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## CHMP Safety Working Party's response to the PDCO regarding the use of PEGylated drug products in the paediatric population

### 1. Introduction

The polyethylene glycols (PEGs) are a family of polymeric chemicals all possessing the same basic structural unit but varying widely in the number of repeating units and in whether these are in a straight chain or branched conformation. PEGs have been in use for many years in the industrial, cosmetic, food and pharmaceutical areas but within recent years they have been more and more often applied as a chemical modifier of biopharmaceuticals. PEGylation of a protein significantly increases its half-life predominantly by decreasing its renal clearance, which opens up for a reduction in the administration frequency.

Repeated parenteral administration of PEGylated proteins to animals has in some cases been associated with cellular vacuolation in macrophages and/or histiocytes in various organs and in renal tubular cells (European Public Assessment Report (EPAR) Cimzia<sup>®</sup>; EPAR SomaVert<sup>®</sup>; Bendele *et al.* 2002). While the vacuolation of macrophages and histiocytes is considered a normal physiological response to remove foreign bodies, it remains to be elucidated why some PEGylated proteins cause vacuolation within the renal tubule cells. Recently, cases of cellular vacuolation of the choroid plexus epithelial cells (ependymal cells) have been observed in repeat-dose toxicity studies conducted with proteins PEGylated with molecules  $\geq 40$  kDa. Immunohistochemical data confirms that the cytoplasmic vacuoles contain PEG.

The ependymal cells of the choroid plexus are the main source of cerebrospinal fluid (CSF) secretion and form the blood-CSF barrier. As such, the choroid plexus is responsible for establishing and maintaining the extracellular milieu throughout the brain and the spinal cord. In addition, the choroid plexus appears to be a source of CSF-borne hormones and growth factors (Redzic and Segal, 2004). Literature data shows that repeated administration of PEGylated proteins may result in extensive cellular vacuolation with distortion of the vacuoles and compression of the nuclei (Bendele *et al.*, 1998). These cellular changes may potentially affect the function of the cell and hence, in the case of the choroid plexus, decrease the production of cerebrospinal fluid. While renal function can be monitored, there is no clinical marker for choroid plexus function. Moreover, many of the PEGylated drug products presently undergoing development are intended for young children and it is presently unclear whether this patient group is more sensitive to drugs affecting the choroid plexus than adults.



In many cases, the PEGylated drug product includes a PEG molecule of more than 40 kDa and presently there is no experience with chronic parenteral treatment of children with this type of molecule. Hence, until more data are available, the risk for ependymal cell vacuolation needs to be addressed before conducting longer term clinical trials in the paediatric population.

## 2. Cases of ependymal cell vacuolation

### 2.1. Discussion

At present, five PEGylated drug products are approved for the European market, namely PegIntron<sup>®</sup>, PegaSys<sup>®</sup>, Neulasta<sup>®</sup>, Somavert<sup>®</sup> and Cimzia<sup>®</sup>. Thus far, only PegIntron<sup>®</sup> has been approved for the treatment of children (down to the age of 3 years). However, several PEGylated drug products are under development for the paediatric population.

Ependymal cell PEG vacuolation has thus far been observed in 3 cases following repeated intravenous or subcutaneous administration of PEGylated proteins to animals, namely with a PEGylated human growth hormone (43 kDa PEG), the PEGylated anti-TNF $\alpha$  Fab fragment certolizumab pegol (Cimzia<sup>®</sup>, 40 kDa PEG) and with 40 kDa PEG alone. In all cases, ependymal vacuolation severity as well as incidence increased with increasing doses and with duration of dosing. Moreover, recovery was very slow following prolonged treatment. For example, following 52-weeks treatment with Cimzia<sup>®</sup>, the ependymal cell vacuolation persisted following a 26-week recovery period.

Based on a review of repeat-dose toxicity studies conducted for PEGylated drug products already approved or under paediatric development, PEG vacuolation within choroid plexus ependymal cells has only been observed if the following conditions were fulfilled: 1) the study was conducted in Cynomolgus monkeys, 2) a PEG moiety size of at least 40 kDa was applied, 3) the study was at least of 6 weeks duration and 4) the administered dose gave rise to at least a monthly PEG exposure of 0.4  $\mu$ mol/kg/month (PEGylated human growth hormone).

It should however also be considered that active transport into the ependymal cell may be a determining factor with respect to which drugs cause ependymal vacuolation. Indeed, plasma-borne hormones, which have receptors on the hypothalamus, undergo facilitated, saturable transport across the ependymal cells (Smith *et al.*, 2004). Active transport across the ependymal cells and into the CSF has thus far been reported for several hormones, e.g., insulin, leptin, prolactin, secretin, thyroid hormone and growth hormone. While it is established that growth hormone may undergo receptor-mediated transport across the choroid plexus ependymal cells, it is at present unclear by which mechanism the PEGylated anti-TNF $\alpha$  Fab fragment (certolizumab pegol) and free 40 kDa PEG accumulate in ependymal cells. However, consistent with the absence of specific transport for 40kD PEG and certolizumab, ependymal vacuolation after treatment with these molecules was only observed at PEG levels 10-fold higher than those of PEGylated human GH.

### 2.2. Conclusion

It is recommended that before conducting clinical trials of more than 4 weeks duration with a PEGylated drug product, the applicant should address:

- whether ependymal cell vacuolation has been observed in the non-clinical studies.
- whether the PEGylated drug product may undergo active transport across the blood-CSF barrier e.g. via a literature review elucidating to what extent there is a physiological role for active transport of the pegylated protein into the CSF.

- the biodistribution of the PEGylated drug product via applying a method enabling detection of the PEG moiety unless the monthly PEG exposure is significantly lower than the cases where ependymal cell vacuolation has been observed ( $\geq 0.4 \mu\text{mol/kg/month}$ ).

In case ependymal vacuolation is observed in the non-clinical studies for a drug product intended for long-term treatment, paediatric development should only be initiated in case a sufficiently large safety margin can be established in a long-term toxicity study applying animals representative for the paediatric population in question and the finding can be demonstrated to be reversible. If feasible, *ex vivo/in vitro data* evaluating the effect of PEG vacuolation on the viability and function of choroid plexus ependymal cells could contribute to the risk assessment process.

### 3. References

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