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Pharmacokinetic-based prediction of real-life dosing of extended half-life clotting factor concentrates on hemophilia

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Abstract

The improvement of clotting factor concentrates (CFCs) has undergone an impressive boost during the last six years. Since 2010, several new recombinant factor (rF)VIII/IX concentrates entered phase I/II/III clinical trials. The improvements are related to the culture of human embryonic kidney (HEK) cells, post-translational glycosylation, PEGylation, and co-expression of the fragment crystallizable (Fc) region of immunoglobulin (Ig)G1 or albumin genes in the manufacturing procedures. The extended half-life (EHL) CFCs allow an increase of the interval between bolus administrations during prophylaxis, a very important advantage for patients with difficulties in venous access. Although the inhibitor risk has not been fully established, phase III studies have provided standard prophylaxis protocols, which, compared with on-demand treatment, have achieved very low annualized bleeding rates (ABRs). The key pharmacokinetics (PK) parameter to tailor patient therapy is clearance, which is more reliable than the half-life of CFCs; the clearance considers the decay rate of the drug concentration–time profile, while the half-life considers only the half concentration of the drug at a given time. To tailor the prophylaxis of hemophilia patients in real-life, we propose two formulae (expressed in terms of the clearance, trough and dose interval between prophylaxis), respectively based on the one- and two-compartmental models (CMs), for the prediction of the optimal single dose of EHL CFCs. Once the data from the time decay of the CFCs are fitted by the one- or two-CMs after an individual PK analysis, such formulae provide to the treater the optimal trade-off among trough and time-intervals between boluses. In this way, a sufficiently long time-interval between bolus administration could be guaranteed for a wider class of patients, with a preassigned level of the trough. Finally, a PK approach using repeated dosing is discussed, and some examples with new EHL CFCs are shown.

Keywords: ABR, FVIII/IX efficacy, FVIII/IX extended half-life, FVIII/IX tailoring, immunogenicity, pharmacokinetics, pharmacodynamics, switch, venous access

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Introduction

During the last 6 years, many new recombinant FVIII (rFVIII) and FIX (rFIX) concentrates have attracted the attention of treaters and patients due to their innovative features. Improvements of the manufacturing procedures of such concentrates, as for example, in the adoption of cultures of human embryonic kidney (HEK) cells,[1,2] more selective immunoaffinity chromatography,[3,4] co-expression of the albumin gene[5–7] or fragment crystallizable (Fc) region of immunoglobulin in HEK cell lines,[8–9] molecule modification[10–12] and/or PEGylation,[13–18] have increased the bioavailability of the rFIX concentrates and, to a lesser extent, rFVIII ones.[19] In this context, pharmacokinetics

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(PK), pharmacodynamics (PD) and pharmacoeconomics (PE) are the tools, which allow the treaters the evaluation of the patients' unmet needs and the way they can be addressed by new CFCs. Both children and adult patients' adherence to therapy could be improved if a tailored access to the new products is allowed. The individualization of the personalized therapy in hemophilia is a key factor not only for improving the efficacy of the drug, but also from an economic point of view. Well-designed PK and population PK studies will help treaters to face these issues, and, probably, also to understand better the immunogenicity of EHL rFVIII concentrates. In this regard, ongoing studies on immunogenicity in previously untreated patients (PUPs) will establish whether the incidence of the anti-FVIII antibodies of EHL CFCs is lower than that of current ones.

Unmet needs

The venous access

The increase of the interval between bolus infusions, necessary to maintain the prescribed trough during the prophylaxis, will make the treatment with the extended half-life (EHL) CFCs more acceptable to hemophilia patients, especially children. The adherence of patients to the current prophylaxis with standard half-life CFCs (usually, three times/week for hemophilia A and two times/week for hemophilia B) can vary largely depending on their age.[20–22] The regimen of primary prophylaxis, which should begin immediately after the first hemarthrosis or within the first three years of life, is generally well-respected by the parents of the youngest patients.[23,24] However, the difficulties of venous access are the major obstacle for the prophylaxes during childhood. Although primary prophylaxis is far less demanding than immune tolerance induction, which requires one daily infusion and in many cases the implantation of a central venous catheter (CVC), such as a Broviac–Hickman (Bard Access Systems) or a Port-a-Cath (Abbot). Unfortunately, these devices, which are very useful for avoiding pains and psychological ill effects due to venipuncture in children, often become ineffective for the occurrence of thrombosis or infections.[25] Moreover, the CVCs require very careful maintenance, which is usually performed at home by the parents themselves.

In contrast with standard half-life CFCs, the EHL CFCs allow the patients to be treated once or twice a week with the new EHL rFVIII concentrates and once every 7–14 days with the new EHL rFIX concentrates. Moreover, these concentrates are expected to achieve the best results in primary prophylaxis in children and home treatment.[26] Instead, the adherence to the treatment significantly falls in adolescence.[20] Indeed, a natural sense of independence from their parents, the hemophilia center, and physicians, often leads to a discontinuous prophylaxis in patients, whose age is between 10 and 20 years. Sometimes, such an interruption can put at risk the results of the primary prophylaxis because of unexpected bleeding and the consequent onset of the hemophilic arthropathy.[27] Also, the teenagers tend to hide the disease, due to the need for replacement therapy.[28] Although these patients do not have problems with venous access and they have practiced self-treatment for many years, EHL CFCs are expected to improve patient adherence to therapy even for this age group. Conversely, adult patients may continue to use traditional concentrates. Indeed, during the hospital stay, especially during the perioperative period, prophylaxis can be done with traditional CFCs, because the replacement therapy of inpatients is currently performed using intravenous lines. Recently, subcutaneous administration of clotting factor concentrates (CFCs) has been explored: the low *in vivo* recovery and the worry of inhibitor development raised concerns about their implementation in clinical practice.[29] On the contrary, subcutaneous injection of new humanized monoclonal antibodies (Mabs) seems to open new therapeutic opportunities. The bispecific Mab binding of FIXa and FX as done by FVIII action reduced significantly the bleeding in hemophilia A.[30] Another approach is based on the subcutaneous administration of Mabs able to bind and inhibit tissue factor pathway inhibitor (TFPI) to increase the thrombin generation in hemophilia patients.[31]

Switch issues

Inhibitor risk. The immunogenicity of the EHL CFCs is not yet well known, nor is the role of PEGylated or fusion proteins. In this regard, the studies required by the United States Food and Drug Administration (US FDA) and European Medicines Agency (EMA) about the immunogenicity of the EHL CFCs are still ongoing in PUPs. Both albumin and Fc are self-proteins, but the molecules within the new EHL CFCs, that is, the rFIX-albumin and rFIX-Fc-fused proteins, could be nonself proteins for the recipients, because of

their quaternary structure. Moreover, although there is a general agreement that the switch from pdFVIII to rFVIII or between different rFVIII concentrates does not involve any inhibitor risk in previously treated patients (PTPs),[32,33] studies that certify the absence of inhibitor risks in the switch from current CFCs to EHL ones in PUPs are still lacking.

Efficacy. In this section, we will quote and compare the outcomes of the major phase III clinical trials of EHL rFVIII and rFIX concentrates, to provide an update discussion about EHL CFCs' efficacy, which we believe is still to be totally established in the real life. Even when the patients are treated with EHL CFCs, they could remain exposed to low levels of rFVIII/IX within the last part of the infusion time-interval, which is usually a longer period with respect to the one of the prophylaxis with current concentrates. Since the risk of bleeding occurs on the last part of the infusion time-interval, the level of rFVIII/IX may be insufficient to ensure an adequate hemostasis.[34] To avoid bleeding, it would be advisable to maintain a higher trough (at least 5 IU/dl)[35] by means of more frequent bolus infusions or a greater initial dose, even if in the first case the advantage of EHL CFCs would be lost or reduced. Unfortunately, as shown in Den Uijl and colleagues' paper,[35] the exact trough ensuring the complete protection from bleeding is not very well known, even if a trough between 12 and 15 IU/ml seems to be able to ensure this goal. The EHL rFIX concentrates, thanks to their long half-life time, are expected to provide a very high steady-state plateau *via* the administration of larger boluses within sufficiently large infusion time-intervals. On the other hand, since the currently available concentrates can ensure a steady-state plateau only after continuous infusion, the replacement therapy could save about 30% of the amount of concentrate, required by the patients. The evidence of such a result can be deduced from the outcomes of phase III studies just by comparing the estimates of the doses computed for the standard and the EHL CFCs (see Tables 1 and 2). The clinical trials have proved how much the prophylaxes with EHL CFCs can be effective if compared with on-demand treatment in PTPs, among children, adolescents, and adults. Tables 1 and 2 compare the medians, average values and standard deviations of the ABR between on-demand treatment and prophylaxis.

The ABR of the PTPs treated on-demand was generally very high. For instance, the Guardian First study included 213 patients from 19 countries worldwide. The range of ABR among the patients treated on-demand before entering the study was 16.90–34.15, and the highest figure (about three bleedings/month) resulted from Russia, due probably to low availability of CFCs in that country.[36] Usually, according also to the experience of treaters, the ABR decreases to low values during continuous cycles of prophylaxis, and consequently, the quality of life of patients increases significantly. The most outstanding results have been achieved in hemophilia B prophylaxis, thanks to the long half-life (70–96 h) and reduced clearance (0.7–3.2 ml/h/kg) of PEGylated and albumin fused rFIX concentrates. In this regard, the increased interval between bolus infusions, up to 14 days, has been extremely well accepted by children and their caregivers. The same results have not been obtained for the new EHL rFVIII concentrates, whose half-life is increased only by about 6 h (for a total of 15–19 h). However, also in this case an improvement is achieved: the prophylaxis of hemophilia A patients will be performed two times a week and not three times, as before. In this way, the patients (especially children) could avoid about 60 venipunctures a year. To summarize, in Tables 1 and 2 the characteristics and the corresponding outcomes of the major phase III clinical trials of EHL rFVIII[37–42] and rFIX[43–45] concentrates are shown.

Pharmacokinetics

Half-life alone is misleading

The real improvement gained by substituting the current CFCs with new EHL CFCs in patients' therapy should be evaluated by comparative PK studies. Indeed, the aim of such a comparison is to accurately and analytically evaluate the difference between the concentrates. The data available from phase I/II provide average values of PK outcomes, though it is well known that relevant inter-patient variability must always be considered.[46–48] Moreover, accurate and repeated single-dose PK studies should be performed in patients before and after the switch between concentrates.[49] However, the PK design is very demanding for outpatients, particularly if they live far from the hemophilia centers, although the availability of an even small number of blood samples could increase their adherence to PK studies. In this regard, the minimal design of blood samples for FVIII/IX can be adapted to the following

Table 1. Comparison of the medians, average values and standard deviations of the ABRs between on-demand treatment and prophylaxis in phase III trials of EHL rFVIII concentrates.

Product	Treatment on-demand			Weekly prophylaxis			
	Patients (*n*)	Dose (IU/kg)	ABR median (Q1–Q3) mean + SD	Patients (*n*)	Dose (IU/kg)	ABR median (Q1–Q3) mean + SD	Ref.
Elocta TM	23	10–50 as required	33.6 (21.1–48.7)	24	65	3.6 (1.9–8.4)	Brand and colleagues[14]
	14		18.36 (10.45–30.46)	27	150	2.03 (0.60–4.39)	Bjørnsdottir and colleagues[15]
BAX 855	17	according to treater	41.58 (31.7–51.1)	101	45 ± 5 2/week	1.9 (0.0–5.8)	Stidl and colleagues[16]
			40.8 ± 16.3			3.7 ± 4.7	
N8-GP	12	according to treater	30.9	175	50 every 4 days	1.3	Turecek and colleagues[17]
BAY 94-9027	20	according to treater	23.4	13	2/week	4.1	Mei and colleagues[18]
				43	45–60 every 5 days	1.9	
				43	60 every 7 days	3.9	
FVIII Single Chain	27	according to treater	19.54 (6.2–46.5) 31.14 ± 35.56	79	20–40 eod	1.93 (0.00, 4.9)	Morfini and Zanon[19]
				47	20–50 2–3/week	0.0 (0.0, 3.3)	

ABR, annualized bleeding rate; eod, every other day; EHL, extended half-life; rF, recombinant factor; SD, standard deviation.

procedure: baseline immediately before the test dose (30–40 IU/kg), and 1 ± 0.5, 8 ± 1, 24 ± 2, 48 ± 2, 72 ± 2 and 96 ± 2 h after the end of infusion.[50,51] Another approach could be provided by population PK. According to this procedure, just two well-spaced (generally, within some 24 h intervals) blood samples are enough to evaluate whether the patient's drug decay agrees with population data.[52] Head-to-head PK studies of phase I/II have been performed with a different sampling designs, which is shorter for the current plasma derived FIX (pdFIX) and rFIX CFCs than for the new EHL CFCs.[43,53,54] However, the collection of blood samples was stopped too early for both pdFIX and rFIX current products, before the baseline value of FVIII/IX was achieved. In particular, the area under the curve (AUC) of the current FIX concentrates extrapolated up to an infinite time, was about 20% larger. Therefore, the clearance (i.e. the volume of plasma per unit of time which is totally cleared of its content of drug) of current rFIX CFCs was about 20% lower. During the switch between concentrates, the PK studies should be performed at the last infusion of the old concentrate and the first infusion of the new one. Usually, both the patients and their parents are very eager to know the difference among the CFCs, and such a drive should be encouraged by the treater; indeed, if the patients are aware of the optimal EHL CFC decay, then the adherence to the new therapy will increase. On the other hand, the treaters must be constantly aware of the patient's behavior under treatment with a new concentrate, especially an EHL one. In this regard, a PK-driven switch is also recommended by the recently issued guidelines of United Kingdom Hemophilia Centers Doctors Organization (UK HCDO)[49] for the use of EHL CFCs.

Treatment of breakthrough bleeding, occurring during prophylaxis, requires a knowledge of the supposed FVIII/IX concentration at the time

Table 2. Comparison of the medians of the ABR between on-demand treatment and prophylaxis in phase III trials of EHL rFIX concentrates.

Product	On-demand			Prophylaxis			Ref.
	Patients (*n*)	Dose (IU/kg)	ABR median	Patients (*n*)	Dose (IU/kg)	ABR median	
Alprolix	27	20–100	18	63	50 weekly at start, afterwards PK-driven	3	Schrijvers and colleagues[20]
				29	100 q10d at start, afterwards PK-driven	1.4	
Novo9-GP	15	according to the treater	15.6	30	10 weekly	2.9	Schrijvers and colleagues[21]
				29	40 weekly	1	
Idelvion	23	35–50	15.43	40	40 × 7 days	1.58	Schrijvers and colleagues[22]
				7	40 × 10 days	1.69	
				21	40 × 14 days	1.61	

ABR, annualized bleeding rate; EHL, extended half-life; PK, pharmacokinetics; rF, recombinant factor.

of the event. As reported by Broderick and colleagues,[55] bleeding usually occurs during the second part of the day due to the increased physical activity of the hemophilia patients in the afternoon. Instead, patients performing weekly prophylaxes with EHL CFCs usually undergo bleeding between the 4th and the 7th day after the infusion. In this regard, the extra dose of EHL CFCs should be calculated on the basis of the difference between the last measured FVIII/IX plasma level and the value of the peak, which is given by the incremental *in vivo* recovery (IVR) of the product. Indeed, also the peak of FVIII/IX plasma concentration (after the end of infusion) and the AUC may affect the bleeding, occurring in patients during every 3rd day after prophylaxis.[56] Usually, it is the weight-based standard dose that is used in regular prophylaxes, but it can lead to an over-or under-treatment of the patient. However, also the peak is quite variable, and the consequent IVR can be misleading to identify the better replacement therapy in hemophilia patients.[57]

Optimal single-dosing: clearance is the key parameter of PK

Although the phase III clinical trials for EHL CFCs can provide the treaters with a prediction of the concentrate dosing for their patients, it is well known that a large inter-patient variability exists, and such a prediction could be quite approximate. Therefore, it is recommendable for real-life treatments and in cases of personalized therapy to know and record the true PK outcomes of each concentrate for each patient. In this regard, UK HCDO recommended performing a PK of the new EHL concentrates during the replacement of the therapy and the follow up of the patients.[49] As shown before, the half-life and trough can be misleading parameters to choose the optimal dose of the drug. Accordingly, also the peak and the AUC should be systematically considered. In this regard, the key parameter of PK, which summarizes all the others (i.e. the peak, AUC, half-life, and trough) is the clearance, which is proportional to the amount of drug removed from the plasma during the infusion time-interval. The clearance Cl (ml/h/kg) is the ratio between the dose D (IU/kg/h) and the AUC ($Cl = D/\mathrm{AUC}$), and better represents the monotonically decreasing behavior of the drug over time in each patient. For example, a flat decay curve for the drug concentration with a low peak and a high value of the trough exhibits a very long half-life, but a small value of clearance. In this regard, the new EHL CFCs should be better defined as reduced clearance CFCs.

However, the evaluation of the clearance from the measurement of the AUC may not be the best solution, since the AUC should be estimated in an

ideally infinite infusion time-interval. As a matter of fact, each time the blood sampling is stopped too early, the value of the clearance is abnormally high. In this regard, to achieve a prediction of the optimal dose for the EHL CFCs, we propose the adoption of compartmental models (CMs) providing us with the exponential time behavior of the drug concentration. The lower sum of squared residuals will show the best model, whether a one-CM or two-CM, fitting the patient's data. The plasma concentration of clotting factors, indeed, is the result of their elimination from the central plasma compartment and of the consequent flow-back from extravascular and extracellular space. In the specific case of the rFIX fused with albumin or Fc, the recycling mechanism, from the cells bearing the Fc neonatal receptor to the plasma compartment, is responsible of the EHL of the rFIX-Fc and rFIX-FP concentrates,[43,54] but seems that it does not affect the time-decay behavior of such drugs in the first part of the infusion time-interval. Moreover, the second part of the decay curve plasma concentration is practically flat for almost all rFIX EHL CFCs, so that it well approximates a linear function with a very small slope.

In the following, we will provide simple formulae for the prediction of the optimal single dose of EHL CFCs, in the context of the personalization of patient treatment. For the sake of clarity of notation, let us observe that * and / denotes, respectively, the operations of multiplication and division, while the symbol exp() is the exponential function. Given the data of the single patient from previous infusions, we propose to analyze the best fitting indexes and identify the CM that better approximates the exponential time behavior of the drug. Afterwards, starting from such an evaluation, the most suitable formula for the optimal single-dosing will be applied. To this end, let us initially consider the expression from the one-CM of $C(t)$, which is defined by the following formula:

$$C(t)=(D/V)*\exp[-(\gamma*t)] \quad (1)$$

where V is the volume distribution, namely the space within which the drug distributes, and $\gamma = Cl/V$ is the decay rate of the concentration–time profile. By inverting Equation (1) as a function of D, we can obtain the prediction of the single dose to be used for the next infusion:

$$D = C(tau)*V*\exp(\gamma*tau). \quad (2)$$

In Equation (2), *tau* denotes the final time at which we measure the last PK outcome, i.e. the time-interval between boluses. Hence, $C(tau)$ corresponds to the trough for the EHL CFCs, that is, to the minimal FVIII/IX level (IU/ml) ensured by the prophylaxes. In this regard, it is worth noting that Cl and V are independent variables, denoted as PK parameters. The volume distribution can be easily derived by measuring the peak of the drug, which is equal to $C_0 = C\ (t = 0)$. The latter, indeed, can be substituted with the value of the drug concentration immediately after the infusion of the EHL CFC. Instead, for the 1-CM the clearance Cl is related with the half-life $t_{1/2}$ according to the following equation:

$$t_{1/2} = (\ln(2)*V)/Cl \quad (3)$$

The half-life is the time required for reducing by half the amount of drug remaining in the body, and it can be directly obtained by the PK outcomes. As a result, from the PK outcomes, we can determine the PK parameters Cl and V, and, consequently, the prediction of the single-dose D. However, Equation (2) does not provide the optimal value of D, that is, D_{opt}, since our goal is to derive the minimum value of D for the longer value of the time-interval *tau* (expressed in hours) between two boluses. Such a result can be achieved just by computing the time-derivative of D, which is then set to zero. We obtain the following relation:

$$Peak = Trough(\text{at final time}\ tau)*\exp(\gamma*tau) \quad (4)$$

Then, being $Peak = D$ * IVR, the optimal value D_{opt} of the single dose is:

$$D_{opt} = (\text{Trough}*\exp(\gamma*tau))/2 \quad (5)$$

where for the IVR has been chosen the well-known value 2. Thus, Equation (5) provides us the minimum dose D_{opt}, where *tau* belongs to the set (60–120h) and the trough (which is chosen by the treater) to the interval (2–15) IU/dl.

Now, let us consider the two-CM, for which the concentration $C(t)$ has a bi-exponential time behavior, given by the relation:

$$C(t)=A*\exp[-(\alpha*t)]+B*\exp[-(\beta*t)], \quad (6)$$

where the parameters $A = [D*(\alpha - k_{2V})] / [V*(\alpha - \beta)]$ and $B = [-D*(\beta - k_{2V})] / [V*(\alpha - \beta)]$ depend not only on D and V (principal volume distribution), but also on the decay rates α and β

(respectively, in the first and second phase of the concentration absorption), and on k_{21}, which is the first-order fractional rate constant for the redistribution of the drug between the principal and peripheral compartments.[58] In this case, the parameters α, β and k_{21} are fixed by the data of the single patient from previous infusions, and, thus, they can be used for the prediction of D_{opt}, minimum value of D in correspondence of the larger value of the time-interval *tau*. Instead, V is given by the ratio between the dose D of the CFC, which has been previously infused to the patient, and its concentration C_0 immediately after the new infusion. As before, by inverting Equation (6) as a function of D, we can obtain the prediction of the single dose from the two-CM at the final time *tau*:

$$D(tau) = [(\alpha - \beta) * V * C(tau)] / \{(\alpha - k_{21}) * \exp[-(\alpha * tau)] - (\beta - k_{21}) * \exp[-(\beta * tau)]\} \quad (7)$$

Then, by computing the time-derivative of D and setting it to zero, we derive the expression for the optimal dose D_{opt}:

$$D_{opt} = \{[(\alpha - \beta)/IVR] * Trough(at\ final\ time\ tau)\} / \{(\alpha - k_{21}) * \exp[-(\alpha * tau)] - (\beta - k_{21}) * \exp[-(\beta * tau)]\} \quad (8)$$

In conclusion, for each value of the trough (chosen by the treater) within the range (2–15) IU/dl and the time-interval *tau* between boluses (from 60 to 120 h), Equation (8) will provide a set of values for the minimum dose D_{opt}. Then, it will be up to the treater to choose the best value among them, with the optimal trade-off between the trough $C(tau)$ and *tau*, also by following the needs of the patient. As general remark, we always recommend taking D_{opt} smaller than 70–90 IU/kg, determining a Cmax in the range 140–180 IU/dl according to an IVR of 2.0 IU/dl/IU/kg.

Repeated dosing

Compared with the single-dose case, the dosing D for a sequence of repeated administrations is simply equal to the clearance Cl multiplied by the prescribed plasma concentration (i.e. the FVIII/IX level) and the interval *tau* between doses, as given in the following:

$$D = Cl * FVIII/IX\ level * tau. \quad (9)$$

Some examples of repeated dosing are shown in Tables 3 and 4. They are based on the values of clearance which have been obtained in phase I/II studies of several EHL CFCs. Given a trough level equal to 0.15 IU/ml, we have computed the value of the dose D for some currently available FIX/FVIII concentrates, by applying Equation (9) with an interval *tau* between infusions fixed to 12 h. In the same way, by fixing the dose D to 10 IU/kg, we have determined the corresponding value of *tau* for different real-life cases. It is worth noting that in case of repeated dosing the value of the dose progressively decreases, because at the steady-state only the amount of the drug, which has been cleared from the plasma compartment, must be replaced. As a final remark, in the extreme case of continuous infusion, the interval *tau* between boluses must be omitted from the calculations. For this reason, continuous infusion is the most cost-effective method for hemophilia replacement therapy. Tables 3 and 4 report the estimates of doses according to different *tau* and different targeted steady-state FIX or FVIII, respectively, concentrations. The values of clearance have been derived from the published papers.

The blood sampling design

Overall, two head-to-head phase I/II studies of EHL concentrates[54,55] have been conducted with a not very accurate blood sampling design. The golden rule of PK, that blood sampling must be prolonged up to achievement of the infused drug baseline concentration, has been completely disregarded as far as the comparator CFCs were concerned. Since the comparison between the clearance of EHL FIX concentrates and that of standard FIX products is not very reliable due to this bias in the PK design, we also considered other studies reporting an increased half-life of nonacog alfa, ranging from 26.8 to 36.0 h, due to their prolonged blood sample design.[59–61] In the crossover study of eftrenonacog,[43] nonacog alfa PK design was prolonged up to 72 h, and the range of the mean residence time (MRT) increased up to 36.0–47.2 h. Similar results of MRT and half-life were observed recently in the multicenter PK Italian study,[62] in which the samples have been collected up to 72 h. Due to the sample timing of nonacog alfa PK (48 h) in two head-to-head studies[54,63] the ratio of clearance between N9-GP and nonacog alfa was 6.99/0.71 ml/h/kg = 9.84 and that between rIX-FP and nonacog alfa was 5.24/0.75 ml7h/kg = 6.99. On the contrary, due to slightly higher clearance of rFIX-Fc and the prolonged sample timing

Table 3. Estimates of different values of *D* and *tau* for several EHL rFIX concentrates by taking into account a sequence of repeated administrations. The results have been obtained by using Equation (9) with a trough level equal to 0.15 IU/ml, and with an interval *tau* between boluses and a value of dose, respectively, equal to 12 h and 10 IU/kg/h.

Phase I/II studies				Estimates of *tau* with trough 0.15 IU/ml and *D* = 10 IU/kg/h			Estimates of *D* with trough 0.15 IU/ml and *tau* = 12 h		Ref.
FIX concentrate	**Trade name**	**Dose (IU/kg)**	**Clearance (ml/h/kg)**	**FIX (IU/ml)**	***Tau* (h)**	**Dose (IU/kg/h)**	***Tau* (h)**	**Dose (IU/kg/12 h)**	
Nonacog alfa pegol	NovoNine GP®	50	0.71	0.15	94	10	12	1.28	Shima and colleagues[30]
pdFIX	n.a.	50	5.48	0.15	12	10	12	9.86	
Nonacog alfa	Benefix®	50	6.99	0.15	10	10	12	12.58	
Eftrenonacog alfa	Alprolix®	50	3.2	0.15	21	10	12	5.76	Schrijvers and colleagues[20]
Nonacog alfa	Benefix®	50	6.3	0.15	11	10	12	11.34	
Albutrepenonacog alfa	Idelvion®	50	0.75	0.15	89	10	12	1.35	Chowdary and colleagues[31]
pdFIX	n.a.	50	4.76	0.15	14	10	12	8.57	
Nonacog alfa	Benefix®	50	5.24	0.15	13	10	12	9.43	

ABR, annualized bleeding rate; EHL, extended half-life; PK, pharmacokinetics; rF, recombinant factor.

of nonacog alfa (72 h) in another study,[43] the ratio of clearance was 6.3/3.20 ml/h/kg = 1.97.

Instead, according to other studies[60,62] with a more extended sampling design, the clearance of nonacog alfa resulted in 4.6 ml/h/kg and 3.01 ml/h/kg respectively. It is easy to understand that the longer the collection time of the samples is, the larger the AUC and the smaller the clearance, respectively, are. Therefore, the same ratios (nonacog alfa clearance/EHL rFIX clearance) respectively for N9-GP, rFIX-Fc and rIX FP[54,43,55] were 6.48, 1.44, and 6.13[60] and 4.24, 0.94, and 4.01, according to the BeneFit study.[62]

As far as the EHL rFVIII CFCs are concerned (Table 4), it is evident that the differences with standard CFCs are not so improved as for those observed for EHL rFIX concentrates (Table 3). The choice of FVIII concentrates is based essentially on the clinical phenotype of hemophilia A patients.[64] Although the trough does play an important role in the prevention of bleeding,[65] also the peak and AUC must be considered[56] as well as the FVIII clearance. On the other hand, it is very well known that the FVIII behavior *in vivo* is essentially determined by its carrier, the Von Willebrand factor (VWF). PEGylation seems to be able to improve the HL of new rFVIII CFCs, reducing the uptake of the FVIII molecule by lipoprotein receptor-related proteins, low density lipoprotein (LRP/LDL) hepatic receptors. Plenty of B-domain deleted FVIII clotting concentrates (Table 4) have been submitted to PEGylation.[39,66,67] A new B-domain deleted rFVIII co-expressed by HEK cells[68] together with Fc, efraloctocog alfa, represented the first human rFVIII, followed very soon by simoctocog alfa.[69] A recycling mechanism by neonatal Fragment crystallizable Receptor (FcRn)[70,71] does not seem effective for the FVIII/VWF complex, the HL clearance of efraloctocog alfa being comparable with that of damoctocog alfa pegol.[67] The fusion with albumin was very successful for rFIX but not for rFVIII. This is probably the reason why ionoctocog alfa was developed.[72]

Discussion

There is no doubt that the new EHL rFIX concentrates represent a revolution in hemophilia B

Table 4. Estimates of different values of *D* and *tau* for several EHL rFVIII concentrates by taking into account a sequence of repeated administrations. The results have been obtained by using Equation (9) with a trough level equal to 0.15 IU/ml, and with an interval *tau* between boluses and a value of dose, respectively, equal to 12 h and 10 IU/kg/h.

Phase I/II studies				**Estimates of *D* with trough = 0.15 IU/ml and *tau* = 12 h**			**Estimates of *tau* with trough = 0.15 IU/ml and *D* = 10 IU/kg/h**		**Ref.**
FVIII concentrate	**Trade name**	**Dose (IU/kg)**	**Clearance (ml/h/kg)**	**FVIII (IU/ml)**	***Tau* (h)**	**Dose (IU/kg/12 h)**	***Tau* (h)**	**Dose (IU/kg/tau)**	
Turoctocog alfa GP	NovoEight GP	75	1.9	0.15	12	3.4	35	10	Powell and colleagues[43]
Turoctocog alfa	NovoEight®	50	4.17	0.15	12	7.5	16	10	
Octocog alfa	Advate®	50	4.24	0.15	12	7.6	16	10	
Damoctocog alfa pegol	BAY 94-9027	25	1.6	0.15	12	2.9	42	10	Collins and colleagues[44]
Octocog alfa-sucrose formulated	rFVIII-FS	25	2.3	0.15	12	4.1	29	10	
Octocog alfa PEG	BAX 855	30	2.76	0.15	12	5.0	24	10	Stidl and colleagues[16]
Octocog alfa	Advate®	30	4.55	0.15	12	8.2	15	10	
Ionoctocog alfa	FVIII-SingleChain	50	2.64	0.15	12	4.8	25	10	Collins and colleagues[49]
Octocog alfa	Advate®	50	3.68	0.15	12	6.6	18	10	
Efraloctocog alfa	Eloctate®	25	1.68	0.15	12	3.0	40	10	Santagostino and colleagues[45]
Octocog alfa	Advate®	25	2.49	0.15	12	4.5	27	10	
Efraloctocog alfa	Eloctate®	65	2.32	0.15	12	4.2	29	10	
Octocog alfa	Advate®	65	3.61	0.15	12	6.5	18	10	
Simoctocog	Nuwiq®	45	4.73	0.15	12	8.5	14	10	Morfini[46]

ABR, annualized bleeding rate; EHL, extended half-life; PK, pharmacokinetics; rF, recombinant factor.

treatment. The dream of each patient is to keep the interval between venepunctures for prophylaxis as long as possible and to be free of bleeding episodes. Some hemophilia B patients are achieving this goal with a weekly infusion of nonacog alfa,[73,74] but they are aiming at 2-week intervals using EHL FIX concentrates. The three EHL rFIX concentrates are not each other equivalent, being the clearance of eftrenonacog alfa quite similar to that of nonacog alfa. The clearance of the other two EHL rFIX concentrates, nonacog alfa pegol and albutrepenonacog alfa, is very small and may allow reduced dosing and very long intervals. The cost effectiveness of these new concentrates is under discussion. In addition, the very important issue of immunogenicity (PUPs studies being ongoing) will be a key factor in the choice of concentrates by patients and their treaters. The long-term toxicity of polyethylene glycol (PEG) has not been very well evaluated. There are a lot of drugs whose HL has been increased by PEGylation, but they are generally used for a limited time, not

more than some months. We now have several B-domain deleted new rFVIII concentrates and one rFIX PEGylated concentrate coming onto the market; accurate short and long-term post-marketing surveillance will be mandatory.

The new EHL CFCs licensed in the USA between 2015–2016 are also going to be introduced in the European Union (EU). The advantages of a reduced number of infusions during prophylaxis will make these products very attractive for all hemophilia patients. No one child or adult, enjoys undergoing frequent venepunctures. The patients' view is based not only on the pain and discomfort of venepunctures, but also on how the treatment can facilitate social activities during working time, sport, relationships, etc. As shown in Table 3, rFIX EHL CFCs, especially nonacog alfa pegol and albutrepenonacog alfa, are demonstrating that even long intervals can maintain a safe trough. These rFIX concentrates will probably be able to change the real-life of hemophilia B patients, allowing a prophylaxis every 1 or 2 weeks. The other key factor in the decision-making process to choose the right therapy for hemophilia is CFC immunogenicity. EHL CFCs are under investigation in the frame of PUP studiesby EMA about the incidence of inhibitors during the first 50 days of exposure. We hope that they will be no more immunogenic than the other current CFCs, or even less immunogenic. For instance, the expected incidence should be less than the 44.5%, as reported for rFVIII CFCs in the Survey of Inhibitors in Plasma-Product Exposed Toddlers study.[75] If it is definitively validated, the adoption of the new EHL CFCs will hugely increase.

Finally, another key factor will be the cost of the new EHL CFCs. Assuming the PD of the EHL rFIX concentrates are like those of the standard ones, the decrease of clearance, and, consequently, the reduced amount of product needed for on-demand treatment or prophylaxis, might push companies to demand a higher price. Considering that the ratio between the doses of the standard rFIX CFCs and new EHL ones to achieve the same plasma level ranges (according to Table 3) goes from 6.98 (9.43/1.35) for albutrepenonacog alfa to 9.83 (12.58/1.28) for nonacog alfa pegol, the cost of new EHL CFCs could be consistently higher. We hope that this will not be allowed by regulatory agencies in the EU, because such an increase in the cost of hemophilia treatment could be untenable for some countries. In the EU, all CFCs are free of charge for patients; consequently, any increase in the health budget will cause an increase in taxation. Conversely, if the cost of the new EHL CFCs is not strictly defined according to their reduced dosing, the cost of hemophilia treatment could decrease. As a matter of fact, the treaters will be able to achieve similar hemostatic efficacy with a smaller dose of the new drugs. The cost of FVIII/IX concentrates represents the major impediment for the spread of hemophilia treatments in developing countries. According to the recent World Federation of Hemophilia Global Survey, about 70% of hemophilia patients worldwide lack any treatment for their bleeding episodes. We hope that the cost of CFCs will decrease in the upcoming years, allowing for the gap between developed and developing countries to be filled.

Conclusion

Accurate individual PK evaluation of new EHL CFCs in hemophilia PUPs or PTPs, in comparison with the standard ones, will allow tailored prophylaxis or on-demand treatment for each patient. This head-to-head approach, as recommended by UK HCDO, will show how the switch to new EHL CFCs will be worthwhile. The increase and personalization of time intervals between bolus infusions, according to the smaller clearance of EHL CFCs, will improve patient adherence to therapy. A balance of these advantages and the cost of the new therapy should be considered by patients and doctors before deciding on the switch.

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Conflict of interest statement

The authors declare that they do not have any conflict of interest related to this paper. Informed consent and ethics approval was not required for this review.

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