



Center for Biologics Evaluation and Research
Office of Therapeutic Products
Office of Plasma Protein Therapeutics
Division of Hemostasis
Hemostasis Branch 2

MEMORANDUM

To: Administrative file for BLA STN 125706/65

From: Andrey Sarafanov, PhD; CBER/OTP/OPPT/DH/HB2

Through: Mikhail Ovanesov, PhD; Branch Chief; CBER/OTP/OPPT/DH/HB2
Zuben Sauna, PhD, Division Director; CBER/OTP/OPPT/DH

Applicant: Mesoblast Inc.

Product: Remestemcel-L [RENUMESC; RYONCIL]

Indication Treatment of acute Graft versus Host Disease (aGvHD) in pediatric patients, when the aGvHD has been refractory to treatment with systemic corticosteroid therapy (SR-aGVHD)

Subject: Consult review of information for extractables and leachables assessment from the container closure system for final drug product

CC: Adriane Fisher, RPM; CBER/OTP/ORMRR
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REVIEW SCOPE

As a consult reviewer, I was asked to provide a review of analytical assessment of leachables from the container closure system (CCS) in the final Drug Product (DP). The active ingredient in DP is ex vivo cultured adult human mesenchymal stromal cells. The Applicant (Mesoblast Inc.) provided the results of an extractables and leachables (E&L) study in Amendment 65, in response to an FDA Complete Response Letter. The Applicant had previously committed

performing an E&L study (Amendment 45, July 21, 2020) to support the use of the CCS (6 mL vials by (b) (4) I was requested to review the following documents:

1. *crl-response-28jan23.pdf*; section 6.5.7 (Other-CMC-7, pages 44-48)
2. *report-leachables-6ml-vial-sim-use-v1nnf520-r1.pdf*
3. *protocol-pr-111-6ml-at-vial-48m-sim-extractables-v1.pdf*.

The additional three documents from the previous submission (Amendment 45) were also provided for my information:

- *report-tox-risk-asess-6ml-vial-leachables-12-dec-2022.pdf*
- *3.2.P.7 Container closure system.pdf*
- *c20453-report-tox-ra-extract-leach-container-closure.pdf*.

I was also asked to address three questions from the Review Team (see below).

BACKGROUND INFORMATION

To ensure my adequate assessment of the E&L information, I performed a brief review of the manufacturing process to identify the major steps from which the leachables are accumulated in DP and the high-risk process components for which the extractables studies are usually required. I also reviewed the DP composition to understand its extraction propensity and possible interference with analytical quantitation of the respective compounds.

Based on my review of the manufacturing process, I determined that the high-risk manufacturing steps are those starting from the (b) (4)

that includes the use of such high-risk leachables materials as (i) (b) (4) The downstream steps also include other high-risk components such as (b) (4) (section 3.2.P.3.3), which may be also sources of potential leachables in the DP. With respect to the composition of the DP, the most important components affecting leachables appearance and detection are (i) Dimethyl Sulfoxide (DMSO, 10%) that facilitates accumulation of leachables from the CCS, cells, and (ii) human serum albumin (HSA, 5%) that may interfere with analytical quantitation of the compounds. In addition, the conditions of DP storage involve a drastic change of temperature (deep freezing of DP to -135 C) which may facilitate leaching of certain compounds (section 3.2.P.1).

The major CCS system components in contact with DP are the vial made from cyclo-olephin copolymer (COC) and the stopper made from thermoplastic elastomer (TPE). Both the vial and stopper comply with (b) (4) articles (section 3.2.P.7) and are widely used for the storage of biologicals. The CCS for Drug Substance (DS) is used in the upstream process steps, after which the intermediate is (b) (4) times which would result in the removal of potential leachables (section 3.2.S.6). Thus, the CCS is not a critical concern from the perspective of E&L assessment.

REVIEW OF INFORMATION

1. *crl-response-28jan23.pdf* (CMC 7, pages 44-48)

The Applicant provided an overview of the background information and the performed E&L studies. They provided considerations for design of the simulated leachables study (which the Applicant refers to as an extractables study) that involved (i) replacement of the actual DP with (b) (4) simulating the ~10% DMSO solution with DP, and (ii) calculation of the Analytical Evaluation Threshold (AET, µg/mL) specific to each method used. The AET values were calculated based on the safety considerations which justified the maximal burden of 120 µg/mL of an organic leachable upon product use. The simulation study involved two arms: (i) when the extraction of the test vials was performed at (b) (4) and (ii) when the same operation was carried out following storage of vials at (b) (4) to model the drastic temperature change.

2. *protocol-pr-111-6ml-at-vial-48m-sim-extractables-v1.pdf*

This document describes the simulated leachables (extractables) study to provide chemical characterization of the 6 mL CCS under the same freezing, storage, and thaw conditions as for DP vial. The study is to span the 48-month shelf-life currently proposed for the DP and, by this, to simulate the clinical use of the vial and stopper across the product shelf-life.

According to the protocol, the test solution (b) (4) is filled in the vial which is then placed under storage at -135 C until testing. Subsequently, the sample vials will be removed from the dry shipper to thaw at room temperature for (b) (4) prior to the analysis for *organic compounds* by (b) (4) and for *elemental compounds*, by (b) (4). For each method, calculation of respective AET (b) (4) included method-dependent uncertainty factors (4-10). The test time points are to be 6, 12, 18, 24, 30, 36, 42 and 48 months. Any chemicals/compounds detected above the AET will require a full toxicological assessment and evaluation for adjusted dose-based threshold (DBT) coupled with a targeted study plan.

3. *report-leachables-6ml-vial-sim-use-v1nnf520-r1.pdf*

This document describes the analysis in detail, including description of the samples, sample preparation and methodology. For each method to analyze organic leachables, specific sets of standards to cover major chemical types of the expected compounds are listed. Additional methods included analysis of non-volatile residue (NVR) upon sample evaporation by (b) (4). Upon analysis, no *organic compounds* above respective AET values, (b) (4), method-dependent, Table 5), were detected. The identified compound (b) (4) and estimated concentrations (µg/vial) of the respective standards were listed in report *crl-response-28jan23.pdf* reviewed above (Tables 4-7).

The respective method-specific (b) (4) limits of detection (LOD, Signal/Noise= (b) (4)) and quantitation (LOQ, S/N= (b) (4)) were determined upon testing the methods for suitability using the sets of the standards covering major chemical classes of the expected compounds as was clarified by the Applicant on May 15, 2023 (STN 125706/78, Question 3). The respective LOQ values (b) (4), method-dependent, Tables 8 and 13) were specified in the response for

Information Request sent on May 5, 2023 (FDA RFI #47). Importantly, these LOQ values were (b) (4) times below the respective AET values, which ensured correctness of the quantitation. (while still being semi-quantitative due to lack of validation of the methods). Using not validated methodology is a common practice in such analysis, as it is not possible to use standards identical to each of the detected numerous compounds, many of these are not identified.

Elemental leachables detected by (b) (4) were also listed; these included (b) (4) with concentrations close to LOD and far below the respective Margins of Safety (MOS). Toxicological risk assessment (TRA) of these compounds concluded their safety in final DP (*report-tox-risk-assess-6ml-vial-leachables-12-dec-2022.pdf*).

REVIEW CONCLUSION AND COMMENTS

I. The analyses of the leachables study provided by the Applicant likely relevant to only initial test point in the ongoing leachables study (aimed to involve multiple test points over the shelf-life according to the stability protocol), but this is not clearly stated in the report. From the analytical perspective, the provided information for assessment leachables from the CCS is acceptable.

However, for the toxicological data review, I recommend considering at least several-fold safety margin (related to analytical “uncertainty factor”) for each compound due to the semi-quantitative nature of the analytical methods used. The semi-quantitative nature of methods implies that the amount of leachables in the final product may potentially be significantly underestimated.

II. Questions from the review committee and my responses are provided below:

Question 1. Please assess whether the (b) (4) used in study *leachables-6ml-vial-sim-use-v1nnf520-r1.pdf* is appropriate to establish the safe use of the 6 mL ready-to-fill closed vials when used for the purpose of BLA 125706.

Comment

The (b) (4) used in the simulation of actual DP use is appropriate, as the propensity of this solvent to extract organic impurities from the CCS can be considered similar or even higher than that of the actual DP matrix containing ~10% of DMSO.

Question 2. The Sponsor stated (page 46 of 85, *crl-response-28jan23.pdf*) that given that no specific chemical standard can be applied to fully quantify a class of chemicals, (b) (4)

(b) (4) was used. Please assess whether the Applicant’s approach is adequate for the purpose of assessing these leachables classes.

Comment

The Applicant’s approach is generally correct for the measurement of organic leachables. However, it should be noted that the use of standards which are not the same as analyzed compounds is considered semi-quantitative, as, when using such approach, the methods cannot be fully validated according to ICH Q2, *Validation of analytical procedures*. Therefore, the quantitation error can be up to several-fold based on my experience. Such error is related to the “uncertainty factor” and “safety margin” parameters used for respective toxicological assessment.

Question 3. Please assess whether the protocol proposed in *protocol-pr-111-6ml-at-vial-48m-sim-extractables-v1.pdf* is appropriate to evaluate extractables in ongoing stability studies.

Comment

This protocol is acceptable for the assessment of leachables from the CCS. However, the leachables appearing in the final DP can also result from other process steps, especially from those which are close to the (b) (4) step when no (b) (4) of the intermediate is performed.

Thus, the correct study design used in such cases simulates the manufacturing process starting from one of the upstream steps. According to my assessment, such a step is the Step (b) (4) that uses materials of high risk of

leachables such as (b) (4) processing (b) (4)

Furthermore, the downstream steps include several other components with a high-risk of leachables such as (b) (4). To ensure adequate simulation of these respective process conditions, it will be sufficient to use only the respective buffers (with the (b) (4) excipients) and perform these operations using respective maximal hold times and temperatures. It is sufficient to use the respective buffers alone, without cells or proteins (human serum albumin from the Plasma-Lyte A solution), as cells and proteins will interfere with the detection of compounds, and they do not affect the extraction propensity of the solution.

Therefore, the study planned by the Applicant to assess the leachables from the CCS is insufficient to cover all leachables in final DP, if a study of cumulative leachables (i.e., leachables from both the upstream process and the CCS), involving the testing at multiple time points over the product shelf-life storage, is not performed in another study arm. Such a study is required for a BLA approval according to CBER policy, which is in turn, based on relevant sections of CFR (§211 (65, 72, 94); §314.70; and §600.11(h)) describing requirements for assessment of impurities in drug products.