported bleeding events, according to the treatment period. A mean of 13.1 bleeding events were reported during the 6-month on-demand period, and 5.0 bleeding events were reported during the 6-month prophylaxis period. Episodes of joint bleeding accounted for approximately 80% of total bleeding episodes. Bleeding was also noted at other sites, including the muscles, other soft tissues, and body cavity. Intracranial and surgical bleeding also occurred. As shown in Panel B, no difference was noted in the treatment (prophylactic) effect on the basis of the order in which patients were randomly assigned to treatment. Panel C shows the mean number of hemarthroses according to the treatment period. A mean of 10.8 joint-bleeding episodes were reported during the on-demand period, and 4.2 joint-bleeding episodes were reported during the prophylaxis period. Error bars indicate standard errors.
ORIGINAL ARTICLE Clinical haemophilia

Randomized comparison of prophylaxis and on-demand regimens with FEIBA NF in the treatment of haemophilia A and B with inhibitors

S. V. ANTUNES,* S. TANGADA,† O. STASYSHYN,‡ V. MAMONOV,§ J. PHILLIPS,¶ N. GUZMAN-BECERRA,† A. GRIGORIAN,‡ B. EWENSTEIN† and W.-Y. WONG†
*UNIFESP, São Paulo, Brazil; †Baxter Healthcare Corporation, Westlake Village, CA, USA; ‡Institute of Blood Pathology and Transfusion Medicine under the Ukrainian National Academy of Medical Sciences, Lviv, Ukraine; §Department of Reconstructive Orthopedic Surgery for Hemophilia Patients, Moscow, Russia; and ¶Wellington Hospital, Wellington South, New Zealand
Fig. 1. Subject disposition flow diagram.
Randomized, prospective clinical trial of recombinant factor VIIa for secondary prophylaxis in hemophilia patients with inhibitors

B. A. KONKLE, L. S. EBBESEN, E. ERHARDTSSEN, R. P. BIANCO, T. LISSITCHKOV, L. RUSEN, and M. A. SERBAN

38 patients screened
1 patient withdrew informed consent

Pre-prophylaxis period
37 patients included in pre-prophylaxis period
A total of 15 patients withdrawn due to the following reasons:
- 13 patients: Insufficient number of bleeds.
- 1 patient: an adverse event reported as sequelae to hepatitis C which caused elevated liver enzymes and thrombocytopenia.
- 1 patient: lack of treatment of bleeds.

Prophylaxis period
22 patients included in prophylaxis period and randomized
11 patients randomized to 90 μg kg⁻¹ rFVIIa
11 patients randomized to 270 μg kg⁻¹ rFVIIa

Post-prophylaxis period
22 patients included in post prophylaxis period - prophylactic period and completed trial
22 patients analyzed
Fig. 2. Number of bleeds per month. The bracketed data are the estimated changes (percentage) in number of bleeds per month (defined as 28 days) for the 90 and 270 μg kg⁻¹ recombinant factor VIIIa treatment groups during the prophylaxis or postprophylaxis period as compared to the preprophylaxis period, and during the prophylaxis period as compared to the postprophylaxis period. ***P ≤ 0.001; **P ≤ 0.01; *P ≤ 0.05.
Treatment – Prophylaxis- Conclusions

• Prophylaxis Reduces Bleeding by about 50-70% in high risk patients
• Decreased Pain and missed work/school
• FEIBA is now FDA Approved for Prophylaxis in inhibitor patients
Global Assays

- Clot Waveform Analysis
- Thrombelastography
- Thrombin Generation
- Euglobulin Clot Lysis Time
- CloFAL Assay
The Thromboelastograph—Yesterday and Today

1948

1999

TEG

ROTEM
Mechanism

oscillating axis (+- 4.75°)

LED light source

counterforce spring

mirror

detector

data processor

ball bearing

cuvette + sample

temperature controlled

cuvette holder

sensor pin

clot formation
• **R time [Reaction time]; Clotting time (CT, ROTEM):** Time from start of sample run to first significant clot. Prolonged by anticoagulation and factor deficiencies

• **K time [Clot formation time]; Rate of Clot Formation (CFTR, ROTEM):** Time to reach a certain level of clot strength (amplitude of 20mm). Prolonged by low fibrinogen level / platelet dysfunction / anticoagulants

• **Angle (α):** Rate of clot formation. Reflects function of fibrinogen, increases with improved platelet function

• **Maximum Amplitude (MA); Maximal Clot Firmness (MCF, ROTEM):** Measurement of maximum strength or stiffness. Influenced by platelet function and number

• **Lysis parameters**

• **G (both TEG and ROTEM):** Calculated by the computer \( G = (5000 \times A/100 \times A_0) \) reflective of the tensile strength of the clot
Additional Parameters

Velocity Profile: First derivative of the TEG thrombelastographic course (clot strength against time), plotted using specialized software which is representative of fibrin polymerization.
Thromboelastography in children with coagulation factor deficiencies

Summary
Hemophilia is traditionally classified according to the levels of the deficient coagulation factor as Severe (<1%), Moderate (1–5%) or Mild (>5%).

<table>
<thead>
<tr>
<th>TEG parameter*</th>
<th>SH (n = 30)</th>
<th>MH (n = 4)</th>
<th>SHI (n = 5)</th>
<th>NC (n = 19)</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>MTG (mm × 100/s)</td>
<td>10·6† (5·4)</td>
<td>11·9 (3·8)</td>
<td>1·5† (0·8)</td>
<td>21·1† (5·8)</td>
<td>&lt;0·006</td>
</tr>
<tr>
<td>TMG (min)</td>
<td>28† (17)</td>
<td>16·9 (3·4)</td>
<td>109·4† (56)</td>
<td>898† (0·6)</td>
<td>&lt;0·009</td>
</tr>
<tr>
<td>R (min)</td>
<td>21·7† (9·6)</td>
<td>13·7 (2·8)</td>
<td>55·95† (30)</td>
<td>7·4† (2)</td>
<td>&lt;0·0001</td>
</tr>
<tr>
<td>K (min)</td>
<td>6·5† (6·9)</td>
<td>3·8 (1·1)</td>
<td>41† (26)</td>
<td>2 (0·5)</td>
<td>&lt;0·0001</td>
</tr>
<tr>
<td>A (degrees)</td>
<td>39·4† (15·6)</td>
<td>45·6 (8)</td>
<td>6·5† (6·9)</td>
<td>62·9† (6·4)</td>
<td>&lt;0·0001</td>
</tr>
<tr>
<td>MA (mm)</td>
<td>59† (7·5)</td>
<td>56·1 (6·9)</td>
<td>11·9† (57)</td>
<td>62·1 (5)</td>
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<tr>
<td>TMA (min)</td>
<td>50·2† (23)</td>
<td>37·1 (4)</td>
<td>−37·9† (160)</td>
<td>27 (5)</td>
<td>&lt;0·0001</td>
</tr>
</tbody>
</table>
Monitoring of bypassing agents

Thrombelastography-Guided Factor VIIIa Therapy in a Surgical Patient with Severe Hemophilia and Factor VIII Inhibitor

A patient with hemophilia and factor VIII inhibitors required urgent evacuation of a spinal cord hematoma. Two large doses of recombinant factor VIIIa (200 µg/kg; followed by 300 µg/kg) were required for hemostasis. Traditional and rotational thrombelastography were used to guide dose and timing of rFVIIIa therapy. With the limitations of prothrombin and partial thromboplastin times as perioperative monitors of rFVIIIa efficacy, this description of thrombelastography supports reports of in vitro use, and may be helpful when large perioperative doses of rFVIIIa are required.

[Evan G. Pivalizza, MB ChB, FFASA*
Miguel A. Escobar, MD†§

Monitoring therapy-Bypassing therapy

Evaluation of thromboelastography for monitoring recombinant activated factor VII ex vivo in haemophilia A and B patients with inhibitors: a multicentre trial
Guy Young, Liselotte S. Ebbesen, Dorthe Viuff, Jorge Di Paola, Barbara A. Konkle, Claude Negrier, John Pasi and Jørgen Ingerslev

Showed faster clot formation and increased speed of clot formation; Unable to differentiate between different concentrations of rFVIIa.
Dose titration of recombinant factor VIIa using thromboelastograph monitoring in a child with hemophilia and high titer inhibitors to factor VIII: a case report and brief review.
Trowbridge CC, Stammers AH, Ciccarelli N, Klayman M:

Lower doses of rFVIIa therapy are safe and effective for surgical interventions in patients with severe FXI deficiency and inhibitors
G. KENET*, A. LUBETSKY*, J. LUBOSHITZ*, B. RAVID*, I. TAMARIN*, D. VARON† and U. MARTINOWITZ*
Haemophilia. 2009 Sep;15(5):1065-73
Monitoring of therapy-combination therapy

Tranexamic acid combined with recombinant factor VIII increases clot resistance to accelerated fibrinolysis in severe hemophilia A

A.-M. HVAS, H. T. SØRENSEN, L. NØRENGAARD, K. CHRISTIANSEN, J. INGERSLEV and B. SØRENSEN
Center for Hemophilia and Thrombosis, Department of Clinical Biochemistry, Aarhus University Hospital, Skejby, Denmark

Combined treatment with APCC (FEIBA®) and tranexamic acid in patients with haemophilia A with inhibitors and in patients with acquired haemophilia A – a two-centre experience

M. HOLMSTRÖM*, H. T. T. TRAN† and P. A. HOLME‡

Haemophilia (2012), 18, 544-549
Thrombelastography-Limitations

Pre-analytical variables associated with other coagulation tests
Needs fresh whole blood
Remains unstandardized

- Activators- TF versus Kaolin
- Dose of Tissue factor (low dose versus high dose)
- Use of Corn Trypsin Inhibitor
- Which Modifications to use for the clinical condition (not one size fits all)
- Which parameters to study (G vs MA vs MRTG)

TEG is a test that I love......

to hate
Treatment

Contact activation (intrinsic) pathway

Damaged surface

XII → XIIa

XIIa → XI → X → IX → IXa → VIII → VIIIa

XIIa → VIIa

VIIa → VII

Tissue factor (extrinsic) pathway

Trauma

TFPI

VIIa → VII

Trauma

Tissue factor

Antithrombin

VIIIa → Xa

Xa → Thrombin (IIa)

VIIIa → Fibrinogen (I) → Fibrin (Ia)

Xa → Thrombin (IIa) → Cross-linked fibrin clot

Active Protein C

Protein S

Protein C + Thrombomodulin

Common pathway

XIIIa → XIII
Treatment – TFPI Inhibitors

• Tissue Factor Pathway Inhibitor (TFPI)
  • Exciting Target for New Therapeutics
  • Many Pharma Companies Developing Products
    • Concizumab – Novo nordisk (IV/SC)
    • Small Molecule & Aptamer – Baxter
    • Antibody Fragment - Pfizer
• Bypasses FVIII and FIX
• Potential for Oral and or Subcutaneous administration
Tissue factor pathway inhibitor-alpha inhibits prothrombinase during the initiation of blood coagulation

Jeremy P. Wood\textsuperscript{a}, Matthew W. Bunce\textsuperscript{b}, Susan A. Maroney\textsuperscript{a}, Paula B. Tracy\textsuperscript{c}, Rodney M. Camire\textsuperscript{b,d}, and Alan E. Mast\textsuperscript{a,e,1}

\textsuperscript{a}Blood Research Institute, Blood Center of Wisconsin, Milwaukee, WI 53226; \textsuperscript{b}Division of Hematology, Children’s Hospital of Philadelphia, Philadelphia, PA 19104; \textsuperscript{c}Department of Biochemistry, University of Vermont College of Medicine, Burlington, VT 05405; \textsuperscript{d}Department of Pediatrics, University of Pennsylvania, Philadelphia, PA 19104; and \textsuperscript{e}Department of Cell Biology, Neurobiology and Anatomy, Medical College of Wisconsin, Milwaukee, WI 53226

Edited by Charles T. Esmon, Howard Hughes Medical Institute, Oklahoma City, OK, and approved September 4, 2013 (received for review May 31, 2013)
Efficacy and safety of a new-class hemostatic drug candidate, AV513, in dogs with hemophilia A

Srinivasa Prasad, David Lillicrap, Andrea Labelle, Sabine Knappe, Tracy Keller, Erin Burnett, Sandra Powell and Kirk W. Johnson

Figure 1. Subcutaneous injections of AV513 improve the clot dynamics in AAV-hemophilia A dogs. In a dose-escalation study, plasma prepared from AAV-hemophilia A dogs before dosing and at the end of each dosing period were evaluated for clotting in a TEG assay. TEG R time represents the time required to initiate a 2-mm clot. (A) Progressive change in TEG R times of individual animals during the treatment period is plotted. (B) A TEG R time averaged from 3 dogs at each dose is represented as a bar graph with error bars representing SD. (P<.017, **P<.019 compared with baseline values.) (C) TEG angle represents the rate of fibrin formation, P values at 0.5, 1.0, and 1.5 mg/kg dose are .04, .002, and .001, respectively. (D) TEG MA represents clot strength, P values at 0.5, 1.0, and 1.5 mg/kg dose are .03, .02, and .015, respectively. Gloria (●), Morag (■), and Angus (★). The boxed area in the graphs represents the clot dynamics values for normal plasma.
Figure 3. Oral administration of AV513 to AAV-FVIII hemophilia A dogs accelerates the plasma clotting time. Plasma prepared from AAV-hemophilia A dogs before and at the end of each dosing period were evaluated for clotting in a TEG assay. A TEG R time averaged from 3 dogs after each dose is represented as a bar graph with error bars representing SD. *P = .05, **P = .01 compared with baseline values.

Figure 4. AV513 has different potency in AAV-FVIII and severe hemophilia A dog whole blood TEG assays. TEG R times were determined in citrated whole blood from dogs with low Factor VIII (AAV-FVIII) or severe, treatment-naive hemophilia A in the presence or absence of added AV513. TEG R times for each dose were averaged from 3 dogs/group, with error bars representing SD.
Target-mediated clearance and bio-distribution of a monoclonal antibody against the Kunitz-type protease inhibitor 2 domain of Tissue Factor Pathway Inhibitor

Lene Hansen *, Lars Christian Petersen, Brian Lauritzen, Jes Thorn Clausen, Susanne Nedergaard Grell, Henrik Agersø, Brit Binow Sørensen, Ida Hilden, Kasper Almholt

Biopharmaceuticals Research Unit, Novo Nordisk AS, Novo Nordisk Park, DK-2760, Måløv, Denmark

Fig. 2. Neutralisation by concizumab of inhibition by TFPI of TF/FVIIa-mediated FXa generation. Confluent layers of endothelial-like cell lines were treated with increasing (0 – 60 nM; 0 – 9 µg/ml) concentrations of concizumab, and the FXa activity in the supernatant was measured after 2 hrs incubation at 37 °C with 50 pM FVIIa and 50 nM FX. A shows neutralisation by concizumab of the inhibition by TFPI of the FX activation activity of TF/FVIIa on the surface of EA.hy926 WT cells (filled circles) or on EA.hy926 AR cells (filled squares). B shows neutralisation by concizumab of the TFPI inhibition of the TF/FVIIa activity on ECV304 cells (filled circles). Results are shown as mean ± SD, (A): n = 4; (B): n = 2.
Drug-drug interaction of the anti-TFPI aptamer BAX499 and factor VIII: Studies of spatial dynamics of fibrin clot formation in hemophilia A

Leonid A. Parunov a,*, Natalia P. Soshitova b, Olga A. Fadeeva b, Anna N. Balandina a,b,c, Konstantin G. Kopylov b,c, Maria A. Kumskova b,c, James C. Gilbert d, Robert G. Schaub d, Kathleen E. McGinness e, Fazoiil I. Ataullakhanyev a,b,c,f, Mikhail A. Panteleyev a,b,c,f

a Center for Theoretical Problems of Physicochemical Pharmacology, 4 Kosygina Street, Moscow 119991, Russia
b National Research Center for Hematology, 4 Novyi Zyzkivski Passage, Moscow 125167, Russia
c Center of Pediatric Hematology, Oncology and Immunology, 1 Samara Makhly, Moscow, Russia
d Amgen Corp., 148 Sidney Street, Cambridge, MA 02139, USA
e Baxter Healthcare Corporation, 148 Sidney Street, Cambridge, MA 02139 USA
f Department of Physics, Moscow State University, 1 Vorobeyevy Gory, Moscow 119991, Russia

Fig. 2. Dependence of clotting parameters on the BAX499 concentration in hemophilia A patients for different time points of factor VIII pharmacokinetics. Averaged clot formation parameter dependence on BAX499 concentrations: (a) lag time, (b) initial velocity, (c) stationary velocity, (d) clot size. The data are means ± S.E., the number of patients is n = 3.
Treatment – TFPI Inhibitors

- Exciting
- Work in single doses in blood samples
- Work in single doses in monkeys
- One failed in a clinical trial – may be specific to the way it was made
- These could be oral or subcutaneous
- Have potential to decrease bleeding in inhibitor patients
Treatment – AT3 siRNA

- RNAi is a way to interfere with gene processing – decreasing protein
- Antithrombin 3 (AT3) is made in the liver
- Decreasing AT3 can increase ability to clot
- Alnylum is producing RNAi against AT3
Treatment RNAi-AT3

**RNA interference (RNAi)**

- **Synthetic siRNA**
  - mRNA degradation
- **Targeted Gene Silencing**
  - mRNA degradation
- **Complementary pairing**
  - Cleavage

**Natural Process of RNAi**

- **RISC**
- **Complementary pairing**
  - mRNA degradation

**ASGPR**
- Highly expressed in hepatocytes
  - 0.5-1 million copies/cell
- Clears serum glycoproteins via clathrin-mediated endocytosis
- High rate of uptake
- Recycling time ~15 minutes
- Conserved across species

**GalNAc-siRNA**
- Trivalent GalNAc carbohydrate cluster has high affinity (nM) for ASGPR
- GalNAc ligand conjugated to chemically-modified, AT-targeting siRNA to mediate targeted delivery
- Can be administered subcutaneously (SC)
(A) Dose-dependent reduction of antithrombin after a single SC administration of ALN-AT3 in wild-type mice (C57BL6). At various time points post-administration, animals were bled and serum was collected. Serum antithrombin levels were analyzed by ELISA. Data points represent group mean, error bars represent standard deviation (N = 5). (B) Treatment of Hemophilia B mice (FIX <1 U/dL) with 30 mg/kg ALN-AT3. At 72 hours post-administration, animals (N = 3) were sacrificed and plasma was collected. Thrombin generation assays were performed using tissue factor = 0.5 pM. Endogenous thrombin potential (ETP) (AUC) for WT mice treated with PBS (N = 2), HB mice treated with PBS (N = 3), and HB mice treated with ALN-AT3 (N = 3). Bars represent group mean, error bars represent standard deviation. HB mouse work courtesy of Y. Dargaud and C. Negrier.
Cynomolgus monkeys were administered ALN-AT3 at two different cumulative weekly dose levels (0.6 mg/kg and 1.5 mg/kg cumulative weekly dose) via SC injection at two different dose intervals (weekly, qw and every other weekly, q2w). Serum was collected at various time points and analyzed for antithrombin protein level by ELISA. AT levels are represented relative to the average of three pre-dose measurements. Dose dependent AT silencing was observed, with 0.5 mg/kg cumulative weekly dose (0.5 mg/kg qw and 1 mg/kg q2w) resulting in approximately 80% AT suppression and 1.5 mg/kg cumulative weekly dose (1.5 mg/kg qw and 3 mg/kg q2w) resulting in approximately >90% AT suppression. Steady-state levels of suppression are achieved by Day 25. Data points represent group mean, error bars represent standard deviation (N = 3).
Treatment-Porcine FVIII

• Pig and Human Factor VIII similar
• Different enough that inhibitors of Human often don’t inhibit porcine
• In the past we used porcine plasma derived FVIII
• Octagen producing ob-1 a recombinant porcine FVIII
Thrombin-Activated Decay Rate of fVIIIa. Human (closed circle) and ovine (open circle) fVIIIa decay was measured by chromogenic Xase assay in which 20 nM fVIII was activated with thrombin and then stopped with desulfatohirudin. Activated fVIIIa in complex with phospholipid vesicles, activated factor IXa, and factor X was measured at 0.5, 3, 5, 8, 15, and 30 minutes to determine residual fVIIIa activity. Half-lives of 1.8±0.09 and 3.5±0.37 minutes were calculated for human and ovine fVIIIa, respectively.

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0049481
Hemophilia A mice were injected with either 100 µl saline or 300 U/kg of VIII in 100 µl sterile saline via tail vein injection (n = 8). After 15 min, bleeding challenge was induced via tail transaction at 2 mm diameter.
Treatment – Porcine FVIII

• Effective
• Perhaps more like normal physiology – better clotting
• Won’t work for everyone – Inhibitors to Porcine FVIII
Eradication of Inhibitors

• Always our Goal
• Standard Immune Tolerance works 60-70% of the time
  • Long
  • Costly
  • Many infusions
  • Central lines
• We need new approaches when standard ITI doesn’t work
  • RESIST Trial – plasma derived factor in ITI
  • Immunosuppressive Regimen
  • Targeting the Unfolded Protein Response
Immune tolerance induction with a high purity von Willebrand factor/VIII complex concentrate in haemophilia A patients with inhibitors at high risk of a poor response

A. Gringeri,* R. Musso,† M. G. Mazzucconi,‡ G. Piseddu,§ M. Schiavoni,¶ P. Pignoloni; and P. M. Mannucci* FOR THE RITS-FITNHEs STUDY GROUP

*A. Bianchi Bonomi Haemophilia and Thrombosis Centre, Department of Medicine and Medical Specialties, University of Milan and IRCCS Maggiore Hospital, Mangiagalli and Regina Elena Foundation, Milan; †Hemophilia Centre, Department

Table 3. Immune tolerance induction (ITI) course and outcomes.

<table>
<thead>
<tr>
<th>Patient code</th>
<th>Inhibitor level at start of ITI (BU)</th>
<th>Dosage (IU kg⁻¹)</th>
<th>Peak inhibitor during ITI (BU)</th>
<th>ITI duration (months)</th>
<th>Current inhibitor titre (BU)</th>
<th>Response</th>
<th>Total follow-up (months)</th>
<th>Present regimen</th>
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<tr>
<td>01</td>
<td>2</td>
<td>50 × 3 week⁻¹</td>
<td>0.5</td>
<td>24</td>
<td>&lt;0.5</td>
<td>Success</td>
<td>38</td>
<td>Prophylaxis</td>
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<td>02</td>
<td>5</td>
<td>50 × 3 week⁻¹</td>
<td>2</td>
<td>11</td>
<td>&lt;0.5</td>
<td>Success</td>
<td>26</td>
<td>Prophylaxis</td>
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<td>03</td>
<td>6</td>
<td>50 × 3 week⁻¹</td>
<td>3</td>
<td>21</td>
<td>1.22</td>
<td>Partial response</td>
<td>23</td>
<td>Tail-off</td>
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<td>5</td>
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<td>7</td>
<td>16</td>
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<td>4</td>
<td>50 × 3 week⁻¹</td>
<td>4</td>
<td>25</td>
<td>&lt;0.5</td>
<td>Success</td>
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<td>Lost to follow-up</td>
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<tr>
<td>10</td>
<td>200</td>
<td>200 day⁻¹</td>
<td>302</td>
<td>28</td>
<td>&lt;0.5</td>
<td>Success</td>
<td>30</td>
<td>Tail-off</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>100 day⁻¹</td>
<td>92</td>
<td>12</td>
<td>&lt;0.5</td>
<td>Success</td>
<td>26</td>
<td>Tail-off</td>
</tr>
<tr>
<td>12</td>
<td>2</td>
<td>50 × 3 week⁻¹</td>
<td>2</td>
<td>9</td>
<td>&lt;0.5</td>
<td>Success</td>
<td>20</td>
<td>Prophylaxis</td>
</tr>
<tr>
<td>13</td>
<td>5</td>
<td>50 × 3 week⁻¹</td>
<td>96</td>
<td>12</td>
<td>70</td>
<td>Discontinued</td>
<td>12</td>
<td>Withdrawn</td>
</tr>
<tr>
<td>14</td>
<td>5</td>
<td>50 e.o.d.</td>
<td>5</td>
<td>23</td>
<td>1.5</td>
<td>Partial response</td>
<td>25</td>
<td>Tail-off</td>
</tr>
<tr>
<td>15</td>
<td>30</td>
<td>100 day⁻¹</td>
<td>30</td>
<td>26</td>
<td>1.5</td>
<td>Partial response</td>
<td>33</td>
<td>Prophylaxis</td>
</tr>
<tr>
<td>16</td>
<td>14</td>
<td>100 day⁻¹</td>
<td>55</td>
<td>12</td>
<td>&lt;0.5</td>
<td>Success</td>
<td>39</td>
<td>Prophylaxis</td>
</tr>
<tr>
<td>17</td>
<td>141</td>
<td>200 day⁻¹</td>
<td>240</td>
<td>30</td>
<td>&lt;0.5</td>
<td>Success</td>
<td>39</td>
<td>Prophylaxis</td>
</tr>
</tbody>
</table>

*e.o.d. = every other day.
Eradication

Haemophilia (2013), 19, 281-286

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ORIGINAL ARTICLE Inhibitors

Use of Haemate® P as immune tolerance induction in patients with severe haemophilia A who failed previous induction attempts: a multicentre observational study

C. ROTHSCILD,* R. D’OIRON,† A. BOREL-DERLON,‡ Y. GRUEL,§ R. NAVARRO¶ and C. NEGRIER**

*Centre de Référence de l’Hémophilie, Hôpital Necker – Enfants Malades AP-HP, Paris, France; †Centre de Référence de...

Table 1. Patient characteristics and outcomes.

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age at study start (year)</th>
<th>FVIII gene mutation ( HGVS nomenclature)</th>
<th>Treatment at time of inhibitor detection</th>
<th>CED at inhibitor detection (days)</th>
<th>Inhibitor titre at the time of detection (BU mL⁻¹)</th>
<th>Number of prior ITI courses (treatment given)</th>
<th>Time from inhibitor detection to start of Haemate® P (years)</th>
<th>Inhibitor titre at start of Haemate® P (BU mL⁻¹)</th>
<th>Duration of ITI with Haemate® P* (months)</th>
<th>Last known inhibitor titre (BU mL⁻¹)</th>
<th>Outcome*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>46</td>
<td>Deletion of exons 6-14 (p.5877Stop)</td>
<td>PD (cryoprecipitate)</td>
<td>Unknown</td>
<td>4</td>
<td>128</td>
<td>3 (Recombinant, Factivity, Recombinate)</td>
<td>27.4</td>
<td>20.6</td>
<td>70.0</td>
<td>4.0 (November 2008)</td>
</tr>
<tr>
<td>2</td>
<td>6</td>
<td>Exon 14 deletion</td>
<td>R (Kogenate)</td>
<td>4</td>
<td>14 Nijmegen</td>
<td>3840</td>
<td>2 (Helixate, Factivity)</td>
<td>5.4</td>
<td>27.0</td>
<td>42.0</td>
<td>22.0 (December 2008)</td>
</tr>
<tr>
<td>3</td>
<td>9</td>
<td>c.1760C&gt;A</td>
<td>R (Kogenate)</td>
<td>7</td>
<td>55</td>
<td>55</td>
<td>3 (Recombinant, Factivity, Recombinate)</td>
<td>8.9</td>
<td>27.0</td>
<td>11.0</td>
<td>19.0 (March 2009)</td>
</tr>
<tr>
<td>4</td>
<td>4.5</td>
<td>c.3406delIT (p.1134delLeuc622)</td>
<td>R (Advate)</td>
<td>17</td>
<td>6</td>
<td>313</td>
<td>2 (Advate, Factivity)</td>
<td>3.4</td>
<td>11.2</td>
<td>26.0</td>
<td>26.0 (March 2009)</td>
</tr>
<tr>
<td>5</td>
<td>3.5</td>
<td>Insertion 1 inversion</td>
<td>R (Recombinant)</td>
<td>9</td>
<td>65</td>
<td>65</td>
<td>1 (Recombinant)</td>
<td>2.8</td>
<td>6.3</td>
<td>45.0</td>
<td>0.7 (February 2009)</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>c.1804G&gt;T</td>
<td>PD (Factivity)</td>
<td>Unknown</td>
<td>Unknown</td>
<td>190</td>
<td>1 (Factivity)</td>
<td>7.8</td>
<td>160.0</td>
<td>&gt;57</td>
<td>0.0 (November 2006)</td>
</tr>
<tr>
<td>7</td>
<td>8.5</td>
<td>c.333Met1A</td>
<td>R (Kogenate)</td>
<td>27</td>
<td>41</td>
<td>200</td>
<td>2 (Kogenate, Factivity)</td>
<td>1.5</td>
<td>11.5</td>
<td>32.0</td>
<td>0.4 (June 2008)</td>
</tr>
<tr>
<td>8</td>
<td>4.9</td>
<td>Insertion 22 inversion</td>
<td>R (Kogenate)</td>
<td>18</td>
<td>2.3</td>
<td>600</td>
<td>2 (Kogenate, Factivity)</td>
<td>3.4</td>
<td>71.0</td>
<td>26.0</td>
<td>13.0 (April 2009)</td>
</tr>
<tr>
<td>9</td>
<td>6.9</td>
<td>Insertion 22 inversion</td>
<td>R (Recombinant)</td>
<td>15</td>
<td>6</td>
<td>200</td>
<td>2 (Recombinant, Factivity)</td>
<td>5.6</td>
<td>52.0</td>
<td>21.0</td>
<td>0.0 (July 2008)</td>
</tr>
</tbody>
</table>

AE, adverse event; BPR, biological partial response; BU, Bethesda units; CED, cumulative exposure days; CPR, clinical partial response; CR, complete response; ITI, immune tolerance induction; Nij, Nijmegen; PD, plasma-derived; R, recombinant.

*As of June 2009.
Eradication – RESIST Trial

- Some believe that vWF containing (plasma derived) FVIII concentrates use improves outcome in ITI
- Ongoing international study to examine efficacy in high Risk Inhibitors and those who have previously failed ITI

Table 1. Immune tolerance studies and major enrollment criteria.

<table>
<thead>
<tr>
<th>International ITI study, ITI-naïve patients</th>
<th>RESIST_{naïve}, ITI-naïve patients</th>
<th>RESIST_{exp}, ITI-experienced patients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Severe haemophilia A (FVIII &lt; 1%) High-responders</td>
<td>Severe haemophilia A (FVIII &lt; 1%) High-responders</td>
<td>Severe haemophilia A (FVIII &lt; 1%) High-responders</td>
</tr>
<tr>
<td>No previous ITI attempt</td>
<td>No previous ITI attempt</td>
<td>Previous failed ITI with any dose with a VWF-free FVIII</td>
</tr>
<tr>
<td>Good prognosis</td>
<td>Poor prognosis (one of the following criteria)</td>
<td>Any age</td>
</tr>
<tr>
<td>Age &lt;7 years</td>
<td>Age &gt;6 years</td>
<td></td>
</tr>
<tr>
<td>Peak titre &lt;200 BU</td>
<td>Peak inhibitor titre &gt;200 BU</td>
<td></td>
</tr>
<tr>
<td>Titre at ITI start &lt;10 BU</td>
<td>Titre at ITI start &gt;10 BU</td>
<td></td>
</tr>
<tr>
<td>Time between INH and ITI ≤2 years</td>
<td>Time between INH and ITI &gt;2 years</td>
<td></td>
</tr>
</tbody>
</table>

ITI, immune tolerance induction; VWF, von Willebrand factor; INH, inhibitor occurrence.
VWF/FVIII concentrates in high-risk immunotolerance: the RESIST study

A. GRINGERI
Department of Medicine and Medical Specialties, A. Bianchi Bonomi Haemophilia and Thrombosis Centre, University of Milan and IRCCS Maggiore Hospital, Mangiagalli and Regina Elena Foundation, Milan, Italy

Fig. 1. Algorithm of enrolment of patients with severe haemophilia A and high-responding inhibitor.
Eradication

What is the Evidence for the Use of Immunomodulatory Agents to Eradicate Inhibitory Antibodies in Patients with Severe Hemophilia A Who Have Previously Failed to Respond to Immune Tolerance Induction?

Michael U. Callaghan¹ and Patrick F. Fogarty²

Table 1. Summary of published cases using immunomodulatory agents for suppression/eradication of refractory inhibitors in hemophilia

<table>
<thead>
<tr>
<th>Immunomodulator(s)</th>
<th>Reference(s)</th>
<th>Patients, no</th>
<th>Tolerized, no</th>
<th>Tolerized, %</th>
<th>Toxicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rituximab</td>
<td>1,7,10</td>
<td>39</td>
<td>13</td>
<td>33</td>
<td>UTI5, herpes zoster¹⁴*</td>
</tr>
<tr>
<td>IVlg (with rituximab)</td>
<td>1,2,5,10</td>
<td>4</td>
<td>1</td>
<td>25</td>
<td>Aspergillus, hepatitis B¹⁰</td>
</tr>
<tr>
<td>Steroid (with rituximab)</td>
<td>3,5</td>
<td>8</td>
<td>5</td>
<td>62.5</td>
<td>Headache/vomiting³</td>
</tr>
<tr>
<td>Cyclophosphamide (with IVlg and immunoadsorption)</td>
<td>8</td>
<td>10</td>
<td>7</td>
<td>70</td>
<td>None reported</td>
</tr>
</tbody>
</table>
Rituximab

Anti-CD20 monoclonal antibody
Target B-Lymphocytes
Initially approved for B-cell lymphoma
REVIEW ARTICLE

Immune tolerance with rituximab in congenital haemophilia with inhibitors: a systematic literature review based on individual patients’ analysis

M. FRANCHINI,* C. MENGOLI,† G. LIPPI,‡ G. TARGHER,§ M. MONTAGNANA,† G. L. SALVAGNO,‡ M. ZAFFANELLO¶ and M. CRUCIANI**

*Immunohematology and Transfusion Center, City Hospital of Parma, Parma; †Department of Histology, Microbiology, and Medical Biotechnology, University of Padua, Padua; ‡Section of Clinical Chemistry, Department of Biomedical and Morphological Sciences, University of Verona, Verona; §Section of Endocrinology, Department of Biomedical and Surgical Sciences, University of Verona, Verona; ¶Department of Mother-Child and Biology-Genetics, University of Verona, Verona; and **Center of Preventive Medicine, HIV Outpatient Clinic, Verona, Italy

Summary. Rituximab, a monoclonal antibody against the pan B-cell antigen CD20, has been successfully used in both adults and children for the management of malignant and non-malignant immune-mediated disorders including acquired haemophilia. On the basis of this positive experience, a number of investigators have recently used this agent in patients with congenital haemophilia and inhibitors refractory to first-line treatments. After a careful electronic and hand search, we have collected 29 studies that included 49 cases. A durable complete remission was obtained in 53% of the cases and no severe adverse events related to rituximab were recorded. A multivariate analysis applied to individual patients’ data identified the diagnosis of a mild/moderate haemophilia and the concomitant treatment with factor VIII concentrates and immunosuppression agents as covariates associated with an increased response to rituximab. Large prospective randomized studies with an adequate follow-up are needed to confirm these preliminary findings.

Keywords: haemophilia, inhibitors, immune tolerance, rituximab
Stress Signaling from the Endoplasmic Reticulum

ER

- BiP
- IRE1
- ATF6
- PERK

SELECTIVE SPlicing

- XBP1

ERAD
- Heat Shock Proteins
- Chaperones

Adaptation

Cell Death

eIF2α

ATF4

CHOP
Quiescent Lymphocyte

ER
IRE1
ATF6 p90
BiP
PERK

BCR
26s Proteasome
Hypothesis

Immune tolerance stimulates memory B-cells which produce antibodies to factor VIII.

Over production of these antibodies results in ER stress in the B-cells leading eventually to apoptosis. Agents that induce CHOP specifically will sensitize these B-cells to ER stress and act as an effective adjuvant to conventional immune tolerance therapy. This could have implications for tolerance to many autoimmune phenomena.
**Reporter Assay**

**A.**
- PERK
- eIF2α-P
- ATF4
- CHOP Promoter
- Luciferase

**B.**
- IRE1
- XBP1
- Luc

**C.**
- Fold (RLU)
- **Z’= 0.84**
- **Z’= 0.88**
- No Tx
- 2.5ug/ml Tm
High Throughput Screen

A. SID-10794  SID-10795  SID-10796

CHOP

XBP1
Proteasome Inhibition

Bortezomib (PS-341)
Licensed for Relapsed or progressive Multiple Myeloma
Specifically Inhibits 26s Proteasome
Reversibly inhibits the chymotryptic-like proteolytic activity of the proteasome, localized within the β5 subunit of the 20S core

Induction of Apoptosis
- UPR induction by interference with ERAD
- Decrease IKBa degradation
  - Less NFKB activity
Stimulated Lymphocyte with Bortezomib

ERAD
Chaperones
Lipid synthesis

ERAD
Chaperones
Lipid Synthesis

Chaperones
Anti-ROS
AA metabolism
Apoptosis (CHOP)

Transcriptional Activation

Selective Splicing

Selective Proteolysis (S1P / S2P)

Selective Translation

Translation Attenuation

BiP

ER

IRE1

ATF6 p90

PERK

eIF2α

eIF2α-P

XBP1

ATF6 p50

ATF4

BCR

FVIII

26s Proteasome
Bortezomib Eliminates Inhibitor Antibodies \textit{ex vivo}

A. Plasma

B. Supernatants

C. Supernatants Re-exposed to VIII

D. Imaging of cell cultures

E. ELISPOT analysis

A. Bortezomib eliminates inhibitor antibodies in plasma, as indicated by the lower OD values compared to controls.

B. Supernatants show similar trends, with bortezomib reducing the OD values.

C. Re-exposure to VIII further reduces OD values in supernatants treated with bortezomib.

D. Images of cell cultures illustrate the effects of bortezomib on cell proliferation.

E. ELISPOT analysis confirms the reduction in inhibitor antibodies in mice treated with bortezomib.
Hemophilia A E16 Inhibitor treated with Bortezomib

1. Injection FVIII Days:
-28, -21, -14, -7

2. Inject Bortezomib 1.3 mg/m2
Day 0, 4, 8, 11

3. Retro-orbital bleed for Plasma
D0, D+28, D+36

4. Anti-FVIII ELISA
Bortezomib eliminates anti-FVIII antibodies \textit{in vivo}.

**Day 0 Anti-FVIII antibodies**

- Control
- Velcade
- Inhibitor
- Inhibitor Velcade

**Day 35 Anti-FVIII**

- Control
- Velcade
- Inhibitor
- Inhibitor Velcade

**Anti-FVIII ab anamnesis**

D +120

- Control
- FVIII
- Bortezomib
- FVIII Bortezomib
Eradication

• Bortezomib is a chemotherapy drug
• It can cause low blood counts
• It can cause neurotoxicity
• We are looking at a number of other drugs from our screen that may be better
• We have some that are more promising
The Hemophilia Federation of America (HFA) is a national nonprofit organization that assists and advocates for the bleeding disorders community.

MISSION

Thank You!

Michael Callaghan
mcllagh@med.wayne.edu

www.hemophiliafed.org