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.3 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, toxicity 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). 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...
| Product * | Generic name | Recombinant protein | PEG size | PEG conjugation | EU-US approval | Approved age group for EU-US | Approved for prophylaxis in EU-US |
|-----------|--------------|---------------------|----------|----------------|----------------|-----------------------------|----------------------------------|
| 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 (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.

*Information obtained from [https://www.ema.europa.eu/en](https://www.ema.europa.eu/en) and [https://www.accessdata.fda.gov/scripts/cder/daf/](https://www.accessdata.fda.gov/scripts/cder/daf/). Accessed October 2019.
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\(^{\text{a}}\): 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.\(^{2,\text{a}}\) 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.

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Andreas Baumann is an employee of Bayer.

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