22 February 2024
EMA/CHMP/275518/2022 Rev. 1*
Committee for Medicinal Products for Human Use (CHMP)

# Overview of comments received on 'Liposomal amphotericin B powder for dispersion for infusion 50 mg product-specific bioequivalence guidance' (EMA/CHMP/559889/2021) – Revision 1

Interested parties (organisations or individuals) that commented on the draft document as released for consultation.

| Stakeholder no. | Name of organisation or individual |
|---|---|
| 1 | Advanz Pharma Ltd. |
| 2 | Assoz.-Prof. Dr.Dr. Vera Hofer, Department of Operations and Information Systems, and Gerhard Gössler, MSc. MSc., Department of Chemistry - Analytical Chemistry for Health and Environment<br>University of Graz, Austria |
| 3 | Russell E. Lewis, Pharm.D, FCCP, Department of Medical And Surgical Specialties, University of Bologna, Bologna, Italy<br><br>Roger Brüggemann, Pharm.D., PhD., FESCMID and FECCM. Radboud University Medical Center, Nijmegen, the Netherlands (Department of Pharmacy and Pharmacology AND Radboudumc Center for Infectious Diseases); Prinses Maxima Center for Pediatric Oncology<br><br>Andreas H. Groll, M.D., FIDSA, FESCMID, FECMM, Center for Bone Marrow Transplantation and Department of Pediatric Hematology/Oncology, University Children's Hospital Münster, Germany |
| 4 | TLC Biopharmaceuticals B.V (Taiwan Liposome Company) |
| 5 | Martin Hoenigl, Jean-Pierre Gangneux, Esther Segal<br>Board of the ECMM |
| 6 | Fresenius Kabi Oncology Ltd |
| 7 | Gilead |

* This revision concerns the addition of specific comments from a stakeholder that were not included in the original version published 22 May 2023.

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# 1. General comments – overview

| Stakeholder no. | General comment (if any) | Outcome (if applicable) |
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| 3 | The product-specific guidance document developed by the PKWP describes a design for pharmacokinetic studies to establish bioequivalence for liposomal amphotericin B. We assume that it does not replace the points listed in the previous EMA/CHMP reflection/guidance document (1) but only specifies comparative pharmacokinetic studies for liposomal amphotericin B as outlined in Section 3.2.4, Clinical Studies, and that the stringent pharmaceutical quality requirements for a generic liposomal product fully remain in place. In addition, as experience with liposomal formulations developed with reference to an innovator is limited, we believe it is important to incorporate recommendations from the reflection/guidance document (1) that are specifically adapted to our current understanding of liposomal amphotericin B PK/PD.<br><br>**References**<br><br>1. EMA/CHMP/806058/2009/Rev. 02 "Reflection paper on the data requirements for intravenous liposomal products developed with reference to a liposomal product. 21 February 2013. | The assumption is correct, demonstration of bioequivalence for liposomal amphotericin B is considered a multidisciplinary approach as was indicated in the "to be noted" box.<br><br>However, to emphasise that the comparative pharmacokinetic study is only part of this process, we have now indicated this at the introduction of this guideline: "The current recommendations for product specific guidance for liposomal amphotericin should be read and followed in line with the Reflection paper on the data requirements for intravenous liposomal products developed with reference to a liposomal product EMA/CHMP/806058/2009/Rev. 02. Demonstration of equivalent efficacy and safety of a liposomal formulation developed to be similar to an innovator product is considered a multidisciplinary approach that in addition to the pharmacokinetic study, it also takes account of quality and non-clinical comparison, and a clinical therapeutic equivalence study, where appropriate." |
| 5 | As the board of the European Confederation of Medical Mycology (ECMM) we want to emphasize that we are in full agreement that there is an unmet need in Europe and beyond for more affordable cheaper lipid associated Amphotericin B formulations.<br><br>At the same time we do have some concerns regarding this bioequivalence guidance for liposomal amphotericin B. | See previous comment, demonstration of bioequivalence for liposomal amphotericin B is considered a part of the package and quality and non-clinical comparison, and a clinical therapeutic equivalence study where appropriate, should also be considered. |

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| | It is well described in literature that the size of liposomes, and also the lipidic composition matter, as they are determining for the release of Amphotericin B outside the liposome (i.e., efficacy and toxicity concerns), including release into tissue. This has also been outlined before by a previous broader EMA guidance on liposomal formulations https://www.ema.europa.eu/en/data-requirements-intravenous-liposomal-products-developed-reference-innovator-liposomal-product-0. For some reasons, however, the size of the liposomes is not mentioned in this current bioequivalence guidance.<br><br>This is even more concerning, when considering that the new guidance does not require assessing clinical efficacy but proposes a study in healthy volunteers only. Given that the size of liposomes may translate in clinical efficacy neither assessing liposome size nor efficacy would actually lay the path for a dramatic failure.<br><br>We are hopeful that EMA shares our concerns and that these issues raised will be included into the totality of evidence approach. | |
| 7 | Find below general information and comments on the production of liposomal amphotericin B based on the learnings from over 30 years of research. We will briefly outline key findings in the literature relevant to this guidance.<br><br>**1. Liposomes as drug carriers**<br><br>A liposome is an artificially prepared vesicle comprising of a lipid bilayer shell and an inner core of aqueous compartment. The drug substance may be encapsulated in the lipid bilayer or inner core. Liposomal drug products may be designed to release drug to a particular target tissue, or to act as a parenteral dosage form for sustained release in the systemic circulation. | See first comments, demonstration of bioequivalence for liposomal amphotericin B is considered a multidisciplinary approach. |

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| | Due to the engineered properties, these nanoparticle drug products have altered pharmacokinetic and pharmacodynamic profiles versus the non-liposomal, non-lipid parent compounds thereby leading to important consequences with respect to biodistribution which can significantly alter the safety profile of the liposomal or liposome associated parent drug compound.<br><br>(*Adapted from AAPS Advances in Pharmaceutical Sciences Series 13. FDA Bioequivalence Standards. Editors: Lawrence X. Yu & Bing V. Li. Section 1.4.7: Bioequivalence for Liposomal Products).*<br><br>**2. Importance of liposome formulation and production process**<br><br>Liposomal or lipid-associated amphotericin B formulations display a different pharmacokinetic profile of the parent drug substance, attributable to the physicochemical characteristics of the final finished product. A detailed assessment of these properties and their potential impact on patient safety and clinical efficacy is therefore paramount.<br><br>In formulating amphotericin B liposomal preparations, the association of the amphotericin B with the lipid is critical and must be carefully controlled to ensure that the decreased toxicity of the amphotericin B in the liposome can be maintained from batch to batch, since this control will have a significant impact on the efficacy and safety of the drug. Published reports have demonstrated that for liposomal amphotericin B formulations alterations in the molar ratios of the drug to phosphatidylcholine and phosphatidylglycerol or variations in the length of the fatty acid chain of the phosphatidylglycerol can significantly alter the single-administration $LD_{50}$ for mice (*Adler-Moore, J. P., and R. T. Proffitt. 1993. Development, characterization, efficacy and mode of action of AmBisome, unilamellar liposomal formulation of amphotericin B. J. Liposome Res. 3:429–450*. *Olson J.A. et al. Comparison of physicochemical, antifungal and toxic* | |

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| | *properties of two liposomal amphotericin B products. AAC 2008; 52(1): 259-268.*).<br><br>The process used for making the liposomes can also affect the product's toxicity. An example of this effect comes from the early development of AmBisome (Gilead Sciences, liposomal amphotericin B) upon which it was reported that liposomes formed by sonication were more toxic in mice than those produced by homogenization even though the liposomes had the same drug-to-lipid molar ratios *(Adler-Moore, J. P., and R. T. Proffitt. 1993. Development, characterization, efficacy and mode of action of AmBisome, unilamellar liposomal formulation of amphotericin B. J. Liposome Res. 3:429–450).*<br><br>Jensen et al. also reported that other stresses on a liposomal product, such as sterile filtration during production, storage, and lyophilization procedures, also must be controlled to produce batches that are reproducible from lot to lot and that sensitive assays have to be developed to monitor any changes. Several examples are provided in this article of significant final liposomal product differences even with essentially identical composition. *(Jensen, G. M., T. H. Bunch, N. Hu, and C. G. S. Eley. 2006. Process development and quality control of injectable liposome therapeutics, p. 297– 310. In G. Gregoriadis (ed.), Liposome technology, 3rd ed., vol. I. Liposome preparation and related techniques. Informa Healthcare, New York, NY.)*<br><br>**3. Liposome use to modulate amphotericin B toxicity**<br><br>The fundamental reason for the incorporation of amphotericin B into a lipid carrier was to avoid rapid clearance, aid in drug delivery to the site of infection and to reduce free amphotericin from binding to mammalian | |

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| | cholesterol, hence reduce relative toxicity compared to Conventional amphotericin B (cAMB) in a clinical setting.<br><br>In 1999, Jensen et al. determined the relative toxicity of different amphotericin B formulations. (*Jensen et al. Determination of the relative toxicity of amphotericin B formulations: A red blood cell potassium release assay. Drug Delivery 1999: 6 (2): 81-88*).<br><br>The investigators quantified the level of reduction of intrinsic toxicity of 3 formulations of lipid amphotericin B products (*amphotericin B colloidal dispersion (ABCD), amphotericin B lipid complex (ABLC), and liposomal amphotericin B (AmBisome))* employing a variety of blood sources and incubation times. Overall, the propensity in each formulation of amphotericin B to partition into the red cell membrane during incubation is measured by determining the concentration of amphotericin B required to achieve 50% potassium release.<br><br>The study demonstrated that the AmBisome liposomal formulation of amphotericin B has a much lower ability to induce potassium leakage in rat red blood cells. The onset of significant potassium leakage occurs at an amphotericin B concentration nearly 300 times higher than for ABLC and nearly 1000 times higher than for d-AmB.<br><br>It is also critical to be able to correlate toxicity with impact on potential patient safety. Increases in the therapeutic index of a drug can be achieved by reduction of toxicity and improvement or retention of efficacy. A large body of preclinical and clinical evidence for AmBisome demonstrates that AmBisome retains essential potency of amphotericin B deoxycholate (d-AmB) while significantly reducing toxicity. It is therefore recommended that analysis such as the potassium release assay, shall form a critical part of the assessment of any liposomal product. | |

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| | **4. Importance of Liposomal Amphotericin B Particle Size**<br><br>In 2008, Olson *et al*. compared the physiochemical, antifungal, and toxic properties of two liposomal amphotericin B products. Reconstituted AmBisome (Gilead Sciences Ltd.) consistently appeared as a clear dispersion with slight opalescence, while the reconstituted comparator product, Anfogen (Genpharma, S.A. Argentina) appeared as a clear dispersion but with notable haze, and in some vials, there were visible particulates. The reconstituted materials in each vial were examined for median particle size and for the upper limit of the range of diameters corresponding to 90% of the particles. The latter measure is referred to as the 90% passing diameter and is an indicator of the presence of particles larger than 100 nm in the small unilamellar vesicle dispersion. For AmBisome, the median particle size ± the standard deviation was 77.8 ± 2.2 nm. The 90% passing diameter averaged 122.0 ± 4.8 nm. For Anfogen, the median diameter was 111.5 ± 96.2 nm, and the 90% passing diameter averaged 273.6 ± 158.5 nm. Even excluding the one vial of Anfogen with a very large median particle diameter, the average 90% passing diameter for Anfogen was 60% greater than that for AmBisome, indicating a substantial presence of particles larger than 100 nm in Anfogen compared to AmBisome. (*Olson et al. Comparison of the physicochemical, antifungal and toxic properties of two liposomal amphotericin B products. Antimicrobial Agents and Chemotherapy 2008; 52 (1): 259-268*)<br><br>Particle size is important for a number of reasons:<br><br>a) The difference in particle size can affect how liposomes distribute in the host and their rate of cellular uptake. (*Olson J.A. et al. Toxicity and efficacy differences between liposomal amphotericin B formulations in uninfected and Aspergillus fumigatus infected mice. Medical Mycology 2015 Feb; 53 (2): 107-118.)* | |

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| | b) The active drug in AmBisome is locked into the liposome and not free to engage with various sub-compartments within the kidney. There is no glomerular filtration due to the size of the liposomes, which may explain the lower renal toxicity of AmBisome. (*Stone N. R.H. et al. Liposomal amphotericin B (AmBisome): A review of the pharmacokinetics, pharmacodynamics, clinical experience and future directions. Drugs 2016 March; 76 (4): 485-500)*<br><br>Sections 1-4 described above summarise how the association between the carrier and the active agent, amphotericin B, can be significantly altered by the processes used to prepare the product, and this association is critical for obtaining the desired therapeutic index of the carrier-drug preparation. By combining particle size determination, *in vitro* testing, *in vivo* single- and multiple-dose toxicity testing, measurement of BUN and creatinine levels, and histopathological evaluation of both uninfected and infected animals, a more accurate assessment of the drug's potential performance in the clinic can be achieved than can be provided by any of these tests alone. *(Olson J.A. et al. Comparison of the physicochemical, antifungal, and toxic properties of two liposomal amphotericin B products. AAC 2008: 52 (1): 259-268)*<br><br>**5. Regulatory guidance on innovator liposomal products**<br><br>The EMA reflection paper on data requirements for intravenous liposomal products developed with reference to an innovator liposomal product {EMA/CHMP 2013} align with the above thinking and emphasize the importance of a number of critical pharmacokinetic and physicochemical properties resulting from their formulation and manufacture (production, product, and process control), that may impact their distribution characteristics and ultimately, therefore, their efficacy and safety profiles. Within the 2013 reflection paper it is notable that pharmacokinetic tests | |

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| | based upon an assessment of plasma concentrations are not considered to correlate reliably with therapeutic performance, even for a new product with identical composition to an existing therapy. Complete characterization of stability and pharmacokinetics, including tissue distribution, is considered critical to establish product efficacy and safety, as conventional bioequivalence testing alone will not identify differences in liposome-cell interactions and liposome distribution characteristics that may result from differences in the manufacturing process between a new product and an existing therapy. These considerations should be taken into account when designing nonclinical and clinical programs for a new liposomal product, including any new follow-on generic product, in order to inform a proper assessment of safety, quality, and efficacy.<br><br>In the above we have outlined some general important points of liposomal amphotericin B production, and their implications for the benefit risk profile, and also highlighted some historical examples where significant differences in toxicity and efficacy have been previously demonstrated between AmBisome® (Gilead Sciences' Liposomal amphotericin B) and other liposomal amphotericin B preparations. (Adler-Moore et al. *Comparison between liposomal formulations of amphotericin B. Medical Mycology 2016*; 54:223-231). Given this, and the potential impact these differences can have on quality of care and patient safety, there is a need to distinguish between these products in both the published literature and in clinical use. | |

## 2. Specific comments on text

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| Line 16 Disclaimer | 7 | **Comment**:<br><br>The disclaimer on line 16 and 17 of the proposed guidance states that "This guidance should not be understood as being legally enforceable and is without prejudice to the need to ensure that the data submitted in support of a marketing authorisation application complies with the appropriate scientific, regulatory and legal requirements."<br><br>A previous document (EMA/CHMP/806058/2009/Rev .02) which was adopted on 23 February 2013 by the EMA listed 'the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product'. This document is still accessible and currently available on the EMA website and hence it is taken to be valid and presently in use. Adoption therefore of the proposed EMA guidance issued on 16 December 2021, specific to liposomal amphotericin B would be in disagreement with currently existing EMA recommendations. In particular, Gilead has noted that the recommendations in that document differ significantly to those in this present document open for consultation. In the 2013 document, it states: 'Contrary to products where the active substance is in the form or a simple solution, liposomal medicinal products have formulation and manufacturing-specific distribution characteristics after intravenous administration and similar plasma concentrations may not correlate with therapeutic performance'. It is therefore of considerable concern in the present document that the EMA is suggesting that demonstration of bioequivalence can be | **Partly accepted**<br><br>The cited 'Reflection paper on the data requirements for intravenous liposomal products developed with reference to an innovator liposomal product' (EMA/CHMP/806058/2009/Rev .02) was finalised by the CHMP in February 2013. It is stated in the Conclusion of the document that '*The experience with liposomal formulations developed with reference to an innovator is limited. As a result, only general recommendations can be given in this reflection paper and companies are advised to seek product-specific scientific advice regarding specific questions on the data requirements to demonstrate comparability of liposomal formulations.'* With this in mind product-specific bioequivalence guidelines were subsequently developed for liposomal doxorubicin (draft for consultation published June 2018) and liposomal amphotericin B (draft for consultation published December 2021) to help applicants meet the expectations of regulators in the European Union, particularly for generic applications.<br><br>Additional text has been added after the disclaimer to emphasise the need to take account of the Reflection paper i.e. *The current recommendations for product specific guidance for liposomal amphotericin should be read and followed in line with the Reflection paper on* |

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| | | achieved by comparison of conventional amphotericin B with a liposomal amphotericin B product. The same 2013 document also states: 'The complete characterisation of the stability, pharmacokinetics (including tissue distribution) of a new liposomal product is critical to establish safe and effective use……differences between the applicant's product and innovator product with regard to manufacturing process steps and formulation may substantially modify efficacy/safety due to changes in specific liposome-cell interactions and liposome distribution characteristics which are not detectable by conventional bioequivalence testing alone'.<br><br>**Proposed change**:<br><br>We therefore propose that it be made clearer in the disclaimer that there would also be a requirement to comply with the guidance outlined in EMA/CHMP/806058/2009/Rev .02 which we believe to still be active.<br><br>Please see also comments in 'General Comments', section 5. **Regulatory guidance on innovator liposomal products** | *the data requirements for intravenous liposomal products developed to be similar to a liposomal product (EMA/CHMP/806058/2009/Rev. 02).*<br><br>In addition, as stated in response to the general comments received, it is also stated that *'Demonstration of equivalent efficacy and safety of a liposomal formulation developed with reference to an innovator product is considered a multi-disciplinary approach that in addition to the pharmacokinetic study, it also takes account of quality and non-clinical comparison, and a clinical therapeutic equivalence study, where appropriate.'* |
| Line 18 Bioequivalence assessment | 1 | **Comment:** Requirement for the partial AUCs for non-liposomal amphotericin<br><br>The draft guideline proposes partial AUCs for specific timepoints (0-24 h and 24-last for non-liposomal form and 0-10 h and 10-last for liposomal form) to be included in the PK assessment of similarity as the main pharmacokinetic variables to "fully characterize the distribution and elimination process". Apart from the fact that these partial AUCs are not, to the best of our | **Partly accepted.**<br><br>Indeed, pharmaceutical comparison is part of the multidisciplinary approach. When there are major differences in composition or quality attributes with the reference product, a comparative pharmacokinetic study is considered not sufficient and additional studies are required as indicated in Reflection paper on the data requirements for intravenous liposomal |

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| | | knowledge, correlated to any meaningful pharmacodynamic parameter, we would like to point out several additional arguments as to why the partial AUCs for amphotericin are not valuable PK parameters for establishing bioequivalence.<br><br>1. **PK aspects**: Partial AUCs have been introduced into valid guidelines to characterize exposure of modified-release drugs, e.g. in case of multiphasic release, or in cases of drugs with long half-lives. It is acknowledged that non-liposomal amphotericin has a very long half-life of approximately 200 h. However, the cut-off time points for partial AUCs proposed in this draft guideline represent only a minor fraction of the total exposure, around 12 % of the total AUC for the non-liposomal form and about 4 % of the liposomal form. Considering these minor percentages, we believe that the biopharmaceutic properties are much better described by the total exposure of both liposomal and non-liposomal forms, in addition to the sameness exercise on the quality level.<br><br>Partial AUCs as primary PK parameters for both liposomal and non-liposomal forms have practical implications in terms of bioequivalence study design. Apart from conventional PK parameters, partial AUCs are additional primary PK parameters that should be accounted for during sample size calculations. Our internal data show approximately 33 % intrasubject variability for the non-liposomal form, which would lead to recruitment of around 250 subjects plus the substitutes to account for dropouts. Such high numbers | products developed with reference to a liposomal product EMA/CHMP/806058/2009/Rev. 02.<br><br>Amphotericin B is released for a prolonged period of time from the liposomes and for that reason we consider $AUC_{0-t}$ and $C_{max}$ insufficient to fully characterise distribution and elimination processes of liposomes. It is recommended to select the cut-off time points for the partial AUC values as such that both partial AUCs represent approximately 50% of $AUC_{inf}$.<br><br>It is however acknowledged that the long half-life of non-liposomal amphotericin is driven by the slow release from the tissues and may not reflect the release from the liposomes.<br><br>Therefore, it is now proposed to demonstrate bioequivalence for the following pharmacokinetic variables<br><br>**Main pharmacokinetic variables:** $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{max}$ for liposomal and non-liposomal amphotericin B and partial AUCs for liposomal amphotericin B (e.g. $AUC_{0-10h}$ and $AUC_{10-tlast}$) |

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| | | of subjects recruited in bioequivalence studies is considered unethical, and presents practical and logistic difficulties, which can further inflate already present analytical error and variability, and lead to an incorrect conclusion of bio inequivalence.<br><br>2. **Analytical aspects**: liposomal drugs, including amphotericin, are in general very fragile analytical samples, which require extremely careful handling. Processing of the sample is prone to error resulting in artificial disruption of the liposomes during the extraction procedure. This can lead to false overestimation of the non-liposomal fraction and false conclusion of bio inequivalence. In light of these difficulties, it is considered that the demonstration of liposome stability is much more reliable when shown *in vitro*, as a part of the sameness exercise on the quality level.<br><br>3. **Regulatory aspects**: the requirement of the draft guideline to include partial AUCs as the main PK parameters is not in line with the Reflection Paper on the Data Requirements for Intravenous Liposomal Products Developed with the Reference to an Innovator Liposomal Product (EMA/CHMP/806058/2009/Rev. 02) and the WHO Notes on the Design of Bioequivalence Study: Amphotericin B (liposomal), which state that partial AUCs should be evaluated descriptively as supportive data. The FDA approach is even more general, as the | |

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| | | FDA guideline on liposomal amphotericin instructs to calculate only total exposure.<br><br>In conclusion, based on the arguments above we are of the opinion that the partial AUCs are of limited value in describing the PK performance of the generic product as currently there are no publicly available data that could substantiate the cut-off time points for partial AUCs from either pharmacodynamic, pharmacokinetic or regulatory points of view. It is acknowledged that partial AUCs in general have their place in regulatory science and clinical development, but in case of this draft guideline we have doubts that the PK performance of liposomal amphotericin can be fully characterized by partial AUCs with the specific cut-off time points, as proposed. Therefore, until more data are generated which would enable rational decision of the partial AUCs truly indicative of the PK performance of the generic product, and correlated to the pharmacodynamically meaningful endpoint, we suggest that the partial AUCs are presented as supportive data, only.<br><br>**Proposed change:**<br><br>Main pharmacokinetic variables: $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{max}$~~., partial AUCs (e.g. liposomal amphotericin B: AUC0-10h and AUC10-tlast; non-liposomal amphotericin B: AUC0-24h and AUC24-tlast)~~<br><br>Supportive pharmacokinetic variables: Partial AUCs (e.g., *liposomal amphotericin B*: $AUC_{0-10h}$ and $AUC_{10-tlast}$; *non-liposomal amphotericin B*: $AUC_{0-24h}$ and $AUC_{24-tlast}$) | |
| Line 18 | 2 | **Comment:** | **1) Not accepted.** |

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| | | The implied test for average bioequivalence (ABE) (90% confidence interval: 80.00–125.00%) in the case of $C_{max}$ might not be appropriate for the following reasons:<br><br>1.) In the case of unencapsulated Amphotericin B, a concentration as low as possible is aimed at. Testing for ABE does not seem to be a sensible approach since a lower acceptance limit of 80% is not useful.<br><br>2.) The classical approach to assess ABE based on responses from a 2x2 crossover design is only suitable for the case where the product-related variability $\sigma_p^2$ (inter-batch variability and intra- as well as inter-individual variability) can be neglected compared to the variability $\sigma_e^2$ resulting from chemical analysis. A relatively high product related variability increases the total variability of the data, and thus can obscure equivalence with respect to the means when applying a classical equivalence test, as proposed e.g. in the GUIDELINE ON THE INVESTIGATION OF BIOEQUIVALENCE (CPMP/EWP/QWP/1401/98 Rev. 1/ Corr **). This can result in a high risk of not passing the test, even for a generic drug that is equivalent in terms of average bioequivalence.<br><br>A simulation study (1000 runs) of a 2x2 cross-over design with 24 subjects in total, equal expected values of 6.75 and an equal $\sigma_e$ of 0.15 (on the log-scale) for both, the test and reference product, and with uncorrelated responses within subjects, yields, in the absence of | Generic products bridge to the positive benefit /risk of the innovator product. Therefore, equivalence should be demonstrated and not inferiority. A lower $C_{max}$ for the test product would indicate a difference between the two formulations thus contradicting being a generic by definition.<br><br>**2) Not accepted.**<br><br>Product related variability for the test product is expected to be within the product related variability of the reference product. The applicant should select representative batches of test and reference product and is expected to select batches which show great similarity.<br><br>Information on non-liposomal and liposomal amphotericin B residual variability currently available shows ~30-35% CV for non-liposomal and 10-15% for liposomal amphotericin B. Therefore, there is no indication for exceptionally high variability in pharmacokinetics and bioanalysis of non-liposomal and liposomal amphotericin B. Assessment of bioequivalence based upon 90% confidence interval of pharmacokinetic parameters ratio using statistical analysis of log transformed pharmacokinetic parameters by ANOVA is recommended. |

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| | | product related variability $\sigma_p$, a probability of about 0.2% for rejecting equivalence, while, in the presence of additional product related variability ($\sigma_p$ assumed to be 0.26 for both products, thus increasing the total standard deviation to 0.3 in both cases) yields a probability of about 41% for rejecting equivalence although the test and the reference product have the same distributions. This problem is aggravated, when mean values are not equal. Simulations with a similar setting as above (a total standard deviation of 0.3 and an expected value for the reference product of 6.75), but with expected values of 6.65 or 6.55 for the test product (i.e. the difference of the logarithmized mean values is not only within [-0.223, 0.223], but also the expected values of the test product are smaller than that of the reference product), yield the following probabilities for rejecting equivalence despite of equivalence with respect to means: | |

| Nr. of Subj. | 24 | 60 |
|---|---|---|
| Expected values for test product | rejection probability | |
| 6.65 | 63% | 29% |
| 6.55 | 97% | 87% |

**Proposed change:**

1.) If the variability in the data is caused mainly by chemical analysis, a non-inferiority test should be used instead of testing for ABE.

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| | | 2.) If there is significant product-related variability, a pure comparison of mean values should be avoided and a range-based approach should be adopted, e.g. a comparison of suitable quantiles of the distributions in question (e.g. the 95%- or the 99.87%-quantile, the latter corresponding to the well-known 6σ philosophy in quality control) should be performed. | |
| Line 18. Requirements for a single, parallel bioequivalence study in healthy volunteers at 3 mg/kg | 3 | **Comment:**<br><br>The recommendation of a single-dose, cross-over study of 3 mg/kg in healthy volunteers would not provide sufficient evidence of bioequivalence for liposomal amphotericin B product. As stated in EMA/CHMP/806058/2009/Rev. 02 reflection document (1) "single and multiple dose studies at different dose levels may be needed to support the claim of similar pharmacokinetics." As the innovator liposomal AMB product exhibits dose and time-dependent non-linear pharmacokinetics in animals and humans with invasive fungal diseases (2-5), a single-dose study of 3 mg/kg in healthy populations would not adequately characterize this pharmacokinetic behaviour for a new liposomal amphotericin B product, in particular as the approved dose range is between 1 and 5 mg/kg (i.e., 1, 3, and 5 mg/kg for the different indications).<br><br>It has also been demonstrated in vitro (6,7) and in vivo (8) the pharmacokinetics and release of AMB from the liposome of the innovator product may be altered by fungi and inflammatory status of the host. | **Not accepted.**<br><br>It is agreed that the pharmacokinetics of liposomal and non-liposomal amphotericin B can be different in critically ill patients compared to healthy subjects. However, this relates to the disease. To demonstrate bioequivalence between two formulations, the study conditions should be standardised in order to minimise the variability of all factors involved except that of the products being tested. Therefore, the studies should be preferably performed in healthy volunteers.<br><br>Regarding the dose, a greater than proportional increase in AUC with increasing dose has been demonstrated for liposomal amphotericin B. Therefore, the highest dose in the non-linear part of the AUC vs. dose curve / lowest dose in the dose linear part is considered the most sensitive and safe dose to detect the differences that may exist between products. Therefore, a dose of 3 mg/kg infused over 120 minutes is recommended. |

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| | | The EMA/CHMP/806058/2009/Rev. 02 reflection document also states that when the active substance might not be tolerated by healthy volunteers, a pharmacokinetic study may be performed in patients. If a single-dose is not feasible in patients, then multiple dose pharmacokinetic studies in patients will be acceptable. The innovator liposomal amphotericin B product is associated with potential risk of complement activation-related pseudoallergy (CARPA) (9) and nephrotoxicity with multiple daily doses or prolonged therapy (10). It is unclear how safety issues could be assessed vs. an innovator formulation with a single-dose parallel study in healthy volunteers.<br><br>**Proposed change:**<br><br>We would recommend, at minimum, a parallel multidose bioequivalence study vs. the innovator liposomal amphotericin B product in patients with severe systemic or deep mycoses at two dosage levels (3 mg/kg and 5 mg/kg) in both adult and pediatric populations.<br><br>**References:**<br><br>1. EMA/CHMP/806058/2009/Rev. 02 "Reflection paper on the data requirements for intravenous liposomal products developed with reference to a liposomal product. 21 February 2013.<br><br>2. Walsh TJ, et al. Safety, tolerance, and pharmacokinetics of a small unilamellar liposomal formulation of amphotericin B (AmBisome) in neutropenic patients. Antimicrob Agents Chemother 1998; 42:2391–8. | |

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| | | 3. Walsh TJ et al. Safety, tolerance, and pharmacokinetics of high-dose liposomal amphotericin B (AmBisome) in patients infected with Aspergillus species and other filamentous fungi: maximum tolerated dose study. Antimicrob Agents Chemother 2001; 45:3487–96.<br><br>4. Würthwein G et al. Population pharmacokinetics of liposomal amphotericin B and caspofungin in allogeneic hematopoietic stem cell recipients. Antimicrob Agents Chemother 2012; 56:536–43.<br><br>5. Seibel NL et al. Safety, tolerability, and pharmacokinet-ics of liposomal amphotericin B in immunocompromised pediatric patients. Antimicrob Agents Chemother 2017; 61:pii: e01477-16.<br><br>6. Walker L, et al. The viscoelastic properties of the fungal cell wall allow traffic of amBisome as intact liposome vesicles. MBio 2018; 9.<br><br>7. Lestner JM, Howard SJ, Goodwin J, et al. Pharmacokinetics and pharmacodynamics of amphotericin B deoxycholate, liposomal amphotericin B, and amphotericin B lipid complex in an in vitro model of invasive pulmonary aspergillosis. Antimicrob Agents Chemother 2010; 54:3432–3441.<br><br>8. Mehta RT, McQueen TJ, Keyhani A, López-Berestein G. Phagocyte transport as mechanism for enhanced therapeutic activity of liposomal amphotericin B. Chemotherapy 1994; 40:256–264. | |

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| | | 9. Szebeni J. Complement activation-related pseudoallergy: a new class of drug-induced acute immune toxicity. Toxicology 2005; 216:106–121.<br><br>10. Stanzani M et al. Retrospective cohort analysis of liposomal amphotericin b nephrotoxicity in patients with hematological malignancies. Antimicrob Agents Chemother 2017; 61.<br><br>11. Wingard JR et al. A randomized, double-blind comparative trial evaluating the safety of liposomal amphotericin B versus amphotericin B lipid complex in the empirical treatment of febrile neutropenia. L Amph/ABLC Collaborative Study Group. Clin Infect Dis 2000; 31:1155–1163. | |
| Line 18. Analyte. Liposomal and non-liposomal drug in plasma/serum | 3 | **Comment:**<br><br>As stated in the EMA/CHMP/806058/2009/Rev. 02 reflection document "comparative human pharmacokinetic investigations should demonstrate not only similarity of exposure of the total, unencapsulated and liposome encapsulated drug, but they should also demonstrate similar distribution and elimination characteristics"<br><br>**Proposed change:**<br><br>Liposomal and non-liposomal amphotericin B should be measured in plasma/serum, whole blood (i.e. specifically mononuclear phagocytes and neutrophils)- key immune cells for invasive fungal diseases that take up the innovator liposomal AMB product (13), and urine. If higher concentrations of non-liposomal amphotericin B are detected in urine vs. the innovator product, this would represent an important signal for altered | **Not accepted.**<br><br>Demonstration of bioequivalence for liposomal amphotericin B is considered a multidisciplinary approach. As indicated earlier when there are major differences in composition or quality attributes with the reference product, a comparative pharmacokinetic study is considered not sufficient and additional studies are required as indicated in Reflection paper on the data requirements for intravenous liposomal products developed with reference to a liposomal product EMA/CHMP/806058/2009/Rev.2.<br><br>However, when the product has the same qualitative composition and very similar quantitative composition in excipients as well as equivalent liposome characteristics as the reference product, comparative |

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| | | drug release characteristics from the liposome versus the innovator product (< 10% of drug excreted unchanged in urine) and greater potential for nephrotoxicity (2).<br><br>1. EMA/CHMP/806058/2009/Rev. 02 "Reflection paper on the data requirements for intravenous liposomal products developed with reference to a liposomal product. 21 February 2013.<br><br>2. Groll AH, Rijnders BJA, Walsh TJ, Adler-Moore J, Lewis RE, Brüggemann RJM. Clinical Pharmacokinetics, Pharmacodynamics, Safety and Efficacy of Liposomal Amphotericin B. Clin Infect Dis 2019; 68:S260–S274. | plasma pharmacokinetics are sufficient to demonstrate similar distribution and elimination characteristics. |
| Bioequivalence assessment / Main pharmacokinetic variables | 4 | **Comment:**<br><br>The requirement for partial AUCs as one of the BE assessment variables for this specific medicinal product (Liposomal Amphotericin B) is questionable.<br><br>1. The primary disadvantage is that partial AUC may be extremely heterogeneous compared to the more robust full AUC. As reported by Walter, an empirical truncation process complicates the interpretation of partial AUC and makes the assessment problematic. One such problematic issue is that the partial area lacks a useful symmetrical property enjoyed by the full AUC. By dividing the full AUC into parts, undue scrutiny and influence may be given to a partial AUC that may have little to no relevance to the overall action of the object of study. [1] DiLiberti has discussed other performance issues with partial AUCs, such as being prone to high within- | **Partly accepted.**<br><br>See previous comments on the partial AUCs.<br><br>Partial AUCs are still proposed for the liposomal amphotericin B but no longer for non-liposomal amphotericin B. |

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| | | subject variability even for reference product itself and overly discriminating. [2]<br><br>2. High intra-group variability is especially pronounced for *non-liposomal amphotericin B* in a parallel design study, which will lead to impracticably large subject number required to demonstrate bioequivalence in partial AUC. Bioequivalence in $C_{max}$, $AUC_{0-t}$ and $AUC_{0-\infty}$ of *non-liposomal amphotericin B* under parallel design should be sufficient to demonstrate similarity between formulations.<br><br>3. Taken available international product-specific bioequivalence guidelines for Liposomal Amphotericin B into considerations, the partial AUCs of *non-liposomal amphotericin B* are either requested for supportive information only in bioequivalence studies in WHO Notes [3] or never requested by US FDA in any version of guidelines. [4-6] The partial AUCs may be submitted as supportive information, as WHO suggests [3], but not considered as main PK variables.<br><br>4. US FDA only adopts the partial AUCs in some product-specific bioequivalence guidance. The partial AUCs should be truncated at median of $T_{max}$ for early exposure or at an alternative time point when clinically relevant to achieve a rapid onset of action (such as analgesic effect) or avoid a toxic side effect (such as hypotensive action from an antihypertensive). [7] For AmBisome, the $T_{max}$ occurs at about the end of infusion (120 minutes per draft guidance) and $AUC_{0-Tmax}$ may be an appropriate indicator to compare | |

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| | | the early exposure from potential burst release which is clinically relevant to toxicities.<br><br>5. In the case of AmBisome®, the treatment requires multiple doses to exhibit its anti-fungal efficacy and $AUC_{0-\infty}$ could serve as an indicator of systemic daily exposure. The selection of cut-off point at 24 hours does not appear to correlate with any significant factor (e.g., $T_{max}$, $T_{1/2}$) identified in the full *non-liposomal amphotericin B* AUC profile, $AUC_{0-t}$ or $AUC_{0-\infty}$. The $AUC_{0-24h}$ of *non-liposomal amphotericin B* only presents about 15% of $AUC_{0-\infty}$ for *non-liposomal amphotericin B*. Also, no clinically relevant PD measurement has been identified within 24 hours of the first dose. Also, $AUC_{24-tlast}$ is similar to $AUC_{0-tlast}$ due to long sampling time covering long elimination phase of *non-liposomal amphotericin B*. Furthermore, the $AUC_{0-\infty}$ is a more relevant parameter to assess pharmacodynamics and evaluate bioequivalence between AmBisome® and its generic candidates in terms of the product property.<br><br>6. It is also noticed that the bioequivalence assessment of other liposomal product (pegylated liposomal doxorubicin HCl) includes partial AUCs of encapsulated drug only but not unencapsulated drug. [8]<br><br>**Proposed change:**<br><br>1. Partial AUCs of *non-liposomal amphotericin B* should be provided as supportive information. | |

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| | | 2. If the Agency decides to include partial AUCs as supportive information, please reconsider to set up a more meaningful cut-off point around $T_{max}$ for *non-liposomal amphotericin B* to demonstrate formulation similarity.<br><br>**Reference:**<br><br>[1] Walter, S. D., The partial area under the summary ROC curve, 2005, Statistics in Medicine, 24(13): 2025–2040<br><br>[2] DiLiberti, C.E., Partial AUCs 2.0 – Improved Metrics for Assessing Bioequivalence on Mixed Release Mode (IR/ER) Drug Products, 2017, FDA/OGD Leveraging QMM Workshop<br><br>[3] WHO Notes on the Design of Bioequivalence Study: Amphotericin B (liposomal) (06 July 2021)<br><br>[4] US FDA, Product Specific Guidances for Generic Drug Development – Amphotericin B, Injectable, Liposomal; RLD: AmBisome (August 2020)<br><br>[5] US FDA, Product Specific Guidances for Generic Drug Development – Amphotericin B, Injectable, Liposomal; RLD: AmBisome (January 2016)<br><br>[6] US FDA, Product Specific Guidances for Generic Drug Development – Amphotericin B, Injectable, Liposomal; RLD: AmBisome (April 2014)<br><br>[7] FDA Bioequivalence Standards. Lawrence X. Yu, Bing V. Li (2014), ISBN: 978-1-4939-1252-0 | |

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| | | [8] EMA, Pegylated liposomal doxorubicin hydrochloride product-specific bioequivalence guidance (EMA/CHMP/800775/2017) | |
| Line 18 | 6 | **Comment:**<br><br>Dose for bioequivalence study in healthy subjects should be < 3 mg/kg as safety data at 3mg/kg in healthy subject is not available in public domain.<br><br>**Proposed change:**<br><br>Lowest feasible dose (< 3 mg/kg) administered over 60-120 minutes in healthy subjects.<br><br>Or<br><br>A fixed dose (in the range of 2-3 mg/kg e.g., 150 mg) in healthy subjects can also be used for bioequivalence assessment. | **Not accepted.**<br><br>The 3 mg/kg dose was selected considering the clinical doses used, the more than dose proportional pharmacokinetics at lower doses, and the safe administration in healthy subjects.<br><br>Based on available information not publicly available, it was considered by clinical experts within EU that 3 mg/kg is a dose that could be safely administered to healthy subjects for a single dose administration. |
| Line 18 | 6 | **Comment:**<br><br>Partial AUCs should not be considered as main pharmacokinetic parameters. This may be used as supportive purpose without applying 90% confidence interval.<br><br>**Proposed change:**<br><br>Main pharmacokinetic variables: $AUC_{0-t}$, $AUC_{0-\infty}$, $C_{max}$, | **Partly accepted.**<br><br>See previous comments on partial AUCs.<br><br>Partial AUCs are still proposed for the liposomal amphotericin B but no longer for non-liposomal amphotericin B. |
| Line 18 | 6 | **Comment:**<br><br>Bioequivalence should be assessed on 90% confidence interval of $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max}$ for liposomal amphotericin B only. Data of $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max}$ for free amphotericin B should | **Not accepted.**<br><br>The release mechanism of amphotericin B and activity of liposomal amphotericin B at the site of the fungal invasion are not fully understood. Therefore, liposomal |

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| | | be provided as supportive data as suggested in other guidances (USFDA and WHO).<br><br>**Proposed change:**<br><br>90% confidence interval: 80.00–125.00% of $AUC_{0-t}$, $AUC_{0-\infty}$ and $C_{max}$ for liposomal amphotericin B only. | and non-liposomal amphotericin B are both considered relevant to conclude on bioequivalence as they best reflect the biopharmaceutical quality of the proposed product. |
| Line 18 | 6 | **Comment:**<br><br>The non-clinical studies (PK, biodistribution, PD etc) requirements should be considered an optional supportive aspect and should not be mandatory requirement for approval. The comprehensive analytical characterization provides more meaningful assessment to ensure similarity with Reference medicinal product. Therefore, the non-clinical studies requirements should be considered optional. | **Accepted.**<br><br>As indicated before, demonstration of bioequivalence for liposomal amphotericin B is considered a multidisciplinary approach. Depending on the sameness in qualitative and quantitative composition as well as equivalent liposome characteristics with the reference product, in addition to the comparative pharmacokinetic study in healthy subjects, additional non-clinical and clinical studies may be necessary or not. |
| Line 18<br>Use of single dose | 7 | **Comment**:<br><br>This draft bioequivalence guidance for liposomal amphotericin B currently recommends a single dose study. There are several reasons why the use of a single dose in amphotericin B bioequivalence studies suggest that they are not satisfactory. Firstly, the pharmacokinetics of liposomal amphotericin B and conventional amphotericin B are markedly different and use of a single dose for comparison is insufficient to characterise them. Dosing in humans demonstrated that pharmacokinetic parameters change with dose, with $C_{max}$ at 1mg/kg/d of 8 ug/mL but at a dose of 5 mg/kg/d the $C_{max}$ is 83 ug/mL. At the | **Not accepted.**<br><br>As stated above, it is agreed that the pharmacokinetics of liposomal and non-liposomal amphotericin B can be different in critically ill patients compared to healthy subjects. However, this relates to the disease. To demonstrate bioequivalence between two formulations, the study conditions should be standardised in order to minimise the variability of all factors involved except that of the products being |

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| | | same doses, the mean AUC are 27 ug.h/mL and 555 ug.h/mL respectively (*Serrano et al. Pharmaceutical Nanotech. 2013; 1: 250-258*). For conventional amphotericin B, data are only available at 1 mg/kg/d as toxicity precludes use at higher doses, and $C_{max}$ is 1.7 ug/mL and AUC is 18.7 ug.h/mL (*Heinemann et al. AAC, 1997; 41: 1275-80*), illustrating the significantly different pharmacokinetic profiles of these two forms of amphotericin B. The predictor of outcomes for polyenes is the ratio of $C_{max}$ /minimum inhibitory concentration (MIC) of the infecting organism (*Lepak & Andes, Cold Spring Harb Perspect Med 2015;5:a019653*), so changes in $C_{max}$ can be significant. Maximising $C_{max}$ is important and to calculate this on the basis of a single dose appears to be insufficient to produce reliable results.<br><br>Secondly, one of the key differences between conventional amphotericin B and liposomal amphotericin B is renal toxicity as demonstrated by the head-to-head non-inferiority study conducted by Walsh T. et al. NEJM 1999. Therefore, use of a single dose may not provide sufficient data to evaluate this safety parameter. Nephrotoxicity is generally low for the first 7-10 days of dosing (*Stanzani et al. AAC. 2017; 61: e02651-16*) so unless multiple dosing is tested over a longer time period, a comparison of the safety of a novel liposomal amphotericin B product should not be established using the proposed recommendations in this document.<br><br>Thirdly, comparing two liposomal formulations of amphotericin B in a single dose experiment is challenging as liposomal amphotericin B also exhibits non-linear pharmacokinetics and | tested. Therefore, the studies should be preferably performed in healthy volunteers.<br><br>Regarding the dose, a greater than proportional increase in AUC with increasing dose has been demonstrated for liposomal amphotericin B. Therefore, the highest dose in the non-linear part of the AUC vs. dose curve / lowest dose in the dose linear part is considered the most sensitive and safe dose to detect the differences that may exist between products. Therefore, a dose of 3 mg/kg infused over 120 minutes is recommended.<br><br>Also as previously stated, the 3 mg/kg dose was selected considering the clinical doses used, the more than dose proportional pharmacokinetics at lower doses, and the safe administration in healthy subjects. |

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| | | these cannot be characterised on the basis of a single dose. In adults, liposomal amphotericin B demonstrates initial disposition into the central compartment (mainly in the liver, spleen and marrow which are rich in mononuclear phagocytes), a lower concentration in the kidney, lung, and brain and then a slow return to the blood from these sites (*Groll et al. Clin Inf Dis. 2019; 68 (S4): S260-274*). In immunocompromised children, the model that best describes the pharmacokinetics is a 2-compartment model (*Lestner et al. AAA 2016; 60: 7340-7346*).<br><br>Finally, liposomal amphotericin B will be used to treat a range of invasive fungal diseases and may be used for several weeks. Although there are no defined durations for specific diseases, treatment for 2-3 weeks is quite common and possibly several months for mucormycosis. Hence testing of a single dose does not represent, in our view how the product would be used if it was approved for clinical use.<br><br>**Proposed change**:<br><br>A dose of 3mg/kg/day for 14 days should be used (this is typical of dosing in a patient with invasive aspergillosis having undergone an allogeneic HSCT). However, it is important to note that this will not provide bioequivalence data for higher dosing such as 5-10mg/kg/day for mucormycosis. | |
| Line 18<br>Cross-over design | 7 | **Comment:**<br><br>The current proposal suggests that a cross-over design in healthy volunteers should be recommended. However, for crossover designs, an adequate washout interval is required | **Partly accepted**<br><br>Although a cross-over design is recommended in line with it being the standard in the EMA 'Guideline on the investigation of bioequivalence' |

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| | | between the two periods so that the drug level at the beginning of each period is almost zero or negligible. (*Adapted from AAPS Advances in Pharmaceutical Sciences Series 13. FDA Bioequivalence Standards. Editors: Lawrence X. Yu & Bing V. Li. Section 1.4.7: Bioequivalence for Liposomal Products*).<br><br>Studies have shown that AmBisome® has distinctive pharmacokinetics in animals and in humans. AmBisome demonstrates non-linear clearance from plasma that appears to be due to saturation of the reticuloendothelial system (RES) and redistribution of the drug into non-RES tissues. Multiple dose studies in rats treated daily with increasing doses of AmBisome (1 to 4 mg/kg) showed parallel increases in high-performance liquid chromatography (HPLC)-measured drug levels in both RES and non-RES tissues. These levels were sustained for at least 30 days after termination of treatment with evidence of only minor reversible histopathological changes. (*Adler-Moore et al. Effect of tissue penetration on AmBisome efficacy. Current Opinion in Investigational Drugs 2003 4(2):179-185*).<br><br>Given the unique pharmacokinetics of liposomal amphotericin B, and continued presence in tissues, we do not feel that a washout period would be possible in an appropriate time period without the prolongation of drug A in the tissues potentially providing an additive effect on the outcomes of drug B in the second arm.<br><br>In contrast, for parallel designs, each treatment would be administered to a separate group of subjects with similar demographics and no washout period is needed. Parallel designs | (CPMP/EWP/QWP/1401/98 Rev. 1), both the draft and final product-specific guideline for liposomal amphotericin B state that '*Given the long terminal elimination half-life of non-liposomal amphotericin B, a parallel design study could be considered'*. |

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| | | are often used for bioequivalence studies conducted in patients or for drugs with a long half-life where crossover studies are difficult or impossible to perform.<br><br>Consequently, we propose that a parallel design study would be more appropriate and would produce more reliable results.<br><br>**Proposed change**:<br><br>Parallel Design: Given the long terminal elimination half-life of liposomal amphotericin B. | |
| Line 18 Use of Healthy Volunteers | 7 | **Comment**:<br><br>The current proposal recommends that a cross-over design study is conducted utilising healthy volunteers.<br><br>Healthy, immunologically competent individuals have a high degree of innate resistance to fungi. Resistance to fungi is based primarily upon cutaneous and mucosal physical barriers. Severity of disease depends on factors such as inoculum, magnitude of tissue destruction, ability of the fungi to multiply in the tissue, and the immune status of the host. (*George S. Kabayashi. Chapter 74: Disease Mechanisms of Fungi in Medical Microbiology , 4th Edition. Galveston (Tx) University of Texas Medical Branch of Galveston; 1996*)<br><br>In most cases, healthy individuals are able to avert the fungal attacks by mounting proper antifungal immune responses. Among the pattern recognition receptors (PRRs), C-type lectin receptors (CLRs) are the major players in antifungal immunity. The fungal recognition by CLRs mainly leads to proinflammatory | **Not accepted**<br><br>See previous comments that it is agreed that the pharmacokinetics of liposomal and non-liposomal amphotericin B can be different in critically ill patients compared to healthy subjects. However, this relates to the disease. To demonstrate bioequivalence between two formulations, the study conditions should be standardised in order to minimise the variability of all factors involved except that of the products being tested. Therefore, the studies should be preferably performed in healthy volunteers. |

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| | | responses and a subsequent activation of adaptive immunity via Th17 responses. However, negative or anti-inflammatory effects have also been noted and both types of responses are necessary to mount a specific immune response. (*Goyal et al. The interaction of human pathogenic fungi with C-Type Lectin Receptors. Front. Immunol. 04 June 2018*).<br><br>Effector mechanisms of innate immunity are performed by phagocytic cells such as neutrophils, macrophages and monocytes, which mediate several protective mechanisms including phagocytosis and the production of reactive oxygen species and hydrolytic enzymes that can directly kill fungal pathogens as well as releasing inflammatory mediators such as cytokines. (*Speakman et al. T-cell antifungal immunity and the role of C-type lectin receptors. Trends Immunol. 2020 Jan; 41 (1): 61-76*). Unfortunately, in many patients who suffer a systemic fungal disease these immune responses are significantly altered. A common example of this being patients undergoing allogeneic haematopoietic stem cell transplantation for a haematological malignancy. Such patients have on average, 21 days of severe neutropenia post HSCT and consequently are unable to mount a sufficient immune response.<br><br>Moreover, in 2018, Louise Walker et al. described in depth, the Mode of Action of AmBisome (Gilead Sciences) which involves the drug perturbation of the fungal cell membrane by selectively binding to ergosterol, thereby disrupting membrane function. It was clearly demonstrated therefore in this research that liposomal amphotericin B requires fungi to be present in the | |

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| | | blood or tissues of the host for it to exert its effect, some of which will become tissue bound. In healthy volunteers this would not be possible and consequently the amount of non-fungal bound AmBisome present in a healthy volunteer may give a false picture of the drug pharmacokinetics. (*Walker et al. The viscoelastic properties of the fungal cell wall allow traffic of AmBisome as intact liposome vesicles. mBio 2018: 9(1): e02383-17.)*<br><br>Furthermore, patients undergoing an active fungal infection will mount an immune response and this will often involve a rise in body temperature due to activation of inflammatory pathways. It is therefore critical to ensure that any bioequivalence investigation also looks at the stability of the liposomal preparation in patients with an elevated temperature >37.8oC. This would not be possible in healthy volunteers.<br><br>Finally, as mentioned above we would recommend the use of a parallel design study to prevent the need for complexities over a washout period due to the prolonged retention of AmBisome® in the tissues. The need to use relevant patients in this bioequivalence work would make any study utilising a crossover design inappropriate in our view. Leaving a patient with a known active infection untreated whilst a washout period was undertaken would impact the patient's prognosis by giving the infection time to progress. In addition, as described a single dose BE is not sufficient for amphotericin B and multiple doses should not be used in healthy subjects due to potential safety concerns. | |

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| | | **Proposed change**:<br><br>We would recommend at a minimum conducting the bioequivalence studies in adult HSCT patients and adult high-risk critically ill patients in a parallel study design. | |
| Line 18 Use of non-liposomal amphotericin B | 7 | **Comment:**<br><br>It is important to note that non-liposomal (amphotericin B deoxycholate/conventional amphotericin B) and lipid preparations of amphotericin B are not interchangeable. This has been recognised by many regulatory authorities globally.<br><br>A number of DHPC's have been requested over the years regarding the potentially fatal risks of medication errors with dosing non-liposomal formulations at the liposomal formulation dose, highlighting the fact that one amphotericin B drug cannot be substituted by another.<br><br>March 2017 ANSM (regulatory agency in France) requested a DHPC to be drafted highlighting the Risks of medication errors with the different formulations of parenteral amphotericin B: Abelcet®, AmBisome® and Fungizone®. ANSM requested the letter mention the following key points:<br><br>• The fact that one amphotericin B drug cannot be substituted by another,<br>• The importance of prescribing with the trade names of drugs in order to avoid any risk of confusion between Abelcet®, AmBisome® and Fungizone®, | **Partly accepted**<br><br>It is not intended that a non-liposomal innovator product be used as the reference (comparator) product. To highlight this, in the final guideline the following has been added at the introduction: "The current recommendations for product specific guidance for liposomal amphotericin should be read and followed in line with the Reflection paper on the data requirements for intravenous liposomal products developed with reference to a liposomal product EMA/CHMP/806058/2009/Rev. 02.<br><br>The proposed change is not accepted accordingly as it is not foreseen that amphotericin B deoxycholate is used as a comparator. |

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| | | • The methods of preparing and administering different amphotericin B drugs.<br><br>On 17th July 2018, the MHRA in the UK published an online alert to remind healthcare professionals of the risk of potentially fatal adverse reactions if formulations are confused. This was published following their receipt of a third case of fatal medication error caused by the administration of Fungizone (a non-lipid-based formulation of amphotericin B) instead of a lipid-based formulation (AmBisome, Abelcet). They remind healthcare professionals that these formulations are not interchangeable, and that prescribers, pharmacists and nurses should be fully aware of the formulation being used and the associated dosing regimen (https://www.gov.uk/drug-safety-update/parenteral-amphotericin-b-reminder-of-risk-of-potentially-fatal-adverse-reaction-if-formulations-confused).<br><br>Previous to this alert, in 2007 the National Patient Safety Agency (NPSA) in the UK issued an alert regarding the death of two patients in an oncology ward at Birmingham Heartlands Hospital after being treated with the wrong formulation of amphotericin B, whereby the dosing had been confused between two different formulations.<br><br>The NPSA at the time stated that "confusion between the formulation can lead to a dose that is too high or too low, leading to either inadequate treatment or a fatal outcome. There were 53 incidents involving the drug(s) between January 2004 and July 2007. Seven resulted in 'low harm' to patients, one resulted in moderate harm, and 43 in no harm." (Nigel | |

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| | | Hawkes. Agency warns about dosing error for amphotericin after patients with cancer die. BMJ 2007 Sept; 335 (7618): 467).<br><br>Following a number of reports of serious medication errors, some leading to death, and after consultation with EMA's safety committee (PRAC), the CHMP and CMDh agreed all marketing authorisation holders of medicines containing liposomal drug delivery systems were requested to submit to EU regulators a variation to change the names of these medicines as soon as possible before the end of September 2019. The qualifier 'liposomal' should be added after the invented name and before the strength to make a clearer distinction between liposomal and non-liposomal formulations of the same active substance to avoid medication errors. Since the two formulations may have different biodistribution and release properties, medication errors can pose serious risks to the health of patients.<br><br>It is therefore critical that the use of a non-liposomal amphotericin B (amphotericin B deoxycholate/conventional amphotericin B) formulation is not used to study bioequivalence at the same dose as a liposomal preparation as this could potentially cause significant harm to patients and/or volunteers in the bioequivalence studies.<br><br>**Proposed change:**<br><br>Parallel Design is required. If amphotericin B deoxycholate is used as a comparator the doses must not be the same. Further detail of the dosing is provided in the section below. | |
| Line 18 Strength | 7 | **Comment**: | **Partly accepted** |

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| | | This draft liposomal amphotericin B bioequivalence guidance currently recommends that with respect to strength, 3 mg/kg should be infused over 2 hours. The background provided is that 3 mg/kg is the usual starting dose and a sensitive dose in the clinical dose range.<br><br>This EMA consultation document also proposes using conventional amphotericin B as the comparator for liposomal amphotericin B preparations. It should be noted that use of conventional amphotericin B at doses of 3 mg/kg/d, or any dose above 1.5 mg/kg/d is not acceptable. A dose of 3 mg/kg/d of conventional amphotericin B could be potentially fatal. As stated in the Fungizone SmPC for conventional amphotericin B:<br><br>"Under no circumstances should a total daily dose of 1.5 mg/kg be exceeded. Fungizone overdoses can result in potentially fatal cardiac or cardiorespiratory arrest …"<br><br>As stated in the section above, there have been multiple cases in the UK where higher doses of conventional amphotericin were used and at least three documented cases of patients who were given conventional amphotericin B at the same or similar dose to liposomal amphotericin B which resulted in fatal outcomes (*O'Dowd, Br Med J. 2007; 335: 274; Hawkes, Br Med J. 2007; 335: 467*; https://www.gov.uk/drug-safety-update/parenteral-amphotericin-b-reminder-of-risk-of-potentially-fatal-adverse-reaction-if-formulations-confused). Hence, the use of 3 mg/kg/d in a comparative study of conventional versus liposomal amphotericin B would be deemed unacceptable. | As stated above, it has been further highlighted that the use of a liposomal comparator product only is foreseen.<br><br>Also as previously stated, the 3 mg/kg dose was selected considering the clinical doses used, the more than dose proportional pharmacokinetics at lower doses, and the safe administration in healthy subjects. |

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| | | In addition, liposomal amphotericin B is used over a range of doses, from 1 – 5 mg/kg/d in various conditions, and up to 10 mg/kg/d in mucormycosis. In a study of AmBisome (Gilead Sciences) in invasive aspergillosis, use of 10 mg/kg/d was associated with increased nephrotoxicity and hypokalaemia (*Cornely et al. CID. 2007; 44: 1289-1297*) and if comparator liposomal amphotericin B products resulted in the release of more free amphotericin B, the potential for increased risk to study subjects' safety is significant. However, to obtain good pharmacokinetic data on a novel liposomal preparation, dosing over a clinically relevant range of doses should be performed.<br><br>A third consideration is that the pharmacokinetics of liposomal amphotericin B are non-linear (*Groll et al. CID. 2019; 68:S260-74*), probably due to dose-related saturation of the reticulo-endothelial system (*Stone et al. Drugs 2016; 76: 485-500*). If only a single dose of liposomal amphotericin B is used this is not reflective of what will happen during routine clinical use; and parameters calculated from that should not be extrapolated to those that would be seen with multiple dosing over a longer duration.<br><br>**Proposed change**:<br><br>Strength: Liposomal amphotericin B infused over 2 hours per day at a range of doses from 1 – 5 mg/kg/d as appropriate for the underlying disease of the study subject. Background: Dosing of liposomal amphotericin B varies considerably depending on the disease and doses from 1-5mg/kg/d are all recommended on AmBisome® prescribing information and on | |

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| | | various international guidelines (for example ECIL-6 guidelines (Tissot 2017) and IDSA Guidelines (Pappas 2016) and consequently should be tested for bioequivalence. Multiple doses are also required to ensure that the non-linear pharmacokinetics can be studied and compared. | |
| Line 18 Number of studies | 7 | **Comment**:<br><br>This current draft bioequivalence guidance recommends that only one study should be performed.<br><br>To fully determine the properties of a generic application of liposomal amphotericin B it will be necessary to carry out at least 2 studies; one in paediatric patients and one in adult patients. Studies of AmBisome® pharmacokinetic (PK) parameters in paediatric patients, dosed at 2.5 – 10mg/kg/d have demonstrated significant variability between patients and are non-linear PK at higher doses (*Lestner et al. AAC. 2016; 60:7340-7346*). However, the non-linear PK profiles were different in paediatric patients to those seen in adults, demonstrating that they need to be studied separately for bioequivalence.<br><br>**Proposed change**:<br><br>One study to examine bioequivalence in adult patients | **Not accepted**<br><br>In line with the EMA Guideline on the investigation of bioequivalence (CPMP/EWP/QWP/1401/98 Rev. 1), the '*in vivo* healthy volunteers' model is regarded as adequate in most instances to detect formulation differences and to allow extrapolation of the results to populations for which the reference medicinal product is approved (the elderly, children, patients with renal or liver impairment, etc.). This is considered the case for liposomal amphotericin B. |
| Line 18 Other Critical Aspects | 7 | **Comment:**<br><br>This current guidance recommends an infusion time of 2 hours to lower the risk of infusion-related reactions. It also states that premedication, as appropriate, may also be given. | **Partly accepted**<br><br>The product-specific guideline recommendation regarding premedication is that it may be given, as appropriate. This would be addressed in the |

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| | | Use of premedication during a clinical study could impact the results related to the incidence of infusion-related toxicity and reflecting the underlying physico-chemical properties and attendant efficacy//toxicity of the product may mask important infusion-related reactions (IRR). Around 70% of patients receiving conventional amphotericin B will have an IRR within the first 7 days (*Goodwin et al. Clin Inf Dis. 1995; 20: 755-761*) and many pre-medications have been used to mitigate these, including diphenhydramine, corticosteroids, paracetamol and heparin. If pre-medication is to be allowed, it should be defined and used in all subjects to ensure equity in the results.<br><br>Drugs which are recommended for use if required for pre-medication with AmBisome (Gilead Sciences) as listed on the SmPC are diphenhydramine, paracetamol, pethidine and/or hydrocortisone. Drugs recommended for pre-medication with conventional amphotericin B as listed on the Fungizone SmPC are aspirin, other antipyretics, antihistamines or anti-emetics, pethidine, hydrocortisone or heparin.<br><br>**Proposed change**:<br><br>Dosing without premedication should be considered. If pre-medication is to be used, the same drug should be used for patients receiving both conventional and liposomal amphotericin B. As paracetamol, diphenhydramine, pethidine and hydrocortisone have been recommended for both formulations, one of these should be selected and used in all patients. | Investigator's Brochure for the study and considered by the approving Ethics Committee, etc. |
| Line 18 Analytes – liposomal | 7 | **Comment**: | **Partly accepted** |

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| amphotericin and non-liposomal drugs | | This draft guidance on liposomal amphotericin B bioequivalence recommends that both liposomal and non-liposomal drugs be used as analytes for study. The background provided on this is that liposomal and non-liposomal amphotericin B are both considered relevant to conclude on bioequivalence as they best reflect the biopharmaceutical quality of the proposed product.<br><br>As has been discussed in sections 1-4 of the general comments and in previous sections, the behaviour of non-liposomal drug, i.e., conventional amphotericin B, is significantly different to the behaviour of liposomal amphotericin B, particularly AmBisome® which is the reference liposomal product. As such, novel liposomal products should not be compared with conventional amphotericin B, but with AmBisome®.<br><br>Differences between conventional and liposomal amphotericin B formulations include marked differences in tissue distribution, pharmacokinetic behaviour, clinical efficacy and toxicity and hence conventional amphotericin B should not be used in our view, as a comparator for novel liposomal products.<br><br>**Proposed change**:<br><br>Analyte – a liposomal amphotericin B formulation should be compared to AmBisome as the reference liposomal amphotericin B product. | As stated above, it has been further highlighted that the use of a liposomal comparator product only is foreseen.<br><br>Of note, reference to measuring the 'non-liposomal drug' as analyte, is in the context of 'free' or 'unencapsulated' drug bound in different states following administration of a liposomal product. However, the term 'non-liposomal drug' is specifically used in the guideline for liposomal amphotericin B as compared to the term 'unencapsulated drug' in the guideline for liposomal doxorubicin (Pegylated liposomal doxorubicin hydrochloride concentrate for solution 2 mg/ml product-specific bioequivalence guidance (europa.eu)). This is because of the difference in structure i.e., amphotericin B is located in the lipid bilayer, so it is an integral part of the lipid bilayer, rather than for doxorubicin which is located inside the liposome and the lipid bilayer is surrounding it.<br><br>As stated above, the release mechanism of amphotericin B and activity of liposomal amphotericin B at the site of the fungal invasion are not fully understood. Therefore, liposomal and non-liposomal amphotericin B are both considered relevant to conclude on bioequivalence as they best reflect the biopharmaceutical quality of the proposed product. |

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| Line 18 Analyte – plasma/serum | 7 | **Comment**:<br><br>This draft guidance on liposomal amphotericin B bioequivalence recommends that serum and plasma are to be used as the sites for an analyte measurement.<br><br>If serum and plasma are to be used as the sites for analyte measurement, it is important to differentiate between the various forms of amphotericin B, which can include total drug, protein-bound drug, liposome-bound drug and unbound amphotericin B in these matrices. Unbound amphotericin B is responsible for most of the toxicity of the drug whereas liposome-bound is less likely to cause toxicity as it acts as a carrier for the amphotericin B which is not available to interact with human cholesterol and cause toxicity. If the liposome is not completely disrupted, it may result in an underestimate of the drug present (*Stone et al. Drugs 2016; 76: 485-500*).<br><br>Currently, there are no accepted assays for measuring protein-bound or liposome-bound amphotericin B, so the only option would be to completely disrupt the liposomes, release all the amphotericin B and measure total amphotericin B. However, that is not an accurate reflection of what is occurring in the body and negates the benefit of liposomal use, which binds the amphotericin B and in so doing, limits its toxicity. Hence, measurement of amphotericin B concentrations will be fraught with difficulties in interpretation and hence comparison between products will also be extremely difficult, if not impossible, to interpret using this method. | **Not accepted**<br><br>To clarify the "tick box" plasma/serum does not indicate that amphotericin should be analysed in both serum and plasma but that blood as matrix should be used. Plasma concentrations of liposomal and non-liposomal amphotericin B plasma should be evaluated.<br><br>For bioequivalence studies, robustly validated and reliable analytical methods allowing clear and consistent distinction between encapsulated and unencapsulated amphotericin B should be used. |

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| | | **Proposed change**:<br><br>Interpretation of measurements of amphotericin B concentrations in serum/plasma is complex and there is no consensus as to what amphotericin B bound in different states means. Thus, this measure of bioequivalence should not be included for liposomal amphotericin B. | |
| Line 18<br>Enantioselective analytical method | 7 | **Comment:**<br><br>This current draft bioequivalence guidance for liposomal amphotericin B states that enantioselective analysis is not required.<br><br>Amphotericin B is a single stereoisomer – enantioselective analysis not required. | **Accepted.**<br><br>The comment supports the wording in the guideline. |
| Line 18<br>Main Pharmacokinetic variables | 7 | **Comment:**<br><br>The main pharmacokinetic variables proposed in this draft bioequivalence guidance are $AUC_{0-t}$, $AUC_{0-\infty,}$ Cmax, partial AUCs (e.g. liposomal amphotericin B: $AUC_{0-10h}$ and $AUC_{10-tlast}$; non-liposomal amphotericin B: $AUC_{0-24h}$ and $AUC_{24-tlast}$). The background/justification are stated as: $AUC_{0-t}$ and $C_{max}$ are considered insufficient to fully characterize distribution and elimination processes of liposomes, which release the active substance over a longer period of time.<br><br>Use of standard PK parameters are insufficient and inaccurate in characterising the distribution and elimination of liposomes. It is known that liposomal amphotericin B (AmBisome, Gilead Sciences Ltd.) accumulates at different concentrations in the | **Accepted**<br><br>As previously stated, it is not intended that a non-liposomal comparator be used and also it has been highlighted that demonstration of bioequivalence for liposomal amphotericin B is considered a part of the package and quality and non-clinical comparison, and a clinical therapeutic equivalence study where appropriate, should also be considered. |

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| | | tissues, with differing concentration-time profiles between these sites and plasma/serum (*Felton et al, Clin Micro Rev. 2014; 27:68-88*). Because of that, measuring amphotericin B in plasma/serum will not accurately reflect its tissue concentration and distribution. Liposomal amphotericin B preferentially accumulates in tissues rich in mononuclear phagocytic cells, such as the liver, spleen and bone marrow (*Groll et al. 2019*) with concentrations in the liver and spleen up to 5-fold higher than those in plasma. In contrast, pleural fluid concentrations may be only 5-25% of those found in the plasma (*Felton et al. Clin Micro Rev. 2014; 27: 68-88*).<br><br>One of the key drivers of the development of liposomal amphotericin B was to reduce the nephrotoxicity associated with the parent compound, hence the stability of the liposome is of fundamental importance to this. In a liposomal preparation where the liposomes are stable, the majority of the amphotericin B will be within the liposome structure – for AmBisome (Gilead Sciences Ltd.) 97% of amphotericin B is bound in the liposome at 4hr and 55% at 168hr (*Groll et al. 2019*). However, if liposomal formulations are measured where the binding of the amphotericin B is less stable, more of that will be released as free amphotericin B. Rather than simply measuring concentrations of amphotericin B, it is preferable to measure concentrations of amphotericin B in a functional assay, such as the red blood cell potassium release assay (*Olsen et al. Med Mycol. 2015; 53:107-118*) as mentioned in section 3 of the earlier general comments. This assay allow to compare the toxicity associated with different liposomal preparations in vitro, | |

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| | | with results reported as the concentration of the preparation which results in release of 50% of potassium from rat red blood cells after 12 hrs incubation. Using this assay, marked differences in the in vitro toxicity of liposomal amphotericin B products have been reported, with greater toxicity for Lambin® (Sun Pharmaceutical Industries Ltd, India) and Anfogen (Genpharma, SA, Argentina) than with AmBisome (Gilead Sciences Ltd; *Adler-Moore et al. Med Mycol. 2016; 54: 223-231*).<br><br>It has also been shown that for AmBisome (Gilead Sciences Ltd.), the liposome structure is able to transit whole through the fungal cell wall and hence the amphotericin B is only released at the fungal cell membrane, its target (*Walker et al. mBio. 2018; 9: e02383-17*). This ensures that the amphotericin B remains bound and hence reducing the potential for toxicity associated with free amphotericin B. Thus, measuring free amphotericin B in plasma does not accurately reflect the complex changes in biodistribution and its attendant effects in safety and efficacy associated with liposomal formulations<br><br>Elimination of amphotericin B is very different for conventional amphotericin B and liposomal amphotericin B, further underlining that conventional amphotericin B is not a suitable comparator in bioequivalence studies. For conventional amphotericin B, most of the drug is excreted unchanged in the urine (21%) and faeces (43%) with over 90% of the drug excreted in a week. In contrast, <10% of liposomal amphotericin B was excreted unchanged and no metabolites | |

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| | | were detected, with 55% of the amphotericin remaining in the liposomes at 1 week (*Groll et al. 2019*).<br><br>As previously discussed, liposomal amphotericin B does not display the same pharmacokinetic behaviour as conventional amphotericin B. For polyenes, the pharmacodynamic relationship which is most predictive of efficacy is $C_{max}$/fungal MIC (minimum inhibitory concentration) and maximising the $C_{max}$ leads to optimal outcomes (*Lepak & Andes, Cold Spring Harb Perspect Med 2015;5:a019653*), hence $C_{max}$ would be an important variable to measure as part of bioequivalence studies. | |
| Line 18<br>To be noted | 7 | **Comment:**<br><br>The current draft guidance states that "Proving equivalent efficacy and safety of a liposomal formulation developed to be similar to an innovator product is considered a totality of evidence approach, which, in addition to the pharmacokinetic study, also takes account of quality and non-clinical comparison, and a clinical therapeutic equivalence study, where appropriate."<br><br>It should be noted that while the qualitative and quantitative composition of alternative liposomal amphotericin B formulations may be identical to that of AmBisome®, even minor alterations in constituents can affect the association between amphotericin B and the liposomal membrane, thereby potentially altering a product's toxicity relative to that of AmBisome®. Similarly, differences in the manufacturing process used to create a liposomal amphotericin B formulation can affect | **Accepted**<br><br>As previously stated, it has been highlighted that demonstration of bioequivalence for liposomal amphotericin B is considered a part of the package and quality and non-clinical comparison, and a clinical therapeutic equivalence study where appropriate, should also be considered. |

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| | | the characteristics of the liposomal delivery vehicle, and thus the toxicity profile of the product (*Adler-Moore et al. Comparisons between different liposomal formulations of amphotericin B. Medical Mycology 2016; 54: 223-231*).<br><br>In addition to liposome components, the method used to manufacture the liposomes may will also affect its toxicity. For example, liposomes formed by sonication were found to be more toxic to mice than those produced by homogenisation, even though the liposomes had the same AMB:lipid molar ratios. Other studies have shown that various stresses on liposomes, such as sterile filtration during production, filtration prior to use, requirements for refrigeration, lyophilisation conditions, and dilution in infusion diluents, can lead to flocculation, aggregation, leakage of the drug, phase separation, and disintegration of the liposomes. Consequently, monitoring of any such changes is required at every manufacturing step to ensure batch reproducibility. (*Adler-Moore et al. Comparisons between different liposomal formulations of amphotericin B. Medical Mycology 2016; 54: 223-231*)<br><br>**Proposed change**:<br><br>As a minimum assessments should be conducted in addition to any formal pharmacokinetic analysis to assess equivalency of a reference liposomal amphotericin B product to a comparator drug. | |