ANNEX No. 1

to Decision No. of the
Eurasian Economic Commission's Board
dated 20 .

# GUIDELINES
## on quality assessment and study of medicinal products developed based on intravenous liposomes

### I. General provisions

1. One of the strategies for developing delivery systems for medicinal products based on intravenous liposomes (hereinafter referred to as liposomal medicinal products) in order to improve targeting specific to each disease, control the rate of release of the active substance and (or) obtain a pharmaceutical formulation suitable for clinical use, is to include (encapsulate) the active substance(s) in the aqueous phase of a liposome or to incorporate (entrap) it to the lipid component. Liposomes are classically defined as artificially prepared vesicles composed of one or more concentric lipidic bi-layers containing one or more aqueous chambers (compartments). They include mono- and multi-lamellar liposomes, multivesicular liposomes, and polymer-coated liposomes.

2. In any medicinal product, a proportion of the active substance can be outside the liposomes, in free form in stock solution.

3. Since liposomal medicinal products have a number of critical pharmacokinetic properties, including their rapid recognition and elimination by the body’s monocytic-phagocytic system and premature release of the active substance (instability) from the liposome, the physico-chemical

properties of the liposomes, such as particle size, membrane fluidity, surface charge and composition are the cause of such critical pharmacokinetic properties *in vivo.* The properties of certain liposomal medicinal products improved after the addition of sterols (e.g. cholesterol), size reduction and surface modification with covalently entrapped polymers (e.g. polyethylene glycol).

4. Contrary to medicinal products where the active substance is in the form of a simple solution, liposomal medicinal products have distribution characteristics after intravenous administration that depend on the formulation and manufacturing process' peculiarities. Therefore, similar plasma concentrations of their active substances may not correlate with therapeutic activity. Even for cases of clearly identical composition of liposomes, changes in their manufacture, control of liposome manufacturing technology and quality control of the finished medicinal product may lead to different therapeutic activity of liposomal medicinal products. The most complete characterization of the stability and pharmacokinetics (including tissue distribution) should be carried out to guarantee the safety and efficacy of a new liposomal medicinal product. This is because differences between the generic product and reference liposomal medicinal product with regard to manufacturing process stages and formulation of liposomal medicinal product may significantly affect safety and efficacy due to changes in the nature of liposome-cell interaction and liposome distribution according to their physical characteristics, which are not detectable by conventional bioequivalence studies. When designing the non-clinical and clinical study programme for the liposomal medicinal products developed as analogues of a specific liposomal reference medicinal product, the aims for developing the latter and data justifying its use should be taken into account.

5. The reference liposomal medicinal product used for comparability studies should be authorized as a liposomal medicinal product in the territories of the Eurasian Economic Union Member States (hereinafter referred to as the Member States); it should be used as a control in all proposed pharmaceutical tests of quality characterization, as well as in pivotal non-clinical and clinical comparability studies.

6. These Guidelines outline the principles for assessing liposomal medicinal products developed as analogues of reference liposomal medicinal products. The requirements for selecting any specific analytical,non-clinical, or clinical strategy are not covered by these Guidelines.

Since there is currently limited data on the pharmaceutical development of generic liposomal medicinal products, these Guidelines provide general recommendations for confirming and assessing the comparability of such generic liposomal medicinal products with reference liposomal medicinal products. To confirm the comparability of certain types of liposomal medicinal products, a specialized scientific advice should be sought in accordance with paragraph 26 of the Rules for Marketing Authorization and Expert Assessment of Medicinal Products for Human Use approved by Decision No. 78 of the Eurasian Economic Commission's Council dated November 3, 2016.

7. The application of these Guidelines will assist the developer (manufacturer) of the liposomal medicinal product to obtain the necessary data on the quality, non-clinical and clinical studies required to support marketing authorization of liposomal medicinal products developed as analogues of the reference liposomal medicinal product. These Guidelines include instructions on the following issues:

the presentation of pharmaceutical data as part of the registration dossier for a medicinal product necessary to confirm the comparability

between generic liposomal medicinal product and reference liposomal medicinal product or to confirm the comparability between modified liposomal medicinal product and the original liposomal medicinal product with a view to justify comparable safety and efficacy of such products;

the need to conduct non-clinical and clinical studies and circumstances which may allow waiving certain studies;

consideration of the design of relevant *in vivo* non-clinical studies and the potential role of *in vitro* models.

8. The principles set forth in these Guidelines may also apply to other new types of “liposomal-like” and vesicular medicinal products that may be under development, including routes of administration other than the intravenous ones. Medicinal products based on phospholipids where the active substance is not released from the phospholipid system, i.e. where such system serves solely as a solvent for the active substance, do not fall into the category of liposomal or liposomal-like medicinal products and are not covered by these Guidelines.

## II. Definitions

9. For the purposes of these Guidelines, concepts and terms shall be used with their following meanings:

«unentrapped, unencapsulated or free active substance» – means an active substance located outside the liposome. Within these Guidelines, free concentration is equivalent to the concentration of unentrapped (unencapsulated) active substance, regardless of whether the active substance is entrapped in plasma or other tissue proteins;

«total concentration of active substance» – means an aggregate concentration of liposome-entrapped and unentrapped (unencapsulated) active substance;

«entrapped or encapsulated active substance» – means an active substance located inside the liposome and separated from the biological matrix by one or more lipid membrane;

«membrane fluidity» – means the ability of most of the lipids and proteins that make up a membrane to diffuse within its lipid bilayer.

## III. General requirements for the pharmaceutical quality of liposomal medicinal products

10. The critical quality indicators of liposomal medicinal products may significantly affect the *in vivo* pharmacokinetic and pharmacodynamic properties, since

a) the rates of active substance release from liposomes may affect pharmacokinetics and pharmacodynamics, as well as safety and efficacy profile of the liposomal medicinal product;

b) an entrapped active substance may not be bioavailable and may be protected from degradation, as well as from metabolism when it is within the liposome;

c) the pharmacokinetics of the encapsulated substance may be controlled by the pharmacokinetics of the carrier (i.e., liposomes), which in turn is affected:

by the physico-chemical properties of that carrier (liposomes);

by the physico-chemical state of the encapsulated active substance; and

by interactions between the components of the liposome and the biological environment, or which is determined by it;

d) the formulation may affect absorption and distribution of the active substance in the tissues.

11. Pharmaceutical comparability between the product subject to authorization and the reference product should be confirmed before starting

non-clinical and clinical development of any liposomal medicinal product. Due to the formulation complexity of liposomal medicinal products, isolated confirmation of pharmaceutical comparability with the reference product cannot replace non-clinical and (or) clinical data, but may justify the reduced scope of such studies. The scope and complexity of non-clinical and clinical studies should be determined by the results of comparability studies at each stage.

## 1. Quality characterization

12. Correct determination of significant physico-chemical properties of a liposomal medicinal product is critical to ensure its quality. The following general parameters should be studied when preparing a registration dossier for all types of liposomal medicinal products:

critical analysis of the lipidic components (description, source and characterization, 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;

conformity of active substance/lipidic components ratio at significant manufacturing stages to the acceptable range to ensure consistent performance of the product;

liposome morphology, mean size and size distribution, aggregates;

fraction of encapsulated active substance (free/encapsulated ratio);

stability of the active substance, lipids and functional excipients in the finished product, including assay of critical degradation products (e.g. lysophosphatidylcholine, oxidated (hydrolytic) moieties);

rates of *in vitro* active substance release from the liposome in physiologically (clinically relevant) media.

13. Reliable and discriminating validated methods for assessing *in vitro* release should be developed to:

monitor the simulated release of the active substance from liposomes in physiologically (clinically relevant) media. If justified, an *in vitro* leakage test in relevant media under different conditions (e.g. range of temperatures and pH values) may be appropriate;

monitor stability when storing; it should be sensitive enough to ensure the batch-to-batch consistency;

stability studies under intended conditions of use;

robustness of process for reconstitution and (or) pharmacy preparation.

14. The quality and purity of the lipid starting materials is an essential factor for the medicinal product's quality; in this regard, the appropriate quality characterization and specification of the lipid starting material is considered especially important. The characteristics that determine the functionality should be properly analyzed in accordance with the article «Excipient Performance» of the Pharmacopoeia of the Union or the Pharmacopeia of the Member States. The scope of information to be provided within the dossier depends on the excipients' complexity. Use of multiple sources (e.g. animal, plant, synthetic sources) or suppliers for the lipid components would require additional characterization and comparability studies.

15. Depending on the specific function of the liposomes (e.g. modifying the active substance's distribution by encapsulation to improve safety profile or modifying liposomal pharmacokinetics by pegylation), the additional parameters below should also be specified in the registration dossier:

maintaining the integrity of the finished liposomal formulation in plasma;

characterization of the phase transition process of the lipid bilayer (for example, temperature and transition enthalpy);

determination of liposomal «surface» charge;

pH value of the internal compartment of liposomes filled with a gradient of pH values;

characterization of physical state of the active substance inside the liposome (e.g. precipitate formation in the case of doxorubicin) (if significant);

distribution of active substance inside the liposome (e.g. on the surface, in a bilayer, in the internal environment);

for conjugated (e.g. pegylated) liposomal products:

quality and purity of the pegylated starting material which are fundamental factors for the medicinal product's quality;

chemical evidence of conjugation (such as PEG-lipid or similar constructs with or without polyethylene glycol);

molecular weight of conjugated lipid and size distribution (dispersion);

location of polyethylene glycol on the surface;

stability of the conjugate.

16. A list of tests to be applied routinely to the liposomal product should be determined; it should be based on the parameters used to characterize the product in accordance with paragraphs 11, 12 and 14 of these Guidelines.

## 2. Establishing pharmaceutical comparability

17. The qualitative and quantitative composition of the developed liposomal medicinal product should be identical to or nearly coincide with the reference product.

18. As a rule, the applicant of a liposomal medicinal product developed by analogy to the reference product does not have access to information about the manufacturing process of the reference product. Extensive test program using state of the art characterization methods should be conducted for both liposomal medicinal products in parallel to obtain evidence that the characteristics of the developed and reference liposomal medicinal products are comparable. This program should include all the significant tests described in Subsection 1, Section III of these Guidelines suitable for the correct characterization of the generic and reference liposomal products, particularly with respect to the characterization of their *in vivo* functional properties.

19. The significance of the selected tests should be analyzed to confirm the equivalence of the liposomal medicinal product's *in vivo* performance. All differences between the products found in the comparability study should be considered, thoroughly assessed and justified from the perspective of their impact on the safety (efficacy).

20. In addition to the characterization studies conducted under normal conditions, comparative stress tests should be conducted for both products in order to compare physical and chemical degradation.

21. All batches of the reference product used in the characterization studies should be analyzed within their shelf life; they should be stored under recommended conditions prior to analysis.

### 3. Pharmaceutical development of the liposomal medicinal product

22. A well-described manufacturing process with satisfactory process controls is required to ensure that a liposomal product is consistently manufactured with acceptable quality. At the same time, it is known that small changes to liposomal medicinal products may significantly affect their performance. Approaches to determining the impact of any manufacturing process change depend on the specific manufacturing process, the product, the scope of knowledge and experience previously obtained by the manufacturer in relation to such process, and on the available data on the development of a liposomal medicinal product. Comparative studies should be conducted when the manufacturing process is changed during development stage and after marketing authorization (e.g. when scaling up).

23. If the results of physico-chemical tests indicate a change in the properties of the liposomal medicinal product, *in vivo* studies may be required aimed at confirming that no changes have affected the safety and efficacy profile.

24. The applicant is advised to consider the basic principles set forth in Section 1.4, Chapter 9.1 of the Rules for Conducting Studies of Biological Medicinal Products of the Eurasian Economic Union approved by Decision No. 89 of the Eurasian Economic Commission's Council dated November 3, 2016 (hereinafter referred to as the Rules for Conducting Studies of Biological Medicinal Products).

## IV. Non-clinical and clinical studies

### 1. General provisions

25. The documentation required to authorize a liposomal medicinal product developed as an analogue of the reference liposomal medicinal

product should be detailed enough to guarantee the justified conclusion on equivalent efficacy and safety compared to the reference product. As a rule, the non-clinical studies to be conducted prior to clinical studies imply comparative examination of pharmacokinetics (including tissue distribution), toxicology and pharmacodynamics. However, the complexity of any specific liposomal medicinal product will determine the possibility of reducing the scope of comparative non-clinical studies and, if appropriate, it may be decided on a case-by-case basis which studies could be excluded.

26. In the comprehensive assessment of a new liposomal medicinal product, data obtained from pharmaceutical, non-clinical, and clinical studies should be analyzed as a whole. For example, if the results of non-clinical studies reveal any significant differences of the liposomal medicinal product developed as an analogue of the reference product, then a critical re-assessment of physico-chemical characteristics of the liposomal medicinal product is recommended in order to find out the possible causes of such differences before starting clinical studies. Differences in data obtained when examining the similarity between the reference and generic liposomal medicinal products can lead to a conclusion that there is no comparability of liposomal medicinal products and shall constitute the ground for the Member States' authorized authorities to draw up the corresponding conclusion when considering an application for marketing authorization of such medicinal products.

27. When the active substance is administered in a liposomal formulation, significant changes in the pharmacokinetic characteristics are detected: i.e., the volume of distribution and clearance may be reduced, while the half-life may be prolonged. The clearance of the liposomal active substance is dependent on:

the clearance of the liposomal carrier itself;

the rate of release of entrapped active substance from the liposomal carrier;

the clearance and metabolism of unencapsulated active substance after its release.

The rate and location of *in vivo* active substance release is a crucial parameter which can affect its toxicity and efficacy.

Therefore, the pharmacokinetics of the developed liposomal medicinal product should always be compared with that of the reference product. Only certain aspects of the conventional approach to bioequivalence study are applicable, and in some cases, additional requirements determined on a case-by-case basis should be introduced.

28. Comparative pharmacokinetic studies should confirm not only the similarity of total exposure of the active substance unencapsulated and encapsulated in the liposome (see below for analytes to be determined in non-clinical and clinical studies), but they should also confirm similar distribution and clearance parameters.

## 2. Methods of analysis

29. In addition to conventional methods for determining the total content of the active substance and its metabolites in the blood (plasma) and tissues, in order to compare with the reference liposomal medicinal product, analytical methods to assay encapsulated and unencapsulated active substance in the blood (plasma) and unencapsulated active substance in tissues should be developed and validated.

30. Separate assay of unencapsulated and encapsulated active substances involves the use of separation methodologies, which require special attention to verify their reliability. For every blood/plasma sample, total content of the active substance should be quantified without separation

between encapsulated and unencapsulated products in order to independently verify the reliability of the separation methodology. Despite the feasibility of assaying unencapsulated, encapsulated and total active substances in the blood (plasma), it is acknowledged that tissue processing is likely to lead to the destruction of liposomes.

31. When determining the content of an unencapsulated active substance in tissues, care should be taken to separate the unencapsulated active substance prior to tissue processing procedures that are likely to result in the destruction of liposomes. In developing the method, special attention should be paid to the influence of all sample preparation procedures, employing methodologies to verify the suitability and interpretability of all bioanalytical results.

32. The methods of analysis used to assay the levels of active substance (total, unencapsulated and encapsulated) and metabolites in the plasma and tissues and their validation should be described. The lower limits of assay and recovery of the active substance from plasma, tissues, and, if applicable, in particular tissues of interest, e.g. in tumors, should be specified.

## 3. Non-clinical studies of liposomal medicinal products

### Non-clinical pharmacodynamic studies of liposomal medicinal products

33. The non-clinical pharmacodynamic studies of liposomal medicinal products should include:

where possible, the development of *in-vitro* tests capable of characterizing any interaction between liposomes and target cells or other cells where the interaction is toxicologically relevant; While it is possible to characterize the pharmacodynamic profile of a liposomal medicinal product only using *in vitro* tests, the scope of data obtained in such tests is limited, and it is highly likely that *in vivo* studies will be required;

confirmation of pharmacodynamic response's comparability using appropriate *in vivo* models and at different doses chosen, taking into account the sensitivity of the model.

## Non-clinical pharmacokinetic studies of liposomal medicinal products

34. Some pharmacokinetic parameters of liposomal medicinal products with regard to their performance in humans may be predicted by animal and, where applicable, cell-based models. However, the choice of relevant species and models to study *in-vivo* release of the active substance from liposomes should be justified with special attention paid to such areas as accumulation and retention in target organs, pharmacokinetics and distribution.

35. In addition to the systemic exposure, similarities in the distribution and elimination should be confirmed. These studies provide pivotal evidence of the comparability of liposomal medicinal products' pharmacokinetics, since it is not possible to have a full picture of the distribution in humans relying on blood (plasma) data alone. For this reason, the studies should be conducted in accordance with the Good Laboratory Practice Rules of the Eurasian Economic Union in the field of medicinal product circulation approved by Decision No. 81 of the Eurasian Economic Commission's Council dated November 3, 2016 in species relevant with respect to the product's pharmacology and safety. The generic liposomal medicinal product should be manufactured using a full-scale (industrial) manufacturing process, and in non-clinical studies, it would be optimal to use the same batch of liposomal medicinal product which will be subsequently examined in pivotal clinical studies.

36. Time points and sampling duration should be carefully selected so as to accurately assay the dynamics of changes in the concentration of

unencapsulated and total content of the active substance and metabolite in the tissues, balancing the need to assay early active substance release from liposomes (e.g. during the first 15 minutes) with the need to examine the persistence of the active substance in certain tissues. If, for analytical considerations, free concentrations cannot be measured, then attempts should be made to compare the metabolite concentrations in the target organs. Since these studies involve the destruction of samples during sampling, the number of animals to be included in the study will depend on the number of sampling time points, the variability of tissue distribution of the active substance between species, and the variability due to the experiment (tissue excision, weighing, homogenization and pre-sampling as well as bioanalytical sources of variability). Careful selection of sampling times will increase the precision of the results obtained. Pilot studies to establish the appropriate dose levels, necessary sampling strategy and the number of animals to be included are advised to avoid failed pivotal studies or the lack of interpretability of their results. Tissues to be analyzed should include tissues that determine safety and efficacy of the product as well as tissues involved in significant processing (elimination) of liposomes. Due to the limited experience in conducting such studies, it is impossible to provide specific criteria for comparability of the active substance's distribution in the tissues.

37. Repeated (replicate) study designs where at least the reference product is replicated are advised, otherwise any differences between generic and reference product will not be interpreted. The use of an appropriately selected internal standard should be considered to decrease the results' variability. The data obtained should be presented in various ways, including providing differences in pharmacokinetic parameters and ratios between pharmacokinetic parameters of products and visual comparisons of the concentration-time profiles for each type of tissue and for each analyte. All

estimates and data displays should include quantification of uncertainty, e.g. confidence intervals. The clinical consequences of all detected differences in tissue distribution of the active substance between generic and reference product should be analyzed.

### Dose of liposomal medicinal products to be examined in non-clinical studies

38. Single- and multiple-dose studies at different dose levels may be required to justify the comparable pharmacokinetics. Doses should be chosen based on concentration of the active substance of a liposomal medicinal product in human blood when administering therapeutic doses of such liposomal medicinal product. To establish the correct dose, allometric equations or physiologically based pharmacokinetic modelling should be used.

### Analytes to be determined in body fluids after administration of liposomal medicinal products

39. The kinetics (including tissue distribution and excretion) of both the unencapsulated active substance and the encapsulated active substance should be studied if feasible.

### Toxicological studies of liposomal medicinal products

40. In general, toxicological studies may not be needed. However, depending on the outcome of pharmaceutical comparability study and nature of toxicity produced by the product, appropriate organ function tests may be required to justify the equivalence in the context of known target organ toxicity, e.g. in the case of suspected toxicity to the heart, a functional test

such as an assessment of cardiac function by measurement of left ventricular end-diastolic pressure in a rat model may be appropriate.

41. To assess the potential for the development of adverse events, it is necessary to envisage the use of *in vitro* and *in vivo* tests for immune reactogenicity, such as determining the activation of complement and (or) macrophages (basophils) and testing for the pseudo-allergic reaction caused by complement activation in sensitive animal models.

## 4. Clinical studies of liposomal medicinal products

### Comparative pharmacokinetic studies of liposomal medicinal products

#### Dose of liposomal medicinal products to be examined in clinical studies

42. Pharmacokinetic parameters are often dose-dependent and therefore, a new liposomal medicinal product and the reference liposomal medicinal product should be compared within the recommended dose range unless linearity has been confirmed. The claimed linearity for the encapsulated, unencapsulated and total active substance should be confirmed unless scientific literature provides appropriate data.

43. In the case of non-linearity, demonstration of bioequivalence at the highest and lowest doses will be sufficient even if different doses are used for varied indications. In such cases, further clinical studies are not performed. In some cases, bioequivalence studies cannot be conducted with certain doses for ethical or other considerations. In these cases, assessment of therapeutic equivalence in each indication requires individual approach.

#### Design issues on studies of liposomal medicinal products

44. Healthy volunteers may not tolerate the active substance. 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 determined in body fluids after administration of liposomal medicinal products in clinical studies

45. The validated bioanalytical method should enable a reliable assay of the total, encapsulated and unencapsulated active substance. Since metabolism of the active substance takes place only after its release from liposomes, assay of at least one metabolite regardless of its pharmacological activity may facilitate the assessment and comparison of the rate of active substance release from the liposomal product. If there are several metabolites, then one should be guided by kinetic considerations when choosing one of them. If one or more metabolites have significant clinical activity, then it might be necessary to compare their kinetics as well.

## Pharmacokinetic parameters to be determined and documented in clinical studies

46. The studied pharmacokinetic characteristics of total, encapsulated and unencapsulated active substance should provide a comparison of the rate of active substance release from liposomes, since this will determine the onset and duration of the therapeutic effect. In addition, conventional pharmacokinetic metrics such as AUC and $C_{max}$ might not give sufficient characterization of release rates in the target tissues. Therefore, examination data for additional pharmacokinetic parameters should be provided to describe other pharmacokinetic processes such as distribution and elimination in addition to the rate and extent of release. If this is significant, the rate and extent of active substance's excretion in urine should be compared.

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

48. When the elimination rates of the unencapsulated and encapsulated active substance are different, which is typical for liposomes releasing 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 to 24 h, 24 to 48 h, etc.). These parameters should be assessed descriptively. This may require further characterization of the integrity of liposomes and their uptake by peripheral tissues/reticulo-endothelial system. Besides, further descriptive parameters could be considered, e.g. inter-compartmental clearance and volume of the peripheral and central compartments. It is recommended to determine the dynamics of the concentration ratio of unencapsulated and encapsulated active substance.

## Acceptance criteria for equivalence indicators of liposomal medicinal products

49. Similarity of the concentration of the total, encapsulated and unencapsulated active substance should be confirmed. As a rule, the confidence intervals for $C_{max}$, $AUC_{(0-\infty)}$ and $AUC_{(0-t)}$ ratios should be within 80 –125%. In special cases, additional parameters include partial AUCs, or acceptance criteria for the pharmacokinetic parameters of the metabolite.

## Assessing liposomal medicinal products' efficacy

50. The need to conduct clinical efficacy study (-ies), in addition to mandatory clinical pharmacokinetic studies, is usually determined on a case-by-case basis depending on the ability of the non-clinical models and clinical

pharmacokinetic data to detect differences between the reference liposomal medicinal product and the product developed by its analogy, and the complexity of the formulation.

51. It is highly likely that additional therapeutic equivalence studies will be required if the formulations differ in terms of qualitative composition. An example of the need to conduct clinical studies including therapeutic equivalence studies are cases of combining polymers with lipids using various linking methods.

52. However, due to the relative insensitivity of clinical studies to detect formulation-dependent differences, this approach is not preferred. In this regard, when developing a liposomal medicinal product as an analogue of a reference medicinal product, all attempts should be made to confirm the equivalence of pharmaceutical quality of products and similarity in non-clinical pharmacokinetic and pharmacodynamic and clinical pharmacokinetic studies. If differences between the reference and the generic liposomal medicinal products are found in data resulting from the studies conducted to justify product similarity, then such liposomal medicinal products are deemed as not similar, and data presented shall constitute the ground for the Member States' authorized authorities (expert organizations) to provide their comments on the studies completed.

## Safety issues of liposomal medicinal products

53. Acute infusion reactions to liposomal medicinal products are common adverse reactions. However, the frequency of such adverse reactions is deemed as comparable unless the generic liposomal medicinal products differ with respect to qualitative composition (e.g. different excipients) or manufacturing process. To prevent a mismatch in the safety profile, it is necessary that the qualitative and quantitative composition of the product

being developed is identical to or closely match the reference product. At the same time, to minimize the possibility of increased frequency of acute infusion reactions, *in vitro* and *in vivo* immune reactogenicity studies outlined in the section on toxicological studies would be required. If there is any sign that a new liposomal medicinal product might cause an increased risk of developing these reactions, the product development should be analyzed until reasons are clarified. Moreover, infusion reactions should be carefully assessed in bioequivalence studies and, again, when any differences are identified, information and data on the product development should be analyzed.

54. As a rule, full-scale clinical studies are not required prior to the authorization of a generic liposomal medicinal product. The clinical safety of generic liposomal medicinal products should be assessed in the process of their circulation in accordance with the acts included in the law of the Eurasian Economic Union, including the Good Pharmacovigilance Practice Rules of the Eurasian Economic Union approved by Decision No. 87 of the Eurasian Economic Commission's Council dated November 3, 2016.

ANNEX No. 2

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# GUIDELINES
# on quality assessment and study of block copolymer micelle medicinal products

## I. General characteristics of block copolymer micelle medicinal products

1. The creation of block copolymer micelles is used when developing technologies for medicinal products manufacturing in order to improve the delivery of poorly soluble, highly-toxic and (or) unstable active substances, to increase tissue targeting and/or to improve the efficacy of cytosolic delivery of macromolecular drugs. Block copolymer micelles are self-assembled micelles typically prepared from AB block copolymers, while other more complex block copolymer compositions are also possible. An active substance can be incorporated into the inner core of the block copolymer micelle product by chemical conjugation or by physical entrapment. Block copolymers with amphiphilic properties spontaneously assemble into polymeric micelles in aqueous media; this self-association is typically driven by hydrophobic interactions. To promote micelle formation and enhance micelle stability, it is allowed to use other physico-chemical processes of molecule self-association (for example, electrostatic interactions

between charged block copolymers and oppositely charged active substances, polymer-metal complex formation and hydrogen bonding between molecules). In specific cases, functional features may also be added to the system, for example, by targeted molecule conjugation to the block copolymer, or by the addition of another homopolymer or an additional stabilizer to stabilize the micelle, to modify the release rate and (or) to increase the loading of the active substance.

2. In block copolymer micelle medicinal products, part of the active substance may be free (i.e., not incorporated in the micelle) in the bulk solution.

3. Block copolymer micelle medicinal products are composed of block copolymer micelles that have a traceable structure: their inner core typically serves as a container for active substance and is surrounded by an outer shell of hydrophilic polymers. The required chemical properties can be conferred to such block copolymer micelles to ensure:

high stability of micelles after dilution and subsequent administration due to a low critical association concentration;

optimal pharmacokinetics (targeted delivery of the active substance);

controlled active substance release, etc.

A slow dissociation kinetics of such block copolymer micelles can be provided by modifying chemical properties.

4. The properties of block copolymer micelles differ from traditional surfactant micelles used to entrap, solubilize or aid the transport of active substances in the body. A block copolymer micelle medicinal product can contain additional components within its core including chemically bound active substance, which in certain cases may be covalently bound.

It has been shown in non-clinical studies that block copolymer micelles are able to preferentially accumulate in solid tumors due to their

microvascular hyperpermeability and impaired lymphatic drainage (the so-called enhanced permeability and retention effect).

5. The particular physico-chemical properties of block copolymer micelles, such as: size, surface charge, composition and stability can be important safety and efficacy parameters for all intended indications for using the micelle medicinal product under development.

6. As block copolymer micelle medicinal products are of nanoscale size, contain several components, and are purposely designed for specific clinical applications, they are considered as nanomedicines.

7. These Guidelines provide general principles for the development and assessment of block copolymer micelle medicinal products. These Guidelines do not establish any product-specific strategies for assessing the quality, non-clinical or clinical characteristics of block copolymer micelle medicinal products.

8. These Guidelines contain basic information for the pharmaceutical development and non-clinical and early clinical studies of block copolymer micelle medicinal products created to modify pharmacokinetics, stability and distribution of incorporated or conjugated active substances *in vivo*. The provisions of these Guidelines for block copolymer micelle medicinal products for intravenous administration may be applied to block copolymer micelle medicinal products for other routes of administration.

9. The active substance may be a low molecular weight chemical entity, a nucleic acid, or a biological or biotechnological entity, including peptides and proteins.

10. Due to the complex structure of block copolymer micelle medicinal products, regardless of the type of chemical bond of the active substance inside them and (or) the use of additional stabilizers, for proper assessment of critical indicators and specific requirements for each block copolymer micelle

medicinal product, a specialized scientific advice should be sought in accordance with paragraph 26 of the Rules for Marketing Authorization and Expert Assessment of Medicinal Products for Human Use, approved by Decision No. 78 of the Eurasian Economic Commission's Council dated November 3, 2016. As part of scientific advice, developers (manufacturers) of block copolymer micelle medicinal products should obtain approval for new methods defining the quality and non-clinical properties of block copolymer micelle medicinal products that are significant for its intended clinical use.

## II. Definitions

11. For the purposes of these Guidelines, concepts shall be used with their following meanings:

«potency (expressed in units)» – means, for a block copolymer micelle medicinal product containing protein molecules as the active substance, the quantitative measure of biological activity based on the medicinal product's attribute linked to its relevant biological properties;

«biological activity» – means the specific ability or capacity of a product to achieve a defined biological effect.

«block copolymer» – means more than two types of polymers connected in series, e.g. an AB block copolymer or an ABA block copolymer (or others). The block copolymer is also called a monomer, the minimum compound from which the block copolymer micelle is prepared. The active substance may be chemically bound to the monomer;

«block copolymer micelle» – means a micelle consisting of block copolymers. Active substances can be incorporated into the inner core of the block copolymer micelle by chemical conjugation (including covalent conjugation) or by physical entrapment;

«block copolymer micelle medicinal product» – means a medicinal product that contains the active substance, block copolymers and, in certain cases, other ingredients.

«active substance» – means a compound which shows the main therapeutic effect;

«quantity (expressed by weight)» – means a physico-chemical measure of protein content in a block copolymer micelle medicinal product containing protein molecules as an active substance;

«free active substance» – means an active substance contained in the medicinal product that is not incorporated within the block copolymer micelle by chemical conjugation or by physical entrapment. Free active substance may be released from the block copolymer micelle medicinal product after its administration. In these Guidelines, the term «free» does not imply active substances that are not associated with plasma and serum proteins.

## III. Examining chemical properties, manufacturing process and quality control of block copolymer medicinal products

### 1. Establishing quality characteristics of block copolymer micelle medicinal products

12. It is necessary to identify the critical quality indicators of block copolymer micelle medicinal products that will have a major impact on the *in vivo* pharmacokinetic and pharmacodynamic properties able to affect the safety and efficacy of the block copolymer micelle medicinal product.

13. It is essential to correctly identify parameters that define significant physico-chemical properties of the block copolymer micelle medicinal product to ensure its quality.

## 2. Description and composition of block copolymer micelle medicinal products

14. The typical components of block copolymer micelle medicinal products are the active substance, the block copolymer and, in certain cases, other components such as stabilizing agents.

15. The critical quality indicators of block copolymer micelle medicinal products should be carefully defined on a product-specific basis. Such critical indicators may be:

content of the block copolymer and active substance in the block copolymer micelle medicinal product. These should be expressed as both the molar ratio and the mass fraction of each;

composition, mean molecular weight and polydispersity of the polymers (homopolymers, copolymers, etc.) used to synthesize the block copolymers (or «block copolymer-active substance» conjugates);

composition, mean molecular weight and polydispersity of the block copolymers used to create the block copolymer micelle.

All the selected value ranges of critical indicators should be justified in detail.

## 3. Quality characterization of block copolymer micelle medicinal products

### Quality indicators of the components included in block copolymers

16. The chemical composition of block copolymers significantly affects the physico-chemical processes that underlie the polymer self-association, and therefore, the size and physico-chemical characteristics, as well as *in vitro* and *in vivo* stability of micelles formed. The key indicators include:

chemical structure of the block copolymers;

chemical nature and stability of chemical linkage in the case of «block copolymer-active substance» conjugate;

impurity profile (e.g. macromolecular impurities).

## Quality indicators of block copolymer micelle medicinal products

17. Quality indicators critical for characterizing a finished block copolymer micelle medicinal product include:

a) quality indicators directly related to the block copolymer micelle:

block copolymer micelle size (mean micelle size and micelle size distribution profile);

morphology and zeta potential;

other surface properties (e.g. targeting ligand);

association (aggregation) number;

concentration dependency of the nano-structure (in some cases, it is expressed as critical micelle concentration or critical association concentration; in this case, these parameters of certain block copolymers are too low to be measured using the current analytical methods);

drug loading;

surface properties;

chemical structure;

physical condition of the pharmaceutical substance;

*in vitro* stability of the block copolymer micelle in plasma and (or) relevant media;

*in vitro* release of the active substance from the block copolymer micelle medicinal product in plasma and (or) relevant media;

*in vitro* degradation of the block copolymer in plasma and (or) relevant media;

b) quality indicators related to the micelle manufacturing process:

validated process of reconstitution;

validated process for ensuring sterility;

c) quality indicators related to the *in vivo* behavior of micelles:

osmolarity;

fraction of active substance that is surface-associated;

rate and location of active substance release;

rate and location of block copolymer degradation.

18. Reliable and discriminating validated *in vitro* release methods that can simulate the release of the active substance from a block copolymer micelle in physiologically (clinically) relevant media should be developed in order to predict *in vivo* stability/ The value of such *in vitro* drug release as a quality control test should be adequately demonstrated. However, it is acknowledged that it may not always be possible to establish *in vitro-in vivo* correlations.

19. If the block copolymer component (not the active substance) has its own biological activity which would affect clinical efficacy and (or) safety, its potency and physico-chemical properties that are critical for its biological activity should be assessed as part of characterization.

20. The developer (manufacturer) should define a list of validated tests to be used on a routine basis with respect to the block copolymer micelle medicinal product; it should be based on the parameters chosen to characterize the medicinal product including those described above, based on specific aspects of the development of the medicinal product.

21. Development of discriminating, bio-relevant *in-vitro* release methods is important for the purposes of:

defining the release of the active substance or «block copolymer-active substance» conjugate from the block copolymer micelle during its circulation;

defining the release of the active substance or «block copolymer-active substance» conjugate from the block copolymer micelle at the targeted place of action. The proposed media should reflect the physiological environment of the block copolymer micelle during its use;

defining the stability when stored.

22. The methods used should be sensitive enough to ensure batch-to-batch consistency. It is particularly important to monitor batch-to-batch consistency in case of presence of «block copolymer-active substance» conjugate in micelles.

## 4. Manufacturing process of block copolymer micelle medicinal products and process control

23. A stable manufacturing process with associated process control procedures is needed to ensure that medicinal product with specified quality indicators is produced on a consistent basis. It is known that small changes to block copolymer micelle medicinal products may significantly influence their performance.

24. The manufacturing process should be controlled to ensure consistency in the medicinal product’s performance in terms of its safety and efficacy. Data showing consistency in quality, and controls for critical steps should be provided.

### Manufacturing process of components containing block copolymers and (or) «block copolymer-active substance» conjugates and process control

25. Detailed descriptions of the synthetic process, extraction, and purification procedures should be provided based on specific aspects of the development of the block copolymer micelle medicinal product.

26. The source and specifications for any starting materials should be provided. In particular, molecular weight and molecular weight distribution

of polymeric starting materials should be clearly described. Impurities such as manufacturing impurities and macromolecular reaction by-products should be listed in detail.

27. Key intermediates in the manufacturing process should be identified and controlled.

28. Biotechnological entities and (or) entities of biological origin that are used as starting materials or active substance should comply with the requirements for medical use contained in the Rules for conducting study of biological medicinal products.

29. To determine the effect of a manufacturing process change, e.g. change in its scale, a careful assessment of all foreseeable consequences for the block copolymer micelle medicinal product including process validation (assessment) should be performed.

## Manufacturing of block copolymer micelle medicinal products and process control

30. In the manufacturing process of block copolymer micelle medicinal products, micelle formation process is critical. If micelle formation occurs spontaneously, the process of micelle formation would be equal to the dispersion process of block copolymer. Where other methods are required for micelle formation, critical quality attributes associated with the process (e.g. micelle size and solution transparency) should be controlled.

31. Block copolymer micelle medicinal products contain highly functional polymers, so the end product testing alone is insufficient to define their quality. In light of this, it is highly recommended that appropriate quality control of intermediates (i.e., the block copolymer) and (or) the manufacturing process, is undertaken based on the quality by design concept.

## 5. Specification of block copolymer micelle medicinal products

32. In order to draw up an appropriate specification for a block copolymer micelle medicinal product, its developer (manufacturer) should seek a specialized scientific advice in accordance with paragraph 26 of the Rules for Marketing Authorization and Expert Assessment of Medicinal Products for Human Use. Additional testing specific to block copolymer micelle medicinal products may be possible.

### Specification of the components composing block copolymers

33. A detailed description of the tests, procedures, and acceptance criteria for block copolymers and (or) «block copolymer-active substance» conjugates should be provided. assessment of the copolymer, e.g. mean molecular weight or its distribution should be carried out. The composition of each component should also be defined.

### Specification of block copolymer micelle medicinal products

34. Since block copolymer micelle medicinal products are functional polymeric structures, the critical quality indicators should be defined for the functions relevant for their intended use. These indicators include particle size, release rate of the active substance from the micelle and potency of the active substance if it is a biotechnological (biological) entity. Where present, the composition regarding average number of targeting molecules conjugated to the polymeric micelle to promote active targeting should be justified.

35. It should be noted that block copolymer micelle medicinal products may be a mixture of block copolymer micelles and block copolymer monomers (bound or unbound with the active substance), depending on the individual characteristics of the block copolymers, the active substance and the test conditions used. In this regard, analytical tests should be performed

under appropriate test conditions and using appropriate methods, taking into account the product’s dosage form. The test concentration should be carefully selected, because dilution of block copolymer micelle medicinal products may result in disassociation of micelles and increase of proportion of monomers.

36. Both the active substance and the block copolymers should be analyzed as part of the analysis of identity and purity of the block copolymer micelle medicinal product.

37. The analyzed parameters include:

a) impurities, including possible synthetic macromolecule synthesis by-products, impurities in the form of undesirable aggregates, precipitates and degradation products;

b) potency, if the active substance is a biotechnological (biological) entity;

c) other indicators:

physico-chemical properties of block copolymer micelle products considered to be critical to product quality. However, it is not required to include all tests to establish the physico-chemical characteristics of block copolymer micelle medicinal products in the specification;

assay of incorporated (or conjugated) and unincorporated (or unconjugated) active substance of block copolymer micelle medicinal product;

assay of block copolymers or weight fraction of active substance.

## 6. Study of block copolymer micelle medicinal products stability

38. Stability studies should be planned and performed in the context of the intended clinical use of block copolymer micelle medicinal product and

the selection of methods and type of stability studies should be justified in the specification.

39. The principles set forth in the Requirements to Stability Studies of Medicinal Products and Pharmaceutical Substances approved by Decision No. 69 of the Eurasian Economic Commission's Board dated May 10, 2018 and in Chapter 8 of the Rules for Conducting Studies of Biological Medicinal Products, shall be applied to the planning of stability studies of block copolymer micelle medicinal products.

40. Stability studies, in general, should confirm the physical and chemical stability of the active substance, block copolymers, “block copolymer-active substance” conjugates (if any), and the resultant micelles.

41. Typical attributes assessed in the framework of stability studies include (but are not limited to):

a) physical stability:

b) mean block copolymer micelle size;

c) release of the incorporated (or conjugated) active substance;

d) secondary aggregation;

e) chemical stability of: active substance;

block copolymer components (e.g. degradation of polymers);

«block copolymer-active substance» conjugates (if any).

42. *In vitro* methods, using conditions relevant to the proposed use, should be used to determine the release rate of the entrapped active substance from the block copolymer micelles;

the rate of release of active substance chemically bound to block copolymer micelles.

### 7. Changes in manufacturing process during development of block copolymer micelle medicinal products

43. If there are changes in critical manufacturing process parameters or equipment used for manufacture, complete characterization of the block copolymer micelle product may be required on a case-by-case basis. Approaches to determining the impact of each process change vary based on the specific manufacturing process, the product, the extent of the manufacturer's knowledge and experience regarding the process and development data provided.

44. It is also necessary to provide for the application of the principles for assessing comparability studies of products before and after changes made in the manufacturing process, similarly to those developed for biological medicinal products as set out in the Rules for Conducting Studies of Biological Medicinal Products.

## IV. Non-clinical studies of block copolymer micelle medicinal products

### 1. General issues of non-clinical studies

45. Significant changes in pharmacokinetic characteristics may occur when an active substance is administered as a block copolymer micelle product, e.g. volume of distribution and clearance may be changed, half-life may be prolonged and tissue distribution changed. If the active substance is administered as a block copolymer micelle product, changes not only in the pharmacokinetic characteristics but also in the pharmacodynamics and safety may also occur. In addition, some block copolymers (not containing an active substance) may exhibit their own biological activity, which would have an impact on clinical efficacy and (or) safety. Cellular uptake of the active

substance entrapped in block copolymer micelle may be limited to the endocytic route.

46. The pharmacokinetic characteristics of the block copolymer micelle medicinal product could be dependent on:

the rate of clearance of the block copolymer micelle containing entrapped or chemically bound active substance;

the rate of dissociation of the block copolymer micelle. This may lead to release of block copolymer monomers (with or without bound active substance) that would have lower molecular weight (smaller size characteristics) exhibiting other clearance characteristics;

the rate of release of entrapped active substance from the block copolymer micelle;

the rate of release of active substance chemically bound to the block copolymer monomer;

the rate of degradation of the block copolymer;

clearance and metabolism of free active substance;

the distribution of the block copolymer micelle;

interaction of the block copolymer micelle with plasma or serum proteins or blood cells.

47. The rate and location of *in vivo* active substance release is a crucial parameter which often determines the toxicity and efficacy of block copolymer micelle medicinal product. In the process of pharmaceutical development of a block copolymer micelle medicinal product, it is necessary to provide for the development of the methodology necessary to define active substance release.

48. All non-clinical studies should be conducted using well-characterized block copolymer micelle product, and the rate of micelle

dissociation and product stability should be known under the chosen test conditions.

## 2. Non-clinical pharmacokinetic study of block copolymer micelle medicinal products

### Analytical methods

49. Validated analytical procedures should be developed, that are capable to measure the concentrations of active substance both in total and in free form in blood, plasma or serum, and the total concentration of active substance in organs and (or) tissues.

### Pharmacokinetics

50. Since the pharmacokinetic behavior of block copolymer micelle medicinal products can be very different from that of the active substance administered without the block copolymer micelle carrier and this can impact significantly on efficacy and safety, pharmacokinetics require an *in vivo* characterization. The choice of appropriate species and models to investigate *in vivo* pharmacokinetics and release of the active substance should be rationalized in respect of proposed clinical use and the composition of the block copolymer micelle.

51. Since physico-chemical parameters such as size, surface-charge and morphology may affect the distribution of block copolymer micelle product, the effect of variability in such parameters on distribution of block copolymer micelle product should be shown to justify the product specification. It is also necessary to assess the following pharmacokinetic parameters specific for a block copolymer micelle for both total and free active substances in blood, plasma or serum:

maximum concentration ($C_{max}$);

half-life ($t_{½}$);

area under the pharmacokinetic curve (AUC).

Pharmacokinetic parameters should be measured at different dose levels of a block copolymer micelle medicinal product and at appropriate time points.

52. Distribution of block copolymer micelle medicinal products in organs and (or) tissues relevant to the proposed clinical use and route of administration should be defined.

One shall consider, however, that the assessment of total amounts of active substance of a block copolymer micelle medicinal product in test-system may be required depending on the chosen analytical method.

A distribution time profile of a block copolymer micelle medicinal product should be defined using multiple time points with the rationale of the time course of the study.

53. Sampling time points and sampling duration should be carefully selected in order to correctly assay the dynamics of changes in both the total and free concentrations of the active substance and its metabolites in blood, plasma or serum, and the total concentration of active substance and metabolites in organs and (or) tissues. Some factors should be considered when planning the sampling schedules, for example, the block copolymer micelle stability after administration, and the profile of localization to specific organs and (or) tissues. In particular, samples taken in the initial distribution phase (e.g. <15 min) are considered very informative for calculating the distribution volume to assess the stability of block copolymer micelles in blood circulation.

Measurement of active substance metabolites in blood, plasma or serum and maybe organs and (or) tissues is especially important when the metabolite is the primary active compound. If one or more metabolites have

substantial clinical activity then it might be necessary to compare their kinetics, and if necessary, toxicokinetics, to determine accumulation following multiple doses.

The pharmacokinetics of a block copolymer micelle medicinal product and the active substance administered in the pure form should be compared. Such comparative studies are also useful to demonstrate a claimed pharmacokinetic advantage of the block copolymer product against the active substance administered in the pure form.

It is necessary to analyze the need of considering the protein and cellular interaction of block copolymer micelles administered intravenously as these factors may influence the distribution, stability and safety of nanomedicines.

54. The metabolic and excretion pathways of the active substance should be determined after administration of the block copolymer micelle product, then it should be fully characterized. Since the metabolic and excretion pathways of the micelle components are important for the correct and safe use of the block copolymer micelle medicinal product, the refusal to study them should be rationalized. If there is no rationale, a detailed establishment of these characteristics of the block copolymer micelle medicinal product is required.

55. If there is a theoretical probability that block copolymer micelle medicinal products can cause drug interactions (e.g. modulating membrane carriers such as p-glycoprotein), the drug-drug interactions of the block copolymer micelle medicinal product should be thoroughly assessed.

### 3. Studies of non-clinical pharmacodynamics

56. The studies of non-clinical pharmacodynamics should include the confirmation of pharmacodynamic response in appropriately rationalized *in*

*vitro* (if possible) and *in vivo* models. *In vivo* assessment should involve an appropriate route of administration, rationalized dose levels and a rationalized dosing regimen depending on proposed clinical use. The suitability of the pharmacological model should be analyzed in respect of the pharmacokinetics of the block copolymer micelle product, and of the pharmacokinetics and pharmacodynamics of the active substance when administered in the pure form.

57. The chemical composition and physico-chemical properties (including size and surface-charge, and the rate of release of the active substance) of a block copolymer micelle product affect pharmacodynamic properties. Some factors that should be considered when designing studies to define the mechanisms of action include:

the behavior of active substance (the location and rate of *in vivo* active substance release);

the behavior of the micelles (block copolymers or other stabilizing components) following administration and (or) cellular entry by endocytosis or other mechanisms.

58. The pharmacodynamic effect of the micelles should be assessed using *in vitro* and *in-vivo* pharmacodynamic models. The applicant shall provide thorough rationale why both *in vitro* and *in vivo* models to assess the pharmacodynamic effects of micelles cannot be used.

## 4. Pharmacological safety studies

59. It is necessary to carry out the basic set (battery) of pharmacological safety studies in accordance with the requirements of the Guidelines on Preclinical Safety Studies to Conduct Clinical Studies and Registration of Medicinal Product, Guidelines for the Pharmacological Safety Studies of Medicinal Products for Human Use approved by the Commission, if

applicable for the developed block copolymer micelle medicinal product (e.g. block copolymer micelle medicinal product that is not classified as anticancer medicinal product).

## 5. Toxicological and toxicokinetic studies

60. For the non-clinical assessment of toxicities of block copolymer micelle products, appropriate toxicological studies of the block copolymer micelle medicinal product should be performed to assess both the toxicological profile and the «exposure-response» relation.

61. In addition to blood, plasma, or serum concentration, the active substance should be measured in the target tissue and organs that are toxicologically significant in terms of the proposed clinical use of block copolymer micelle medicinal products.

## 6. Additional studies

62. Depending on the physico-chemical and (or) pharmacokinetic characteristics of the block copolymer micelle product and (or) the block copolymer used for its manufacture, target organ function assessment may be necessary.

63. Certain block copolymer micelle medicinal products have the potential to induce infusion reactions. Depending on the characteristics of the block copolymer micelle medicinal product, it is necessary to consider studies aimed at studying complement activation, hematotoxicity, antigenicity and (or) immunotoxicity.

## V. The first clinical study of block copolymer micelle medicinal products in humans

64. Block copolymer micelle medicinal products are designed, inter alia, to change the active substance distribution in human body. In this regard, when designing clinical studies for the first time in humans, based on the characteristics of the developed block copolymer micelle medicinal product, the provisions of Chapters 5.3 and 5.4 should be considered. Rules for conducting studies of biological medicinal products.

When designing clinical studies it is mandatory to analyze non-clinical pharmacokinetic data, and provide for the investigation during clinical studies of the following pharmacokinetic data specific for block copolymer micelle medicinal product (namely, block copolymer micelle, active substance, intended clinical use and route of administration of the block copolymer micelle medicinal product) obtained for total active substance and for free active substance in blood, plasma or serum:

maximum concentration ($C_{max}$);

half-life ($t_{½}$);

area under the pharmacokinetic curve (AUC).

65. Pharmacokinetic data should be obtained using time points and duration of sampling to quantitatively characterize the time profile of block copolymer micelle medicinal products in a correct manner according to the total content of active substance, free active substance and metabolites.

A sufficient number of samples to adequately describe the plasma concentration-time profile should be collected. Frequent sampling at early time points are considered useful for providing reliable information about the initial distribution process. Generally the sampling schedule should also cover the plasma concentration time curve long enough to provide a reliable estimate of the total extent of exposure.

66. Distribution of block copolymer micelle medicinal products in target lesion and major organs, specifically, total amounts of active substance in target lesion and major organs and their time profiles at multiple time points over an adequate period of time should be studied.

67. The starting dose for first-in-human studies should be chosen in compliance with the Guidelines on Preclinical Safety Studies to Conduct Clinical Studies and Registration of Medicinal Product and guidelines of the Union Member States, and after a thorough analysis of all relevant non-clinical studies, including critical product attributes, pharmacological dose-response dependence, pharmacokinetics and pharmacological (toxicological) profile.

68. Dose-limiting toxicity in humans can be determined in a way similar to study procedures that apply to other medicinal products, except for hypersensitivity reactions because these reactions are not always dose-dependent.

69. Potential critical quality attributes for each block copolymer micelle product should be identified and used to assess the constancy of properties. Consistency of the quality attributes should be confirmed between the block copolymer micelle medicinal products used for non-clinical studies and those used for first-in-human studies; and analytical procedures should be developed before the start of first-in-human studies.

70. If the manufacturing process used to prepare block copolymer micelle product used previously for non-clinical studies is changed before the start of first-in-human studies, comparability of the old and new manufacturing processes of block copolymer micelle medicinal product should be confirmed or otherwise rationalized.

71. Stability data that confirm the block copolymer micelle medicinal product's stability throughout the first-in-human clinical studies are required.

ANNEX No. 3

to Decision No. of the
Eurasian Economic Commission's Board
dated 20

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# GUIDELINES
## on quality assessment and study of medicinal products for parenteral administration coated with nanoparticles

## I. General provisions

1. The surface of a medicinal product for parenteral administration coated with nanoparticles (hereinafter referred to as the nanomedicine product) is created artificially and interacts with biological environment, the specific properties of which depend on the intended clinical use and the route of administration of a nanomedicine product into the body, e.g. blood plasma after intravenous administration.

2. Most nanomedicine products include as an integral component of their structure either a non-covalent or covalently bound coating. The coating is, as a rule, used to minimize aggregation and improve stability of finished dosage form of a nanomedicine product (e.g. iron saline solutions for treatment of anaemia) or, in certain cases, to minimize reticulo-endothelial system clearance after intravenous administration with the aim of prolonging circulation time of the substance in plasma and providing an opportunity for improved delivery to specific cellular targets (e.g. pegylation as in case with pegylated liposomes).

3. Coatings are also used to improve haemato-compatibility and limit antigenicity. These phenomena can arise due to the specific physico-chemical composition of a nanomedicine product or due to the surface adsorption of biomolecules from the biological environment to which they are located, e.g. plasma protein interaction to form what was called “protein corona”.

4. It is evident that presence of a coating has the potential to affect the critical properties of nanomedicine products in terms of safety and efficacy. The physico-chemical nature of the coating, the uniformity of surface coating, and the coating stability (both in terms of attachment and tendency to degradation) will govern the pharmacokinetics, the biodistribution of the product and its intracellular pathway. In addition, the infusion-related reactions observed clinically for certain coated nanomedicine products (e.g. iron solutions and pegylated liposomes) may be due to the physico-chemical properties of the coating material, specific biomolecular interaction with the coated nanomedicine products (e.g. complement activation) and (or) cell interaction. In some cases, a coating material may cause new biological responses, not observed for coating material or the unmodified surface separately.

5. On-going research is rapidly leading to the development of more complex surface modifications and some nanomedicine products are already in clinical development (e.g. liposomes designed for receptor-mediated targeting). In this regard, the use of ligands (e.g. proteins) to promote receptor mediated targeting require a thorough study of the chemical mechanism used for their attachment. Control of ligand orientation is important as heterogeneity will affect the pharmacokinetics and biodistribution of a nanomedicine product. In addition, random orientation may lead to a new model of biomolecule association with the nanomedicine product's surface, which may in turn affect its safety.

6. Both covalently bound and non-covalently associated coatings in terms of their potential impact on the safety and efficacy of nanomedicine products should be carefully studied during the pharmaceutical development of nanomedicine products. These Guidelines provide general principles for the development and assessment of nanomedicine products.

According to paragraph 26 of the Rules for Marketing Authorization and Expert Assessment of Medicinal Products for Human Use approved by Decision No. 78 of the Eurasian Economic Commission's Council dated November 3, 2016, when developing each specific type of nanomedicine products, the applicant may apply to the authorized authorities or expert organizations of the Member States for scientific and pre-authorization advice in accordance with the Member States' legislation.

7. General issues to consider during the development of nanomedicine products with covalently bound or non-covalently bound coating include:

how the coating affects the stability of the nanomedicine product's finished dosage form (e.g. polymer-coated liposomes);

how the coating affects the pharmacokinetics and biodistribution of the nanomedicine product's active substance (e.g. polymer-coated liposomes);

the effect of the coating (physico-chemical characteristics) on biomolecule interactions (including opsonization), and cellular interaction in the biological environment of the intended use;

the potential of the coating material to induce non-specific (as a rule, charge-dependent and hydrophobic interactions) and (or) receptor-mediated cellular targeted delivery (e.g. polysaccharides (glucose) receptors). For example, alteration in the pattern of liver cell localization (Kupffer cells: hepatocyte ratio); the potential of the coatings to affect the metabolic pathway of the encapsulated active substance.

## II. Submission of nanomedicine product information in the registration dossier

8. The study and description of quality performances, non-clinical and clinical data are important for the correct assessment of the safety, quality and efficacy of coated nanomedicine products. To assess the safety, quality and efficacy of a nanomedicine product, the registration dossier should include the following:

complete description of the coating material, including its composition and control of coating quality performances. If the coating agent itself (or when a specific ligand is added to specific targets) includes a complex molecule (e.g. protein or antibody), then additional characteristics may be required for their compatibility and reproducibility;

complete validation of the coating step, including description of the chemicals responsible for adherence of non-covalent coatings or conjugation of covalently bound coating material. It is also important to define the physico-chemical properties of the surface to which the coating adheres or is covalently bound;

the assessment of the potential impact of coating heterogeneity on the safety and efficacy of the product;

the orientation and conformational state of any ligand should be defined in ligands involved in the active formation of target-specific residues on the nanomedicine product's surface;

the assessment of the coating stability (stability during storage and during use) and the ability of the coating to detach and (or) be degraded. Premature detachment of the coating material and (or) degradation of the coating may be important to reveal new functional groups on the nanomedicine product's surface. The potential consequences of this process

in terms of efficacy and safety of the nanomedicine product should be studied and analyzed;

*in vitro* determination of the physico-chemical stability of the coating in respect of intended use, under conditions relevant to the route of administration, pharmacokinetics and biodistribution, and target disease;

the study results and analysis of *in vivo* impact of various coating materials (surface coatings) on pharmacokinetics and biodistribution.

The biodistribution of the released material from the nanomedicine product's coating and its metabolites should be studied.

Control and assurance of the quality of coated nanomedicine products cannot just be based upon a set of test specifications on the final product. It requires a well-defined and controlled manufacturing process supplemented with an appropriate control strategy (adequate in process controls for the critical steps of the manufacture of the product including the coating process).

9. When developing nanomedicine products, the potential impact of the coating on the efficacy and safety of the product should be carefully studied and analyzed. This information is critical when assessing studies designed to:

to demonstrate the clinical efficacy and safety of the first use of a nanomedicine product in humans;

to study the clinical efficacy and safety of a nanomedicine product at the pre- and post-authorization stage when making changes to its manufacturing process;

to demonstrate the comparability (equivalence) of a nanomedicine product developed by analogy to the reference nanomedicine product.

## III. Submission of information on generic iron-based nano-colloidal products for intravenous administration

10. When comparing generic colloidal iron-based nanomedicine products for intravenous administration (hereinafter referred to as iron-based nano-colloidal products), quality characterization on its own does not sufficiently guarantee the comparability (equivalence) of two medicinal products (reference and generic), even if their similarity was established on the basis of quality analysis. To prove the comparability (equivalence) of generic iron-based nano-colloidal products, an approach based on the comprehensive assessment of data from quality studies, non-clinical studies, and human pharmacokinetic studies is used.

11. Iron-based nano-colloidal products used to treat iron deficiency consist of a polynuclear core containing iron atoms, mainly present in the iron (III)-oxyhydroxide form, stabilized by a carbohydrate complex, which is responsible for nano-colloidal structure.

12. Upon parenteral administration, the nano iron complexes come into cells via the endocytosis, i.e. via cells of the reticulo-endothelial system. After intravenous administration of various iron-based products, it was noted that they are found in liver macrophages or hepatocytes.

13. The release of iron depends on the size and surface properties of the colloidal iron complex and matrix. In addition, the susceptibility of carbohydrates to intracellular degradation (high rate of degradation) may also affect the release of iron. Transfer and (or) accumulation of iron-based products in cells of any type may cause risks of use.

14. The complexity to fully characterize and define iron-based complex particles using only methods of quality analysis, and the uncertain nature of the relationship of qualitative performances with *in vivo* pharmacokinetics of iron-based nano-colloidal products, require additional studies. Comparing the

quality of the reference and generic iron-based nano-colloidal products and demonstrating the similarity of plasma iron concentrations (standard bioequivalence study) is insufficient to confirm the comparability (equivalence) of the *in vivo* metabolic pathways of these products, and their toxicological and pharmacological effects. In this regard, non-clinical data are required in addition to human clinical pharmacokinetic data. The required scope of additional non-clinical and clinical data depends on the accuracy with which the results of physico-chemical and non-clinical studies may predict differences that could affect the efficacy and safety of the medicinal product. Further clinical studies may be required if the results of quality studies, human pharmacokinetic studies and non-clinical studies do not provide sufficient evidence of product similarity.

15. This section provides guidance on the submission of comparative data from quality studies, non-clinical and clinical pharmacokinetic studies necessary for marketing authorization of generic intravenous iron-based nano-colloidal product, considering the following issues:

the pharmaceutical data necessary to confirm the comparability (equivalence) between generic and reference products, from that it may be concluded about similar comparative safety and efficacy;

the selection of the types of non-clinical and clinical studies necessary to confirm the data proving the similarity of the products.

16. The principles outlined should be considered when establishing data requirements, on the basis of which changes are made to the manufacturing process and the control system of existing iron-based nano-colloidal products.

## 1. Quality study

17. An extensive comparability study with a reference medicinal product is required to confirm highly similar quality profiles of the generic and reference medicinal product of an iron-based nano-colloidal product. It should include a comprehensive direct analysis of generic and reference iron-based nano-colloidal products using sensitive methods to determine not only similar properties, but also potential differences in quality attributes.

18. The potential impact of any differences in quality attributes on the safety and efficacy of the generic iron-based nano-colloidal product should be properly established. If such significant quality differences are confirmed, the iron-based nano-colloidal product should not be considered as generic one, and its marketing authorization application as a reference medicinal product would be preferable. To eliminate the established differences, the applicant could make changes to the manufacturing process, which will allow getting marketing authorization for iron-based nano-colloidal product as a generic product.

19. The description of chemical and physical properties is an important means for establishing the comparability of the generic iron-based nano-colloidal product to the reference product. It is necessary to ensure consistent quality of the complex iron-based products through the well-defined and controlled manufacturing process and comprehensive description of the product properties. The results may vary depending on the methods used, and where possible, two or more complementary analytical methods should be used to confirm the comparability and ensure the consistency of characteristics.

20. The quality attributes of iron-based nano-colloidal products, which may have a significant impact on the efficacy and safety, include:

a) the stability of the iron-carbohydrate complex (the fraction of labile iron released at the time of product administration and the short term stability in plasma), as labile iron has direct toxic effects and may influence on pharmacokinetics and product distribution in the body;

b) the physico-chemical properties of the carbohydrate matrix associated with:

the potential for anaphylactic (pseudo-anaphylactic) reactions;

the influence on the pharmacokinetics and distribution in the body;

the formation of specific products of carbohydrate coating degradation;

c) the physico-chemical properties of the iron and iron-carbohydrate complex, including size and of the iron core, and the size of the iron-carbohydrate complex and its size distribution.

## Description of the quality attributes of the generic iron-based nano-colloidal product

21. The correct determination of the parameters that define relevant physico-chemical properties of an iron-based nano-colloidal product is extremely important to ensure its quality.

22. When drawing up the registration dossier for any type of these products, it is necessary to provide information on the following general parameters:

quality standard for carbohydrates used in the manufacture of the active pharmaceutical substances and finished products (description, source and characterization, manufacture, assay, impurity profile, and stability characteristics);

structure and composition of carbohydrate matrix;

spectroscopic properties (e.g. $^{1}$H-NMR, $^{13}$C-NMR, IR spectrometry, optical spectroscopy, mass spectrometry, XRD analysis);

identification and control of key intermediates in the manufacturing process;

size of the iron core;

amount of labile iron released from the product when administered;

polymorphic form of the iron comprising the core;

impurities, e.g. ratio of divalent and trivalent iron;

morphology, e.g. microscopic assessment of the surface;

ratio of bound carbohydrate to iron;

particle size, particle distribution based on size, charge and surface properties of the iron-carbohydrate complexes;

degradation path for the iron-carbohydrate complex;

developed reliable and discriminatory method for determining degradation kinetics of iron-based nano-colloidal products (if justified). It should be taken into account that the study of

the degradation of iron-based nano-colloidal products in acid is not a reliable and discriminatory method;

stability on storage of the product;

in-use stability (including the product after re-constitution with recommended for use diluents) with consideration to instructions for administration, set out in a typical summary of product characteristics, e.g. concentration.

23. The quality of the finished product depends to a large extent on the quality and purity of the carbohydrate starting materials; therefore, the appropriate characterization and specification of the starting materials is extremely important. In some cases, the carbohydrate starting material is further modified. Often the carbohydrate is activated to enable binding. Processing at high temperatures or perhaps even moist heat sterilization of the finished product (if possible) may modify the composition of the

carbohydrate matrix. The different types of carbohydrate and the levels presented in the starting material should be controlled. The starting materials referred to in the Pharmacopoeia of the Eurasian Economic Union or the Pharmacopoeias of the Eurasian Economic Union Member States should comply with the pharmacopoeial requirements in terms of quality indicators. At the same time, to confirm compliance with the reference product, more detailed specifications for individual parameters are required. When using components from different sources, an additional description of characteristics and a comparability study would be required.

24. A list of the required routine tests for the iron-based products should be approved, taking into account the relevant pharmacopoeial monographs. This list should be based on the parameters used to characterize the dosage form as described above. The analytical methods used in characterization and control testing should be developed to ensure that integrity and stability of the iron complex is maintained during analytical testing, e.g. the absence of changes in the complex size upon dilution.

25. To ensure the safety of intravenous iron-based products and to eliminate the risks associated with labile iron, an important step is to develop methods to determine labile iron *in vitro*, which may be used as a means to demonstrate product similarity, to guarantee the batch uniformity and to determine the impact of changes in manufacturing processes.

26. The measurement of labile iron may be carried out by different methods, but the following methods are indicative of labile iron:

kinetic studies of iron (III) reduction during degradation of an iron-based nano-colloidal product under acid with UV detection. These studies should also be part of the specification for iron-based nano-colloidal products. Acceptance limits (both upper and lower) should be set based on

the performance of batches in which the release of the required volume of labile iron was established in tests *in vitro*;

*in vitro* labile iron transfer studies aimed at measuring direct labile iron transfer from an *in vitro* transferrin product, which are performed by adding iron-based nano-colloidal product to transferrin solution or to blood serum (human or animal). These studies may be used to confirm the comparability of the generic product to a reference iron-based nano-colloidal product. *In vitro* labile iron transfer studies should be included in the medicinal product specification initially. Until manufacturing experience increases, the intervals between repeated studies should be shortened.

## Establishing pharmaceutical comparability between generic and reference iron-based nano-colloidal products

27. The composition of the developed generic iron-based nano-colloidal product should be identical or closely identical to the composition of the reference iron-based nano-colloidal product with regard to its qualitative and quantitative characteristics.

28. Several different batches of the reference medicinal product should be used to provide a comprehensive analysis and to generate a representative quality profile.

29. The manufacturing date of the different batches of reference medicinal product should also be considered when establishing the target quality profile.

30. The chemical composition of the carbohydrate should be described and compared to the composition of the reference iron-based nano-colloidal product as part of the assessment of the chemical similarity of the products. Any differences in the composition of the carbohydrate matrix may increase the data requirements to demonstrate the similarity between the generic and

reference iron-based nano-colloidal product and may also lead to serious concerns with the regulatory body when considering the similarity of the chemical composition.

31. As a rule, the applicant does not have access to information about the manufacturing process of the reference iron-based nano-colloidal product. Therefore, comprehensive studies using state of the art methods for characterization should be applied to both products in parallel in order to provide a complete assurance that their characteristics are comparable. Such studies should include all tests to adequately characterize the generic and iron-based nano-colloidal products. The suitability of the selected tests should be considered to establish the equivalence of the medicinal products *in vivo* effect. Any differences between the products established during comparability studies should be examined, thoroughly assessed and justified with regard to their potential impact on the safety (efficacy).

32. A well-defined manufacturing process with proper control is required to assure that an acceptable product is produced on a consistent basis. The critical process parameters of the manufacturing process should be defined based on a suitable control strategy.

33. Some critical characteristics associated with *in vivo* performance cannot be accurately estimated using only one single criterion (e.g. particle size, shape, surface area and its properties). In this case, where possible, two or more complementary analytical methods based on different principles should be used to confirm better comparability of the two products.

34. In addition to the characterization studies conducted under normal conditions, accelerated stability tests of products should be conducted in order to compare physical and chemical degradation.

35. 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.

36. Any differences from the reference iron-based nano-colloidal product identified during comparability studies should be examined and thoroughly analyzed. When deciding on the need for further studies required to confirm that the developed generic and reference iron-based nano-colloidal products have a similar therapeutic effect, one shall be guided by the principles set forth in Chapter 9 of the Rules for Conducting Studies of Biological Medicinal Products of the Eurasian Economic Union approved by Decision No. 89 of the Eurasian Economic Commission's Council dated November 3, 2016.

37. Approach to determine the impact of any changes depends on the specific manufacturing process, the product, the manufacturer’s experience and competence, and the collected development data. Comparative studies should be performed when changes are introduced into the manufacturing process during development stage and also after marketing authorization for the medicinal product, e.g. for manufacturing scale up. Comparative studies of the developed generic and reference iron-based nano-colloidal products should also be performed if manufacturing site of generic iron-based nano-colloidal products is changed.

## 2. Non-clinical studies of iron-based nano-colloidal products

### Analytical methods

38. To compare the generic iron-based nano-colloidal product with the reference one, the development and validation of analytical methods to quantify analytes in the blood (plasma) and in tissues are required. The effect of all sample processing procedures in the development of analytical methods

to quantify analytes in the blood (plasma) and in tissues should be thoroughly studied, using methodologies to verify the suitability and interpretability of all bioanalytical results.

39. The lower limits of quantitation and recovery in plasma, tissues and, where necessary, in individual tissues under study should be stated according to the form provided in the table.

Table

Depots involved in the distribution of iron-based nano-colloidal products for intravenous administration for the treatment of iron deficiency

| | Type of depot (approximate list) | Limit of quantitation | Recovery level |
|---|---|---|---|
| 1. | Plasma (or serum) and red blood cells | | |
| 2. | RES: macrophages | | |
| | spleen | | |
| | liver (Kupffer cells) | | |
| 3. | Pharmacological target tissues<br>bone marrow | | |
| 4. | Toxicological target tissues<br>kidneys<br>liver (hepatocytes)<br>lungs<br>heart | | |

Biodistribution studies of iron-based nano-colloidal products

40. Non-clinical studies are planned to confirm the comparability between generic and reference iron-based nano-colloidal products. Studies should be conducted in accordance with the Good Laboratory Practice Rules of the Eurasian Economic Union in the field of medicinal product circulation approved by Decision No. 81 of the Eurasian Economic Commission's

Council dated November 3, 2016, unless otherwise justified (e.g. the need to use specialized test systems).

41. Non-clinical studies of generic and reference iron-based nano-colloidal products are performed after a detailed description of their characteristics. The generic iron-based nano-colloidal product should be produced using the full-scale (manufacturing) process and should be preferably taken from the same batch that would be used for the clinical studies.

42. It is believed when administered by parenteral route, iron nanoparticles are recognized by the reticulo-endothelial system (liver, spleen, lymph nodes, bone marrow, lungs, etc.) and undergo phagocytosis by macrophages, but may also be processed by endothelial or epithelial cells (e.g. hepatocytes) through endocytosis. Internalization of iron can take place in different ways, depending on the surface properties of the nanoparticles and protein adsorption (corona formation). Consequently, phagocytosis caused by opsonization-like phenomena will take place in different ways and with different rates, which most probably will result in a significant inter-species variability.

43. Some pharmacokinetic aspects of iron-based nano-colloidal products in humans can be modelled by animal and cell-based models. Moreover, biodistribution studies in a suitable animal model are of fundamental importance to assess distribution, metabolism and excretion of these nanoparticles and of their *in vivo* degradation or solubilization products. Particular attention should be paid to the distribution, accumulation and retention of at least three “depots”: plasma, reticular endothelial system and target tissues (organs). These studies should provide an important evidence of comparability of the distribution, metabolism and *in vivo* elimination of iron-

based nano-colloidal products, since it is impossible to fully study the product distribution in human body, based only on data on blood (plasma).

44. Analysis of the distribution in rodents should start with a dose finding study to establish appropriate dose levels, which can be accurately measured (to determine the sensitivity of the method) or to determine the best sampling strategy of time points to reflect the incoming iron and release of iron from the respective tissue. Suitable time points should be carefully analyzed and selected in such a way as to fully describe the concentration-time profile for all the tissues studied. Previous information on the biodistribution of the reference iron-based nano-colloidal product may also be used in the research design process. Early sampling time points (e.g. earlier than 24 hours) should also be included in the study to confirm the comparability with respect to the clearance of the reticulo-endothelial system in the early stages.

45. A main distribution study including one or two genders with one to two dose levels and single administration may be sufficient.

46. When choosing organs and target tissues for measuring analyte content, it should be considered at least the organs identified from the distribution pattern of the reference product and the generic iron-based nano-colloidal product, and by three depots, according to the form of the table. For the depot of the reticulo-endothelial system, the spleen is the recommended organ for the measurement of iron concentrations. Other methods to measure distribution such as the use of imaging technologies may be acceptable if proven to be effective.

47. Since the coated nanoparticles will be gradually degraded, total iron measurements will not reflect the physiological level of iron or the oxidation status. However, the time-dependent release of iron stored in a particular depot reflects the product degradation process and its biological relevance.

Therefore, measurement of the time-dependent overall iron content in various tissues may be sufficient to establish the degradation profile of the nanoparticle.

48. The distribution of the generic iron-based nano-colloidal product in individual depots should be established in addition to the level of tissues or organs on the cellular level. It is obvious, to establish a model of the cellular distribution of iron is an important step, that is, where the product is distributed in the liver: to Kupffer cells or to hepatocytes.

49. Iron concentration in tissues may be measured for example via inductively coupled plasma mass spectrometry (ICP-MS) or inductively coupled plasma atomic emission spectrometry (ICP-AES) or via photometry. In addition, a histological detection of iron in tissues is used as an additional method. In any case, a less sensitive method may be used, since the increase in the concentration of iron during intravenous administration, as a rule, is quite substantial. Presenting the data on the substance amounts per gram of tissues as well as presenting the data on the substance percentage in the dose (with a mass balance determination) is preferable.

50. Development of additional and more accurate methods for analyzing degradation process of the nanoparticles is recommended. For example, cell and tissue culture systems could be used to study the mechanism of absorption of nanoparticles and their degradation products or solubilization products by the reticulo-endothelial system, macrophages or hepatocytes (cells) of Kupffer.

51. When confirming the similarity of iron-based nano-colloidal products due to the lack of experience in assessing the results of comparative non-clinical studies of biodistribution and the experience of using statistical methods of analysis, it is necessary that the data resulting from comparative non-clinical studies be complex (longitudinal data for many endpoints in

multiple compartments). Nevertheless, it is recommended to strive for the use of quantitative statistical methods developed to confirm the equivalence. In addition, the study sponsor should clearly define and justify comparability criteria for distribution and clearance to be compared with the reference iron-based nano-colloidal product before starting the studies. The clinical implications of any of the established differences in the tissue distribution of iron-based nano-colloidal products between the generic and reference iron-based nano-colloidal products should be thoroughly discussed in the clinical report.

52. The data from the biodistribution study could be analyzed by non-compartmental analysis, taking into account the sparse (destructive) sampling to derive the summary parameters $C_{max}$ (or maximum amount), $t_{max}$ (time of maximum concentration or amount) and AUC (or area under the amount-time curve). Modelling with physiologically based pharmacokinetic model or empirical models could be used also to supplement the non-compartmental analysis of concentration (or amount) of substance in fluid (tissue). General parameters ($C_{max}$, $t_{max}$, AUC) should be obtained from both types of analyzes: model-based and non-compartmental analysis (refer to notes).

Toxicity studies are not sensitive enough to distinguish between generic and reference iron-based nano-colloidal products. For this reason, they are not suitable for this purpose and lead to unnecessary use of laboratory animals. In the case of specific concerns on the safety of iron-based nano-colloidal products, appropriate safety endpoints included in the design of the biodistribution study may be sufficient to address these concerns.

## 3. Clinical studies of iron-based nano-colloidal products

### Pharmacokinetic studies of iron-based nano-colloidal products

53. The pharmacokinetics of the iron-based nano-colloidal products should always be compared with the pharmacokinetics of reference product. Single-dose parallel or crossover design study is recommended here. The main variables in this study are the $AUC_{(0-t)}$ and $C_{max}$ of total- and transferrin-bound iron. Baseline correction is recommended to decrease inter-individual variability. In addition, other significant endpoints may be included. The development and validation of analytical methods should be aimed to confirm absence of impact from sample processing procedures and ensure the usage of methodologies to verify the conformity and interpretability of all bioanalytical results.

54. If a replicate design is used, the acceptance ranges for $C_{max}$ can be expanded in accordance with the Rules for Conducting Bioequivalence Studies of Medicinal products within the Eurasian Economic Union, approved by Decision No. 85 of the Eurasian Economic Commission' Council dated November 3, 2016. Otherwise, the 90% confidence interval should be in 80-125% range. The sampling period should be sufficiently long to confirm that the iron levels return to the previous baseline level. Analysis of the results of the study should be carried out in conjunction with the analysis of the results of *in vitro* quality control tests.

### Studies of the efficacy and safety of iron-based nano-colloidal products

55. Provided that the totality of the data obtained (quality comparison, non-clinical data and data of the human pharmacokinetic study) confirms the similarity, no further studies of therapeutic equivalence to confirm comparable efficacy and safety are generally required.

56. Differences between the reference and generic iron-based nano-colloidal products, which may affect the efficacy and safety of the generic iron-based nano-colloidal product, are the basis for the authorized authority (expert organization) to refuse in marketing authorization of this medicinal product.

57. If there are significant differences in the results of quality studies, non-clinical studies and human pharmacokinetic studies, which may establish the lack of similarity between the reference and generic iron-based nano-colloidal products, the data obtained in further studies of therapeutic equivalence cannot be considered as the basis for approval the marketing authorization by the authorized authority of this generic iron-based nano-colloidal product.

58. If there are minor differences in the results of the studies between the two iron-based nano-colloidal products, a study of therapeutic equivalence may be necessary to resolve the issue on how these differences may affect the efficacy and safety.

59. When choosing a clinical study to confirm the differences, the applicant may seek a specialized scientific advice in accordance with paragraph 26 of the Rules for Marketing Authorization and Expert Assessment of Medicinal Products for Human Use.

60. Clinical studies should be conducted at least 3 months and performed in a group of patients with anemia of a similar aetiology (e.g. patients with chronic renal failure). As the endpoints of a clinical study, an assessment of the following parameters should be included:

ferritin;

transferrin saturation;

haemoglobin;

total iron dose administered during the study;

total erythropoietin dose administered during the study.

61. Safety end points in such a study should focus on short term safety, studying the most common adverse events and also markers that could indicate adverse events in the safety profile:

anaphylactoid reaction rate;

non-transferrin bound iron (free);

overall adverse event rates;

markers of oxidative stress and free radical activity.

## Pharmacovigilance and risk management plan for iron-based nano-colloidal products

62. The main safety concerns of iron-based nano-colloidal products are associated with side effects, such as hypersensitivity reactions (anaphylactic, anaphylactoid), as well as iron overdose leading to organ damage.

63. The rate of hypersensitivity reactions during a short-term study of pharmacokinetics does not reflect the actual rate of these reactions during post-marketing studies. Hypersensitivity reactions after intravenous administration of iron-based nano-colloidal products are of special safety concern. Therefore, additional risk minimization measures should be included in the risk management plan for all intravenous nano-colloidal products, including the submission of cumulative annual safety reporting.

64. The risk of iron overdose leading to organ damage is inherent to all iron-based nano-colloidal products. The risk of iron overdose can be significantly reduced through strict adherence to indications (contraindications) and by avoiding off-label use or error indication of iron-based nano-colloidal products.

65. The risk management plan for an iron-based nano-colloidal product developed on the basis of the reference product should, in general, be

consistent with the plan for the reference product regarding important identified and potential risks and missing information.