

CBER CMC BLA Review Memorandum

BLA STN 125706

Remestemcel-L-rknd

Reviewer/Title/Affiliation

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1. BLA#: STN 125706**2. APPLICANT NAME AND LICENSE NUMBER**

Mesoblast, Inc.

3. PRODUCT NAME/PRODUCT TYPE

USAN/Proper/Non-Proprietary Name: Remestemcel-L-rknd

Proprietary Name: RYONCIL

4. GENERAL DESCRIPTION OF THE FINAL PRODUCT

Remestemcel-L is an allogeneic, off-the-shelf cellular therapy product comprised of mesenchymal stromal cells (MSCs) cryopreserved in a suspension of Plasma-Lyte A supplemented with human serum albumin (HSA) solution and 10% dimethyl sulfoxide (DMSO). The drug product (DP) is distributed in 6 mL cryovials with a label claim of 25 million cells per vial. The potency of each DP lot is measured by cell concentration, cell viability, and the level of TNFR1 in MSC (b) (4) as measured by a commercial (b) (4). The proposed indication is treatment of pediatric subjects younger than 18 years of age with steroid-refractory graft-versus-host disease (SR-aGVHD), and the recommended treatment plan is a four-week course of twice weekly infusions of 2 million cells/kg.

5. MAJOR MILESTONES

Initial Modules Received	May 29, 2019
Module 3 Received	January 31, 2020
Application Filed	March 30, 2020
Mid-Cycle Communication	June 1, 2020
Late-Cycle Communication	July 23, 2020
Advisory Committee Meeting	August 13, 2020
Cut-off Date for Amendment Review	August 20, 2020
PDUFA Action Date:	September 30, 2020

6. CMC/QUALITY REVIEW TEAM

Reviewer/Affiliation	Section/Subject Matter
Matthew Klinker, PhD BLA Chair; CMC Reviewer OTAT/DCGT/CTB	Environmental Analysis (1.12.14) Labeling (1.14) DS General Information (3.2.S.1) DS Manufacturer(s) (3.2.S.2.1) DS Description of Manufacturing Process (3.2.S.2.2) DS Characterization (3.2.S.3) DS Specifications/Justification (3.2.S.4.1, 3.2.4.5) DS Batch Analyses (3.2.S.4.4) DS Reference Standards (3.2.S.5) DP Description and Composition (3.2.P.1) DP Pharmaceutical Development (3.2.P.2.1) DP Manufacturer(s) (3.2.P.3.1) DP Description of Manufacturing Process (3.2.P.3.2) DP Controls of Critical Steps and Intermediates (3.2.P.3.4) DP Process Validation and/or Evaluation (3.2.P.3.5) DP Control of Excipients (3.2.P.4) DP Specifications/Justification (3.2.S.5.1, 3.2.5.6) DP Batch Analyses (3.2.S.4.4) DP Characterization of Impurities (3.2.P.5.5) DP Reference Standards (3.2.P.6) Facilities and Equipment (3.2.A.1) Batch Records (3.2.R)
Steven Bauer, PhD CMC Reviewer OTAT/DCGT/CTTB	DS Stability Summary and Conclusions (3.2.S.7.1) DS Post-approval Stability Protocol (3.2.S.7.2) DS Stability Data (3.2.S.7.3)
Heba Degheidy, MD, PhD CMC Reviewer OTAT/DCGT/CTTB	DP Analytical Procedures/Validation (3.2.P.5.2, 3.2.P.5.3)
Alyssa Kitchel, PhD CMC Reviewer OTAT/DCGT/CTB	DS Controls of Critical Steps and Intermediates (3.2.S.2.4) DS Process Validation and/or Evaluation (3.2.S.2.5) DS Manufacturing Process Development (3.2.S.2.6)
Elizabeth Lessey-Morillon, PhD CMC Reviewer OTAT/DCGT/CTB	DS Control of Materials (3.2.S.2.3) DS Analytical Procedures/Validation (3.2.S.4.2, 3.2.S.4.3) DP Control of Materials (3.2.P.3.3)
Bao-Ngoc Nguyen, PhD CMC Reviewer OTAT/DCGT/CTB	DS Container Closure (3.2.S.6) DP Container Closure (3.2.P.7) DP Stability (3.2.P.8)

7. INTER-CENTER CONSULTS REQUESTED

Reviewer/Affiliation	Section/Topic	In agreement with consult recommendations?
Samanthi Wickramasekara, PhD CDRH/OSEL/DBCMS	DS and DP Container Closure Extractables and Leachables (3.2.S.6, 3.2.P.7)	YES

Reviewer/Affiliation	Section/Topic	In agreement with consult recommendations?
Hainsworth Shin, PhD CDRH/OSEL/DBCMS	DS and DP Container Closure Toxicological Risk Assessment (3.2.S.6, 3.2.P.7)	YES
Arifa Khan, PhD CBER/OVRR/DVP/LR	DCB Adventitious Viral Agents Testing (3.2.S.4.3)	YES

8. SUBMISSION(S) REVIEWED

Date Received	Submission	Comments/ Status
6/4/2019	125706/0.0	<ul style="list-style-type: none"> Rolling BLA Submission Initiated Module 4 and Draft Labeling/Proprietary Name
12/31/2020	125706/0.2	Module 5
1/31/2020	125706/0.3	Module 3 – Application Complete
3/17/2020	125706/0.13	Response to DMPQ IR#7
3/27/2020	125706/0.15	Response to CMC IR#9
4/20/2020	125706/0.19	Response to CMC IR#15
5/6/2020	125706/0.23	Response to CMC IR#17
5/14/2020	125706/0.26	Response to DBSQC IR#16
5/29/2020	125706/0.27	Response to CMC IR#18 (CDRH)
6/4/2020	125706/0.28	Response to DMPQ IR#19
6/5/2020	125706/0.29	Applicant Slides from Mid-cycle communication
6/5/2020	125706/0.30	Response to DBSQC IR#20 (Lot Release Protocol)
6/15/2020	125706/0.32	Response to CMC IR#24 (Mid-cycle follow-up, partial)
6/18/2020	125706/0.34	Response to CMC IR#23
6/24/2020	125706/0.35	Response to DMPQ IR#21
6/29/2020	125706/0.36	Response to DMPQ IR#26 (Partial)
6/30/2020	125706/0.37	Response to DBSQC IR#25
7/1/2020	125706/0.38	Response to DMPQ IR#21
7/2/2020	125706/0.39	Response to CMC IR#18 (CDRH)
7/10/2020	125706/0.41	Draft Table of Contents for Applicant's Advisory Committee Briefing Document
7/14/2020	125706/0.42	Response to DMPQ IR#26 (Partial)
7/21/2020	125706/0.44	Response to CMC IR#33 (Labeling)
7/21/2020	125706/0.45	Response to CMC IR#32 (CDRH)
7/27/2020	125706/0.46	Response to DMPQ IR#28
7/28/2020	125706/0.48	Response to CMC Question from Late-Cycle Meeting
8/12/2020	125706/0.49	Response to CMC IR#24

9. REFERENCED REGULATORY SUBMISSIONS

Submission	Holder	Referenced Item	Letter of Cross-Reference?	Comments/Status
MF5 (b) (4)	Lonza Walkersville, Inc	Manufacturing Facility for Lonza Walkersville (LWI)	Yes	Defer to DMPQ Reviewer

Submission	Holder	Referenced Item	Letter of Cross-Reference?	Comments/Status
MF5 (b) (4)	Lonza Bioscience Singapore Pte, Ltd	Manufacturing Facility for Lonza Bioscience Singapore (LWI)	Yes	Defer to DMPQ Reviewer
MF2 (b) (4)	Mesoblast, Ltd	Manufacture of MSCs	N/A	Describes manufacturing process used to make remestemcel-L under IND
IND 7939	Mesoblast, Inc	Development History of remestemcel-L	N/A	IND under which clinical studies submitted with this application were conducted

10. REVIEWER SUMMARY AND RECOMMENDATION

A. EXECUTIVE SUMMARY

The subject of BLA 125706 is remestemcel-L, a cryopreserved suspension of allogeneic culture-expanded MSCs derived from bone marrow aspirate collected from healthy human donors. Mesoblast, Inc. (“the Applicant”) requests a license to market remestemcel-L in the US under the proprietary name RYONCIL for the treatment of SR-aGVHD in pediatric subjects. SR-aGVHD is a serious and life-threatening complication of hematopoietic stem cell transplants, and there are no drugs approved for this condition for patients younger than 12 years old.

The manufacturing process consists of two stages of expansion in culture. First, adherent cells from the starting material are cultured in cell factories through (b) (4) passages. At the end of this first stage the cells are harvested, pooled, and dispensed into (b) (4) cryobags for long-term storage. Each batch of this drug substance intermediate is referred to as a donor cell bank (DCB) lot. In the second stage, the contents of a (b) (4) DCB (b) (4) are thawed and the cells are expanded through an additional 3 passages. The cells are harvested at the end of the second stage as the DS, then formulated with Plasma-Lyte A supplemented with HSA solution and 10% DMSO to make the DP. The DP is then dispensed into 6 mL cryovials and cryopreserved.

The remestemcel-L development program was acquired by the Applicant in 2013 from Osiris Therapeutics, Inc. (OTI), who had been developing remestemcel-L under IND 7939 since 1998. OTI conducted multiple randomized clinical trials for a variety of conditions, including a randomized placebo-controlled study of SR-aGVHD in subjects of all ages. All of these trials failed to meet their primary endpoint and remestemcel-L is not approved for any indication in the US. After acquisition, the Applicant conducted MSB-GVHD001 (“Study 001”), a single-arm trial that enrolled 55 pediatric subjects with SR-aGVHD between 2015 and 2017. The clinical data from Study 001 is the primary basis of efficacy for this BLA application.

Before acquisition by the Applicant, remestemcel-L DCB and DP lots were manufactured and stored at the Lonza Walkersville facility (LWI), and the Applicant took

control of all unused DCB and DP material at the time of acquisition. The DP lots used in Study 001 were made from DCB material made at LWI in 2008-2009 and expanded through the second stage of manufacturing at the Lonza Singapore facility (LBSS) in 2015-2016. The proposed commercial product is also made from DCB material made in 2008-2009 that is thawed and expanded at LBSS to make the DP, and the Applicant initiated a new manufacturing campaign at LBSS in late 2019 to qualify the manufacturing process and make product intended for initial commercial release.

Our areas of focus during review were in understanding the product's activity and establishing controls that would ensure that commercial DP lots have a level of potency similar to the DP lots used in the primary clinical trial (Study 001). The application as originally submitted identified two lot release assays as tests for potency: (1) an (b) (4) for TNFR1 performed on MSC (b) (4), and (2) an *in vitro* bioassay intended to measure the capacity of each lot of product to inhibit activation of allogeneic T cells (inhibition of IL-2R α assay). Through interactive review, we engaged the Applicant on several deficiencies regarding these assays:

1. The potency of lots made in 2019-2020 for initial commercial release appeared to be reduced relative to lots used in Study 001 as measured by the inhibition of IL-2R α assay. We asked the Applicant to address this reduced apparent potency, and they attributed this difference to variability in the assay. Furthermore, they indicated that the assay was too variable to be used as a quantitative assay for lot release, and proposed to designate this as a qualitative assay for activity rather than an assay for potency. Even if only qualitative, the inhibition of IL-2R α assay could be considered a secondary assay in a potency assay matrix as recommended by FDA guidance, if the TNFR1 assay was found to be an appropriate quantitative test for potency. The Applicant later agreed to revise the specification for the inhibition of IL-2R α assay to the minimum value of DP lots used in Study 001, ensuring that if the application were approved that lots with inhibition of IL-2R α results below those of DP lots used in Study 001 would not be released for commercial use. See review of Module 3.2.P.2.3 for a more thorough discussion of this topic.
2. The data provided in the original submission to support a relationship between TNFR1 levels and the potency or activity of the product were not convincing. At the mid-cycle communication, the Applicant provided results of a re-analysis of previous clinical and product data purporting to show that TNFR1 levels were associated with survival outcomes, however we did not agree with the Applicant's conclusion because of severe limitations in the analytic approach. See review of Module 3.2.P.2.3 for a more thorough discussion of this topic.

At the mid-cycle communication (Amendment 32, dated June 15, 2020), the Applicant committed to repeating experiments performed in 2005 that were the basis of the rationale supporting the use of the TNFR1 assay as a test for potency. The results of these new experiments were submitted to the BLA late in the review cycle and showed that TNFR1 knockdown had no detectable effect on the capacity of remestemcel-L to inhibit *in vitro* T cell activation, directly contradicting the results obtained in 2005. The Applicant acknowledged that these results show that the immunomodulatory effect of

remestemcel-L on T cells is TNFR1-independent and provided some additional data to support their claim of a TNFR1-dependent immunomodulatory effect on monocytes/macrophages, but the data provided were not adequate to support the Applicant's conclusion. The Applicant has therefore not provided a scientific rationale for this assay that is supported by data, nor demonstrated the relevance of TNFR1 levels to product potency.

Potency assay issues were the subject of the CMC portion of the meeting of the Oncologic Drugs Advisory Committee (ODAC) held for this application on August 13, 2020. We asked the committee to provide their opinions and suggestions regarding characterization and potency assays for remestemcel-L. Members of ODAC noted that assessing the current potency assay is difficult because of the complex mechanism of action for remestemcel-L, and one member suggested the addition of a transcriptome analysis to lot release testing. While the committee discussed the challenges of defining potency for this product, they did not directly address the adequacy of the TNFR1 assay, and therefore, the feedback provided did not materially affect the review team's position that this assay may not be adequate to assure consistent lot-to-lot potency.

Without an appropriate laboratory test for potency, the Applicant cannot ensure that each lot has a sufficient level of potency to mediate the desired clinical effect or characterize the product's stability and establish a meaningful shelf life. Additionally, the Applicant will need to implement a major manufacturing change when their current stock of DCB material is depleted and they begin manufacturing new DCB lots, and analytical methods alone are unlikely to be sufficient to demonstrate product comparability without a robust and scientifically sound potency assay. The Applicant indicated in Amendment 15 (dated March 27, 2020) that their remaining DCB material could produce enough product to treat fewer than (b) (4) subjects, so this manufacturing change is likely to occur in the near future should this application be approved.

The basis for the approval of a biologics license application should include a demonstration that the biological product that is the subject of the application has been shown to be "safe, pure, and potent" [42 USC 262(a)(2)(C)(i)(I)]. The potency of biological products can be demonstrated by "appropriate laboratory tests or by adequately developed and controlled clinical data" [21 CFR 600.3(s)]. We recommend that this application not be approved because the Applicant neither has an appropriate laboratory test for potency nor has provided adequately controlled clinical data to indicate potency. In their resubmission, the Applicant should provide data demonstrating that the product attributes measured by potency assays used for lot release testing and establishing stability have a statistically meaningful relationship to clinical outcomes, surrogate markers of *in vivo* activity, or a relevant product activity as measured by an *in vitro* biological assay.

B. RECOMMENDATION

We recommend that the BLA not be approved and that the following Complete Response items be issued:

I. COMPLETE RESPONSE

After review of your application, we have concluded that we cannot grant final approval because of the deficiency outlined below.

1. All lots of remestemcel-L, Drug Product (DP), are tested for potency using an (b) (4) to measure the amount of TNFR1 in the mesenchymal stromal cell (MSC) (b) (4). As this is a non-biological analytical assay for potency, the product attribute measured by this assay should have a demonstrated relationship to a relevant product-specific biological activity. The information you provided in your application was insufficient to establish that your analytical assay for potency measures a product attribute related to the specific ability or capacity of the product per 21 CFR 600.3(s) for the following reasons:
 - a. You provided analyses in Amendment 32 (dated June 15, 2020) purporting to show an association between TNFR1 results and survival outcomes. We do not agree that these analyses adequately support your conclusion, as an association was only observed when analyzing data pooled from multiple clinical studies, where differences in study populations and manufacturing processes for the DP used in these studies severely limit the interpretability of these analyses. You cite manufacturing changes made in 2009 as the source of increased TNFR1 levels and better clinical outcomes, but these changes were not identified in the summary of manufacturing process development you provided in your original submission of this application, and it is not clear how many of these changes were maintained when manufacturing was transferred to the Lonza Singapore facility (LBSS). Furthermore, at the time of implementation these changes were reported in an annual report to IND 7939 as “minor updates” to the manufacturing process, and were reported to have been implemented to simplify the process rather than improve the quality of the product. You also did not provide an explanation supported by data for how this process “optimization” could lead to production of a more consistent product, nor a justification for grouping lots made at LBSS with an updated manufacturing process with lots made at the Lonza Walkersville facility (LWI) in 2009 in this analysis without demonstrating product comparability. As such, the interpretability of these results is severely limited.
 - b. The data provided in your application do not establish a scientific rationale for your assay for product potency, and in fact suggest that the attribute measured by this assay is not related to the immunomodulatory activity of the DP. The basis for selecting TNFR1 as a marker of potency was a series of experiments conducted in 2005 using a previous version of the remestemcel-L product. These initial experiments showed that knockdown of TNFR1 reduced the capacity of MSCs in the precursor product to inhibit

T cell proliferation (Report R-045-05). In Amendment 32 (dated June 15, 2020), you committed to repeating these knockdown experiments using DP made using the current manufacturing process and to provide these results before the late-cycle meeting on July 23, 2020. You stated in this amendment that you anticipated “that the data will show that knockdown or neutralization of TNFR1 will impair the ability of MSC from the current manufacturing process to inhibit T cell proliferation *in vitro*.” The results provided in Amendment 49 (dated August 11, 2020), however, refuted this hypothesis, and in Report MR-128 you stated that these results instead demonstrate that “the immunomodulatory effects of remestemcel-L on activated T cell proliferation *in vitro* are independent of TNFR1 activity and expression.” We agree with this interpretation, and therefore the scientific rationale on which TNFR1 level was selected as an attribute related to product potency does not appear applicable to remestemcel-L made using the proposed commercial manufacturing process.

- c. In light of these results demonstrating that TNFR1 is dispensable for remestemcel-L’s immunomodulatory effect on T cells, you proposed an alternative mechanism of action for remestemcel-L at the meeting of the Oncologic Drugs Advisory Committee (ODAC) on August 13, 2020, in which remestemcel-L exerts immunomodulatory effects on macrophages rather than directly on T cells. The data you provided in Amendment 49 in support of this mechanism show that TNFR1 knockdown reduces the capacity of MSCs to respond to TNF- α , but these results do not adequately demonstrate that TNF- α -dependent factors are required for the purported effect on macrophages, or that a dependence on TNFR1 signaling is relevant in the context of an inflammatory milieu containing multiple cytokines that may activate the immunomodulatory activity of MSCs. Therefore, you have not adequately demonstrated that TNFR1 levels are related to the product’s immunomodulatory activity toward monocytes and/or macrophages.

Because of these deficiencies, you have not demonstrated that TNFR1 levels are relevant to product activity or related to clinical outcomes, and therefore it is not clear that this test can be considered an appropriate test for potency per 21 CFR 610.10. All biological products regulated under section 351 of the Public Health Service Act must meet prescribed requirements of safety, purity and potency per 21 CFR 601.2. An appropriate assay for potency is necessary to provide assurance of a consistent manufacturing process and establish product stability.

Additionally, you will need to implement a major change to the manufacturing process to continue production of donor cell banks (DCBs) when your current stock of DCB material is depleted. If you intend to leverage previous clinical results to support the safety and/or efficacy of the post-change product, you will need to conduct a convincing comparability exercise. If you have not identified product attributes that are associated with potency, however, it is unlikely that analytical methods alone will be sufficient to demonstrate product comparability to support such a change. Previous clinical results may not be considered

relevant to the post-change product if comparability cannot be demonstrated by analytical methods, and therefore new clinical trials may be necessary to support the safety and efficacy of the post-change product.

Please identify all assays that you consider tests for product potency and provide a justification for how controlling the attributes measured by these assays is adequate to ensure that each lot of remestemcel-L has acceptable levels of product activity. Additionally, please provide data demonstrating that the product attributes measured by potency assays used for lot release testing and establishing stability have a statistically meaningful relationship to clinical outcomes, surrogate markers of *in vivo* activity, or a relevant product activity as measured by an *in vitro* biological assay. If the product attributes measured are related to an *in vitro* activity of the product only, please also include a scientific rationale explaining the relevance of the *in vitro* activity to the clinical effect of the product.

Non-CR comments:

2. During review of your application, you agreed to revise the release specifications for TNFR1 and inhibition of IL-2R α assays to reflect the more stringent values observed among DP lots used in Study 001 compared to the initial specifications. Given the distribution of values obtained from vials within a single DP lot as observed in your PPQ multiple sampling analyses, testing a single vial from each lot may not be appropriate because the revised specifications fall within the expected range of values for a given lot, which may result in the rejection of entire lots, if the vial randomly chosen for testing happens to fall on the low end of this distribution. We therefore recommend that you develop a more thorough lot sampling approach that includes testing multiple vials from each lot, and that you revise your specifications for these assays to account for the distribution of values obtained from multiple vials within a lot. For each assay you should choose a minimum acceptable result for each vial, then perform a statistical analysis to determine how to set your specifications to ensure that the frequency of vials below this minimum value is acceptably low.
3. In Amendment 32 (dated June 15, 2020), you acknowledged that your inhibition of IL-2R α assay is not suitable as a quantitative assay due to variability and attributed this variability to differences in lots of (b) (4) [REDACTED]. If you intend to continue using this assay for DP lot release, we recommend that you continue to develop this assay and revise the testing procedure as appropriate to improve robustness to (b) (4) [REDACTED] variability and provide more consistent results.
4. In the Chemistry, Manufacturing, and Controls Information Request #23, we stated that assays established as stability-indicating for the DP may not be stability-indicating for the DCB, and recommended that you establish these assays as stability-indicating for the DCBs in addition to the DP. In Amendment 34 (dated June 18, 2020), you indicate that (b) (4) [REDACTED]

(b) (4).” Please note that assays that are not established as stability-indicating specifically for DCB material may not be relevant for use in future comparability exercises performed after changes are made to the DCB manufacturing process.

5. (b) (4)

[REDACTED]

6. During validation of the (b) (4) for TNFR1 and IL-2R α that are used for DP lot release testing, you evaluated the effects of (b) (4) different lots of each (b) (4), however, this evaluation was performed using results obtained from different DP lots. We recommend that you characterize variability in performance between lots of (b) (4) by using (b) (4) lots to test the same test article (i.e., the same cell (b) (4)).
7. In Amendment 34 (dated June 18, 2020) you committed to submitting results from additional studies to address deficiencies in the validation of your (b) (4) assays by August 31, 2020. You provided your response in Amendment 52 (dated September 1, 2020) and Amendment 54 (dated September 14, 2020), however these amendments were not reviewed due to receipt late in the review cycle. Additional information may be requested after review of the materials submitted.
8. In Amendment 45 (dated July 21, 2020), you committed to submitting results from additional extractable and leachable studies to support the use of the 6 mL (b) (4) Vials as container closure for the DP. As these reports were not submitted before the action date for this application they were not reviewed, and additional information may be needed after the results are reviewed.
9. In your response to FDA late-cycle meeting materials (dated July 23, 2020), you committed to providing an updated assessment of DP stability using the agreed-upon revised DP specifications. You provided your response in Amendment 55 (dated September 18, 2020), however this amendment was not reviewed due to receipt late in the review cycle. Additional information may be requested after review of the materials submitted.

II. SIGNATURE BLOCK

Reviewer	Concurrence	Signature
Matthew Klinker, PhD Biologist OTAT/DCGT	Concur	
Heba Degheidy, MD, PhD Staff Fellow OTAT/DCGT	Concur	
Alyssa Kitchel, PhD Biomedical Engineer OTAT/DCGT	Concur	
Elizabeth Lessey-Morillon, PhD Biologist OTAT/DCGT	Concur	
Bao-Ngoc Nguyen, PhD Staff Fellow OTAT/DCGT	Concur	
Steven Bauer, PhD Supervisory Biologist OTAT/DCGT	Concur	
Melanie Eacho, PhD Chief, Cell Therapy Branch (CTB) OTAT/DCGT	Concur	
Steven Oh, PhD Deputy Division Director OTAT/DCGT	Concur	
Raj Puri, MD, PhD Division Director OTAT/DCGT	Concur	

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INTRODUCTION

PRODUCT DEVELOPMENT NARRATIVE

Osiris Therapeutics, Inc. (2002-2013)

Product development was initiated by Osiris Therapeutics, Inc. (OTI) under IND 7939. (b) (4) in 2002 OTI began manufacturing an unrelated and non-HLA-matched allogeneic version of the product called “prochymal” that is the precursor of the proposed commercial product remestemcel-L. The OTI manufacturing process developed through several stages, and in 2007 manufacturing began under contract at the Lonza Walkersville (LWI) facility. At LWI manufacturing used a two-step process where MSCs were isolated from bone marrow aspirates (BMAs) and expanded through (b) (4) passages before cryopreservation as a drug substance intermediate called donor cell banks (DCBs), then thawed and further expanded through Passage (b) (4) and cryopreserved as the DP. This two-stage culture expansion is used throughout product development as well as in the proposed commercial manufacturing process.

OTI conducted two Phase 3 randomized, placebo-controlled studies in patients with acute graft-vs.-host disease (aGVHD): (1) Protocol 265 for adult patients in combination with corticosteroids, and (2) Protocol 280 for patients of all ages with steroid-refractory aGVHD (SR-aGVHD). In 2009 OTI began a rolling BLA submission (BLA 125334) while completing these studies, but both Protocol 265 and 280 failed to meet their primary endpoints and OTI withdrew the application before it was complete.

In anticipation of their licensing application, OTI applied to USAN for a proper name and prochymal was designated “remestemcel-L” by USAN in 2009. After the failure of their randomized control trials to meet primary endpoints, OTI maintained Protocol 275, an expanded-access protocol (EAP 275) under which pediatric patients with SR-aGVHD received remestemcel-L in addition to standard of care.

Mesoblast, Inc. (2013-present)

In 2013, the remestemcel-L development program was acquired by the Applicant, Mesoblast, Inc. (MSB). This acquisition included all remaining remestemcel-L DP and DCB lots, as well as responsibility for the ongoing EAP 275. MSB continued enrolling subjects under EAP 275 using DP made by OTI, and reanalyzed data from the small number of pediatric patients (n=28) enrolled in OTI’s Protocol 280. This reanalysis also used overall response (OR) rate on Day 28 rather than the rate of a complete response (CR) ≥ 28 days within 100 days of the first infusion as used originally in Protocol 280. Based on results from pediatric patients enrolled in 275 and 280, MSB proposed a single-arm study in pediatric patients to support licensure. This study (MSB-GVHD001, or Study 001) would use Day 28 OR as the primary endpoint, and would be considered to have demonstrated efficacy if the results excluded a Day 28 OR of 45% as the null hypothesis.

MSB used DP lots manufactured by OTI to treat subjects under EAP 275, but at this time had not manufactured any new product since acquisition. As the remaining DP lots

acquired from OTI would not be sufficient to complete the proposed study, MSB proposed to manufacture new DP lots from DCB material acquired from OTI. The new manufacturing would occur at Lonza's Singapore facility (LBSS) using DCB material produced in 2008-2009 at LWI and acquired from OTI. As several years had passed and some materials and equipment used had become obsolete or unavailable, the new manufacturing process would necessarily be different from that used at LWI. As product characterization was not sufficient to establish that DP made using the updated manufacturing process at LBSS was comparable to DP made at LWI, FDA recommended that Study 001 be conducted using only product made with the updated manufacturing process.

The LBSS facility produced new DP lots during a 2015-2016 manufacturing campaign from DCB material made by OTI. MSB then enrolled 55 pediatric subjects with SR-aGVHD into Study 001, with all but 8 subjects receiving product made using the updated manufacturing process at LBSS. Study 001 met its primary endpoint (excluding an overall response rate of 45% at Day 28), and the results of this study are the primary evidence of efficacy for this application. They also cite results from EAP 275, and subgroup analysis of pediatric subjects enrolled in Protocol 280 to support their claims of product efficacy. These studies are summarized in the table below.

Table 1 Summary of Pediatric SR-aGVHD Clinical Trials for Remestemcel-L

	Protocol 280	Protocol 275	MSB-GVHD001
Design	Randomized, double-blind, placebo-controlled	Expanded Access Only	Single Arm
Population	Adult and Pediatric	Pediatric	Pediatric
Subjects Receiving Product	163 (14 pediatric, 149 adult)	241	54
Treatment	Standard of Care + DP or Placebo	Standard of Care + DP	DP
Day 28 OR	Pediatric subjects: 64.3% All Subjects: 58%	65.1%	69.1%

COMMERCIAL MANUFACTURING SUMMARY

The proposed commercial manufacturing process for remestemcel-L has two steps:

- (1) BMA is collected from healthy human donors, and MSCs are isolated from the BMA and expanded in culture through two passages. At the (b) (4) passage, MSCs are harvested and cryopreserved as a drug substance intermediate. Each lot of this drug substance intermediate is a donor cell bank (DCB) that is stored in (b) (4) which are subsequently thawed for further manufacturing use.
- (2) (b) (4) DCB is thawed and expanded in culture through three additional passages. At the (b) (4) passage, the cells are harvested, formulated, distributed into cryovials, and cryopreserved as the DP. These vials are stored in the vapor phase of a liquid nitrogen freezer until distribution for use.

The first step in manufacturing (production of the DCBs) was performed at LWI while under contract with OTI using a process that has since been retired, while the second step in manufacturing is ongoing at LBSS and uses DCB material made at LWI in 2008-2009. The Applicant indicated in 125706/0.15 (received on March 27, 2020) that they currently held (b) (4) cryobags of DCB material made using the retired process and estimated that (b) (4) vials of commercial DP could be manufactured using this remaining DCB material. In Study 001, a subject received product from an average of (b) (4) vials, so the material on-hand could produce enough product to treat approximately (b) (4) patients, if approved. The Applicant intends to produce more DCB material using an updated manufacturing process, however this new process will be evaluated sometime after approval when the Applicant submits these manufacturing changes as a pre-approval supplement to the BLA.

Reviewer Note: As the first step of the manufacturing process is no longer ongoing, it is impossible to inspect the facility and observe this part of the manufacturing process. We therefore cannot confirm that DCBs were manufactured in accordance with cGMP regulations. After discussing with DMPQ reviewers, we determined that these DCBs may be acceptable for use in the commercial manufacturing process if each DCB lot is properly controlled for safety and quality.

MODULE 3 ORGANIZATION

The application describes both the retired manufacturing process, and the manufacturing process ongoing at LBSS for producing DP from DCB material acquired from OTI.


MODULE 3.2.S: The drug substance module describes the manufacturing process used at LWI in 2008-2009 to produce the DCB lots that are used as starting material in the current manufacturing process at LBSS. The endpoint of the process described in Module 3.2.S is not a true drug substance, but the cryopreserved DCB which the Applicant refers to as a drug substance intermediate.

MODULE 3.2.P: The drug product module describes the ongoing commercial process occurring at LBSS. This process expands material from (b) (4) remaining DCB lots made at LWI while under contract with OTI in 2008-2009 as the starting material. After expansion, the DS is then formulated as the DP and dispensed into cryovials that are distributed for commercial use.

Reviewer Note: Although Module 3.2.S is almost entirely dedicated to information regarding production of DCBs, it does also include some information regarding manufacturing process development (Module 3.2.S.2.6) and DP characterization (Module 3.2.S.3). Information regarding DP but provided in Module 3.2.S is reviewed under the section in which it was submitted.

3.2.S DRUG SUBSTANCE


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49 pages determined to be not releasable: (b)(4)

3.2.P DRUG PRODUCT

3.2.P.1 Description and Composition of the Drug Product

The remestemcel-L DP is a cryopreserved suspension of culture-expanded mesenchymal stromal cells (MSCs) stored and distributed in 6 mL vials. Each vial is labeled as containing 3.8 mL of volume at a concentration of 6.68×10^6 cells/mL for a total of 25×10^6 total cells. The remestemcel-L DP is formulated as described in the table below.

Table 24 Remestemcel-L DP Formulation

Component	Concentration	Function	Quality Standard
MSCs	(b) (4)	Drug Substance	See Module 3.2.P.5.1.
25% Human Serum Albumin (HSA) Solution	20% v/v (5% w/v HSA)	Stabilization and protection of cells	(b) (4) FDA-approved pharmaceutical
Dimethyl Sulfoxide (DMSO)	10% v/v	Cryoprotectant	(b) (4)
Plasma-Lyte A	70% v/v	Diluent providing physiological osmolarity and pH	(b) (4) FDA-approved pharmaceutical

The container closure used to store the DP is a 6 mL (b) (4) Closed Vial manufactured by (b) (4). The closed vials and caps are supplied sterile and filled by (b) (4). The vial is composed of cyclo-olefin co-polymer (b) (4) and the stopper is composed of thermo plastic elastomer (b) (4). The DP is stored and transported in the vapor phase of liquid nitrogen at $\leq 135^\circ\text{C}$ until thawed for administration.

Remestemcel-L is administered intravenously by a qualified health professional. Dosing is based on the patient's body weight at the time of infusion with a target dose of 2×10^6 MSCs/kg. The administering professional calculates the volume of DP needed for the target dose and thaws the appropriate number of DP vials. The calculated volume is withdrawn from the vials and transferred aseptically into an infusion bag. An additional 40 mL of Plasma-Lyte A is then added to the infusion bag, and after gentle mixing the contents of the bag are infused.

The target concentration is a (b) (4) excess of the label claim, and the target volume is (b) (4) in excess of the label claim. These overages are in accordance with (b) (4) (b) (4), which recommends volume excess of (b) (4) for a target volume of (b) (4) for a target volume of (b) (4).

3.2.P.2 Pharmaceutical Development

3.2.P.2.1 Components of the Drug Product

3.2.P.2.1.1 Drug Substance

The DS is comprised of culture-expanded adult human MSCs that have been harvested at (b) (4) in the manufacturing process as described in Module 3.2.P.3.3. As the DS is composed of living cells, it must

be maintained at a physiological osmolality and temperature, and exposure to compounds with known or potential effects on cell viability should be well-controlled to minimize these effects. Excipients present in the DP have been selected to maintain cellular viability of the DS throughout formulation, cryopreservation, and administration of the DP to patients.

3.2.P.2.1.2 Excipients

Plasma-Lyte A: An FDA-approved pharmaceutical manufactured according to the USP monograph “Multiple Electrolytes Solution Injection Type 1”. This excipient comprises 70% of the DP formulation by volume and is used to resuspend the thawed DP immediately prior to infusion. Plasma-Lyte A provides an aqueous solution for suspending the MSCs at a physiological osmolality and pH, helping to maintain viability of the DS.

Human Serum Albumin (HSA) Solution (25%): An FDA-approved pharmaceutical for the restoration or maintenance of blood volume. It is manufactured in accordance with the relevant (b) (4) for albumin (human) and 21 CFR 640 Subpart H – Albumin (Human). This excipient comprises 20% of the DP formulation by volume. It is composed of (b) (4) human albumin dissolved in an aqueous buffer containing (b) (4) as stabilizing agents. The protein content of this excipient stabilizes (b) (4)

Dimethyl Sulfoxide (DMSO): DMSO is manufactured according to the (b) (4) “Dimethyl Sulfoxide.” This excipient comprises 10% of the DP formulation by volume and acts as a cryoprotectant that improves cell survival and function during freezing and cryopreservation.

3.2.P.2.2 Drug Product

3.2.P.2.2.1 Formulation Development

Target DS and excipient concentrations have not changed since initiation of product development by OTI in 2003, however a new container (b) (4) were implemented when DP manufacturing moved to LBSS. DP lots produced during OTI’s development (2003-2009) were filled into (b) (4) containers (b) (4) for storage, with a nominal fill of (b) (4)

This (b) (4) concentration overage is maintained for the proposed commercial product.

3.2.P.2.2.2 Overages

The DP is formulated with a target concentration of (b) (4) cells/mL, which is (b) (4) greater than the label claim. Studies performed during product development assessed

the recovery of cells after DP is formulated for infusion as occurs in the clinical setting which informed this chosen overage. The Applicant performed a similar study using DP made using the proposed commercial manufacturing process as LBSS, and found that cell concentrations and recovery were above specification throughout the (b) (4) hold time tested. The target fill volume is (b) (4), which is (b) (4) above the label claim of 3.8 mL.

3.2.P.2.2.3 Physicochemical and Biological Properties

The biological properties of remestemcel-L are consistent with the scientific literature regarding characterization of MSCs. See review of Module 3.2.S.3 for additional discussion of the properties of remestemcel-L DCB and DP material.

3.2.P.2.3 Manufacturing Process Development

The Applicant identified three sequential versions of the DP manufacturing process that were used during product development:

DP Process 1 (2003-2007): The first process was used for production initially at OTI's production facility in Baltimore, MD, but was later transferred to LWI with some relatively minor process improvements. DP1 was used to produce 360 released DP lots.

DP Process 2 (2008-2009): The second process was performed exclusively at LWI. DP2 differs from DP1 in that it uses some (b) (4)

. DP2 was used to produce 217 released DP lots.

DP Process 3 (2014-2016): When MSB acquired the remestemcel-L development program in 2013 no DP had been produced since 2009. In the intervening time materials and equipment used in DP2 were no longer available or obsolete, and as a result the DP2 process could not be restarted. MSB transferred manufacturing to LBSS and implemented an updated process with some substantial changes relative to the DP2 process. DP3 was used to produce the 31 DP lots used in Study 001.

The proposed commercial manufacturing process is DP3 with a few process improvements that were implemented prior to PPQ. Study 001 used mostly DP lots made using DP3, however data from previous studies using mostly DP2 product (Protocol 275) or DP1 product (Protocol 280) are cited as supportive studies. The transition from DP2 to DP3 represents the most significant manufacturing change (described in Module 3.2.P.2.3, Table 5) and included changes to materials, equipment, and reagents used in addition to changes to the process itself. Many of these changes are improvements or replacements of outdated equipment.

Quality Target Product Profile (QTPP) and Critical Quality Attributes (CQAs)

In anticipation of this application, the Applicant initiated a failure modes and effects analysis (FMEA) to identify process parameters for evaluation in subsequent process limit evaluation (PLE) studies. The results of these PLE studies (Report MR-082), along with manufacturing experience and historical experimental data, were used to develop a QTPP for remestemcel-L. The QTPP was subsequently used to develop CQAs relevant to the safety and efficacy of remestemcel-L.

Table 25 Critical Quality Attributes for Remestemcel-L

(b) (4)

Based on results from PLE studies and manufacturing experience, the Applicant then evaluated process parameters for their known or potential effects on CQAs and identified critical process parameters (CPPs) and implemented process range limits.

Table 26 Critical Process Parameters for Manufacturing Remestemcel-L

(b) (4)

PRODUCT COMPARABILITY THROUGH DEVELOPMENT

MSB conducted a comparability study to demonstrate that DP made at LBSS using DP3 was comparable to DP made at LWI using DP2 (Report MR-041). This report is included with the present application, but was previously submitted to FDA for review under IND 7939 (Amendment 413). MSB used historical manufacturing data to generate tolerance intervals for most lot release assays, and then tested (b) (4) DP lots made using DP3 and compared the results to the historical data. Additionally, (b) (4) were assessed in the new lots. While the Applicant's position is that these results demonstrated comparability between DP2 and DP3 product lots, the product reviewer for IND 7939 (Steve Bauer) did not agree (see letter to Applicant dated December 21, 2015). All (b) (4) new DP lots failed to show any (b) (4), and one lot was notably different in the (b) (4) assay. Given these differences, and the small number of lots tested in the comparability exercise, this report is not sufficient to demonstrate comparability between DP2 and DP3 products.

MSB initially intended to use both DP2 and DP3 product in Study 001, however FDA recommended that only product made using DP3 be used as the comparability of the two product versions had not been established. The first eight subjects enrolled in Study 001 received DP2 product, but all subsequent subjects (46/54) received DP3 product. MSB manufactured 31 DP lots using the DP3 process to provide material in support of Study 001.

Although the Applicant has not conducted any further comparability studies, the application includes analysis of lot release testing results for DP lots produced using all three version of the process in Module 3.2.P.2.3. Although not a formal comparability report, in some ways the DP3 product appears different from DP1 and DP2 products:

(b) (4)

Reviewer Comments: Any comparability exercise for MSC-based products is limited by the inherent heterogeneity and variability of the product, and the lack of well-established quality attributes with a relationship to potency. The Applicant's position upon submission of this application was that product lots throughout development are comparable, however considering all available data, the Applicant has not convincingly demonstrated that product made using the proposed commercial process (DP3) is comparable or highly similar to product lots made using older processes. Analyses excluding the eight subjects in Study 001 (total 54 subjects) who received DP2 product do not affect the result or interpretation of Study 001, however this lack of comparability makes interpreting the relevance of results from studies 275 and 280 more challenging.

Most DP lots used in Study 001 were produced during (b) (4) manufacturing campaign in 2015-2016. In 2019 MSB initiated a process performance qualification (PPQ), then began a new manufacturing campaign that will continue through the end of August 2020. However, the Applicant indicates that “further process improvements and material updates” were implemented prior to PPQ and will be included in the commercial manufacturing process. These changes include (b) (4)

These changes appear to be relatively minor and are unlikely to affect the safety or quality of the product, however product made in 2019-2020 has an apparent reduced potency relative to DP lots made in 2015-2016 and used in Study 001. This issue is discussed in more detail below.

POTENCY ASSAY DEVELOPMENT AND RATIONALE


Potency assays should have a demonstrated relationship to the activity of the product. Ideally this connection is made with clinical or *in vivo* effects in humans, but could instead link to the biological activity of the product as measured by *in vitro* methods. FDA guidance suggests that a matrix approach using multiple assays for potency may be necessary to assess the potency of cell therapy products that may have complex or not fully characterized mechanism(s) of action. The original submission of this application proposed a two-assay matrix for measuring lot-to-lot potency: (1) a quantitative (b) (4) to measure the levels of TNFR1 in product lots, and (2) a quantitative bioassay measuring the capacity of product lots to inhibit T cell activation *in vitro*. Both assays were inherited from OTI, and although the testing facilities and some of the details in the procedures are different, the assays and specifications upon submission of this application were essentially unchanged since these assays were first implemented early in product development.

Development of TNFR1 Potency Assay

Rationale: OTI conducted experiments in 2005 to identify potential surrogate markers of potency. Informed by the pathophysiology of aGVHD and contemporary knowledge of the immunomodulatory properties of MSCs, OTI designed experiments to identify markers that were associated with MSC-mediated inhibition of T cells *in vitro*.

(b) (4)

(b) (4)



Reviewer Comment: During review of this application, we expressed our concern that the quality attributes of the product had not been linked to potency, and that controlling these attributes might therefore not be adequate to control the potency of the

commercial product. The Applicant performed several analyses of previously submitted data in an attempt to link TNFR1 to clinical outcomes.

TNFR1 and Clinical Outcomes: At the mid-cycle communication and in a follow-up amendment to the application (125706/0.32), the Applicant provided results from analyses of overall survival outcomes and TNFR1. These analyses combined data from Study 001 with results from Protocol 280 (a failed placebo-controlled study for SR-aGVHD conducted by OTI) and EAP 275 (an expanded access protocol used to treat pediatric subjects with remestemcel-L). The Applicant concluded that differences in the mean TNFR1 values of DP lots administered in these studies may account for the difference in outcomes as summarized in the table below.

Table 27 Product Attributes and Clinical Outcomes in Data Pooled from Studies 275, 280, and 001

Protocol	TNFR1 (pg/mL)	IL-2R α Inhibition	Day 28 OR	Day 100 OS	(b) (4)
Protocol 280	205.7	65.1%	58%	52%	
EAP 275	241.3	68.7%	65%	66%	
MSBGVHD001	321.8	81.3%	70%	74%	

(b) (4)

A combined analysis is difficult to interpret because the studies differed in the allowance of concomitant medications (allowed in 275 and 280, but not 001) and the unexplained inclusion of adult subjects in Protocol 280 (only 14/163 of subjects receiving the product were pediatric). Additionally, the Applicant acknowledges that there is no association of TNFR1 results and the primary clinical endpoint (Day 28 OR) in the pooled dataset, and that there is no association with any clinical outcome when only data from Study 001 is considered. Finally, significant manufacturing changes were made during development, and product characterization is not sufficiently advanced to determine that DP lots used in all three studies are similar. Because of these limitations this analysis is not sufficient to demonstrate a relationship between TNFR1 results and clinical outcomes.

Using the same pooled analysis dataset, the Applicant also attempted to link a manufacturing change to increased TNFR1 levels and improved survival outcomes. At the mid-cycle communication and the Oncologic Drugs Advisory Committee (ODAC) meeting for this application, the Applicant argued that manufacturing changes made in 2009 while OTI was still manufacturing product at LWI using DP2 had led to both higher TNFR1 levels and better overall survival of study subjects. Among DP lots administered to study subjects in the pooled dataset (280, 275, and 001), DP lots made after these changes had higher levels of TNFR1 (Avg. = 331 pg/mL) than DP lots made before the changes (Avg. = 213 pg/mL). Subjects receiving only DP lots made after the change were also more likely to survive to Day 100 than those who received only DP lots made before the changes (75% vs 58%, $p=0.0026$).

Table 28 Applicant's Analysis of DP Potency and Clinical Outcomes as Presented to ODAC

Protocol	TNFR1 (pg/mL, SD)	IL-2R α Inhibition (SD)	Day 28 OR	Day 180 OS
Only "original" process (n=348)	213 (32)	56% (25)	63%	58%
Only "optimized" process (n=92)	331 (39)	79% (6)	70%	75%
p-value	<0.0001	<0.0001	0.2643	0.0026

Although the Applicant presented these changes as "manufacturing enhancements that optimized [the] process" at the ODAC meeting (see slide #25 in the Applicant's morning slide deck), these changes and their impact on product potency were not mentioned in the original materials submitted for this application. At the time these changes were implemented, OTI reported them in an annual report under their master file for remestemcel-L manufacturing (MF (b) (4) [REDACTED], received on April 11, 2011), describing them as "minor updates to the DP manufacturing procedure." Indeed, the changes implemented at that time were made to streamline and simplify the manufacturing process rather than improve product quality. The changes included (b) (4) [REDACTED]

[REDACTED] Most of these changes were not carried over into DP3 as implemented at LBSS six years later, and the changes made in transferring the process to LBSS were more substantial than any of the changes made in 2009, and therefore there is little justification for grouping DP2 lots made after these changes with DP3 lots as the Applicant has done. The Applicant has also not provided a detailed analysis of which changes made at that time were maintained in DP3 or a plausible explanation for why these changes may have improved product potency. At the ODAC meeting the Applicant referred specifically to the reduced trypsinization time as potentially the key change, but this parameter was identified as a non-critical process parameter (NCP) in the material originally submitted to this application (Table 8, Module 3.2.P.2.3). Durations of up to (b) (4) [REDACTED] were tested during process limit evaluation experiments in 2018 (Document PD.1012R, Report MR-082 in Module 3.2.P.2) from which the Applicant concluded that extended duration of trypsinization was "not detrimental to final product yield or CQA of DP."

The figure below shows variation in TNFR1 results for remestemcel-L DP lots by manufacturing date. The values are color coded by DP process development stage (blue for DP1, orange for DP2, and black for DP3). The gray bar indicates the time during DP2 manufacturing in which the changes made to the manufacturing process identified by the Applicant were in place.

Figure 6 TNFR1 Values for Remestemcel-L Lots by Manufacturing Date

(b) (4)

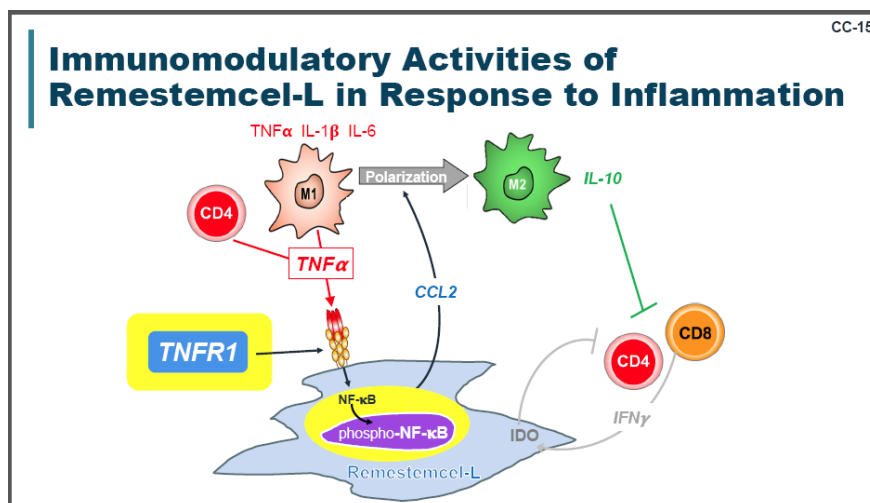
Reviewer Comment: The Applicant maintains that the above analyses link TNFR1 to clinical outcomes, but the pooled analysis approach has several limitations as described above and therefore we do not agree with their interpretation.

(b) (4)

Additional experiments in Report MR-128 showed that TNFR1 expression affects the capacity of remestemcel-L to secrete cytokines and chemokines in response to stimulation with TNF- α , but they do not demonstrate that this deficit affects the activity of the product.

Despite these inconclusive results, the Applicant presented an updated model of the mechanism of action for remestemcel-L at the ODAC meeting reflecting a newly proposed central role of macrophages. In this new mechanism, remestemcel-L senses TNF- α through TNFR1, then secretes CCL2, which acts on macrophages to increase secretion of IL-10, and this macrophage-derived IL-10 finally acts on T cells to limit their activation. This figure below is a recreation of slide #15 in the Applicant's morning slide deck.

Figure 10 New Proposed Mechanism of Action for Remestemcel-L as Presented by the Applicant at ODAC Meeting




Reviewer Comment: The use of TNFR1 as a surrogate marker for potency was predicated on early knockdown experiments showing that loss of TNFR1 result in a reduction in immunomodulatory activity as measured by the capacity to inhibit T cell proliferation *in vitro*. In the current version of this product, however, loss of TNFR1 does not affect this activity. The data provided to support a macrophage-centric mechanism of action are not adequate. Therefore, the rationale for using TNFR1 as a surrogate measure of potency is not clear and the Applicant should provide additional data and justification for continuing to use this assay as a surrogate for potency, or better characterize their product to identify alternative attributes as surrogate potency markers.

Development of the Inhibition of IL-2R α Assay

(b) (4)

(b) (4)




3.2.P.2.4 Container Closure System

When MSB initiated manufacturing at LBSS using the updated process in 2015, a new container was selected for use. Rather than the (b) (4) bags, new DP lots are distributed into 6 mL closed vials. The nominal fill is 25 million cells in 3.8 mL, with a target concentration (b) (4) above the target and an overfill of (b) (4). This container is the same that will be used in the proposed commercial manufacturing process. See review of Module 3.2.P.7 for more information regarding the appropriateness of the product container.

3.2.P.2.5 Microbiological Attributes

The DP is composed of living cells and therefore cannot be terminally sterilized before release, however the manufacturing process and controls are designed to ensure that reagents and materials used in production are sterile and to avoid the inadvertent introduction of contaminating microbes during the manufacturing process. Each lot of DP is tested for sterility by (b) (4) testing both the final formulated product (b) (4) and an in-process sample. Mycoplasma testing occurs on a sample collected (b) (4)



The container closure system has been tested for its ability to maintain integrity during cryopreservation and shipping using (b) (4) vials were shipped to the testing facility in (b) (4) shipments, and all (b) (4) vials maintained integrity. Additionally, vials from (b) (4) DP lots were tested for container integrity after at least (b) (4) months in cryostorage and all vials tested maintained integrity.

3.2.P.2.6 Compatibility

The general process of preparing the DP for intravenous infusion is as follows:

- (1) Calculate the volume required for the intended dose based on the patient's weight.
- (2) Thaw the appropriate number of DP vials in a 37°C water bath for 5 to 8 minutes.

- (3) Using a 5 mL syringe and 18-gauge needle, remove the required volume of DP from a vial. Each vial used requires its own syringe and needle.
- (4) Dispense the DP into an empty infusion bag.
- (5) Collect 40 mL from a bag of Plasma-Lyte A in a 60 mL syringe, then dispense the 40 mL of Plasma-Lyte A into the infusion bag containing the dispensed DP.
- (6) Infuse the diluted DP within 5 hours of formulation.

The Applicant conducted a post-formulation stability study to support the proposed 5-hour hold time of the product as prepared for infusion (Report MR-107). This study used product from (b) (4) DP lots made during PPQ. Vials were thawed in a manner intended to mimic the process as would occur at clinical sites, and the volume required to treat a 50 kg patient was pooled and diluted with Plasma-Lyte A as described in the product's preparation instructions. After formulation the contents of the bag were sampled in triplicate at 30, 90, 180, and 300 minutes timepoints, and the cell viability and concentration were determined using the same assays in place for product release.

(b) (4) lots manufactured during PPQ were each tested (b) (4) times.

At Time 0, all (b) (4) lots showed a reduction in the cell concentration relative to the release testing results, but the concentrations observed were all above the concentration specification. Concentration did not appear to appreciably decline over the course of the experiment, suggesting that cells are not clumping or attaching to the infusion bag. Recoveries were also fairly stable over the timepoints tested, suggesting that the dose administered after 300 minutes is not substantially reduced relative to a dose administered at an earlier timepoint. Finally, cell viability also appeared to be stable, with slight reductions observed initially relative to the release values, but no further reduction appeared to occur over subsequent timepoints and all values obtained were above the product's specification of (b) (4) viability.

Reviewer Comment: This study is somewhat limited as a stability study, but it does demonstrate that the viability and recovery of the MSCs in not substantially affected by the routine formulation process.

Overall Reviewer's Assessment of Section 3.2.P.2:

- The proposed commercial manufacturing process has been developed over more than 15 years and is a modernized version of previous processes, however the CQAs and potency assays/specifications were not refined during development. The Applicant agreed to more stringent specifications to provide better assurance that commercial product would be similar to product lots used in Study 001.
- During interactive review of this application, the Applicant demoted the inhibition of IL-2R α assay from a quantitative assay for potency to a qualitative assay of "activity" because of the apparent reduction in this attribute in commercial lots relative to clinical lots. This assay may be, however, acceptable provided that another assay for potency is in place. Even as a qualitative assay, it may be appropriate to include the inhibition of IL-2R α

assay in a potency assay matrix with another assay that is quantitative and measures an attribute clearly linked to product potency.

- The Applicant's analyses to support a relationship between TNFR1 results and overall survival have severe limitations and do not convincingly demonstrate that this attribute has a link to clinical outcomes, but demonstrating such a link is not a requirement for licensure.
- The rationale on which the TNFR1 potency assay was based is not applicable to the current version of the product, as TNFR1 knockdown does not affect the product's *in vitro* immunomodulatory activity. The experiments provided do not adequately support the revised mechanism of action presented at the ODAC meeting, and therefore the justification for using this as a surrogate marker for potency is not clear. The Applicant should provide additional data to support this mechanism of action, or otherwise link TNFR1 to a reasonable *in vitro* activity that may be related to *in vivo* activity of the product.
- Given the limited supply of remaining DCB material, the Applicant will have to re-establish DCB manufacturing within the next few years. As product attributes associated with potency and/or specific activity have not been established for this product, it is in the Applicant's interest to develop more relevant and robust assays to be used to for lot release and as part of a comparability exercise. If analytical methods are not sufficient to convincingly demonstrate comparability, then an additional clinical trials may be required to support the safety and efficacy of the product made from new DCB material.

Major Deficiency: The relevance of TNFR1 levels as a surrogate marker for potency is not clear, and therefore this assay cannot be considered an appropriate laboratory test for potency. The Applicant should provide additional data and justification to demonstrate that controlling this attribute of the product ensures acceptable levels of product activity in each lot.

Additionally, the product attributes identified as CQAs in the BLA may not be sufficient to establish stability or demonstrate comparability between DP lots made using new DCB material and DP lots used in previous clinical trials. The Applicant should better characterize the product to identify potentially more meaningful CQAs including attributes for potency so that comparability may be demonstrated by analytical methods. If no new CQAs are identified, it is likely that a new clinical trial may be needed to ensure the safety and efficacy of the post-change product because demonstrating comparability by analytical methods will not be feasible or sufficient by the Applicant. See CR Item #1.

3.2.P.3 Manufacture**3.2.P.3.1 Manufacturer(s)****Table 29 Drug Product Manufacturers**

Facility Name	Address	Registration	Responsibilities
Lonza Bioscience Singapore Pte. Ltd. (LBSS)	35 Tuas South Ave 6, 637377 Singapore	FEI: 3009725845 DUNS: 936939342	Manufacturing, primary packaging, OC in-process and batch release testing, drug product storage
Integrated Commercialization Solutions (ICS) Amerisource Bergen	(b) (4)	(b) (4)	Secondary packaging, drug product storage and distribution
(b) (4)	(b) (4)	(b) (4)	QC batch release testing, stability testing
(b) (4)	(b) (4)	(b) (4)	QC batch release testing, stability testing
(b) (4)	(b) (4)	(b) (4)	QC batch release testing
(b) (4)	(b) (4)	(b) (4)	QC stability testing
(b) (4)	(b) (4)	(b) (4)	QC in-process and batch release testing
(b) (4)	(b) (4)	(b) (4)	QC stability testing
Mesoblast International Sarl	21 Biopolis Road #01-22 Nucleos (South Tower) 138567 Singapore	DUNS: 595372361	QA review and bulk batch release
Mesoblast, Inc.	505 Fifth Avenue New York, NY 10017	DUNS: 616697426	Final packaged product batch release

3.2.P.3.2 Batch Formula

The pooled and washed MSCs are first resuspended at approximately (b) (4) viable cells/mL in a solution of (b) (4) v/v Plasma-Lyte A and 5% v/v HSA solution. An (b) (4) volume of cryoprotectant solution comprised of (b) (4) v/v Plasma-Lyte A, (b) (4) v/v DMSO and (b) (4) v/v HSA solution is then added to the cell suspension to make the final formulated drug product. Formulation for a batch varies depending on the final yield of viable cells, with a typical batch producing ~120 vials of DP.

Table 30 Drug Product Batch Formula

Component	Nominal Amount in DP vial	Nominal Amount in typical batch
MSCs	(b) (4)	(b) (4)

25% HSA Solution	20% v/v	(b) (4)
DMSO	10% v/v	
Plasma-Lyte A	70% v/v	

Overall Reviewer's Assessment of Sections 3.2.P.3.1 and 3.2.P.3.2:

The information provided is acceptable.

3.2.P.3.3 Description of Manufacturing Process (ELM and MK)**Manufacturing Overview**

The manufacturing process for remestemcel-L occurs in the following stages:

- (1) DCB thawing and culture expansion through 3 passages (LBSS)
- (2) Harvest of DS, formulation of DP, vial filling, and cryopreservation (LBSS)
- (3) Packaging vials into cartons and cryopreservation until distribution (ICS)

At each passage the number of (b) (4)

Manufacturing Process in Detail

(b) (4)

Control of Materials Used in DP Manufacturing

DCB Lots Available for DP Manufacturing

Of the DCB lots manufactured by OTI at Lonza Walkersville prior to acquisition by MSB, material from (b) (4) DCB lots is available for commercial manufacturing. Those (b) (4) DCB lots are summarized in the table below.

Table 31 DCB Lots Available for Commercial Manufacturing

DCB Batch Number	Bone Marrow Aspirate Donor Number	Date of Manufacture
(b) (4), (b) (6)		

MSB Control of Materials

The Applicant uses a risk-based assessment of materials used in the manufacturing of the DP by process use, contact time with the product, amount used, and potential case of failure.

Risk-based assessment of the material. Material criticality is assigned through assessment of key parameters such as evaluation of if the material makes product contact, of animal origin, material of construction, process use, contact time with the product, amount used, and potential case of failure. The level of risk is assessed by reviewing current controls, suitability, compliance with compendial standards, and evaluating if the material makes product contact, of animal origin, material of construction. The table below summarizes the reagents used in manufacturing remestemcel-L DP.

Table 32 Reagents Used in Manufacture of Remestemcel-L DP

Component	Manufacturer	Animal Origin	Excipient
Fetal Bovine Serum	(b) (4)	Bovine	No
(b) (4)	Lonza Walkersville Inc.	No	No
Recombinant Trypsin	(b) (4)	No	No
Plasma-Lyte A	Baxter Healthcare (NDC code: 0338-0221-04)	No	Yes, Reviewed in <u>3.2.P.4.1</u>
Human Serum Albumin Solution (25%)	(b) (4)	Human	Yes, Reviewed in <u>3.2.P.4.1</u>

Component	Manufacturer	Animal Origin	Excipient
Dimethyl sulfoxide (DMSO) (b) (4)	(b) (4)	No	Yes, Reviewed in <u>3.2.P.4.1</u>

Reagents Not of Human or Animal Origin

Recombinant Trypsin is used in passaging cells during the drug product manufacturing and manufactured by (b) (4). The recombinant trypsin is derived from (b) (4) without materials of animal origin. The Applicant provides a COA from the manufacture details testing to (b) (4) standards for trypsin and (b) (4) testing. The Applicant also tests the formulated trypsin for (b) (4) standards. The formulated reagent is stored (b) (4) and stored until manufacture's expiration date or (b) (4) years from date of manufacture, whichever is less.

(b) (4) is manufactured at LBSS, or LWI and is in compliance with ISO 13485/FDA Quality System Regulations. The (b) (4) is detailed in the provide COA for the (b) (4) from LWI or the current testing for the (b) (4) manufactured at LBSS.

Plasma-Lyte A is manufactured by Baxter Healthcare Corporation and is an FDA approved product (NDC Code: 0338-0221-04), indicated as a replacement intravenous infusion, as a source of water, electrolytes and calories. It is tested by the manufacturer to USP multiple electrolytes type 1 requirements. A COA is provided (3.2.P.4.1: Specifications). Plasma-Lyte is used in the (b) (4) Solution, and as an excipient in Cryoprotectant Solution (reviewed in section 3.2.P.4.1).


(b) (4) -DMS is manufactured by (b) (4) and used in the Cryoprotectant Solution and is an excipient in the final DP formulation. It is pharmaceutical grade, sterile, manufactured per GMP regulations, and complies with both (b) (4). A COA is provided (3.2.P.4.1: Specifications). The Applicant also tests the reagent to current DMSO (b) (4) testing standard. DMSO as an excipient reviewed in section 3.2.P.4.1.

Reagents of Human or Animal Origin

The FBS as a growth media (b) (4)

HSA (25%) is a component of (b) (4)

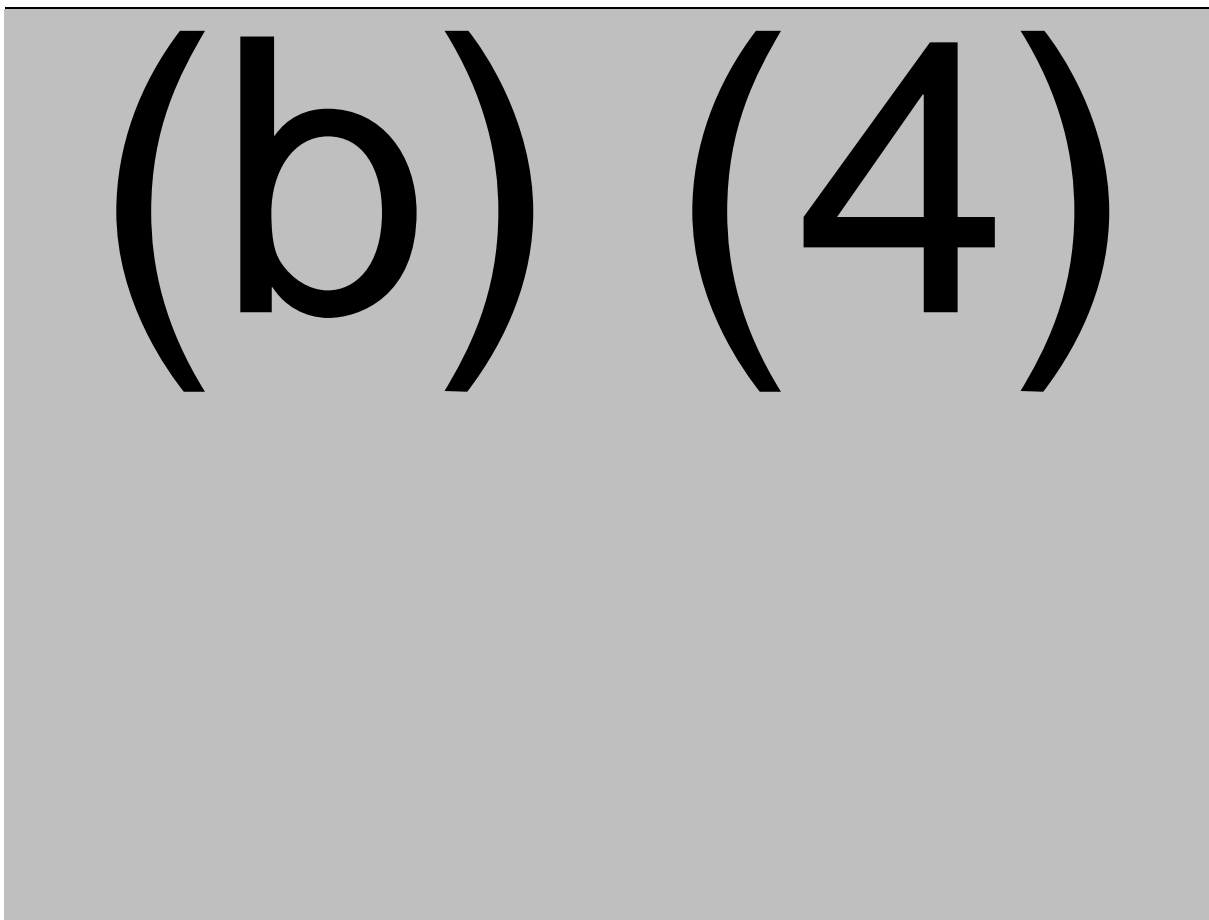
(b) (4)



Process Solutions and Media used in the Manufacture of DP


The Applicant provides COAs for the in-house formulated solutions and media formulation used in manufacturing the drug product, and the composition of these solutions are summarized in the table below.

Table 33 Composition of Solutions/Media Formulations Used in Manufacture of Remestemcel-L DP




(b) (4)

(b) (4)



(b) (4)

A large section of the document is redacted with grey bars. The redaction covers approximately seven lines of text, starting from the first line below the header and ending just above the Overall Reviewer's Assessment box.

Overall Reviewer's Assessment of Sections 3.2.P.3.3:

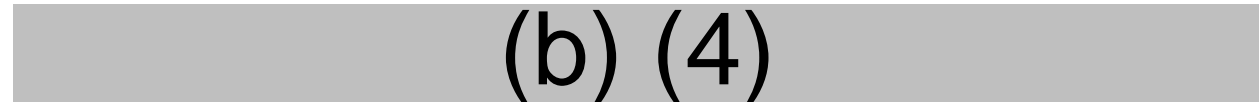
The information provided is acceptable. The sponsor has provided adequate information for all reagents used in the manufacturing of the drug product, including COAs, details regarding reagent testing, and process controls.

3.2.P.3.4 Controls of Critical Steps and Intermediates

A DP process control strategy (PCS) was developed using a risk-based approach and evaluation of historical manufacturing experience. The purpose of the PCS is to control product CQAs by identifying critical process parameters (CPPs) that affect the quality of the final product. The process parameters identified by the Applicant as being CCPs are described in the table below:

Table 34 Critical Process Parameters for DP Manufacturing

(b) (4)

A single line of text is redacted with a grey bar.

(b) (4)

The Applicant conducted a failure modes effects analysis (FMEA) to systematically assess process parameters identify parameter targets to be tested in several process limit evaluation (PLE) studies to identify.

Overall Reviewer's Assessment of Sections 3.2.P.3.4:

The experiments performed during the PLE study support the Applicant's identified CPPs, and the control strategies in place to monitor CPPs are adequate.

(b) (4) during culture were identified as non-critical process parameters, however these are likely critical to manufacturing success so it is not clear how the Applicant justifies considering them non-critical. Despite classification as non-critical these parameters both parameters are continuously monitored by qualified equipment and therefore this mischaracterization does not affect overall manufacturing control. The information provided is acceptable.

3.2.P.3.5 Process Validation and/or Evaluation

Process performance qualification (PPQ) was conducted using full-scale manufacturing runs under protocol (Report MR-100). All PPQ manufacturing runs were performed at LBSS in May-August 2019 using the DP3 manufacturing process with minor changes (see review of Module 3.2.P.2.3, *Manufacturing Process Development*). PPQ used DCB material from (b) (4) DCB lots derived from (b) (4) different BMA donors, producing a total of (b) (4) DP lots. Several parameters of the manufacturing process were challenged for some of the PPQ lots to evaluate the effects of variability within the manufacturing process. (b) (4) lots (b) (4) were manufactured using the (b) (4), with (b) (4) other lots (b) (4) were manufactured using (b) (4). The parameters used for these challenge lots are described in the table below.

Table 35 Lots Challenged During PPQ

(b) (4)

(b) (4)

Overall Reviewer's Assessment of Section 3.2.P.3.5:

The PPQ approach is reasonable and appears consistent with relevant regulatory requirements and FDA guidance. While the PPQ results were successful in the context of the original specifications, if the revised specifications are used then the process appears not as well controlled and will routinely product DP lots that do not meet specification. With the extensive manufacturing experience gained during product of the DP lots used in Study 001 and the manufacturing campaign ongoing during review of this application, the Applicant has extensive experience manufacturing this product, and the process design includes reasonable control of materials and CCPs. The variability observed may therefore be inherent to the cell type being manufacturing, and ensuring product quality may require stringent testing controls to ensure that only lots of the desired quality are release for commercial use.

The Applicant's sampling strategy for routine lot release may have been adequate when specifications were well below the observed values, but with specification closer to the expected values multiple vials should be sampled to obtain a distribution of values for each lot rather than a single value that may fall anywhere within this distribution.

Minor Deficiency: During review of your application you agreed to more stringent specifications for the TNFR1 assay and inhibition of IL-2R α assay that are closer to the values observed among DP lots. Given the distribution of values obtained from vials within a single DP lot as observed in your PPQ multiple sampling analyses, testing a single vial from each lot may not be appropriate because the revised specifications may fall within the expected range of values for a given lot, which may result in the rejection of entire lots if the vial randomly chosen for testing happens to fall on the low end of this distribution. We therefore recommend that you develop a more thorough lot sampling approach that includes testing multiple vials from each lot and revise your specifications for these assays to take into account the distribution of values obtained from multiple vials within a lot. For each assay you should chose a minimum acceptable result for each vial, then perform a statistical analysis to determine how to set your specifications to ensure that the frequency of vials below minimum acceptable result is acceptably low. See CR Item #2.

3.2.P.4 Control of Excipients

3.2.P.4.1 Specifications

Table 36 Excipient Specifications

Excipient	Manufacturer	Specification	NDC#
Plasma-Lyte A	Baxter Healthcare	USP monograph for Multiple Electrolytes Injection Type 1	0338-0221-04
Human Serum Albumin (25% solution)	(b) (4)	(b) (4) for Human Albumin Solution	(b) (4)
DMSO	(b) (4) DMSO)	(b) (4) for Dimethyl Sulfoxide	N/A

Plasma-Lyte A: Each shipment of Plasma-Lyte A undergoes 100% physical inspection, and the CoA(s) are examined upon receipt. The CoA confirms that each lot is tested for identity in accordance with the (b) (4), and is subject to specifications for concentration of (b) (4)

Each lot is also tested for sterility for release.

The Plasma-Lyte A vendor is qualified by more testing each of the (b) (4) lots received at the manufacturing facility, which includes full identity testing according the (b) (4)

The Applicant routinely tests every incoming lot for identity using a subset of the analytes used in the (b) (4)

(b) (4)

HSA (25% Solution): Each shipment of HSA solution undergoes 100% physical inspection, and the CoA(s) are examined upon receipt. (b) (4) tested by the vendor for identification by (b) (4)

Testing conforms to the corresponding (b) (4)

(b) (4). The manufacturing facility routinely tests (b) (4) for identity by (b) (4).

Table 38 HSA Solution Testing and Specifications

Test	Vendor Testing	LBSS Testing
Identification	CoA	Routine
(b) (4) Testing	CoA	--

DMSO: Each shipment of DMSO undergoes 100% physical inspection, and the CoA(s) are examined upon receipt. (b) (4) tested by the vendor for identification by (b) (4) methods described in the (b) (4)

Table 39 DMSO Testing and Specifications

Test	Vendor Testing	LBSS Testing
(b) (4)	CoA	Routine
	CoA	Routine
	CoA	--
	CoA	Routine
	CoA	Routine
	CoA	--
	CoA	Routine
	CoA	Routine
	CoA	Routine
	CoA	Routine
	CoA	Routine
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	CoA	Routine
	CoA	--
	CoA	Routine
	CoA	Routine
	CoA	--
	CoA	--

3.2.P.4.2 and 3.2.P.4.3 Analytical Procedures and Validation of Analytical Procedures

All procedures used to test excipients are in accordance with either the relevant (b) (4), and no validation reports for these methods were provided with the application.

3.2.P.4.4 Justification of Specifications

Specifications for excipients are in accordance with (b) (4).

3.2.P.4.5 Excipients of Human or Animal Origin

HSA Solution is the only excipient with a potential risk transmission of infectious disease as it is derived from human plasma. Donors from which this excipient is derived individually tested negative for reactivity against HBsAg, HIV-1/2, and HCV. Each plasma pool tested negative for antibodies against HBsAg and HIV-1/2, and negative by PCR for HAV, HBV, HCV, Parvo B19, and HIV-1/2. The excipient is sourced from FDA-approved collection centers in the United States, and is a licensed as a human drug by FDA.

Overall Reviewer's Assessment of Section 3.2.P.4:

All excipients are produced and tested according to (b) (4). The information provided is acceptable.

3.2.P.5 Control of Drug Product

3.2.P.5.1 and 3.2.P.5.6 Specification(s) and Justification of Specification(s)

The product specifications provided in the original submission of this application were identical to those used during product development which were unchanged since 2009. During review of the application the Applicant agreed to revise their initial specifications, and the table below reflects the final agree-upon specifications for DP release.

Table 40 Final Specifications for Remestemcel-L

Attribute	Assay	Specification	Sample	Testing Facility
In-Process Sterility (Safety)	(b) (4)	Negative	(b) (4)	(b) (4)
Mycoplasma (Safety)	(b) (4)	Negative	(b) (4)	(b) (4)
Sterility	(b) (4)	Negative	Filled DP Vial	(b) (4)
Purity	Endotoxin (b) (4)	(b) (4)	Filled DP Vial	(b) (4)
Identity	(b) (4)	(b) (4) CD166 ⁺	Filled DP Vial	(b) (4)

Attribute	Assay	Specification	Sample	Testing Facility
Identity	(b) (4)	(b) (4) CD105 ⁺	Filled DP Vial	(b) (4)
Identity	(b) (4)	(b) (4) CD45 ⁺	Filled DP Vial	(b) (4)
Potency	TNFR1 (b) (4)	(b) (4)	Filled DP Vial	(b) (4)
Activity	IL-2R α Inhibition	(b) (4) Inhibition of IL-2R α in (b) (4)	Filled DP Vial	(b) (4)
Potency	Cell Viability	(b) (4) Viability	Filled DP Vial	(b) (4)
Potency	Cell Concentration	$\geq 6.68 \times 10^6$ cells/mL	Filled DP Vial	(b) (4)
Appearance	(b) (4)	(b) (4)	Filled DP Vial	(b) (4)
Purity	Residual BSA (b) (4)	(b) (4)	Filled DP Vial	(b) (4)
Purity	Residual Trypsin	(b) (4)	Filled DP Vial	(b) (4)
Appearance	Visual Inspection and (b) (4) Sampling	(b) (4)	Filled DP Vial	LBSS

Justification for Specifications:

Sterility, Mycoplasma, and In-Process Sterility: The Applicant indicates that these assays are performed in compliance with relevant (b) (4) chapters and therefore negative results are adequate to ensure that the DP is free from microbial contamination.

Phenotype/Identity: The Applicant cites recommendations from the (b) (4) that more than (b) (4) of cells express positive identify markers for MSCs such as CD166 and CD105, and fewer than (b) (4) of cells express the common leukocyte marker CD45. No information regarding how these recommendations were developed was provided, however.

Reviewer Comment: Manufacturing information provided show that DP lots have little variation in these markers and routinely show frequencies of CD166⁺ and CD105⁺ cells greater than (b) (4). Most DP lots report (b) (4) CD45⁺ but the limit of detection for this assay has not been reported.

Endotoxin: The Applicant cites (b) (4) as recommending a limit of (b) (4) for parental drugs. Based on the proposed dosage, the Applicant calculates that the specification limits the amount of endotoxin a patient would receive to 10-fold below this limit.

TNFR1 Potency Assay: The Applicant cites several experiments conducted by OTI during development to show that reduced TNFR1 expression in MSCs leads to a reduction in the immunomodulatory activity as measured by T cell proliferation (Reports RR-048-05, RR-070-06, and RR-045-05). In these experiments OTI knocked down expression of TNFR1 in MSCs and showed that more efficient knockdown of expression correlated with reduced immunomodulatory capacity. These results were used to construct a polynomial regression model to estimate the level of TNFR1 that would provide a (b) (4) reduction in activated T cell proliferation. The Applicant adjusted this value for the increased cell concentration of the DP and the adjusted value was the initial proposed specification for this assay.

Reviewer Comments:

- The reports cited also show that the biological variation of TNFR1 expression among unmanipulated MSC lots did not have a strong link to *in vitro* immunomodulatory activity.
- The Applicant explains that the initial specification proposed was based on an expected (b) (4) inhibitory activity for T cell proliferation but did not indicate why (b) (4) is an appropriate threshold.
- The Applicant repeated TNFR1 knockdown experiments during review of this application and found that the previous results did not hold for the current version of the product (i.e., that TNFR1 knockdown did not affect the immunomodulatory activity of the product).
- During the review period, the Applicant agreed to revise this specification upward:
 - o At the mid-cycle meeting, the Applicant proposed a revised specification of (b) (4), based on their analysis of TNFR1 levels and survival outcomes.
 - o At the late-cycle communication, the Applicant agreed to revise the specification further to (b) (4), which was the lowest value for any DP lot used in Study 001.

IL-2R α Inhibition Potency Assay: In the original application the Applicant cited an observed correlation between the results of this assay and a reduction in the frequency of activated T cells in a subset of subjects enrolled in Study 001 (Module 3.2.S.3.1.7.3.1). The initial specification was chosen based on a review of published studies looking at MSC-mediated inhibition of T cell activation, but no justification was provided to support the proposed specification.

Appearance: The Applicant cites (b) (4). Additionally, the appearance of the product should confirm that cells and HSA are present in the formulation, and that the DP is free from (b) (4).

Cell Viability: The Applicant cites FDA guidance document “Guidance for FDA Reviewers and Sponsors 2008: Content and Review of Chemistry, Manufacturing, and Control (CMC) Information for Human Somatic Cell Therapy Investigational New Drug Applications (INDs).” No supporting data is included in this application or was provided in the cited guidance document, so it is unclear how this threshold was chosen.

Cell Concentration: The chosen specification ensures an overage in concentration at least (b) (4) above the label claim.

Reviewer Comment: In Module 3.2.S.2.2.2 the Applicant indicates that short-term stability studies showed as much as a (b) (4) reduction in cell recovery after (b) (4) Plasma-Lyte A at (b) (4) minutes, therefore it is not clear that this specification is high enough to ensure delivery of the target dose throughout the entire proposed hold times.

Residual BSA and rTrypsin: The justification for the specifications chosen for these assays is the ability of their manufacturing process to consistently meet them.

Visual Inspection and (b) (4) Sampling: The Applicant cites (b) (4)

The original submission did not contain information regarding the number of vials sampled for each test, nor how the samples were shipped to each testing facility. In response to our request for additional information (IR#15) they provided this information in Amendment 15, and this information is summarized in the table below.

Table 41 Drug Product Sampling for Release Testing

Test(s)	Number of Samples/DP Lot	Testing Facility	Shipping Conditions
Mycoplasma	(b) (4)	(b) (4)	(b) (4)
In-Process Sterility	(b) (4)	(b) (4)	(b) (4) shipping box"
Sterility	(b) (4) vials (including (b) (4))	(b) (4)	(b) (4) Cryoshipper to (b) (4)
Endotoxin	1(b) (4)	(b) (4)	(b) (4) Cryoshipper to (b) (4)
Appearance	(b) (4)	(b) (4)	(b) (4) Cryoshipper
Cell Viability Cell Concentration TNFR1 (b) (4)	(b) (4)	(b) (4)	(b) (4) Cryoshipper
(b) (4)	(b) (4)	(b) (4)	(b) (4) Cryoshipper
IL-2R α Inhibition	(b) (4)	(b) (4)	(b) (4) Cryoshipper
Residual BSA	(b) (4)	(b) (4)	(b) (4) Cryoshipper

Test(s)	Number of Samples/DP Lot	Testing Facility	Shipping Conditions
Residual Trypsin	(b) (4)	(b) (4)	(b) (4) Cryoshipper
Visual Inspection (b) (4)	(b) (4) DP Vials	LBSS	Not Applicable

Overall Reviewer's Assessment of Sections 3.2.P.5.1 and 3.2.P.5.6:

The revised specifications are acceptable, however there is likely variation between DP lots that is not captured or controlled by lot release testing.

3.2.P.5.2 and 3.2.P.5.3 Analytical Procedures and Validation of Analytical Procedures (MK and HD)

Review responsibilities for DP analytical procedures and their validation were shared among the product office division (DCGT), the Division of Biological Standards and Quality Control (DBSQC), and the Division of Manufacturing and Product Quality (DMPQ). The table below indicates the review divisions responsible for each of the analytical procedures used for DP lot release testing.

Table 42 Review Responsibilities for DP Analytical Procedures

Procedure/Test	Description	Review Division
In-Process Sterility/DP Sterility	(b) (4)	DBSQC
Mycoplasma		DBSQC
Endotoxin		DBSQC
Phenotype		DCGT
Potency		DCGT
Activity		DCGT
Appearance		DBSQC
Cell Viability/Concentration		DCGT
Residual Bovine Serum Albumin		DBSQC
Residual rTrypsin		DBSQC
Visual Inspection/(b) (4) Sampling		DMPQ

Reviewer Comment: The analytical procedures reviewed by DCGT are discussed below. Please see DBSQC and DMPQ review memos for review of procedures reviewed by these respective divisions.

Cell Viability/Concentration (Potency)

Procedure: (b) (4)

(b) (4)

Overall Reviewer's Assessment of Sections 3.2.P.5.2 and 3.2.P.5.3:

The procedures as described are acceptable, however addition studies should be performed to address minor deficiencies in assay validation described below.

Minor Deficiency: Additional robustness data needed to validate potency and activity assays. See CR Item #6.

Minor Deficiency: The additional information provided in 125706/0.52 and 125706/0.54 containing (b) (4) validation data was not reviewed. This material will be reviewed upon submission of your complete response, and additional deficiencies may be identified at that time. See CR Item #7.

3.2.P.5.4 Batch Analyses

The Applicant provided release testing data from (b) (4) full-scale lots made at the LBSS facility during the 2015-2016 manufacturing campaign. Many of these lots were used in Study 001, but other were used for stability, extended characterization, or other process development studies. The results are summarized in the table below.

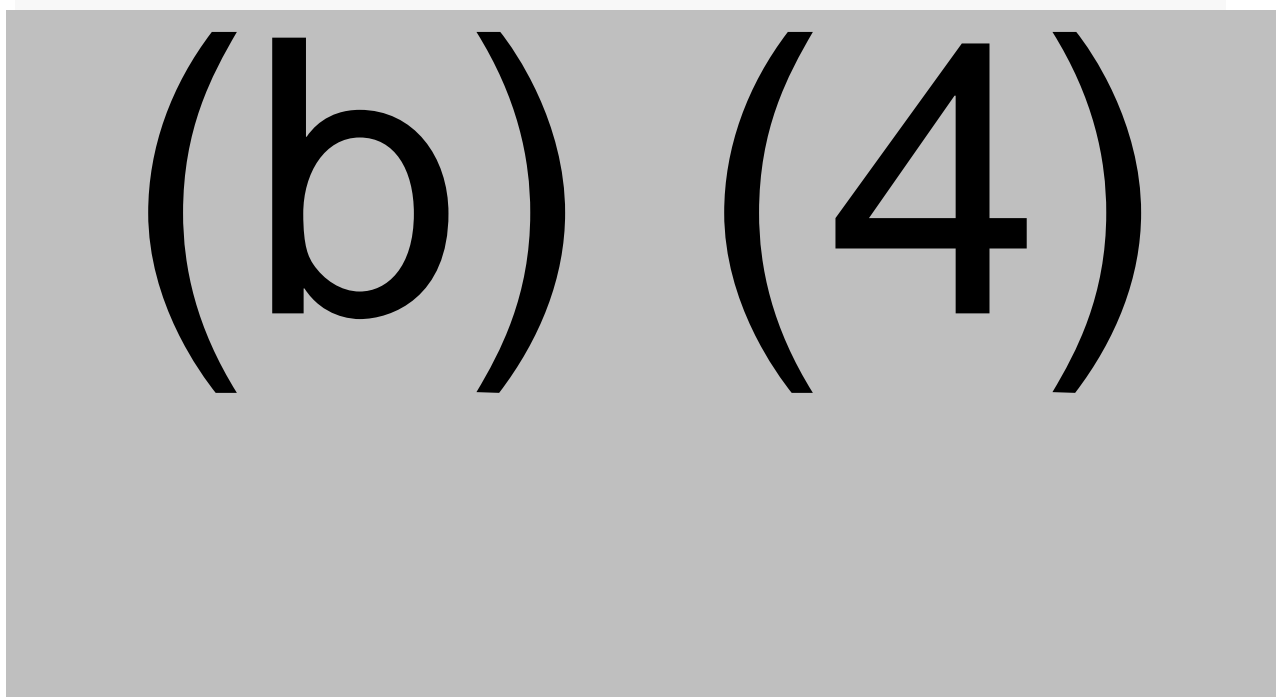
Table 47 DP Batch Analyses Summary

Assay	Specification	Batch Analysis Average
% CD106+	(b) (4) CD166+	(b) (4)
% CD105+	(b) (4) CD105+	(b) (4)
% CD45+	(b) (4) CD45+	(b) (4)
Viability	(b) (4) Viability	(b) (4)
TNFR1	(b) (4)	(b) (4)

Assay	Specification	Batch Analysis Average
Inhibition of IL-2R α	(b) (4)	(b) (4)
Residual BSA	(b) (4)	(b) (4)

Manufacturing ceased at LBSS in May 2016, but was started again in 2019 for PPQ studies and continued through review of this application in anticipation of approval. The original submission contained results from (b) (4) DP lots made during PPQ, and after the mid-cycle communication the Applicant provided additional data from (b) (4) DP lots made during review of this application (125706/0.32). The DP lots used in Study 001 were mostly made during the 2015-2016 manufacturing campaign ((b) (4) lots), but (b) (4) lots of DP were made at LWI while still under OTI. The figures below compare the attributes of the DP lots used in Study 001 with commercial and PPQ lots made in 2019-2020.

Figure 16 DP Potency in Clinical and Commercial Remestemcel-L Lots



Reviewer Comment: The apparent reduction in inhibition of IL-2R α observed in DP lots made in 2019-2020 was discussed throughout review of this application. Please see the *Development of the Inhibition of IL-2R α Assay* part of this memo (Module 3.2.P.2.3, *Manufacturing Process Development*) for review of this issue.

3.2.P.5.5 Characterization of Impurities

Table 48 Product-Related Impurities

Impurity Type	Control Strategies
Non-MSC Cells	<ul style="list-style-type: none"> (b) (4) assay to assure high frequency of cells expressing MSC markers, low frequency of cells expression CD45 (b) (4) DP) Species identification by (b) (4) (b) (4)
Dead Cells	Viability specification (DP)
Cells with Chromosomal Abnormalities	Karyology (DCB)
Viral Adventitious Agents	<ul style="list-style-type: none"> Donor screening and testing (BMA) Assays for detection of viral contamination (DCB)

Table 49 Process-Related Impurities

Impurity Type	Control Strategies
Viral Adventitious Agents	Control of materials of animal and human origin (DCB and DP)
(b) (4)	Diluted during manufacturing process to levels below detection
(b) (4)	Diluted during manufacturing process to levels below detection
Trypsin	Limited by Specification (b) (4) DP)
FBS	Limited by Specification (b) (4) DP)
Endotoxin	Limited by Specification (b) (4) DP)
Microbial Contaminants	Limited by Specification (b) (4), DP and in-process)
Particulates	<ul style="list-style-type: none"> Evaluated for appearance (b) (4) DP) Visual Inspection and (b) (4) Sampling (DP)

Overall Reviewer's Assessment of Sections 3.2.P.5.4 and 3.2.P.5.5:

The information provided in this section is adequate, and impurities in the DP are adequately controlled. Batch analyses showed that product attributes were more consistent among DP lots used in Study 001 than DP lots made in 2019-2020 for commercial use, but revised specifications ensure that only lots consistent with DP lots used in Study 001 are released for commercial use.

3.2.P.6 Reference Standards or Materials

Although the MSC research community has established cellular attributes that define MSCs, there is no accepted reference standard for MSCs. Reference standards were used in several analytical procedures used to test DCBs for release at the time of manufacturing, however, and are summarized in the table below.

Recombinant IL-2R α : This standard is identical to that reviewed in Module 3.2.S.5.

(b) (4)

(b) (4)

is determined to ensure that results will be within the range of the standard curve of the (b) (4). No information regarding the stability or expiration date of qualified (b) (4) lots was provided in Module 3.2.S.

Recombinant TNFR1: (b) (4)

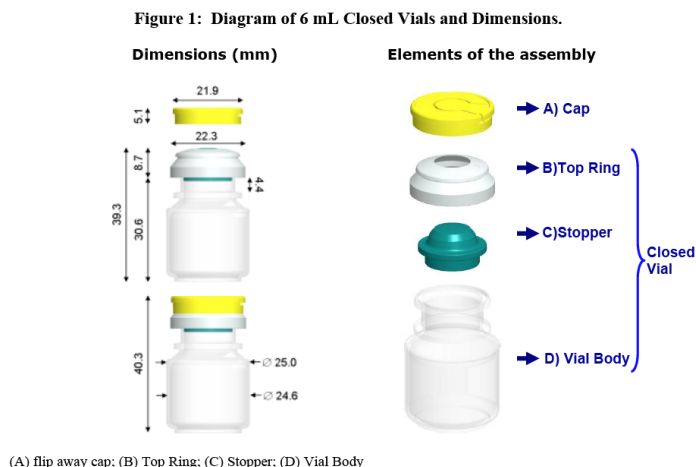
Endotoxin: (b) (4) was used in endotoxin assay used for release testing of DCB lots at the time of manufacture. The endotoxin standard is used for up to (b) (4)

3.2.P.7 Container Closure System (BN)

Primary Container Closure for DP

The primary container closure for the remestemcel-L DP is a 6.0 mL ready-to-fill closed vial manufactured and supplied sterile by (b) (4). The container consists of a vial body (cyclo-elefin copolymer), a stopper (thermoplastic elastomer), a top ring (acrylonitrile butadiene styrene, non-product contacting), and yellow flip away cap (high density polyethylene, non-product contacting). The figure below contains a diagram of the container closure.

Figure 17 Diagram of DP Container Closure Vials



The DP is dispensed into sealed vials at LBSS using a (b) (4) Filling Station, with a target fill volume of (b) (4) of the formulated DP. The stopper is (b) (4) DP is dispensed into the vial, then the (b) (4) by a (b) (4) on the stopper and the closed vial is capped using a snap-fit plastic cap. After filling and inspection, the closed DP vials are cryopreserved at $\leq -135^{\circ}\text{C}$ in liquid nitrogen vapor phase until shipment to the Applicant's secondary distribution center (ICS) and subsequently to clinical sites for administration.

Each lot of closed vials and caps are supplied with a Certificate of Conformity indicating that the lot complies with the manufacturer's specifications, which are summarized in the table below.

Table 50 Compliance and Specifications for DP Container Closure

Test Item	Compliance/Specification
Vial Body: Cyclo-Olephin Copolymer (COC)	(b) (4)
Stopper: Thermoplastic Elastomer (TPE)	
Endotoxin	
Particles	
Cap: High density polyethylene (HDPE)	
Cap Color	
Cap Functionality	
Vial and Cap Conformity	
Sterility (vials and caps)	


The container and closure components have been evaluated by the manufacturer and meet regulatory compliance requirements, including biocompatibility per (b) (4) for containers, and (b) (4) for elastomeric closures for injections. The vial is tested for endotoxins per (b) (4) with a specification of (b) (4). The closed vials and caps are sterilized by (b) (4) that has been validated by the manufacturer.

LBSS also performs identity testing on incoming lots using (b) (4) before their use in manufacturing. Additional qualification testing is performed on the (b) (4) incoming lots from each unique vendor including (b) (4) testing per (b) (4), endotoxin testing per (b) (4) and sterility testing per (b) (4).

DP vials are packed in fibreboard cryoboxes for shipping transport and placed in racks in a fully charged liquid nitrogen dry vapor shipping dewar at $\leq -135^{\circ}\text{C}$. The temperature within the shipping dewar is monitored throughout transport. The shipper's inner lid is secured with a serialized zip tie and the outer lid with a non-serialized zip tie. Shipping records are included with the shipper.

DP Container Closure Integrity and Shipping Validation

The applicant performed a shipping validation study of the DP filled in the 6mL vials as well as integrity testing of the vials (see test report MR-084 in 3.2.P.2). The study is meant to validate the packaging and shipping procedure of the DP (b) (4)

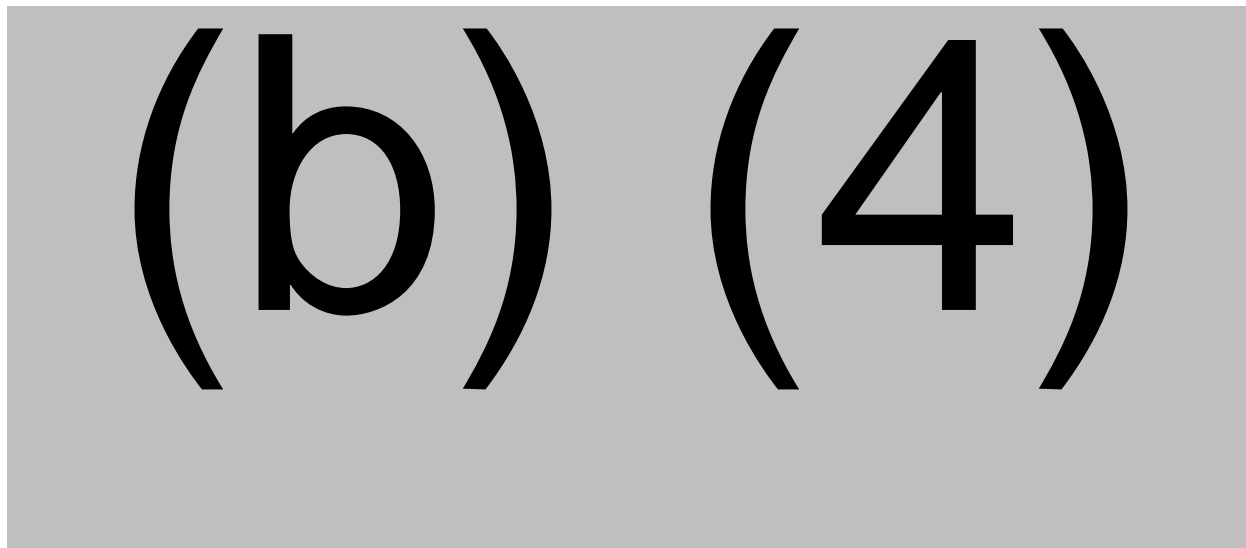


Compatibility of Primary DP Container Closure

The applicant conducted extractable testing (Report C17266.1) on the DP container closure. The applicant states that while the cellular product has direct patient contact, but that the container closure has indirect contact with the patient. Therefore, the sponsor assumes that the container closure has indirect contact with the patient. The applicant also provided a toxicological risk assessment (TRA) for the DP container closure system, based on the extractables and leachables test reports. The TRA was conducted by (b) (4), to determine if leachables or extractables from the container closure could cause toxicities in patients, including carcinogenic and noncarcinogenic systemic risks.

The table below summarizes the conditions used to test the DP container closure in the extractable and leachable studies.

Table 51 Summary of Extractable and Leachable Studies for DP Container Closure



The extractable study identified 82 chemicals which were grouped into 11 groups based on likely similar mechanisms of toxicity in the TRA. Of these, 5 chemicals and one chemical group were identified with a margin of safety (MOS) <1, with a MOS ≥1 indicating an acceptable risk from exposure. No chemicals were identified in the leachable study at a concentration above 1 µg/vial. The Applicant concludes that the chemicals and chemical group identified with MOS <1 in the extractable study present an acceptable risk based on conservative assumptions used in determining MOS and the absence of these compounds from the leachable profile.

INTERACTIVE REVIEW

A request was made for an inter-center consult review from CDRH, and two CDRH reviewers provided consult reviews of the extractable and leachable studies as well as the associated TRA. Their initial review identified deficiencies which were communicated to the Applicant in IR#18 on May 4, 2020. The Applicant's response to IR#18 on May 29, 2020 (125706/0.27) was also reviewed by the CDRH consult reviewers and additional comments for the Applicant were issued in IR#32 on July 13, 2020. The Applicant's response to IR#32 (125706/0.45, July 21, 2020) described additional studies the Applicant committed to performing to address the remaining deficiencies.

Following ISO standard 10993-1: "Biological evaluation of medical devices," to study the biocompatibility of the DP container closure system, the Applicant assumed that the container closure has direct contact with the DP, but does not have direct contact with the patient receiving the product. Therefore, the Applicant concludes that the container closure has indirect contact with the patient through direct contact with the DP. Given these assumptions, the Applicant estimates that the extractables worst-case exposure will be at 100% bioavailability, meaning full exposure to the compound for (b) (4). Based on this information, the Applicant conducted a toxicological risk assessment

(TRA) and leachables study on the DP container closure system to ensure that no chemicals transfer into the drug product from the packaging. Specifically, the Applicant conducted extraction studies using exaggerated and clinical (b) (4) conditions, which identified and categorized potential extracted chemicals as unlikely or likely to cause unacceptable health effects using a margin of safety method. The Applicant concluded in its TRA that the amounts of chemical compounds, based on the margin of safety, found during the extraction process did not pose a risk to patients.

Reviewer Comment: Dr. Hainsworth Shin (CDRH/OSEL/DBCMS) reviewed the TRA and initially had concerns about the extraction methods and assumptions made by the Applicant. The Applicant assumes that in a worst-case scenario, any extractable compounds will be present after administration in the patient for up to (b) (4). Based on Dr. Shin's experience with medical devices, he utilized an assumption that the container closure acts as an implanted device in relation to the biological drug product, and therefore would have an extended exposure (b) (4) once administered. However, in the case of the drug product, it is administered as a one-time bolus injection, rather an implantable device. Therefore, the Applicant's worst-case scenario is reasonable from the perspective of exposure to potential extractables. As part of the extractables study, the Applicant used a margin of safety method to determine the risk of extractables to patients. In response to IR#18, the Applicant indicated that it was referencing a margin of safety method cited by the US Environmental Protection Agency (US EPA) for toxic materials. Based on this method, the Applicant did not find several compounds to be of concern. However, Dr. Shin concluded that the method is not sufficient for medical devices and recommends that additional studies or justification are provided to demonstrate the safety of some of the extractable compounds identified.

For the leachables study, the Applicant conducted a study at which the leachables from the DP container closure were assessed for (b) (4). The leached chemicals were then screened, and some were analyzed using (b) (4).

Reviewer Comment: Dr. Samantha Wickramasekara (CDRH/OSEL/DBCMS) reviewed the leachables study for the DP container closure and concluded that the time and temperature parameters were not representative of the storage conditions of the DP in the container closure system, mainly in that it lacked freeze/thaw cycles. Dr. Wickramasekara indicated that the risk for leachables is highest during abrupt temperature changes, such as freeze and thaw steps. In addition, not all samples were analyzed using (b) (4), which is necessary to detect all possible leachable compounds under clinical and worst-case conditions. Therefore, Dr. Wickramasekara recommends that the leachables study is conducted utilizing appropriate and relevant leachables parameters with sufficient analysis using (b) (4).

In response to IR#32, the Applicant agreed to conduct additional extraction and leachables studies to assess the toxicological risk of the container closure.

Phase 1 (12-week completion): *Extractions will be performed in solvents of varying polarities under exaggerated time and temperature conditions using (b) (4) as the guidance.*

- As the extracts are projected to have an AET less than the instrumental LOD, it is necessary to concentrate the extracts for (b) (4) analysis in order to meet the requirement that LOD be less than AET.
- (b) (4) analysis will be performed for volatile and semi-volatile compounds.
- (b) (4) analysis will be performed for semi-volatile to non-volatile compounds (using (b) (4))
- (b) (4) analysis will be performed for approximately (b) (4) verified by (b) (4)
- (b) (4) analysis will be performed for residual solvents taking guidance from (b) (4)

Mesoblast estimates that following completion of this phase, a report would be available for submission to the agency by November 6, 2020.

Phase 2 (4-week completion): Results of the Phase 1 Extraction study will undergo toxicological risk assessment (TRA), followed by analytical method development and validation for specific chemicals of concern. The analytical method optimized in the development portion of the project will be validated as a Category II Assay (determination of impurities in bulk drug substances or degradation compounds in finished pharmaceutical products).

Mesoblast estimates that following completion of this phase, a report would be available for submission to the agency by December 11, 2020.

Phase 3 (16-week completion): This study is designed to develop methods based on the chemicals found in the extractables studies of the 6 mL (b) (4) Vial, including the D28092 C17266 and earlier extractable study. Analytical methods will be optimized which are appropriate for each applicable analytical technique using the drug product solution. After the developed and optimized methods are complete, validated protocols will be created and validated methods will be used to evaluate the leachables throughout the stability system.

Mesoblast estimates that following completion of this phase, a report and protocol would be available for submission to the agency by April 10, 2021.

Phase 4 (8-week completion): This study is designed to assess the targeted leachables of the fluid path of the 6mL (b) (4) Vial by quantitative analysis. Extractions will be performed in a solvent typical for the test article under clinically relevant conditions including freeze-thaw cycling. Data will be processed using (b) (4) calibration curves of standards or appropriate surrogates for each targeted compound as determined in the method validation project. Compounds of interest will be determined by the compounds of potential concern (CoPCs) identified during extractables analyses and the associated Toxicological Risk Assessment. Results of the Phase 4 targeted leachables study will undergo an additional toxicological risk assessment (TRA).

Mesoblast estimates that following completion of this phase, a report would be available for submission to the agency by June 11, 2021.

Reviewer Comment: According to Dr. Wickramasekara's review, the Applicant has provided a sufficient response to address the concerns regarding the leachables study. The Applicant has provided an acceptable plan to study the extractables and leachables for the DP container closure system. Therefore, based on the information submitted in this response, the proposed study plan will provide sufficient information about the potential risk of the DP container closure system to patients.

Overall Reviewer's Assessment of Section 3.2.P.7:

In this section, the Applicant described the container closure system for the DP, which consists of 6 mL vials from (b) (4). To evaluate the long-term effects of storing the DP in the container closure, the Applicant conducted extractables and leachables testing as well as a toxicological risk assessment. These test reports were reviewed by CDRH consults, who identified several deficiencies. While the assumptions of the CDRH consult reviewers (i.e., exposure profile of container closure to subject) are not all applicable to this submission, some of the identified deficiencies still apply and the deficiencies identified by the consult reviewers were communicated to the Applicant. In response, the Applicant proposed a 4-phase study to determine the extractables and leachables of the DP container closure, however the study would not be completed before the action date for this application. The overall approach described by the Applicant to characterize and assess the safety of the DP container closure system is acceptable. Considering the safe use of this container closure throughout Study 001 and the limited extractable and leachable studies already performed, the review team concluded that the remaining deficiencies do not present a significant safety risk to those receiving the product and therefore do preclude approval. The Applicant should confirm commitment to completing and submitting the information outlined in the 4-phase study for review as part of the BLA.

Minor Deficiency: The Applicant committed to providing additional extractables and leachables studies after the PDUFA action date in 1254706/0.45. See CR Item #8.

3.2.P.8 Stability (BN)

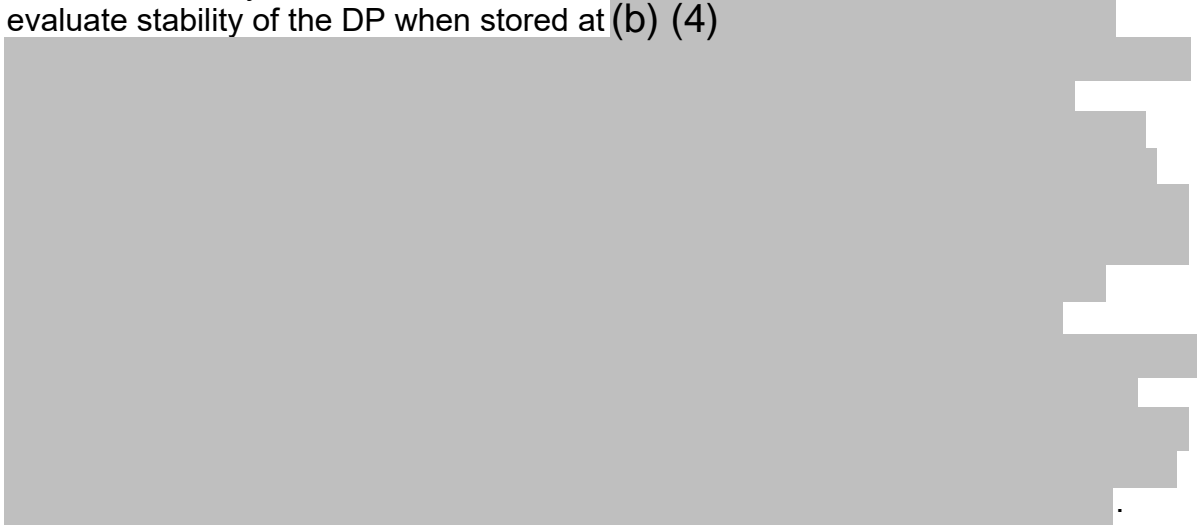

3.2.P.8.1 Stability Summary and Conclusion and 3.2.P.8.3 Stability Data (BN)

The Applicant provides stability data for the DP when stored in the final container closure (a 6 mL closed vial from (b) (4)). Each vial is filled with (b) (4) of the DP was filled into the vial. The container closure, fill volume, and DP formulation used in these studies are identical to the proposed commercial product. The Applicant proposes a shelf life of 48 months when stored at < -135°C in liquid nitrogen vapor.

The Applicant conducted a long-term viability study using (b) (4) batches of DP (across three different studies):

- 1) A long-term, real-time stability study (SP-011) was conducted, storing the (b) (4) lots of DP at < -135°C in liquid nitrogen vapor for 48 months. The Applicant evaluated potency (TNFR1, IL-2Rα), phenotype (CD166⁺, CD105⁺, CD45⁺), cell viability, endotoxin, sterility, and appearance at multiple timepoints (0, 3, 6, 9, 12, 18, 24, 36,

and 48 months). The results support a shelf life of 48 months when stored at $< -135^{\circ}\text{C}$ in liquid nitrogen vapor.

- 2) Long-term, real time stability study (SP-013) studied (b) (4) manufactured DP lots as part PPQ. The lots were manufactured at LBSS and were stored at $< -135^{\circ}\text{C}$ in liquid nitrogen vapor for 60 months. The (b) (4) lots were taken from (b) (4) separate DCBs (b) (4) from (b) (4) donor, and (b) (4) of the other donors). This study is on-going, and the Applicant provided data up to 6 months in 125706/0.15. The data collected thus far supports a shelf life of 6 months when stored at $< -135^{\circ}\text{C}$ in liquid nitrogen vapor.
- 3) Long-term stability study SP-008 was conducted on DP lots used in the Phase 3 clinical study. The lots were manufactured from (b) (4) different DCB lots. (b) (4) of the lots have finished the 48-months stability study, while one was only studied until the 24-month time point. The data support a shelf life of 48 months when stored at $< -135^{\circ}\text{C}$ in liquid nitrogen vapor.
- 4) Accelerated study SP-015 used DP lots manufactured during PPQ, and intended to evaluate stability of the DP when stored at (b) (4)

- 5) Short-term post thaw formulation study SP-014 evaluated the viable cell concentration of a dose of DP following thaw, dilution with Plasma-Lyte A, and held at room temperature at 5 hours to represent the clinical post-thaw formulation procedure and maximum clinical administration infusion time limit. The study used (b) (4) DP lots manufactured at LBSS as part of the PQQ. The (b) (4) lots were manufactured using (b) (4) different DCB lots. The dose was formulated at a clinical dose intended for administration of a subject weighing 50 kg. After thawing, the DP (approximately (b) (4) cells) was diluted in 40 mL Plasma-Lyte A (formulated DP). The formulated DP was then held at room temperature and samples were taken at 0, 30, 90, 180, and 300 minutes post formulation. At the 300-minute timepoint, the cell suspension was sampled (b) (4) . At each of the timepoints, the samples were tested for cell concentration and viability. Table 10 summarizes the stability data for this study to support the proposed post-thaw viability of the cells for 5 hours after formulation.

The Applicant provided a statistical evaluation of the stability studies performed (Report 190704) that describes the analysis conducted according to ICH Q1E stability guidance for the (b) (4) lots of DP manufactured by the current manufacturing process. The Applicant states that at the time of the analysis, up to 36 months of data was available for the (b) (4) DP lots as utilized in study SP-011. According to the report, the Applicant concluded that as ICH Q1E stability guidance allows for extrapolation up to two times the period covered by long-term data, but no more than 12 months beyond the last time-point for which data are available. Therefore, the maximum allowable shelf life for this study is 48 months, as proposed by the Applicant. The Applicant states that after the completion of study SP-011, the data demonstrates that the DP can meet all end of shelf-life specifications when stored at $\leq -135^{\circ}\text{C}$ in liquid nitrogen vapor phase for 48 months.

Reviewer Comment: All stability studies were analyzed using the Applicant original specifications. The Applicant committed to re-evaluate these results using the revised specifications agreed upon during review of this application, but this re-evaluation was not provided in time for review.

3.2.P.8.2 Post-Approval Stability Protocol and Stability Commitment (BN)

The Applicant provided a protocol describing their intended post-approval stability studies (Study SP-016). At least one DP lot produced each year will be placed into the long-term stability program, as well as the first DP lot made from each DCB lot. Testing will continue out to 60 months, and with each lot tested as for lot release and (b) (4).

The Applicant states that the impact of any significant manufacturing process changes on stability will be assessed via a change control process. Additional final product from (b) (4) donors may be required to be placed in both Long-Term and Accelerated (b) (4) stability programs. Additionally, the Applicant states that data from at least 6 months on long term stability and (b) (4) accelerated will be required for comparability to current marketed product.

Overall Reviewer's Assessment of Section 3.2.P.8:

The Applicant has conducted several stability studies to demonstrate that the DP remains stable and meets predetermined specifications, including viability, sterility, endotoxin, identity, potency, and cell concentration, for a proposed 48-month shelf life. The Applicant utilized the same manufacturing process for the stability studies as the process used for the Phase 3 clinical study, which is identical to the proposed commercialization process. The Applicant has also demonstrated that the 5-hour shelf life of the formulated DP when kept at room temperature is acceptable, as the formulated DP is able to maintain the cell viability and cell concentration during these conditions.

As part of the post-approval stability study commitment, the Applicant has proposed one long-term study ($\leq -135^{\circ}\text{C}$ in LN2 for 60 months). The proposed product characteristics and specifications are identical to the final product release specifications.

While the studies provided supported the Applicant's proposed shelf life and hold times using their original specifications, these results should be re-evaluated using the revised specifications agreed upon during review of this application.

Minor Deficiency: Please provide an updated analysis of product stability using product specifications as revised during review of this application and any additional assays implemented for lot release. See CR Item #9.

3.2.A APPENDICES

3.2.A.1 Facilities and Equipment

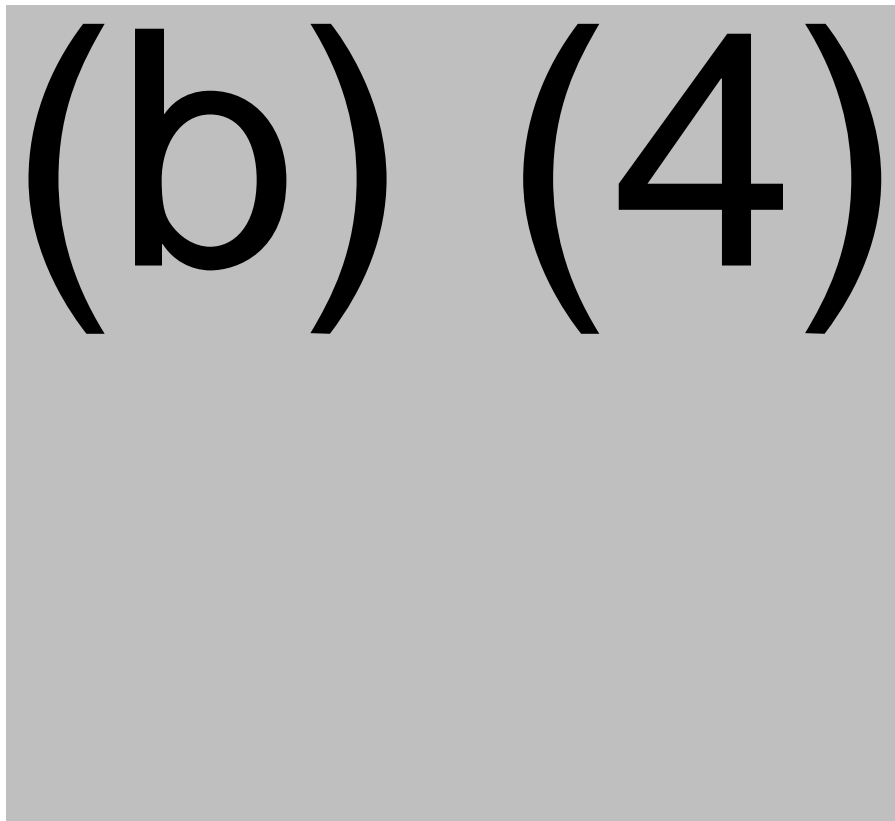
The Applicant provided summaries of the three main manufacturing facilities: (1) LWI, where DCB were manufactured in 2008-2009, (2) LBSS, where DP is manufactured from DCB material for the commercial process, and (3) ICS, where the DP vials are packaged into secondary cardboard cartons.

LWI Facility Summary

DCBs were manufactured at LWI in 2008-2009, but no additional manufacturing will occur at this site. The is still an active manufacturing facility, but does not appear to manufacture cell therapy products at this time. The facility is approximately (b) (4) square feet and included manufacturing, support, storage, laboratory, and office areas. The facility is located in Walkersville, MD, USA.

The area used for (b) (4) was the Production/Final Fill suite that consisted of (b) (4) ISO (b) (4) rooms. Most DCB lots were cryopreserved in an adjacent (b) (4) room, although some lots were cryopreserved in a similar (b) (4) room in an adjacent building if the lot size was large or the equipment in the adjacent room was undergoing maintenance. The figure below shows a diagram of the main production suite.

Figure 18 Layout of LWI Production and Filling Suite



The diagram below shows the production and filling suite and surrounding rooms. Products flow from the (b) (4) suites to the (b) (4) room for cryopreservation, then through the (b) (4) rooms to the warehouse for storage.

Figure 19 Product Flow in LWI Building (b) (4)



Equipment used in the production of DCBs at LWI included Class (b) (4)

Reviewer Comment: The Applicant provided a general overview of how relevant systems at LWI were controlled and maintained, however the status of the facility regarding cGMP compliance cannot be established because production is no longer occurring at this facility. For review purposes, we considered the cryopreserved DCB material acceptable for production based on the extensive testing each DCB was subject to at release and additional requalification tests performed by MSB prior to using DCB lots in manufacturing.

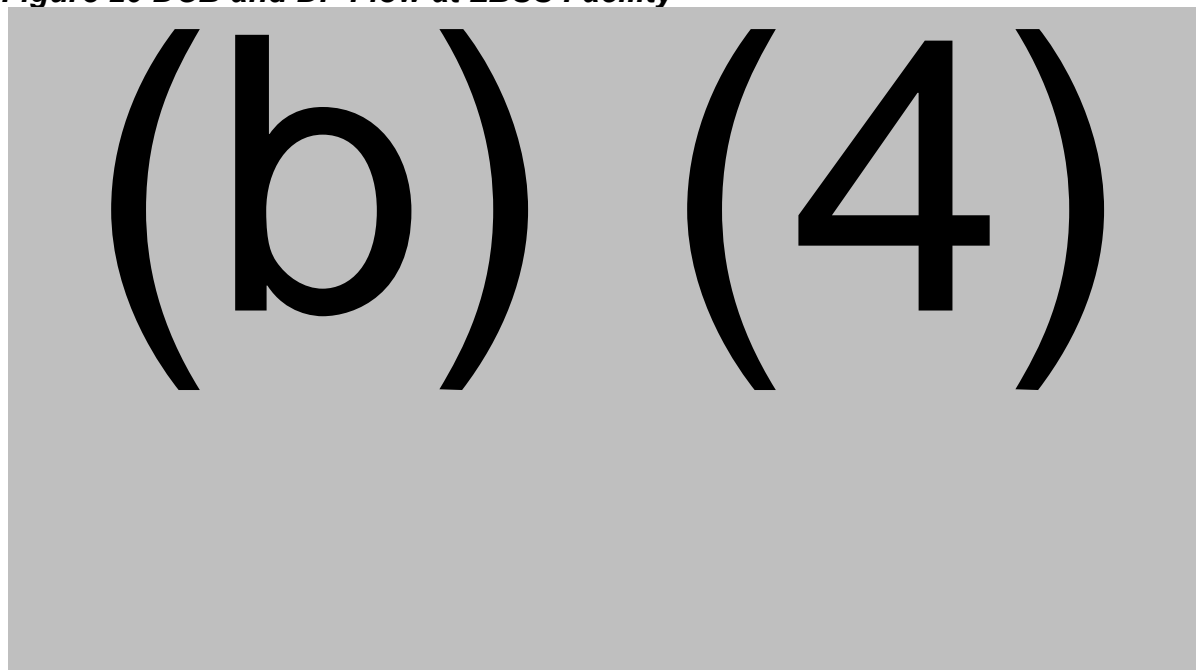
LBSS Facility Summary

The remestemcel-L DP is made from DCBs at Lonza's LBSS facility, a multi-product facility for production of cell therapy products located in Tuas Biomedical Park in

Singapore. The facility has approximately (b) (4) square feet of space, and includes cGMP production areas as well as office, warehouse, and laboratory areas. It is a (b) (4) - story building with office, laboratory, and surface areas on the (b) (4) floor, and the production area consisting of the (b) (4)

for cryopreservation of the final product. The building is adjacent to Lonza Biologics Tuas (LBT), a contract manufacturing facility for the production of monoclonal antibodies. While LBT has been inspected by FDA personnel in the past year, the LBSS facility has a GMP certificate issued by the Health Sciences Authority of Singapore but has not been previously inspected by FDA personnel. The figure below is a diagram of the (b) (4) -floor production and support areas, showing directions of flow for DCB and media reagents into the production suite (orange), formulated product (b) (4) for cryopreservation (blue).

Figure 20 DCB and DP Flow at LBSS Facility




Equipment used at LBSS include (b) (4)

Reviewer Comment: Production of DP lots was ongoing though most of the review period for this application, however travel restrictions in place to reduce the spread of the pandemic SARS-CoV2 virus prevented travel to Singapore to inspect the LBSS facility during review. DMPQ and DCGT both recommend that the product not be approved without an inspection to confirm the cGMP-compliance status of LBSS, and comment regarding the inability to inspect this facility will be included as a CR item. More in depth review of the facility information provided is deferred until an inspection becomes imminent.

ICS Facility Summary

The final stage of manufacturing occurs at Integrated Commercialization Solution (ICS), and contract facility located in (b) (4), USA. Here, the cryopreserved DP vials are packaged into cardboard cartons and stored in a liquid nitrogen freezer until distribution. The facility was designed as a multi-product storage facility for cell therapy product, small molecules, biologics, and drug-device combination products. The facility is approximately (b) (4) square feet, and includes segregated areas for storage of products at various temperature. Within the facility is a (b) (4) square foot area dedicated for cGMP-classified activities, such as labeling/relabeling, repackaging, and serialization of product arriving from production facilities to be stored on-site.

The Applicant did not provide a floor diagram for this facility, but describes the flow of product through the facility as (b) (4). Incoming cryoshippers are (b) (4)



The inventory of each (b) (4) is maintained by the (b) (4) inventory control system. When an order is placed a pick ticket is generated, and after verification of the product code and expiration date is confirmed the product cartons are transferred into a liquid nitrogen vapor container. A (b) (4) employee then verifies the contents are consistent with the request, and then the product is placed into a qualified shipping Dewar and picked up by a courier for delivery to the clinical site.

Reviewer Comment: The information provided for the ICS facility is somewhat limited, but the inventory control system and controls in place to prevent cross-contamination or product mix-ups appear to be adequate.

Overall Reviewer's Assessment of Section 3.2.A.1:

The information provided regarding manufacturing facilities is acceptable. Further review of the LBSS facility will occur upon scheduling of a pre-license inspection.

3.2.A.2 Adventitious Agents Safety Evaluation

Please see review of Modules 3.2.S.2.3 for discussion of control of BMA starting material and materials used in manufacturing the DCB, and review of Module 3.2.P.3.3 for discussion of materials used in production of the DP.

Viral Clearance Studies

Not applicable.

Overall Reviewer's Assessment of Section 3.2.A.2:

Donor eligibility determination and DCB testing for adventitious viral agents are acceptable. Materials used in manufacturing remestemcel-L are adequately controlled to prevent introduction of viral contaminants.

3.2.A.3 Novel Excipients

Not applicable.

3.2.R Regional Information (USA)**❑ Executed Batch Records**

The Applicant provided batch records for a DCB lot (b) (4), (b) (6) and DP lot (b) (4) . The records submitted in the original application, however, consisted only of documentation of the manufacturing process and did not include all relevant documentation that should be reviewed for lot release. In CMC IR#19 we requested all documentation required for review for lot release, and the Applicant responded to this request in 125706/0.15 by providing documentation for donor eligibility determination, reports for in-process and lot release testing, and review by the quality unit for release.

Documentation for DP manufacturing is kept at LBSS, but compiling lot release testing results and other documentation for review occurs by Mesoblast's quality team in Singapore. Before inspection of the LBSS facility occurs, the inspection team should clarify where this review occurs and notify LBSS or Mesoblast Singapore personnel that batch records should be available for review during the inspection.

❑ Method Validation Package

Validation reports were provided in Module 3.2.S.4.3 and 3.2.P.5.3. See review of these sections for discussion of method validation.

❑ Combination Products

Not applicable.

❑ Comparability Protocols

No comparability protocols were provided in this application; however, the Applicant did submit a draft comparability protocol to IND 7939 prior to completing this application.

Deficiencies identified in this protocol will be communicated to the Applicant under IND 7939.

Other eCTD Modules

Module 1

A. Environmental Assessment or Claim of Categorical Exclusion

The Applicant claims categorical exclusion from the requirement to provide an environmental assessment under 21 CFR 25.31.

B. Labeling Review

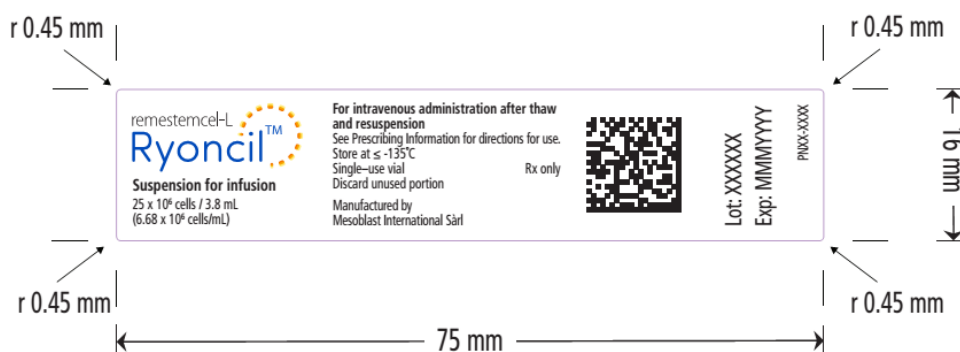
Full Prescribing Information (PI):

Prescribing information was reviewed and revisions were made to the draft provided by the Applicant. The revised PI was not, however, provided to the Applicant as a decision to CR this application was made before review of the revised PI by OTAT management. Review of PI will continue at the time a complete response is received.

Carton and Container Label:

The Applicant provided draft labeling for the 6 mL vial container closure as well as the outer carton. As DP vials must be labelled before cryopreservation, the Applicant received approval of the proprietary name prior to completing this application. Manufacturing of DP occurred during review of this application at the Applicant's risk, and as the non-proprietary name suffix was not approved until later in the review cycle it is not included on the vial label. The figure below is a recreation of the vial label.

Figure 21 Proposed Label for Remestemcel-L Vials



DP vials are distributed into secondary cartons at ICS, with each carton containing either one or four vials. The figure below shows a panel from the proposed 4-vial carton.

Figure 22 Proposed Labeling for Remestemcel-L Secondary Carton (4-vial)**Modules 4 and 5****Analytical Procedures and Validation of Analytical Procedures for Assessment of Clinical and Animal Study Endpoints**

Clinical outcomes were analyzed in relation to product potency as reviewed and discussed in Module 3.2.P.2.3. No analytical methods used in pre-clinical studies were reviewed.