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EMOFILIA B ED INIBITORE

Isabella Cantori

Centro Regionale

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Macerata

inside blood commentary

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GENE THERAPY

Comment on Crudele et al, page 1553

A natural choice for hemophilia B

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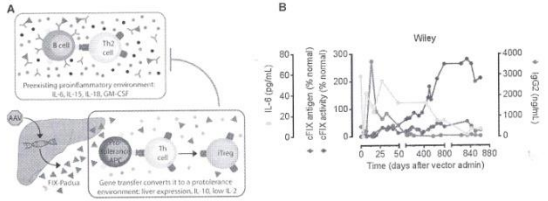
In this issue of *Blood*, Crudele et al describe a novel study of adeno-associated virus (AAV) vector-mediated gene therapy that induced immune tolerance to factor IX (FIX) in a hemophilia B (HB) dog with previously formed anti-FIX inhibitor antibodies (IAs).[1]

The discovery of a naturally occurring gain-of-function in FIX has prompted the development of highly effective, immune-tolerizing gene therapy for HB. The mutation in question was described in an Italian family with a unique form of X-linked thrombophilia, caused by the substitution of leucine for arginine at position 338 (R338L; R384L including the activation peptide) in FIX, which increased the specific activity of FIX by 5- to 10-fold and was termed FIX-Padua.[2]

IAs complicate replacement therapy in hemophilia generally, although IA formation is much more frequent in hemophilia A than in HB. The fundamental issue remains that no effective treatment is available to eliminate IAs, especially in HB where immune tolerance induction frequently fails and has been complicated by allergic reactions and nephrotic syndrome.[3] A novel development, led by Finn et al, has been the administration of gene therapy to overexpress coagulation factor VIII in dogs with hemophilia A, thereby overwhelming the IA response and establishing immune tolerance.[4] Subsequently, 2 groups showed that overexpression of FIX could similarly induce immune tolerance to FIX through a mechanism dependent on regulatory T-cell activation in HB mice.[5,6] Given that an AAV vector-mediated FIX-Padua gene therapy was highly effective and nonimmunogenic in HB mice[7] and dogs,[8] it remained for Crudele et al to demonstrate that it would induce immune tolerance in an HB dog with preformed IA.

The AAV vector encoding FIX-Padua was administered at dosages approximately equivalent to a recent successful clinical trial involving patients with HB.[9] Notably, the specific activity of FIX-Padua was 8- to 12-fold higher, in comparison with wild-type FIX, reaching 25% to 40% of normal without provoking anti-FIX antibodies in 2 naive HB dogs. The third HB dog treated (Wiley) had previously formed antibodies that increased after vector administration, signifying a transient anamnestic response, followed by disappearance of anti-FIX by day 70. Remarkably, FIX activity continued to



AAV liver gene therapy eradicates FIX inhibitors in a hemophilia B dog. (A) Expression of hepatocyte-restricted FIX Padua (green triangle) leads to a protolerance cytokine profile including IL-10 secretion (blue circles), which may modify the preexisting inflammatory environment including high IL-6 levels, facilitating tolerance induction of anti-FIX antibodies (red). (B) Time course of preexisting inhibitor eradication following AAV liver gene therapy in a hemophilia B dog (Wiley). The increased specific activity of FIX Padua results in high levels of FIX activity, with an activity-to-antigen ratio of ~8. Adapted from Figures 2 and 3 in the article by Crudele et al beginning on page 1553.

Regular Article

GENE THERAPY

AAV liver expression of FIX-Padua prevents and eradicates FIX inhibitor without increasing thrombogenicity in hemophilia B dogs and mice

Julie M. Crudele,[1] Jonathan D. Finn,[1] Joshua I. Siner,[1] Nicholas B. Martin,[1] Glenn P. Niemeyer,[2] Shangzhen Zhou,[1] Federico Mingozzi,[1] Clinton D. Lothrop Jr,[2] and Valder R. Arruda[1,3]

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Key Points

- Liver-restricted expression of FIX-Padua induces immune tolerance to the transgene in hemophilia B inhibitor dog models.
- Long-term toxicity studies show no increased risk of thrombogenicity of FIX-Padua in mice and dogs.

Emerging successful clinical data on gene therapy using adeno-associated viral (AAV) vector for hemophilia B (HB) showed that the risk of cellular immune response to vector capsid is clearly dose dependent. To decrease the vector dose, we explored AAV-8 ($1\text{-}3 \times 10^{12}$ vg/kg) encoding a hyperfunctional factor IX (FIX-Padua, arginine 338 to leucine) in FIX inhibitor-prone HB dogs. Two naïve HB dogs showed sustained expression of FIX-Padua with an 8- to 12-fold increased specific activity reaching 25% to 40% activity without antibody formation to FIX. A third dog with preexisting FIX inhibitors exhibited a transient anamnestic response (5 Bethesda units) at 2 weeks after vector delivery following by spontaneous eradication of the antibody to FIX by day 70. In this dog, sustained FIX expression reached ~200% and 30% of activity and antigen levels, respectively. Immune tolerance was confirmed in all dogs after challenges with plasma-derived FIX concentrate. Shortening of the clotting times and lack of bleeding episodes support the phenotypic correction of the severe phenotype, with no clinical or laboratory evidence of risk of thrombosis. Provocative studies in mice showed that FIX-Padua exhibits similar immunogenicity and thrombogenicity compared with FIX wild type. Collectively, these data support the potential translation of gene-based strategies using FIX-Padua for HB. (*Blood*. 2015;125(10):1553-1561)

Review Article

Toward optimal therapy for inhibitors in hemophilia

Christine L. Kempton[1,2] and Shannon L. Meeks[1]

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Treatment of patients with hemophilia A and B has undergone significant advances during the past 2 decades. However, despite these advances, the development of antibodies that inhibit the function of infused clotting factor remains a major challenge and is considered the most significant complication of hemophilia treatment. This chapter reviews current tools available for the care of patients with inhibitors and highlights areas where progress is imminent or strongly needed. For management of bleeding, bypassing agents remain the mainstay of therapy. Recombinant factor VIIa and activated prothrombin complex concentrates are similarly effective in populations of patients with hemophilia and inhibitors; however, individuals may show a better response to one agent over another. Recent studies have shown that prophylaxis with bypassing agents can reduce bleeding episodes by ~50%-80%. The prophylactic use of bypassing agents is an important tool to reduce morbidity in patients before they undergo immune tolerance induction (ITI) and in those with persistent high titer inhibitors, but cost and lack of convenience remain barriers. Because of the significant burden that inhibitors add to the individual patient and the health care system, inhibitor eradication should be pursued in as many patients as possible. ITI is an effective tool, particularly in patients with severe hemophilia A and good risk profiles, and leads to a return to a normal factor VIII response in ~60% of patients. However, for the group of patients who fail to respond to ITI or have hemophilia B, new and improved tools are needed. (*Blood*. 2014;124(23):3365-3372)

bjh review

Inhibitor development in haemophilia B: an orphan disease in need of attention

British Journal of Haematology, **138**, 305–315

Donna DiMichele

Additional morbidity issues of allergic phenotype in up to 60% of patients → anaphylactic reactions, nephrotic syndrome

Greater risk in patients with F9 large deletions and other major gene rearrangements

Poor ITI outcome

Seminars in Thrombosis & Hemostasis Vol. 39 No. 7/2013

Challenges in the Management of Hemophilia B with Inhibitor

Angelika Batorova, MD, PhD[1] Anna Morongova, MD[1] Giuseppe Tagariello, MD[2]
Denisa Jankovicova, MD[1] Tatiana Prigancova, MD[1] Julia Horakova, MD, PhD[3]

Dimensioni del problema

CHAPTER 15

Table 15.1 Comparison of inhibitors in hemophilia A and B.

Area	Inhibitors in factor VIII deficiency	Inhibitors in factor IX deficiency
Inhibitor development in severe disease	High incidence—approximately 20–30% of patients develop inhibitors	Low incidence—approximately 2–4% of patients develop inhibitors
Inhibitor development in moderate to mild disease	Between 3% and 13% of patients develop inhibitors	Extremely rare in mild to moderate hemophilia B
Impact of race	Increased incidence in African-Americans	No conclusive evidence of racial difference in incidence
Presence of infusion-associated reactions	Infusion reactions rare	Approximately 60% of patients with inhibitors have associated infusion reactions when exposed to exogenous factor IX
Success of immune tolerance	Successful in 60–70% of patients	Difficult to achieve with only 15–30% reported success rate
Complications of immune tolerance	Well tolerated	Nephrotic syndrome well-documented complication, especially in patients with history of infusion reactions

A.Tandra and A.D. Shapiro

100 pz HB con INH in USA, 8-10 pz nel registro italiano

>80% degli inibitori: high responder

Reazioni allergiche nel 60% dei pz con INH sottoposti a ITI

Sindrome nefrosica nel 38% dei pz Sottoposti a ITI

Successo dell'ITI: 15-30%

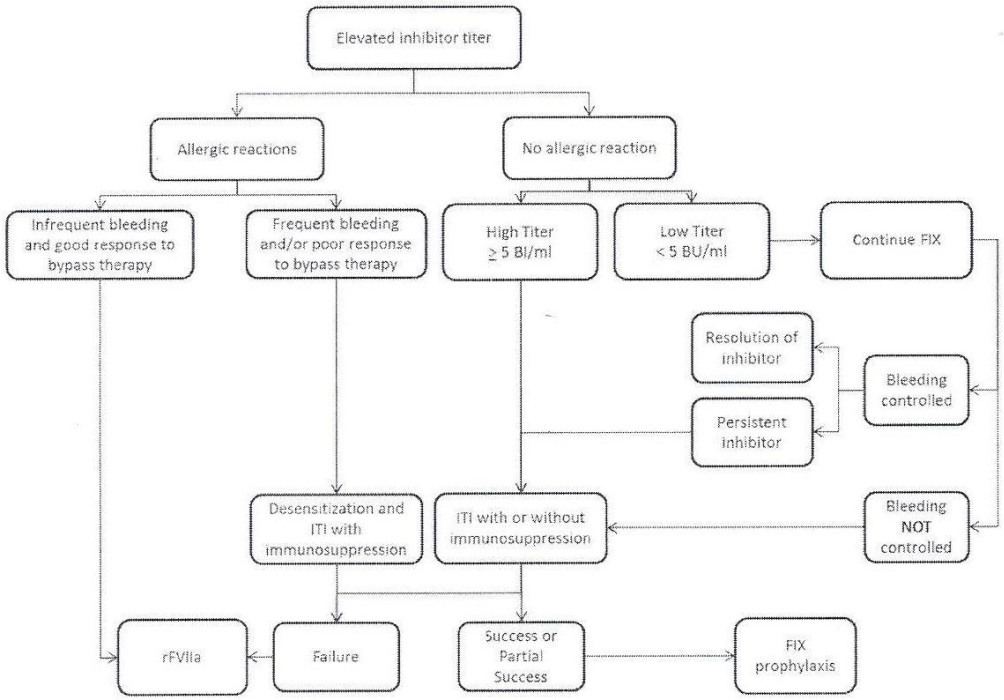

Figure 2. Proposed algorithm for ITI in patients with severe hemophilia B.

RECOMMENDATION

Principles of treatment and update of recommendations for the management of haemophilia and congenital bleeding disorders in Italy

Angiola Rocino[1], Antonio Coppola[2], Massimo Franchini[3], Giancarlo Castaman[4,5], Cristina Santoro[6], Ezio Zanon[7], Elena Santagostino[8], Massimo Morfini[9] on behalf of the Italian Association of Haemophilia Centres (AICE) Working Party (see appendix 1)

[1]Haemophilia and Thrombosis Centre, San Giovanni Bosco Hospital, Naples; [2]Regional Reference Centre for Coagulation Disorders, Federico II University Hospital, Naples; [3]Department of Transfusion Medicine and Haematology, Carlo Poma Hospital, Mantua; [4]Haemophilia and Thrombosis Centre, Department of Haematology, San Bortolo Hospital, Vicenza; [5]Centre for Bleeding Disorders, Careggi University Hospital, Florence; [6]Haematology, Department of Cellular Biotechnology and Haematology, Sapienza University of Rome, Rome; [7]Haemophilia Centre, Department of Cardiologic, Thoracic and Vascular Sciences, University of Padua Medical School, Padua; [8]Angelo Bianchi Bonomi Haemophilia and Thrombosis Centre, IRCCS Ca' Granda Foundation, Maggiore Hospital Policlinico, Milan; [9]Italian Association of Haemophilia Centres (AICE), Italy

Blood Transfus 2014

169) Castaman G, Bonetti E, Messina M, et al; Italian Association of Hemophilia Centers. Inhibitors in haemophilia B: the Italian experience. Haemophilia 2013; **19**: 686-90.

ITI treatment is reported to be less effective in eradicating inhibitors in haemophilia B patients. Data from the North American Registry show that only 5/16 patients achieved immune tolerance and eradication of the inhibitor. Additionally, 65% of patients suffered adverse events during ITI, which often caused discontinuation of the treatment[142]. Similarly, the ITI success rate was only 15% in the ISTH Registry, with 38% of patients developing nephrotic syndrome during treatment[168]. For this reason, international expert groups do not recommend ITI regimens in patients with haemophilia B with inhibitors[76-78]. Nevertheless, recent data collected in Italy on a low-dose ITI regimen in five out of eight patients with inhibitors listed in the AICE database, interestingly show complete success and inhibitor eradication in four cases[169]. However, given the lack of data from a larger number of patients, ITI in patients with haemophilia B with inhibitors is not recommended but when ITI is attempted, precautions suggested by international groups of experts[76-78] should be adopted.

Caso clinico

Rayan nato l' 08/04/2010, Emofilia B grave

Alla nascita (Salesi di Ancona) sanno che il nonno è emofilico e la madre portatrice, ma fanno diagnosi alla comparsa di ematomi nel periodo del gattonamento.

Viene inviato al Centro Emofilia di Milano (nemo….), dove viene impostata terapia con FIX al bisogno.

Studio molecolare: mutazione p.Glu26Stop: sostituzione di una Guanina con una Timina al nucleotide 6451 nell'esone 2 del gene del FIX, per cui l'aa ac. Glutammico viene sostituito con un codone di stop al codone 26

Luglio 2011: ematoma gluteo dx, trattato a Milano con Benefix 500 U.I./die per 2 giorni

Settembre 2011: viene messo in profilassi dopo il primo emartro (18 mesi ca. di età), 500 U.I. x2/settimana

Si sospetta ma non è certa una reazione allergica all' infusione di fattore, no inibitore.

5/03/2012 ricovero al Salesi per versamento nel recesso del quadricipite dx e all' interno del muscolo gemello mediale del ginocchio fino al terzo medio della gamba.

Benefix 500 U.I. ogni 24 ore per 3 volte.

Dimesso con terapia antibiotica e paracetamolo.

17/03/2012 Viene inviato dal Salesi al nostro Ambulatorio (i genitori sanno del nostro Centro dai colleghi di Milano) per emartro ginocchio dx.

Viene stilato Piano terapeutico: Benefix 1000 U.I. ogni 24 ore

20/03/2012 nuovo controllo:

F IX < 1%; ricerca inibitore: risultato dubbio

Nuovo Piano Terapeutico: Benefix 1000 U.I. ogni 12 ore

23/03/2012 il PS del Salesi ci informa che c'è un peggioramento dell' emartro

Attuato ricovero presso U.O. Pediatria di Macerata
Solo nella notte trovato accesso venoso: si passa a 3000 U.I. di Benefix ogni 12 ore

Si evidenzia inibitore: 4 UB

Dopo 8 ore dall' infusione il F IX è indosabile

si decide per agente bypassante :Novoseven 90 mcg/Kg ogni 2 ore, e dopo 24 ore ogni 3 ore.

Viene fatta anche una trasfusione di sospensione di emazie per anemia grave (Hb 7.2 g/dl)

29/03/2012 controllo ecografico : significativa distensione del recesso sottoquadricipitale con riduzione della componente ematica, iperplasia sinoviale.

«Profilassi» con Novoseven 1 mg/die

31/03/2012 Viene dimesso da Macerata e si decide per il ricovero a Milano per il posizionamento di un CVC

Continua Novoseven e il titolo dell' inibitore risulta essere in data 02/04 di 32 UB, riconfermato il 12/04 (31 UB)

03/04/2012 viene posizionato un port-a-cath. Miglioramento e dimissioni il 20/04/2012

26/04/2012 si riscontra un nuovo emartro al ginocchio dx.

Da luglio 2013 a marzo 2014 5 episodi di emartro.

Milano non ha iniziato l' ITI. I genitori si sono rivolti ad altro Centro

Il Dott. Castaman a Vicenza ha iniziato l'ITI nell'**aprile 2014**, quando l'INH era pari a 1-2 U.B.
Dose iniziale: 2.000 U.I./die di Benefix (p.c. 18 Kg)
Monitoraggio continuo di creatinina, esame urine, elettroliti sierici e urinari.
INH dopo un mese: 200 U.B, dopo tre mesi 500 U.B.
In seguito a comparsa di **eritema cutaneo al tronco** si è ridotta la dose a 1.000 U.I./die.

Progressiva riduzione dell'inibitore fino a 4 U.B. tanto che un emartro è stato trattato con successo con 2000 U.I. di Benefix, poi 1000 U.I. ogni 12 ore per 3 somministrazioni.

L'inibitore risale a 6 U.B., il bambino sta bene e continua la somministrazione di Benefix.

Marzo 2015 comparsa di proteinuria e di microematuria: sospesa temporaneamente l'infusione con monitoraggio della funzionalità renale.

Dopo 10 giorni e due controlli negativi ha ripreso Benefix 1000 U.I. a giorni alterni.

Maggio 2015 ultimo controllo : F IX < 0,1% INH 6 U.B.

Novembre 2015: si trasferisce a Parigi e viene seguito dal Centro Emofilia di Bircey

Continua a infondere Benefix 1000 U.I. a gg. alterni per un mese poi passa a 1500 U.I. a gg. alterni, preceduto da **Novoseven 2 mg**

Successivamente, in occasione del cambio di CVC (Port-a-cath), passa a Benefix 1500 U.I. a gg alterni, preceduto sempre da Novoseven 4 mg.

Eventi emorragici

Nell'ultimo anno ha avuto solo un emartro, tre mesi fa, alla articolazione bersaglio (ginocchio dx);

ha trascorso 5 giorni in semi-immobilità, con bendaggi di acqua e soluzione alcolica.

E' stato trattato con Novoseven 4 mg ogni 2 ore il primo giorno, ogni 3 ore il 2° giorno, poi ogni 4-6-12-24 ore nei giorni successivi, per un totale di 5 giorni.

Attualmente il dosaggio dell'inibitore è pari a **0.6%**

Il recupero in vivo del FIX va dal 17% al 30%

Programma futuro:

Appena INH < 0.5% si passerà a infondere Benefix quotidianamente

CONSIDERAZIONI

Patologia tra le più rare

Sindrome nefrosica
Allergia
ITI più complessa

Delicatissimo equilibrio tra rischi elevati e benefici non certi
Difficoltà organizzative e gestionali

NECESSARIE LA RETE CLINICA, L' INFORMAZIONE, LA COOPERAZIONE

Grazie per l'attenzione