Received: 2 August 2019 | Revised: 11 October 2019 | Accepted: 21 October 2019

DOI: 10.1111/hae.13875

Haemophilia WILEY

LETTER TO THE EDITOR

PEGylated biologics in haemophilia treatment: Current understanding of their long-term safety

PEGylation has proven to be a valuable tool to prolong the half-life of proteins in drug delivery. Covalent binding of one or more polyethylene glycol (PEG) molecules—either with the attachment of small (5-10 kDa) PEG groups, or site-directed attachment of large (≤60 kDa) PEG molecules via linkers—increases the hydrodynamic radius of a protein, improving drug stability and reducing clearance receptor interaction.[1,2]

At least 12 PEGylated biopharmaceuticals have been approved in Europe and the United States, across multiple indications. PEGylated products have a clinical track record of >20 years, and no long-term PEG-related safety signals have been identified in humans. Most of the approved products are used to treat chronic diseases, including hepatitis, immunodeficiency disorders, renal failure and autoimmune diseases.[1] Short-term effects of PEG immunogenicity on safety, by detection of either pre-existing or PEGylated biologic-induced anti-PEG IgM and IgG antibodies, have been reported, but will not be the focus of this letter.

Improved pharmacokinetics (PK) and pharmacodynamics conferred by PEGylation also prolong the half-life of coagulation factors in the treatment of haemophilia A and B. Extending protection from bleeds while reducing infusion frequency has been a goal in the development of coagulation factor products. It can be achieved by reducing factor clearance (prolonging terminal half-life), for example by linking human recombinant FVIII (rFVIII) or FIX proteins to other molecules such as the Fc part of an antibody (efmoroctocog alfa [Elocta/Eloctate®]) or to PEG (rurioctocog alfa pegol [Adynovate®], nonacog beta pegol [Refixia®], damoctocog alfa pegol [Jivi®]; Table 1).[3] The resulting half-life prolongation is substantially higher for FIX compared with FVIII products.

Polyethylene glycol molecules have a simple, repetitive structure and are chemically inert, with low toxicity. They are uncharged, water-soluble, non-reactive and do not have any specific receptors or targets in the body.[1,4] However, the accumulation of large (>20-30 kDa) PEG molecules in renal tubular and choroid plexus epithelial cells is a concern because of their increasingly reduced clearance with higher molecular size.[4] In addition, cellular vacuolation in certain tissues and cell types has been observed in non-clinical toxicology studies for about half the PEGylated biologics.[4,5]

How can we address these concerns when discussing PEGylated biologics in haemophilia treatment? Predictions for safe long-term prophylactic use in humans must be based on scientific data. There are four aspects to consider when predicting long-term safety of these compounds in clinical use: (a) regulatory requirements; (b) non-clinical safety (toxicology); (c) pharmacokinetics; and (d) clinical experience.

A maximum acceptable administrable monthly dose of PEG (eg as part of a PEGylated molecule) has been defined for the paediatric population by the Committee for Medicinal Products for Human Use (CHMP) Safety Working Party's paper. CHMP stated that vacuolation in critical cells and tissues like renal tubular endothelium or the choroid plexus was observed in toxicology studies with individual PEGylated biologics following certain conditions (cynomolgus monkeys, PEG ≥40 kDa, toxicology study duration ≥6 weeks and cumulative PEG dose >0.4 µmol/kg/month).[6] Therefore, they suggested that before commencing any clinical studies lasting ≥4 weeks, PEGylated products should be assessed in non-clinical settings for ependymal cell vacuolation, the presence of active transport mechanisms for PEG across the blood-cerebrospinal fluid (CSF) barrier and whole-body biodistribution (if the PEG dose is not <0.4 µmol/kg/month).[6] With the recently approved PEGylated rFVIII damoctocog alfa pegol, the maximum PEG-60 exposure resulting from maximum doses used in clinical trials (60 IU/kg, twice weekly) is 32 µg/kg/month. The potential for vacuolation at 0.4 µmol/kg of PEG equals 24 000 µg/kg/month, providing a 750-fold safety margin between the damoctocog alfa pegol clinical dose and the threshold for vacuolation as defined by CHMP. According to CHMP, if ependymal vacuolation was observed in non-clinical studies, reversibility must be demonstrated.[6] To date, EMA has only approved PEGylated FVIII/FIX products for children ≥12 years old, likely because of the uncertainties regarding the long-term safety of PEG administration in children.

Non-clinical safety studies should be performed before clinical use. The toxicity of PEGylated drugs usually reflects the toxicity of the parent (unconjugated) drug molecule.[4] Data from non-clinical toxicology studies with marketed PEGylated biologics have shown that vacuolation is mainly a cellular response to high concentrations of foreign materials including large PEG molecules. Since PEG is inert, no direct effect on cellular function is expected with any PEGylated molecules, unless vacuolation is accompanied by pathologic effects such as tissue degeneration, inflammation, necrosis or

TABLE 1 Approved PEGylated FVIII and FIX products [2,3,10]

Product	Generic name	Recombinant protein	PEG size	PEG conjugation	EU/US approval[a]	Approved age group for EU/US[a]	Approved for prophylaxis in EU/US[a]
BAY 94-9027 (Jivi®)	damoctocog alfa pegol	BDD-rFVIII	60 kDa branched	Maleimide linker to cysteine amino acid in A3 domain	Yes/Yes	≥12 y/≥12 y	Yes/Yes
N8-GP (Esperoct®)	turoctocog alfa pegol	B-domain truncated rFVIII	40 kDa (glycoPEGylation)	O-linked glycan in truncated B-domain	Yes/Yes	≥12 y/all ages	Yes/Yes
BAX 855 (Adynovate® / Adynovi®)	rurioctocog alfa pegol	rFVIII	20 kDa branched	Amino acids localised in B-domain	Yes/Yes	≥12 y/all ages	Yes/Yes
N9-GP (Refixia® / REBINYN®)	nonacog beta pegol	rFIX	40 kDa branched (glycoPEGylation)	O-linked glycan at Asn157 or Asn167	Yes/Yes	≥12 y/all ages	Yes/No

Abbreviations: Asn, asparagine; BDD, B-domain deleted; rFIX, recombinant factor IX; rFVIII, recombinant factor VIII.
[a]Information obtained from https://www.ema.europa.eu/en and https://www.accessdata.fda.gov/scripts/cder/daf/. Accessed October 2019.

cellular distortion.[4] In the absence of changes in cell morphology, or changes in surrounding tissue, cellular vacuolation observed with high PEG doses has not been linked to changes in organ function and is therefore not considered adverse. Nor have there been any reports of PEG-related adverse events with PEGylated drugs in humans. Although no changes in physiology or function have been reported, it remains unknown whether vacuoles caused by more prolonged or lifelong exposure to higher molecular weight PEG may have functional consequences.[4] One major concern is the possible long-term effect of PEG with chronic administration of PEGylated biologics. These risks are assessed by chronic toxicology studies. Usually, an immune response is observed when running toxicology studies with a human protein in experimental animals, thereby limiting the possibility to address concerns associated with the long-term use of such products. In order to overcome this situation, immunodeficient athymic rats have been used to evaluate PEGylated biologics in addition to existing toxicology programmes.[7,8] In such studies, possible long-term effects of the PEGylated product can be investigated without interference by the immune system, and thus, a more relevant risk assessment for chronic effects can be performed.

In a recent study with damoctocog alfa pegol, no vacuolation was detected in immunodeficient rats after chronic administration up to 26 weeks.[8] This was likely because of the low doses used (still up to 30 × higher than the human dose) reflecting the low dose-ranges needed for therapeutic efficacy of FVIII products. In recent rat studies, it was proposed that PEG-40 blood concentrations >100 μg/mL may trigger tissue vacuolation.[9] This is 1000-fold higher than concentrations observed in clinical studies with damoctocog alfa pegol (60 kDa PEG blood concentration: maximum 0.1 μg/mL).[2]

Understanding PK, metabolism and biodistribution of PEGylated proteins is important for drug safety. PK properties of PEGylated proteins are initially driven by the two major parts of the molecule: the protein itself and its conjugated PEG. When PEG remains after protein catabolism, its biodistribution and PK properties are governed by PEG-related mechanisms. The primary excretion mechanism for PEG molecules up to 60 kDa is urinary. The rate of cellular uptake and excretion is determined by PEG size, PEGylated protein characteristics, existing non-specific uptake or receptor-mediated cellular uptake, PEG dose and dosing frequency, and turnover kinetics of the cells involved in PEG uptake.[5]

Due to the low clearance of PEG, its concentration in blood and tissue levels rises until a steady state is reached. Since excretion processes have been demonstrated, including for large PEGs up to 60 kDa, once a steady state is reached, there are no further increases in blood and/or tissue concentrations.[2] Time to reach steady state increases with PEG size (which determines clearance and thus elimination half-life). However, the total PEG dose administered and whether the PEG level at steady state is associated with any possible adverse effects are of greater clinical relevance. Steady state is driven by PEG dose, which again depends on the individual PEG load of the molecule (usually very low for PEGylated FVIII products). For example, the total amount of PEG administered for damoctocog alfa

pegol (~2.8 μg/kg/week) is ~80-fold lower than for nonacog beta pegol (230 μg/kg/week) and ~250-fold lower than for certolizumab pegol (Cimzia®; 725 μg/kg/week of PEG 40 kDa).

Another important PK parameter to evaluate long-term safety is distribution behaviour. In rats, the 60 kDa PEG moiety of damoctocog alfa pegol distributed slowly from blood to tissues, with no irreversible binding to any tissues and no penetration of the blood-brain barrier.[2]

Finally, predictions from non-clinical studies must be validated by human data. Based on PK data from rat distribution studies, the human plasma steady-state concentrations of PEG (40 or 60 kDa) were simulated for patients receiving nonacog beta pegol or damoctocog alfa pegol.[2,10] Plasma steady-state concentrations in patients receiving therapeutic doses of both compounds were similar to predictions based on non-clinical PK studies, suggesting that organ and tissue concentration models can accurately predict results in humans. Additionally, there was a clear relationship between PEG dose and plasma steady-state levels. Combined with the demonstrated excretion mechanism of large PEGs up to 60 kDa, a very low PEG intake is not expected to have long-term safety consequences, confirmed by clinical data on the use of damoctocog alfa pegol for >5 years.[2] Moreover, no long-term PEG-related safety concerns have been reported in patients after chronic treatment with other PEGylated proteins, including nonacog beta pegol and certolizumab pegol, even though the PEG-40 doses and the expected plasma, organ and tissue exposures were considerably higher than for PEG-60 from damoctocog alfa pegol. In conclusion, the long-term safety risks of PEGylated biologics must be individually investigated using the described strategy.

ACKNOWLEDGEMENTS

Andreas Baumann wrote the paper. Medical editing support was provided by Anila Syed of Sudler Medical Communications.

DISCLOSURE

Andreas Baumann is an employee of Bayer.

Andreas Baumann

Bayer AG, Berlin, Germany

Correspondence
Andreas Baumann, Bayer AG, 13342 Berlin, Germany.
Email: andreas.baumann@bayer.com

ORCID

Andreas Baumann https://orcid.org/0000-0003-3034-4288

REFERENCES

1. Turecek PL, Bossard MJ, Schoetens F, Ivens IA. PEGylation of biopharmaceuticals: a review of chemistry and nonclinical safety information of approved drugs. *J Pharm Sci*. 2016;105(2):460-475.
2. Baumann A, Piel I, Hucke F, Sandmann S, Hetzel T, Schwarz T. Pharmacokinetics, excretion, distribution, and metabolism of 60-kDa polyethylene glycol used in BAY 94–9027 in rats and its value for human prediction. *Eur J Pharm Sci*. 2019;130:11-20.
3. Mancuso ME, Santagostino E. Outcome of clinical trials with new extended half-life FVIII/IX concentrates. *J Clin Med*. 2017;6(4):39.
4. Ivens IA, Achanzar W, Baumann A, et al. PEGylated biopharmaceuticals: current experience and considerations for nonclinical development. *Toxicol Pathol*. 2015;43(7):959-983.
5. BaumannA,TuerckD,PrabhuS,DickmannL,SimsJ.Pharmacokinetics, metabolism and distribution of PEGs and PEGylated proteins: quo vadis? *Drug Discov Today*. 2014;19(10):1623-1631.
6. European Medicines Agency. CHMP Safety Working Party's response to the PDCO regarding the use of PEGylated drug products in the paediatric population. 2012; http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2012/11/WC500135123.pdf. Accessed August 14, 2017.
7. Rasmussen CE, Nowak J, Larsen JM, et al. Long-term safety of PEGylated coagulation factor VIII in the immune-deficient Rowett nude rat. *J Toxicol*. 2017;2017:8496246.
8. Ivens IA, Banczyk D, Gutberlet K, Jackman S, Vauleon S, Frisk AL. Nonclinical safety assessment of a long-acting recombinant PEGylated factor eight (BAY 94–9027) with a 60 kDa PEG. *Toxicol Pathol*. 2019;47(5):585-597.
9. Jacobsen H, Bjørnsdottir I. Learnings from recent regulatory submission with 40 kDa PEGylated coagulation factor IX (N9-GP) PK and safety. 2017. Paper presented at: Hertfordshire, UK: BioSafe European Annual General Membership Meeting; November 14–15, 2017.
10. Bjørnsdottir I, Sternebring O, Kappers WA, et al. Pharmacokinetics, tissue distribution and excretion of 40kDa PEG and PEGylated rFVIII (N8-GP) in rats. *Eur J Pharm Sci*. 2016;87:58-68.