PB 954

Factor VIII Inhibitor Testing Using a Validated Chromogenic Bethesda Assay in HAVEN 1 (BH29884), a Phase 3 Trial of Emicizumab in Persons with Hemophilia A with Inhibitors

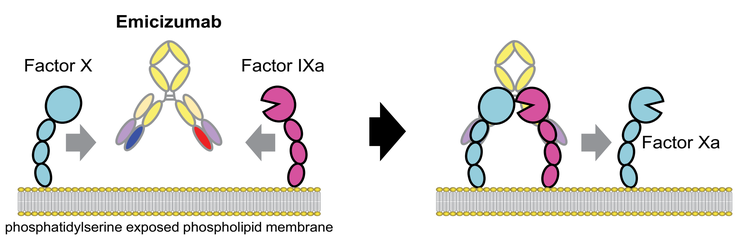
Adamkewicz JI,[1] Schmitt C,[2] Asikanius E,[2] Xu J,[1] Levy GG,[1] Kim B,[1] Calatzis A[3]

[1]Genentech Inc., South San Francisco, United States; [2]F. Hoffmann-La Roche Ltd, Basel, Switzerland; [3]Roche Diagnostics International Ltd, Rotkreuz, Switzerland

BACKGROUND

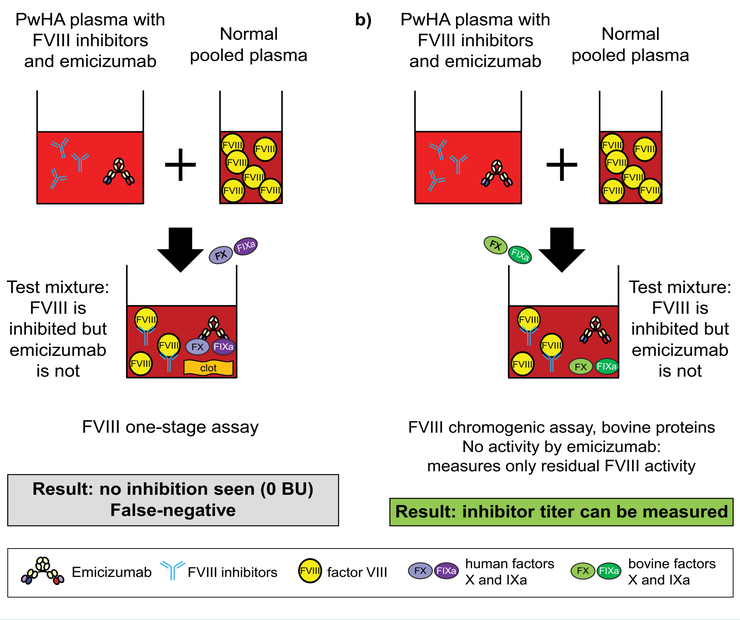
- Emicizumab (ACE910), a novel bispecific antibody, is under investigation for prophylactic treatment of persons with hemophilia A (PwHA) (**Figure 1**)
  - In a Phase 3 trial of adolescent and adult PwHA ≥12 years of age with inhibitors (HAVEN 1; ClinicalTrials.gov NCT02622321), once-weekly subcutaneous emicizumab prophylaxis significantly reduced the number of bleeds over time compared with no prophylaxis and compared with prior prophylactic bypassing agent regimens (Oldenburg et al, ISTH 2017, Abstract ASY 01.1)

Figure 1: Emicizumab mode of action



- Factor VIII (FVIII) inhibitors in PwHA are commonly measured using a mixing-clotting method (Bethesda assay), in which residual FVIII activity is quantified by a one-stage FVIII activity test (**Figure 2a**)
- Emicizumab is not inactivated by heat treatment nor by FVIII inhibitors, and therefore interferes with standard Bethesda assays, leading to false-negative results
- The Chromogenic Bethesda assay (CBA)[1] uses a chromogenic FVIII activity assay containing bovine components that are insensitive to emicizumab, allowing detection and quantification of FVIII inhibitors in PwHA receiving emicizumab (**Figure 2b**)

Figure 2: Nijmegen-Bethesda assay vs. Chromogenic Bethesda assay for use with emicizumab



a) Nijmegen-Bethesda assay: emicizumab drives clotting in one-stage activity assay even in the presence of FVIII inhibitors, leading to false-negative test result

b) Chromogenic Bethesda assay: emicizumab does not drive activity in chromogenic assay with bovine proteins, so assay correctly measures residual FVIII activity and any inhibitors present

OBJECTIVES

- To validate the CBA for use in the presence of emicizumab
- To measure FVIII inhibitor titers and assess change over time for PwHA with inhibitors enrolled in the HAVEN 1 Phase 3 trial (Study BH29884; ClinicalTrials.gov NCT02622321)

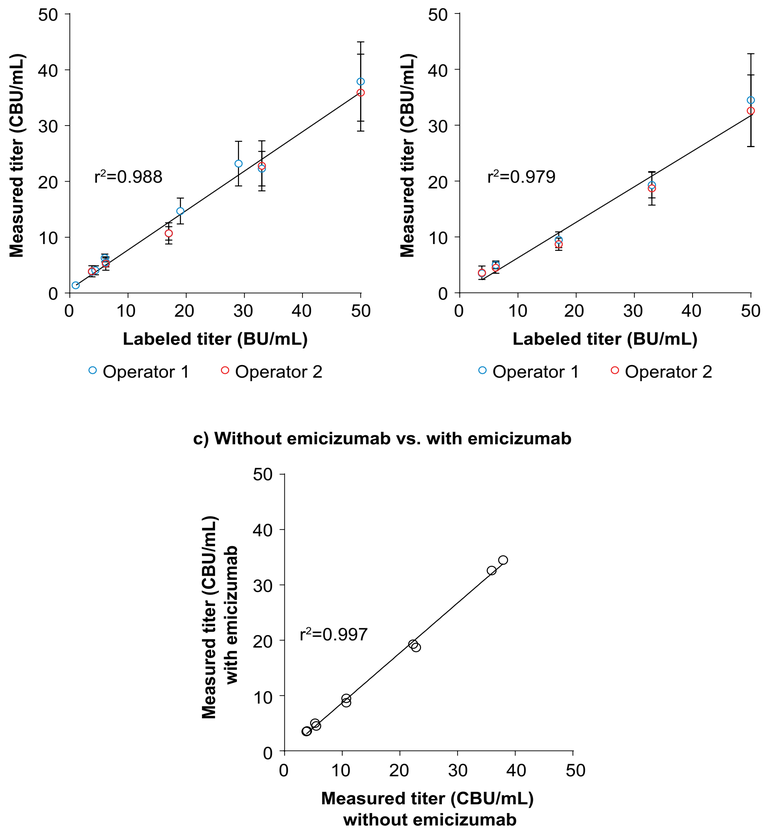
METHODS

- The CBA was implemented according to the methodology of Miller et al.[1]
  - Citrate plasma samples were thawed, heat inactivated for 30 minutes at 56°C, and clarified by centrifugation before being mixed in different ratios with imidazole-buffered normal pooled plasma (Precision BioLogic, Dartmouth, NS, Canada)
  - After incubation, mixed samples were tested for residual FVIII activity using the Siemens chromogenic FVIII assay on STA-R Evolution (Stago, Parsippany, NJ)
  - Inhibitor titers in Chromogenic Bethesda Units (CBU) were calculated based on the mean of all sample dilutions for which residual activity was between 25%–75%
- For validation, plasma samples from PwHA with and without inhibitors (George King Bio-Medical, Overland Park, KS; Affinity Biologicals, Ancaster, ON, Canada), as well as normal donor plasma (Medpace Reference Laboratories, Cincinnati, OH, internal donor pool) were tested with and without the addition of emicizumab (100 µg/mL) (**Figure 3**, **Figure 4**, and **Table 1**)
- In the HAVEN 1 study, FVIII inhibitor titers were measured in a central lab (Medpace Reference Laboratories) by CBA using citrated plasma collected from study participants at baseline (pre-treatment), after 6 weeks, and after 24 weeks on emicizumab prophylaxis
  - The HAVEN 1 study was approved by all local site ethics committees; informed consent/assent was obtained
  - Samples were frozen at time of collection and thawed immediately prior to analysis
  - PwHA enrolled in HAVEN 1 had a history of high titer inhibitors and documented use of bypassing agents, but positive FVIII inhibitor titer at time of enrollment was not required
- For data analysis and reporting purposes:
  - Values ≥0.6 CBU/mL were considered positive[2]
  - Values <0.6 CBU/mL were considered negative and set to 0 for analysis and reporting
  - FVIII inhibitor titers >45 CBU/mL were right-censored

RESULTS

ASSAY VALIDATION

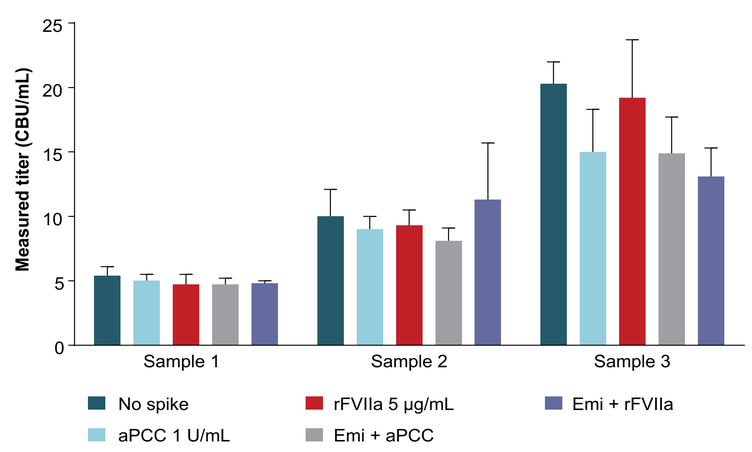
Figure 3: Accuracy of CBA without and with emicizumab



Commercially sourced frozen citrate plasma samples from PwHA with inhibitors were tested by CBA without (**a**; n=10) and with (**b**; n=5) addition of 100 µg/mL emicizumab (without vs. with addition of emicizumab shown in **c**). Data points represent mean ± SD of at least 6 replicates by 2 different operators over 3 days. In some cases, error bars are too small to be visible

CBA results correlated well with the supplier-labeled titers (**a**; y = 0.7058x + 0.7526) and (**b**; y = 0.6375x – 0.0358). However, CBA titers without and with emicizumab were consistently lower than the supplier-labeled values

Figure 4: Accuracy of CBA without and with emicizumab and bypassing agents



Commercially sourced frozen citrate plasma samples from three PwHA with inhibitors were tested by CBA without or with spiked addition of Emi (100 µg/mL), aPCC (1 U/mL), rFVIIa (5 µg/mL), Emi + aPCC, or Emi + rFVIIa. Data bars represent mean ± SD of two replicates from each of two different operators (n=4)

No significant effects were seen. There was a trend for spiked samples to have lower measured titer than the unspiked sample, possibly due to dilution effects (buffer-only spike in control was not included)

aPCC, activated prothrombin complex concentrate; Emi, emicizumab; rFVIIa, activated recombinant factor VII

Specificity of CBA

- Plasma samples from four normal donors and two PwHA without inhibitors were tested without additives or after spiking with emicizumab (100 µg/mL). Samples were tested in 20 replicates over 3 days with no false-positive results; addition of emicizumab had no effect on the negative samples (data not shown)
- Similarly, plasma samples from three PwHA without inhibitors were spiked with buffer alone, emicizumab (100 µg/mL), aPCC (1 U/mL), rFVIIa (5 µg/mL), Emi + aPCC, or Emi + rFVIIa, and tested by one operator over two runs. No false-positive results were seen (data not shown)

Table 1: Precision of CBA without and with emicizumab

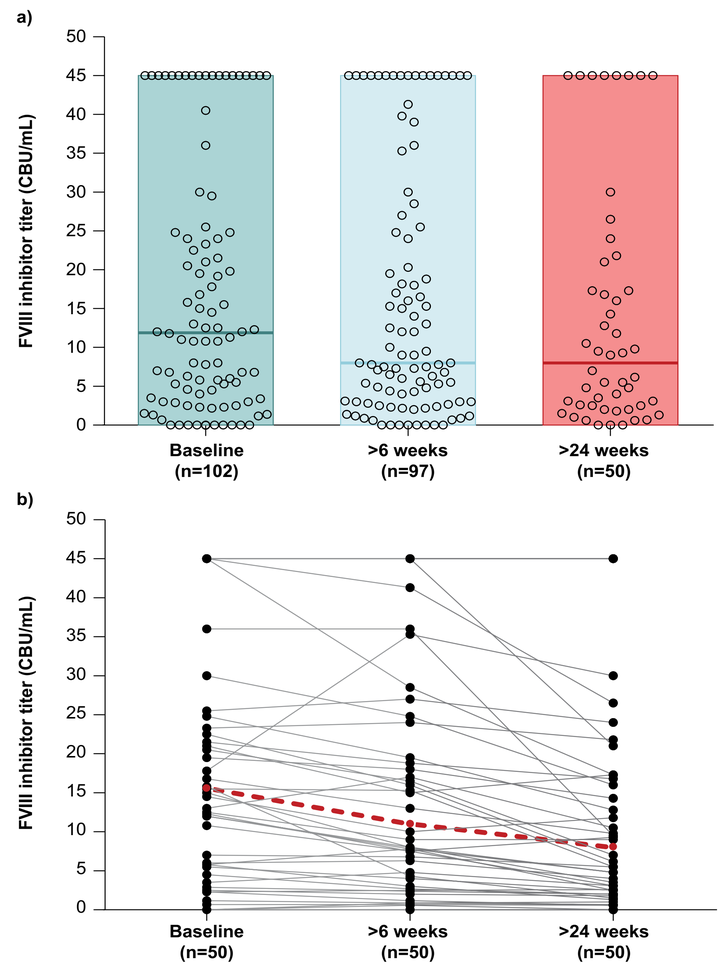
Vendor-assigned titer, BU	3.8	6.2	17	33	50	
						Mean, %CV
Without emicizumab						
Operator 1, %CV	13.4	13.6	11.4	14.0	18.7	14.2
Operator 2, %CV	24.4	22.8	18.0	19.7	24.1	21.8
With emicizumab						
Operator 1, %CV	18.3	13.4	14.5	12.1	19.2	15.5
Operator 2, %CV	32.5	21.3	12.7	16.3	19.6	20.5

Data include both between-run and between-day precision. Values are coefficient of variation (%CV) for a total of 10 independent replicates over 5 days for each of 2 operators

HAVEN 1

- HAVEN 1 data are presented for PwHA with inhibitors who received at least one dose of emicizumab prophylaxis by the time of data cutoff (October 25, 2016)
- Inhibitor titers ranged from negative (ie, <0.6 CBU/mL) to >45 CBU/mL at each time point
- Median titers at baseline, after 6 weeks, and after 24 weeks on emicizumab prophylaxis were 11.9 (n=102), 8.0 (n=97), and 8.0 (n=50) CBU/mL, respectively, with all patients included (**Figure 5a**)
- For the subset of patients with data available from all 3 time points (n=50), the median titers were 15.25, 11.5, and 8.0 CBU/mL, respectively (**Figure 5b**)
- The minor downward trend of median inhibitor titer over time is not due to emicizumab treatment, which is not expected to have any effect on anti-FVIII titer, but rather likely as a consequence of effective treatment with emicizumab
  - Bleed rates on emicizumab prophylaxis were greatly reduced compared to pre-study and no-prophylaxis regimens (Oldenburg et al, ISTH 2017, Abstract ASY 01.1), which may lead to reduced usage of bypassing agents (including aPCC, which contains traces of FVIII antigen)

Figure 5: FVIII inhibitor titers measured by CBA in patients receiving emicizumab prophylaxis in HAVEN 1



a) Results are shown for all patients who received at least one dose of emicizumab prophylaxis. The box plots show median (heavy line) and range (top and bottom). Dots represent titer measurements for individual patients. A total of 10, 8, and 3 patients, respectively, had a negative inhibitor test at each time point while 19, 17, and 8 patients had a titer >45 CBU/mL

b) Results are shown for 50 patients with FVIII inhibitor titer data from all 3 time points. Black dots represent titer measurements for individual patients, with lines connecting measurements for the same patient at different time points (red dots and connecting dashed lines represent medians). Dots at 45 CBU/mL represent 11, 9, and 8 patients at the 3 time points, respectively, while dots at 0 CBU/mL represent 4, 2, and 3 patients whose inhibitor tests were negative

CONCLUSIONS

- The Chromogenic Bethesda Assay is a robust method for determining FVIII inhibitor titer and is accurate in the presence of emicizumab
- FVIII inhibitor titers were measured successfully in HAVEN 1 for PwHA with inhibitors who received weekly emicizumab prophylaxis
- A slight trend toward lower median FVIII inhibitor titer over time may be due to reduced use of bypassing agents for breakthrough bleeds in the presence of effective emicizumab prophylaxis

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JIA, **JX**, and **GGL** are employees of Genentech Inc.; **BK** is a former employee of Genentech, Inc.; **CS** and **EA** are employees of F. Hoffmann-La Roche Ltd; **AC** was an employee of Roche Diagnostics International Ltd at the time of this work

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Joanne I. Adamkewicz

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