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Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product

Final

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¹ The QWP has been involved in the discussions and agreed to the draft.



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1. Introduction

Development of drug delivery systems to improve disease-specific targeting, to control drug release rates and/or to produce a pharmaceutical formulation suitable for clinical use is desirable. One of the strategies has been encapsulation of the active substance(s) in the aqueous phase of a liposome, or incorporation or binding to the lipid components. Liposomes are classically described as artificially prepared vesicles composed of one or more concentric lipidic bi-layers enclosing one or more aqueous compartments. They include, but are not limited to, mono- and multi-lamellar liposomes, multi-vesicular liposomes, polymer-coated liposomes.

In any given product, a proportion of the active substance could also be extra-liposomal, free in bulk solution.

Early parenteral liposomal products were found to have a number of critical pharmacokinetic properties including rapid recognition and removal by the monocyte phagocyte system (MPS) and premature drug-release (instability). It was also recognized that the physicochemical properties of the liposomes, such as particle size, membrane fluidity, surface-charge and composition were relevant determinants of such in vivo behaviour. Some formulations were found to benefit from the addition of sterols (e.g. cholesterol), size reduction and surface modification with covalently linked polymers (e.g. polyethylene glycol [PEG]).

Contrary to products where the active substance is in the form of a simple solution, liposomal medicinal products have formulation and manufacturing-specific distribution characteristics after intravenous administration and similar plasma concentrations may not correlate with therapeutic performance. Even for cases of ostensibly identical composition, variation in production and product and process control technology can lead to products with different therapeutic performance. The complete characterisation of the stability, pharmacokinetics (including tissue distribution) of a new liposomal product is critical to establish safe and effective use. This is because differences between the applicant's product and innovator product with regard to manufacturing process steps and formulation may substantially modify efficacy/safety due to changes in specific liposome-cell interactions and liposome distribution characteristics which are not detectable by conventional bioequivalence testing alone. The aims for developing the innovator and the evidence supporting its use should be taken into account when designing the non-clinical and clinical programme for the liposomal products developed with reference to that particular innovator.

The reference liposomal product used for comparability investigations should be or have been authorised in the EEA and should be used as a comparator in all proposed quality characterization pivotal and non-clinical and clinical comparability studies.

This document discusses the principles for assessing liposomal products developed with reference to an innovator liposomal product but does not aim to prescribe any particular analytical, nonclinical or clinical strategy.

ICH and EU Guidelines

This reflection paper should be read in connection with the following documents:

- Directive 2001/83/EC, as amended
- Part II of the Annex I of Directive 2001/83/EC, as amended
- CHMP/437/04 Guideline on similar biological medicinal products

- Annex II to Note for Guidance on Process Validation CHMP/QWP/848/99 and EMEA/CVMP/598/99 Non Standard Processes (CPMP/QWP/2054/03)
- Guideline on similar medicinal products containing biotechnology-derived proteins as active substances: quality issues
- ICH topic Q5E – Comparability of biotechnological/biological products
- ICH topic S6 - Note for guidance on Pre-clinical Safety Evaluation of Biotechnology-Derived Pharmaceuticals (CPMP/ICH/302/95)
- ICH topic E9 statistical principles for clinical trials - Note for guidance on statistical principles for clinical trials (CPMP/ICH/363/96)
- ICH topic E10 - Note for guidance on choice of control group in clinical trials (Guideline on the choice of the non-inferiority margin (CPMP/EWP/2158/99)
- Points to consider on switching between superiority and non-inferiority (CPMP/EWP/482/99)
- Guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98, rev 1 corr *)

2. Scope

This reflection paper is intended to assist in the generation of relevant quality, non-clinical and clinical data to support a marketing authorisation of intravenous liposomal products developed with reference to an innovator liposomal product. Hence, this document should facilitate a decision on the following issues:

- pharmaceutical data needed as evidence of product comparability between test and reference or after changes to a liposomal product, to support comparative safety and efficacy
- Necessity of pre-clinical and clinical studies and circumstances which may allow to waive certain studies
- Consideration of the design of relevant in vivo non-clinical studies and the potential role for in vitro models.

The principles outlined in this reflection paper might also be considered to be applicable to other novel types of “liposome-like” and vesicular products which may be under development including those to be administered by routes other than intravenous administration. Systems that may use phospholipids but where drug release is unaffected; i.e., where the lipidic system serves only as a solvent for the active agent do not fall in this category and are outside of the scope of this document.

3. Discussion

3.1. Pharmaceutical Quality

The critical quality attributes of liposomal formulations may have a major impact on the in vivo pharmacokinetic (PK) and pharmacodynamic (PD) properties, since some or all of the following may occur to varying degrees:

- the active substance release rates from liposomes can affect PK and PD and therefore the safety and efficacy profile of the medicinal product
- an entrapped active substance may not be biologically available and may be protected from degradation, in addition to metabolism whilst it is entrapped in the liposome

- the PK of the encapsulated substance may be controlled by the PK of the carrier (i.e. the liposomal formulation) which is influenced/determined by the physicochemical properties of the liposomes, by the physico-chemical state of the encapsulated drug substance and by interactions between the components of the liposome and the biological environment.
- the formulation may affect uptake and tissue distribution

Pharmaceutical comparability between the applicant's product and the innovator product should be established before progressing to non-clinical and clinical investigations. Due to the complexity of liposomal formulations, establishing pharmaceutical comparability to the reference product alone cannot replace the need for non-clinical and/or clinical data but may justify reduction in the amount of such studies. The extent and complexity of clinical and non-clinical studies should be driven by the results of the comparability work at each stage.

3.1.1. Quality characterisation

Correctly defining relevant physicochemical properties of a liposomal product is critical to ensure its quality. The following general parameters should be addressed in the submission of all types of liposomal products:

- critical discussion of the lipidic components (description, source and characterisation, manufacture, assay, impurity profile, isomers and stability characteristics);
- quality, purity and stability characteristics of other critical excipients;
- identification and control of key intermediates in the manufacturing process;
- active substance/lipidic moiety ratio at relevant manufacturing steps to be within acceptable range to ensure consistent formulation performance;
- liposome morphology, mean size and size distribution, aggregates;
- fraction of encapsulated active substance (amount of free/entrapped);
- stability of the active substance, lipids and functional excipients in the finished product, including quantification of critical degradation products (e.g. lyso phosphatidylcholine, oxidated/hydrolytic moieties);
- *in vitro* drug substance release rate from the liposome in physiologically/clinically relevant media. Reliable and discriminating validated *in-vitro* release methods should be developed to:
 - monitor the simulated release of the active substance from the liposomes in physiologically/clinically relevant media. If justified an *in vitro* leakage test in relevant media under multiple conditions (e.g. range of temperatures and pH values) could be appropriate.
- monitor stability on storage, and be sensitive to ensure batch to batch consistency.
- stability studies under proposed in-use conditions;
- robustness of process for reconstitution and/or pharmacy preparation.

The quality and purity of the lipid starting materials is essential for the later quality of the drug product, therefore the appropriate characterization and specification of the lipid starting material is considered as vital. Functionality-related characteristics as described in the Ph. Eur. monograph 5.15 'Functionality-related characteristics of excipients' should be adequately addressed. The level of information to be provided with the relevant submission depends on complexity of the excipients. The principles of the Guideline on Excipients in the Dossier for Application for Marketing Authorisation of a

Medicinal Product (EMA/CHMP/QWP/396951/2006) should be considered. Use of multiple sources (e.g. animal, plant, synthetic sources) or suppliers for the lipid components would require additional characterisation and comparability studies.

Depending on the specific function of the liposomal formulation (e.g. modification of the distribution of active substance by encapsulation for improved safety profile or modification of liposomal pharmacokinetics by pegylation), the additional parameters below should be also considered in the submission:

- maintenance of liposomal formulation integrity in plasma;
- characterisation of lipid bilayer phase transition behaviour (e.g. temperature and enthalpy of transitions);
- determination of liposomal 'surface' charge;
- pH of internal compartment for pH-gradient loaded liposomes;
- if relevant characterisation of physical state of the active substance inside the liposome (e.g. precipitation in the case of doxorubicin);
- distribution of drug substance within liposome (e.g. surface, bilayer, interior, etc.);
- for conjugated (e.g. pegylated) liposomal formulations:
 - quality and purity of the PEG starting material is essential for the later quality of the medicinal product;
 - details of linkage chemistry (such as PEG-lipid or similar constructs with or without PEG);
 - molecular weight of conjugated lipid and size distribution;
 - disposition of PEG at surface;
 - stability of conjugation.

A list of tests to be applied routinely to the liposomal product should be defined and should be based on the parameters used to characterise the formulation as described above.

3.1.2. Establishing pharmaceutical comparability

The qualitative and quantitative composition of the developed product should be identical or closely match the reference product.

It is acknowledged that normally the applicant of a liposomal formulation developed with reference to an innovator product will not have access to information about the manufacturing process of this reference product. Therefore, extensive investigations using state of the art characterisation methods should be applied to both products in parallel in order to demonstrate with a high level of assurance that the characteristics are comparable. Such studies should include all the relevant tests mentioned in the *Quality Characterisation* section suitable to adequately characterise the test and reference liposomal products and particularly relate to their performance in vivo. The relevance of the selected tests for equivalent performance of the drug product in vivo should be discussed. Any differences between the products identified in the comparability investigations should be addressed and thoroughly evaluated and justified with regard to implications on safety/efficacy.

In addition to the characterisation studies conducted under normal conditions, comparative stress test studies of both products, should be conducted in order to compare physical and chemical degradation.

All batches of the reference product used in the characterization studies should be analyzed within their shelf-life period and should be stored under the recommended storage conditions prior to analysis.

3.1.3. Pharmaceutical development of the applicant's product

A well-defined manufacturing process with satisfactory process controls is required in order to assure that an acceptable product is produced on a consistent basis. At the same time it is known that small changes to liposomal products can significantly influence their performance. Approaches to determining the impact of any process change will vary with respect to the specific manufacturing process, the product, the extent of the manufacturer's knowledge and experience with the process and development data provided. Comparative investigations (see *Quality Characterisation section*) should be undertaken when a change is introduced into the manufacturing process during development but also after marketing authorisation (e.g. for scale up).

In vivo studies may be necessary to demonstrate that any changes do not affect the safety and efficacy profile of the product when results from physicochemical testing indicate a change in the properties of the product.

It is recommended that the applicant should consider the basic principles as outlined in section 1.4 of ICH Q5E (Note for Guidance on Biotechnological/Biological Products Subject to Changes in their Manufacturing Process).

3.2. Non-Clinical and Clinical Requirements

3.2.1. General Aspects

The documentation required to support regulatory approval of a liposomal formulation developed with reference to an innovator product should be detailed enough to warrant the conclusion of equivalent efficacy and safety compared to the innovator product. In general, the non-clinical studies to be performed prior to clinical studies should include comparative investigation of pharmacokinetics (including tissue distribution), toxicology and pharmacodynamics. However, the complexity of the particular liposomal formulation will determine whether comparative non-clinical studies could be reduced and if appropriate, it may be decided on a case-by-case basis which studies could be waived.

In the comprehensive evaluation of the new liposomal product the body of evidence obtained in quality, non-clinical and clinical studies must be considered as a whole. If e.g. any relevant differences are found in non-clinical studies for the liposomal formulation developed with reference to the innovator then critical re-assessment of physico-chemical characteristics of the product is advised in order to clarify possible explanations for such differences before proceeding with clinical investigations. Differences between the innovator and the test product in the data generated to support product similarity would negate the similarity approach and could be a source of serious regulatory concern.

Significant changes in pharmacokinetic characteristics are evident when an active substance is administered in a liposomal formulation, i.e. volume of distribution and clearance may be reduced and half-life prolonged. The clearance of the liposomal active substance is dependent on:

1. the clearance of the liposomal carrier itself,
2. the rate of release of entrapped drug from the liposomal carrier, and
3. the clearance and metabolism of unencapsulated drug upon its release.

The rate and location of in vivo drug release is a crucial parameter which can affect toxicity and efficacy.

Therefore, the pharmacokinetics of the developed liposomal product should always be compared with the innovator's product. Only certain aspects of the conventional bioequivalence approach are applicable and in some cases additional requirements should be set on a case-by-case basis.

Comparative human pharmacokinetic investigations should demonstrate not only the similarity of exposure of the total, unencapsulated and liposome encapsulated drug (see below for analytes to be measured in non-clinical and clinical studies) but they should also demonstrate similar distribution and elimination characteristics.

3.2.2. Methods of Analysis

For comparison to a reference liposomal product, analytical methods developed and validated to quantify encapsulated and unencapsulated drug in blood/plasma and unencapsulated drug in tissue will be necessary, in addition to traditional methods for total drug and metabolite in blood/plasma and tissues. Individual quantification of unencapsulated and encapsulated drug involves separation methodologies that require special attention to verify their reliability. For every blood/plasma sample, total drug levels should be quantified without separation of encapsulated and unencapsulated drug as an independent verification of the reliability of the separation methodology. While it might be feasible to quantify unencapsulated, encapsulated and total drug in blood/plasma, it is acknowledged that tissue processing is likely to disrupt liposomes. For unencapsulated drug in tissues, care should be taken to separate the unencapsulated drug prior to tissue processing steps that are likely to result in destruction of liposomes. Careful attention should be paid to the impact of all sample processing procedures during the course of method development, employing methodologies to verify the suitability and interpretability of all bioanalytical results.

The methods of analysis used to quantify the levels of drug (total, unencapsulated and encapsulated) and metabolite in the plasma and tissues and their validation should be described. The lower limits of quantitation and recovery in plasma, tissues and, where relevant, in particular tissues of interest e.g. in tumours, should be stated.

3.2.3. Non-Clinical Studies

Non-clinical pharmacodynamic studies

The non-clinical pharmacodynamic studies should include

- where possible the development of in-vitro tests capable of characterising any interaction between liposomes and target cells or other cells where the interaction is toxicologically relevant is encouraged. While it is possible to characterise the pharmacodynamic profile from such studies alone, it is recognised that the current state of knowledge on in vitro tests is limited and it is highly likely that in vivo studies will be needed at present,
- demonstration of the similarity in pharmacodynamic response using appropriate in-vivo models and at various dose levels chosen taking into account the sensitivity of the model.

Non-clinical pharmacokinetic studies

Some pharmacokinetic aspects of liposomal products with regard to their performance in humans can be predicted by animal and, where applicable, cell-based models. However, the choice of appropriate species and models to investigate the in-vivo release of the drug from liposomes should be justified

with special emphasis on areas such as accumulation and retention in target organs, pharmacokinetics and distribution. In addition to the systemic exposure, similarities in the distribution and elimination should be demonstrated. These studies provide pivotal evidence of the comparability of disposition of liposomal drug products, as it is not possible to have a full picture of the distribution in man from blood/plasma data alone. As such, the studies should be conducted in accordance with the principles of Good Laboratory Practice (GLP) in species relevant with respect to the pharmacology and safety of the product. The test product should be produced using the final manufacturing process and would ideally be from the same batch used for the pivotal clinical studies. Sampling time points and sampling duration should be carefully selected so as to accurately quantify the time course of unencapsulated and total drug and metabolite in tissues balancing the need to quantify early drug release from liposomes (e.g. over first 15 min) and persistence of drug in particular tissues. If due to analytical reasons free concentrations cannot be measured then attempts should be made to compare the metabolite concentrations in the target organs. As these studies involve destructive sampling, the number of animals to be included will depend on the number of sampling time points, between animal variability in distribution of drug to tissues and variability as a result of experimental procedures (tissue excision, weight, homogenisation and sampling as well as bioanalytical sources of variability). Careful selection of sampling times will increase the precision of derived parameters. Pilot studies to establish the appropriate dose levels, necessary sampling strategy and the number of animals to be included are advised to avoid failed or uninterpretable pivotal studies. Tissues for analysis should include those associated with the safety and efficacy of the drug as well as those involved in significant processing/elimination of liposomes.

There is insufficient regulatory experience of such studies to support specific decision criteria for comparability of tissue distribution. Replicate study designs where at least the reference product is replicated are advised, as otherwise any differences between test and reference product are uninterpretable. The use of an appropriately selected internal standard should be considered to decrease the variability of the results. A variety of data displays should be utilised including, but not limited to, PK parameter differences and ratios between treatments and visual comparisons of amount vs time profiles for each tissue and each analyte. All estimates and data displays should include quantification of uncertainty, e.g. confidence intervals. The clinical implications of any noted differences in tissue distribution between test and reference product should be discussed.

Dose to be investigated

Single and multiple dose studies at different dose levels may be needed to support the claim of similar pharmacokinetics. Doses should be chosen based on blood levels observed at therapeutic doses in man. To establish the correct dose, the use of allometric equations or PBPK modelling is recommended.

Analytes to be measured

The kinetics (including tissue distribution and excretion) of both the unencapsulated drug and the encapsulated drug should be investigated if feasible.

Toxicological studies

In general, toxicity studies may not be needed. However depending on the outcome of pharmaceutical comparability investigations, and nature of any toxicity produced by the product, appropriate organ function tests may be required to support equivalence in the context of known target organ toxicity e.g. in the case of suspected toxicity to the heart, a test of function such as an assessment of cardiac

function by measurement of left ventricular end-diastolic pressure in a rodent model may be appropriate.

Use of in vitro and in vivo immune reactogenicity assays such as complement (and/or macrophage/basophil activation assays) and testing for complement activation-related pseudoallergy (CARPA) in sensitive animal models should be considered to evaluate the extent of potential adverse event.

3.2.4. Clinical Studies

Comparative pharmacokinetic studies

Dose to be investigated

Pharmacokinetic behaviour is often dose-dependent and hence, the pharmacokinetics of the new formulation and the reference should be compared over the recommended dose range unless linearity has been demonstrated. Demonstration of such linearity with encapsulated, unencapsulated, as well as total drug substance, would be required unless appropriate literature data are provided.

In the case of non-linearity, demonstration of bioequivalence at the highest and lowest doses would suffice even if different doses are used for different indications. In such cases further clinical studies are not needed. In some cases, bioequivalence studies cannot be carried out with certain doses due to ethical or other reasons. In these cases, assessment of therapeutic equivalence in each indication requires individual consideration.

Design considerations

It is probable that the active substance might not be tolerated by healthy volunteers. In such a case, a pharmacokinetic study may be performed in patients. If a single-dose study is not feasible in patients then multiple dose pharmacokinetic studies in patients may be acceptable.

Analytes to be measured

The validated bioanalytical method should reliably quantify total, encapsulated and unencapsulated drug substance. Since metabolism of the active substance takes place only after release from the liposomes, quantification of at least one metabolite regardless of its pharmacological activity may facilitate the assessment and comparison of active substance release rate from the liposomal formulation. If there are several metabolites then the choice of metabolite should be justified on kinetic grounds. If one or more metabolites have significant clinical activity then it might be necessary to compare their kinetics as well.

Pharmacokinetic parameters to be measured and reported

The evaluated pharmacokinetic characteristics of total, encapsulated and unencapsulated drug substance should be compared to allow assessment of the rate at which the active substance is released from the liposomes, since this will determine the onset and duration of the therapeutic effect. However, conventional pharmacokinetic metrics such as AUC and C_{max} might not give sufficient indication of the rate of release at the target sites. Therefore, evaluation of additional pharmacokinetic parameters should be provided to describe other pharmacokinetic processes such as distribution and elimination in addition to rate and extent of release. When relevant, the rate and extent of excretion of the active substance in urine should be compared.

Early sampling time points, during and immediately after infusion of the product, should be included to ensure comparability with regard to early clearance by the reticulo-endothelial system.

When the elimination rates of the unencapsulated and encapsulated active substance is different, that is for liposomes which release the active substance over a longer period of time, then additional pharmacokinetic parameters are needed such as clearance, volume of distribution, terminal half-life and partial AUCs (e.g. 0-24h, 24-48h etc). These parameters should be evaluated descriptively. This may enable further characterisation of the integrity of liposomes and their uptake by peripheral tissues/reticuloendothelial system. Additionally, further descriptive parameters could be considered e.g. inter-compartmental clearance and volume of the peripheral and central compartments.

It is recommended that the ratio of unencapsulated to encapsulated drug concentration over time should be determined.

Acceptance criteria

Similarity should be demonstrated for the total, encapsulated and unencapsulated drug. Generally, the 90% confidence intervals of C_{max}, AUC_{inf} and AUC_t ratios should be within 80 - 125%. In special cases, additional metrics might include partial AUCs, or acceptance criteria for the PK parameters of the metabolite.

Assessment of efficacy

In general, the necessity for a clinical efficacy trial(s) besides the obligatory clinical pharmacokinetic studies is decided on a case-by-case basis depending on the ability of the non-clinical models and clinical PK data to detect differences between innovator and the liposomal product developed with reference to it, and the complexity of the formulation.

It is highly likely that additional therapeutic equivalence studies will be required if the formulations differ in terms of qualitative composition. As an example clinical studies including therapeutic equivalence studies might be required in cases when polymers are attached to lipids by means of different linking methods. However, due to the relative insensitivity of clinical efficacy trials to detect formulation dependent differences, this is not the preferred approach. Therefore, when developing a liposomal product with reference to an innovator product all attempts should be made to demonstrate equivalence of pharmaceutical quality of formulations and similarity in non-clinical pharmacokinetic and pharmacodynamic and clinical pharmacokinetic studies. Differences between the innovator and the test product in the data generated to support product similarity would negate the similarity approach and could be a source of serious regulatory concern.

Safety issues

Acute infusion reactions are relatively common with liposomal formulations. However, the frequency of such side effects is expected to be comparable unless the investigative products differ with respect to qualitative composition (e.g. different excipients) or production methods. However, it is recommended in this reflection paper that the qualitative and quantitative composition of the developed product should be identical or closely match the reference product. Still, to minimize the possibility of increased frequency of acute infusion reactions, use of in vitro and in vivo immune reactogenicity assays are required which are discussed in the toxicological studies section. If there is any sign that a new liposomal product might be associated with increased risk in this regard then the product development should be re-evaluated until reasons are clarified. Furthermore infusion reactions should be carefully evaluated in bioequivalence studies, and again, should any differences be noted, the product development should be re-evaluated. It is not anticipated that full-scale clinical trials are necessary at

the time of authorisation, however the clinical safety of similar liposomal products should be closely monitored in accordance with current EU legislation and pharmacovigilance guidelines.

4. Conclusion

The experience with liposomal formulations developed with reference to an innovator is limited. As a result, only general recommendations can be given in this reflection paper and companies are advised to seek product-specific scientific advice regarding specific questions on the data requirements to demonstrate comparability of liposomal formulations.

Glossary

Entrapped or encapsulated active substance

The active substance is inside the liposome and is separated from the biological matrix by one or more lipid membrane.

Unentrapped, unencapsulated or free active substance

The active substance is outside of the liposome compartment. In the context of this reflection paper free concentration is the same as unentrapped (unencapsulated) concentration regardless of whether the active substance is associated to plasma or other tissue proteins.

Total concentration

Total = Entrapped + unentrapped