To the editor:

Metoclopramide treatment in DBA patients: no complete response in a French prospective study

In a paper by Abkowitz et al,[1] metoclopramide was identified as a potential new therapeutic approach for Diamond-Blackfan anemia (DBA). In order to further assess the drug efficacy, we performed a prospective study in patients included in the French DBA registry. Patients older than 2 years of age and dependent on regular transfusions were randomized at inclusion to be treated either at day 1 (arm B) or 6 weeks later (arm A); the comparison of responders in these 2 groups was planned to provide a more accurate determination of the delay of response. All patients were dependent on regular transfusion. All patients received metoclopramide for at least 16 weeks: 10 mg × 3/d in adults and 0.2 mg/kg × 3/d in children (weight < 50 kg). Packed red blood cell transfusions were prescribed for hemoglobin (Hb) values lower than 80 g/L. The definition of response relied on Hb levels, reticulocyte counts, and the intervals between transfusions that were recorded for the year before inclusion. Ten adults and 23 children have been included (Table 1). The treatment had to be stopped in 2 patients because of side effects: depression (8th week of treatment) and anaphylaxis (14th week of treatment). Other side effects were mild: most patients complained of asthenia and drowsiness, which both decreased after the first week of treatment; amenorrhea (2 patients) and bulimia (3 patients) were also observed. No increase in transfusion need occurred. None of the patients experienced a complete response. Two patients exhibited a partial response with an increase in the mean transfusion interval: from 3 to 8 weeks (patient 4) and from 4 to 5 weeks (patient 28). In these 2 patients, total duration of treatment with metoclopramide was 12 and 27 months, respectively. Last, it has to be emphasized that the 2 French responder adult patients previously included in the pilot study are still on treatment and transfusion independent more than 5 years after the initiation of metoclopramide. To date, only one other responder patient has been reported.[2] Obviously, only very rare transfusion-dependent DBA patients will respond to metoclopramide and, until now, we have no clue how to identify such patients. It is of note, however, that patients 1, 2, and 3 from the Abkowitz et al[1] study were not strictly resistant to steroids. Actually, patient 1, who is followed by one of us, does need the association of metoclopramide with low-dose prednisone (10 mg) to maintain the response. Lastly, our 2 partial responders are known to have previously experienced a response to steroids (Table 1). If the sensitivity to metoclopramide is actually associated with the sensitivity to steroids, the low response rate observed among

Table 1. Patient characteristics

Patient no.	Sex	Age, y	Rps19	History of response to steroids	Ferritin level at inclusion, ng/mL*	Arm	Response
1	F	24	Mutated	R	1201	B	N
2	M	3	Mutated	R	1368	A	N
3	F	7	Mutated	R	837	A	N
4	F	3	Wt	SHT	767	B	PR
5	F	26	Wt	SHT	861	A	N
6	M	23	Mutated	R	8060	B	N
7	M	9	Wt	S	503	A	N
8	F	15	Mutated	R	1857	B	N
9	F	8	Wt	R	1061	B	N
10	M	3	Mutated	R	1229	A	N
11	F	8	Wt	S	1347	A	N
12	F	6	Wt	S	2568	B	N
13	F	12	Mutated	R	2014	A	N
14	M	4	Wt	R	1599	A	N
15	F	43	Mutated	S	736	B	N
16	M	12	Wt	R	2963	B	N
17	M	4	Wt	SHT	2032	A	N
18	M	13	Mutated	SHT	5319	B	N
19	M	4	Wt	R	714	A	N
20	M	7	Mutated	SHT	1125	B	N
21	F	25	Mutated	S	4000	A	N
22	M	16	Mutated	S	592	A	N
23	M	35	Wt	R	2569	B	N
24	F	19	Wt	R	1381	B	N
25	F	12	Wt	R	1063	B	N
26	F	14	Wt	R	567	A	N
27	M	21	Wt	R	793	A	N
28	F	36	Wt	S	1990	B	PR
29	F	8	Mutated	SHT	687	A	N
30	M	3	Wt	R	2131	A	N
31	F	38	Mutated	R	2290	B	N
32	F	20	Mutated	R	936	B	N
33	M	2	Wt	R	1670	A	N

F indicates female; M, male; Wt, wild type; R, strictly resistant to steroids; SHT, patient responder to steroids with a high threshold (>0.5 mg/kg/d); S, patient good responder to steroids in whom steroids were discontinued either because of side effects or loss of sensitivity; N, no response; PR, partial response.

transfusion-dependent patients may be related to the proportion of patients who may be classified as strictly resistant to steroids (20/33 in our cohort). Metoclopramide, which is cheap and nontoxic, may be tested in all patients with regular transfusion need, as a slight decrease in transfusion requirement may be of clinical value in patients with severe hemochromatosis. A synergy in patients with high steroid threshold remains to be prospectively tested.

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We thank our colleagues from the SHIP (Société d'Hématologie et d'Immunologie Pédiatrique) for inclusion of their patients in this study, Assistance-Publique Hôpitaux de Paris for promotion of the study, and Maria Daniela Arturi and DBA foundations for financial support.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

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To the editor:

Correction of the bleeding time in von Willebrand factor (VWF)–deficient mice using murine VWF

Recently, Pergolizzi et al[1] reported in *Blood* about correction of the bleeding tendency in von Willebrand factor (VWF)–deficient mice via hydrodynamic delivery of murine VWF (mVWF) cDNA. The complete correction of the bleeding time was surprising since the multimer pattern was abnormal in these mice following treatment.[1(Fig2)] The mVWF cDNA used by Pergolizzi et al[1] originates from University Medical Center (UMC) Utrecht (P.J.L., P.G.d.G) and was distributed to several laboratories shortly after completion, which took place before the cDNA was fully analyzed. In vitro analysis, however, revealed that the distributed cDNA resulted in low expression levels in stably transfected baby hamster kidney (BHK) cells and HK293T cells, with greater than 90% of intracellular retention. Moreover, purified recombinant mVWF protein displayed impaired collagen binding and lacked multimers exceeding 6-mers (not shown), suggesting that the cDNA encoded a dysfunctional VWF protein. Dysfunction was confirmed in vivo. First, intravenous injection of purified mVWF protein did not correct the bleeding time in VWF-deficient mice (> 600 s versus 93 ± 32 s for wild-type littermates). Second, no VWF multimers could be detected when expression of mVWF was established via hydrodynamic injection of 100 μg cDNA (Figure 1A, lanes 7 and 9; ie, similar conditions used by Pergolizzi et al[1]). Again, no correction of bleeding time was obtained in any of these mice (Figure 1B), despite antigen levels between 22% and 65%. These findings prompted us to compare our original cDNA sequence (Balb/c) with that of other murine strains (C57Bl6/J, A/J, and Casa/Rk mice; kindly provided by D. Motto, University of Michigan). We detected 1 striking difference, the presence of an Arg at position 799, whereas a Cys was present in the other sequences. Moreover, the presence of Cys799 (located within the D′ region) is conserved among species as well (human, pig, canine), suggesting a structural or functional role for this residue. Since the D′ region is critical for multimerization, absence of Cys799 could explain the lack of multimerization we observed. After replacing Arg799 with Cys, a new analysis was performed. In vitro expression resulted in greater than 75% of the protein being secreted as fully multimerized mVWF molecules (not shown). Multimerized mVWF was also observed upon hydrodynamic delivery of corrected VWF cDNA in VWF-deficient mice, although the balance between high and low multimers was different from normal mice (Figure 1A, lanes 2-3, 5). This approach further resulted in expression levels between 148% and 392%, 24 hours after injection, and between 146% and 281% after 48 hours, all of which are significantly higher than levels obtained with the original sequence mVWF/Arg799. Finally, expression of mVWF/Cys799 resulted in a (partial) correction of the bleeding time both

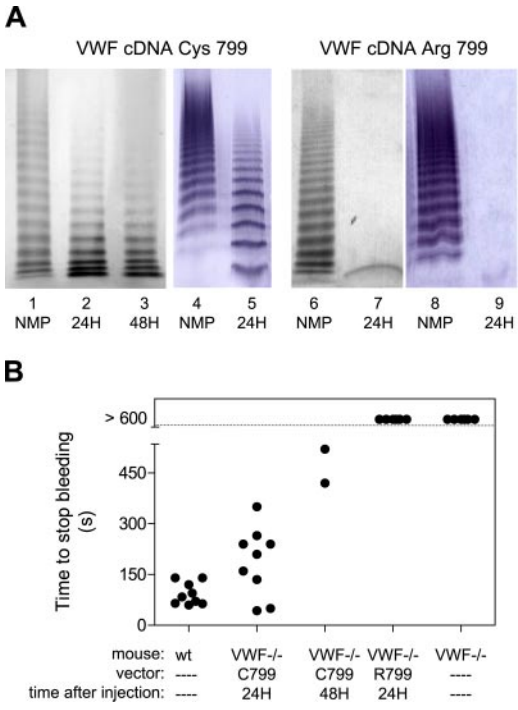

Figure 1. Analysis of recombinant mVWF. (A) Multimeric pattern of recombinant murine VWF after hydrodynamic delivery of 100 μg mVWF cDNA. After 24 hours (lanes 2, 5, 7, and 9) or 48 hours (lane 3), plasma was collected and examined for multimeric composition. Lanes 1, 4, 6, and 8 represent normal pooled mouse plasma (NMP). Lanes 2, 3, and 5 represent samples from mice injected with vector mVWF/Cys799, whereas lanes 7 and 9 represent those injected with vector mVWF/Arg799. For comparison, data of 2 laboratories have been included: lanes 1-3 and 6-7 originate from the Institut National de la Santé et de la Recherche Médicale (INSERM) Unité (U) 770 (Le Kremlin-Bicêtre, France), whereas lanes 4-5 and 8-9 originate from the Laboratory for Thrombosis Research (Kortrijk, Belgium). (B) Mice were examined for their bleeding time at 24 hours or 48 hours after hydrodynamic delivery of 100 μg mVWF cDNA using a tail-cut bleeding model. For wild-type mice, bleeding stopped at 93 ± 32 seconds (n = 9). Twenty-four hours after injection with 100 μg mVWF/Cys799-cDNA, the bleeding time was 188 ± 101 seconds. Bleeding did not stop (> 600 seconds) in noninjected mice or mice injected with 100 μg mVWF/Arg799-cDNA.