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Reflection paper on non-clinical studies for generic nanoparticle iron medicinal product applications

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The present document reflects the current thinking of the CHMP. The principles spelled out in this reflection paper will be reviewed in light of the experience gained with regulatory submissions and contribution from stakeholder.

Keywords	*Iron nanoparticles, non-clinical comparability studies, tissue distribution*

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1. Introduction

For generic products containing a small molecule it is generally accepted that once the molecule is in the blood - free in solution or bound to plasma proteins – the further fate of the molecule in the generic product will not be different from the molecule in the reference product. The physicochemical features (including the size of the molecules) will be identical and regarding their tissue uptake, metabolism and finally excretion no differences are expected. This postulate is the basis of regulatory approval of generic products, which is based on a comparison of physicochemical features and plasma concentrations of the active ingredient in the generic and in the reference medicinal product. Accordingly, for products with small molecules in solution for intravenous use specific bioequivalence studies are not required. For nanoparticle medicinal products this approach is generally not valid.

2. Discussion

Specific characteristics of nanoparticles

Any variation in mean/median size and size distribution and/or the accuracy of methods employed for nano-sizing may result in the generic product displaying different physicochemical properties leading to a different biopharmaceutical profile in respect of pharmacokinetics and biodistribution. This has the potential to significantly impact on safety/efficacy in comparison to the reference product. In addition, in blood as well as in tissues, the active ingredient may be present in the form of nanoparticles of different size and/or stability and/or nanoparticle aggregates. This can impact on safety, antigenicity, infusion reactions etc. Also differences in the rate of degradation/solubilisation can be expected according to nanoparticle size (aggregation state) and surface functionality leading to the potential for differences in the concentration of free 'soluble' molecules and nanoparticle associated molecules present in any compartment with time.

The situation is even more complex for coated nanoparticles which are developed for example to minimise aggregation. In this case either coat or core or both may be different for the generic and reference medicinal product. This may profoundly affect the pharmacokinetics and biodistribution of the product as well as the kinetics of the active ingredient release and, consequently, pharmacological and toxicological effects. A pharmacokinetic comparison of different products based on the measurement of plasma concentrations may only reflect the clearance from plasma but may well fail to detect in which extent the nanoparticles are taken up by different target organs. Additional measurement of target tissue concentration will in general be needed.

Thus, for iron-based nanoparticle medicinal products, physico-chemical characteristics comparison as well as pharmacokinetic measurements in humans based on plasma concentration may not be sufficient to ensure a comparable safety and efficacy between the reference product and the generic. Comparative measurement of target tissues concentrations would usually require non-clinical studies.

Rationale for non-clinical comparability studies for nanoparticle iron medicinal products

Nanoparticle iron (NPI) medicinal products for parenteral use with the active ingredient iron are coated nanoparticles consisting of an iron core and a complex carbohydrate coat with an average particle diameter in the iron-sucrose formulation of approximately 22 nm (1). Differences in tissue distribution and toxicological profiles have been described for nanoparticle iron preparations with different carbohydrate coat (2) and differences in toxicity have been described for nanoparticle iron preparations with the same carbohydrate coat but differences in the manufacturing process (3).

When considering a generic NPI there are several important factors related to the exact nature of the particle characteristics, as described above, that can influence the kinetic parameters and consequently the toxicity. The following factors should be considered in a comparative way:

- Fraction of free iron in the product and short term stability of the nano-particles in plasma, as free iron has well known direct toxic effects.
- Uptake of the nanoparticles in the reticuloendothelial system (RES).
- Degradation of the nanoparticles in the cells of the RES and release of iron from the RES.
- Uptake of iron in pharmacological and toxicological target tissues after degradation of the nanoparticles.
- Direct uptake of the nanoparticles or partially degraded nano-particles in target tissues circumventing the RES.

Based on the steps outlined above at least three compartments, plasma, RES and target tissues need to be considered (Table 1).

Table 1. Relevant compartments for the distribution of parenteral NPI

1. Plasma
2. RES: macrophages e.g. in spleen, lymph nodes, liver (Kupffer cells)
3. Target tissues 3.1 Pharmacological target tissues e.g. bone marrow 3.2 Toxicological target tissues e.g. kidney, liver (hepatocytes), lungs, heart

For each of the above three compartments it will be necessary to show the distribution of the generic and reference medicinal product. For the RES, spleen and/or lymph nodes are the recommended organs for the measurement of iron concentrations.

Selection of target organs and tissues for the measurement of iron content should include at least the organs identified from the distribution pattern of the reference product and the generic (see Table 1).

Time points for sampling of target tissues should be selected to cover the main time-concentration profiles. Other methods to measure distribution such as imaging technologies may be acceptable if shown to be appropriate.

Standard analytical measurements of iron in biological samples may not discriminate different forms or oxidation status of iron or partially degraded nanoparticles. As the coated nanoparticles will be gradually degraded, total iron measurements will not reflect this. As a detailed experimental elucidation of the form of iron in the tissue samples may exceed what can reasonably be expected in an application for a generic product, overall iron content of tissue samples may be sufficient. Any additional more sophisticated analyses, however, of the degradation process of the nanoparticles will be welcome and are encouraged. The use of cell or tissue culture system for mechanistic purposes is encouraged, e.g. to study the uptake and degradation of the nanoparticles in macrophages. In addition histochemical studies on iron distribution within an organ and investigations on the oxidation status of the iron may also be helpful.

The studies should be designed to be sensitive enough to detect potential differences between the reference and generic NPI medicinal product. The demonstration of the sensitivity of the assays to detect potential differences between the reference and generic products is paramount and should be appropriately demonstrated.

3. Conclusion

For the comparison of generic and reference NPI medicinal products data on time dependent plasma levels alone are of limited value as they may conceivably fail to detect relevant differences in the tissue distribution of iron. The appropriate measurement of organ or tissue distribution of iron in humans may be not feasible. Comparative data from non-clinical studies on the time-dependent iron content in the major target organs may be used to support the claim of essential similarity of generic and reference NPI medicinal products.

4. References

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