I concur with this review memo. A. Wensky. 4/13/2023

FOOD AND DRUG ADMINISTRATION
Center for Biologics Evaluation and Research
Office of Therapeutic Products
Office of Pharmacology/Toxicology
Division of Pharmacology/Toxicology 1
Pharmacology/Toxicology Branch 3

BLA NUMBER:	STN #125738.000
DATE RECEIVED BY CBER:	01-Jun-2022
DATE REVIEW COMPLETED:	12-Apr-2023
PRODUCT:	OMISIRGE (omidubicel-onlv)
APPLICANT:	Gamida Cell Ltd.
PROPOSED INDICATION:	Indicated to reduce the time to neutrophil recovery and the incidence of infection in adult and pediatric patients (12 years and older) with hematologic malignancies undergoing myeloablative conditioning regimen followed by umbilical cord blood transplantation.
PHARM/TOX REVIEWER:	Kate Dabirsiaghi
PHARM/TOX BRANCH CHIEF:	Allen Wensky
PRODUCT (CMC) REVIEWERS:	Elizabeth Lessey-Morillon, Sukhanya Jayachandra, Archana Siddam, Heba Degheidy
CLINICAL REVIEWERS:	Najat Bouchkouj
PROJECT MANAGER:	Cara Pardon
DIVISION DIRECTOR:	Iwen Wu (Acting)
OFFICE DIRECTOR:	Iwen Wu

EXECUTIVE SUMMARY:

OMISIRGE (omidubicel-onlv) is a cryopreserved, allogeneic hematopoietic cellular therapy consisting of two cell fractions; a (b) (4) selected cultured fraction (CF) and an (b) (4) non-cultured fraction (NF), which are both derived from the same subject-specific cord blood unit (CBU). In the CF, hematopoietic progenitor cells (HPCs) are expanded in the presence of (b) (4) NAM is intended to inhibit HPC differentiation and to increase HPC migration, bone marrow (BM) homing, and engraftment efficiency. The NF consists of hematopoietic mature myeloid and lymphoid cells. OMISIRGE is indicated to reduce the time to neutrophil recovery and the incidence of infection in adult and pediatric patients (12

years and older) with hematologic malignancies undergoing a myeloablative conditioning regimen followed by umbilical cord blood (UBC) transplantation.

In vitro pharmacology studies evaluating the effects of NAM on the phenotype of (b) (4) cells indicated an increase in non-differentiated early progenitor cells following in vitro culture and increased migratory potential of CD34+ cells towards (b) (4) in a (b) (4) assay.

In vivo pharmacology, pharmacokinetic, and toxicology studies were conducted to evaluate the activity, distribution, and safety of the product in immunocompromised, irradiated mice. Studies evaluated (b) (4) cells cultured with (b) (4)], (b) (4) in nonobese diabetic/severe combined immunodeficiency (NOD/SCID) mice. Intravenous dose levels of 1.2 x 10^6 to 1 x 10^7 cells/mouse (approximately 4.8 x 10^7 to 4 x 10^8 cells/kg) showed an increased overall engraftment (as measured by (b) (4) cells in the BM) compared to (b) (4) cells cultured with cytokines only or unmanipulated cells. Cell distribution data showed the expected widespread distribution of cells following intravenous administration, and engraftment was observed through 6 weeks post-infusion, which was the longest time point evaluated. In the toxicology study, there were no deaths or adverse findings related to the test article.

The genomic integrity was evaluated using (b) (4) analysis. Results showed no difference in the occurrence of chromosomal abnormalities between the CF, NF, and control cells and no evidence of clonal aneuploidy.

Carcinogenicity and developmental and reproductive toxicity studies were not conducted with omidubicel-onlv. These studies are not warranted based on the product characteristics and safety profile.

PHARMACOLOGY/TOXICOLOGY RECOMMENDATION:
There are no nonclinical deficiencies identified in this submission. There are no outstanding requests for additional nonclinical data for evaluation of OMISIRGE. The nonclinical information provided in the BLA submission supports approval of the licensure application.

Formulation and Chemistry:
OMISIRGE is a cryopreserved, allogeneic hematopoietic cellular therapy consisting of two cell fractions; a CF and NF, which are both derived from the same subject-specific CBU. The CF is manufactured from a thawed CBU that undergoes (b) (4) selection for (b) (4) cells. The NF is manufactured from (b) (4) cells that are eluted during the (b) (4) selection process.

1. The CF consists of allogeneic, hematopoietic $CD34^+$ progenitor cells. After (b) (4) (b) (4) At the end of the (b) (4) process, besides $CD34^+$ cells, the CF contains (b) (4) progenitor cells and (b) (4) less differentiated, early progenitor cells, which are mainly myeloid cell subsets at different stages of maturation.

The CF consists of other cell populations as well, including more differentiated myelomonocytic cells, dendritic cells, and granulocytes.

2. The NF consists of allogeneic, hematopoietic mature myeloid and lymphoid cells. The NF is the fraction of (b) (4) cells collected in the flow through effluent during the (b) (4) separation process.

The CF and NF components are collectively referred to as omidubicel-onlv. The CF contains at least 8 x 10^8 total number of viable cells (TNVC) with a minimum of 8.7% $CD34^+$ cells and a minimum of 9.2 x 10^7 $CD34^+$ cells. The NF contains at least 4 x 10^8 TNVC and 2.4 x 10^7 $CD3^+$ cells. Both fractions are cryopreserved at the end of their manufacturing process and thawed and diluted before intravenous infusion using the infusion solution. The fractions are administered separately, with the CF being administered first, then the NF being administered within one hour of the CF.

Abbreviations

ALL	Acute lymphocytic leukemia
AML	Acute myelogenous leukemia
BM	Bone marrow
CBU	Cord blood unit
CF	Cultured fraction
(b) (4)	(b) (4)
cGy	Centigray
CML	Chronic myelogenous leukemia
(b) (4)	(b) (4)
(b) (4)	(b) (4)
(b) (4)	(b) (4)
(b) (4)	(b) (4)
(b) (4)	(b) (4)
gDNA	Genomic DNA
HPC	Hematopoietic progenitor cell
HSA	Human serum albumin
HSC	Hematopoietic stem cell
HSCT	Hematopoietic stem cell transplantation
(b) (4)	(b) (4)
MDS	Myelodysplastic syndrome
(b) (4)	(b) (4)
NAM	Nicotinamide
NF	Non-cultured fraction
ng	Nanograms
NOD/SCID	Nonobese diabetic/severe combined immunodeficiency
(b) (4)	(b) (4)
pg	Picograms
POC	Proof-of-concept

QA	Quality assurance
(b) (4)	(b) (4)
(b) (4)	(b) (4)
SCF	Stem cell factor
SRC	SCID repopulating cell
TBI	Total body irradiation
TNC	Total nuclear cell
TPO	Thrombopoietin
TNVC	Total number viable cells
UCB	Umbilical cord blood

Related File(s)

IND #14459: Allogeneic Unrelated Umbilical Cord Blood Cells (b) (4) Selected with (b) (4) in the presence of Nicotinamide along with (b) (4) Negative Fraction (b) (4) formerly NiCord); and Allogenic Unrelated Umbilical Cord Blood; Chemotherapy; To treat patients with hematological malignancies; Gamida Cell Ltd.

Table of Contents

INTRODUCTION

Allogeneic hematopoietic stem cell transplantation (HSCT) is a potentially curative therapy in which hematopoietic stem cells (HSCs) from a donor are infused into a recipient with a life-threatening hematologic malignancy or other blood disorder. HSCT is considered to be a definitive therapy for subjects with high-risk hematologic malignancies including acute myelogenous leukemia (AML), acute lymphocytic leukemia (ALL), myelodysplastic syndrome (MDS), chronic myelogenous leukemia (CML), and lymphomas.

For subjects who do not have an available matched donor, UCB has been used as an alternative source of stem cells. Omidubicel-onlv has been developed to improve the outcomes in these subjects and is intended to serve as a graft source for the treatment of hematologic malignancies. Omidubicel-onlv is manufactured using a NAM-based technology that is intended to overcome the induction of accelerated proliferation, differentiation, cellular stress, and signaling pathways that are typically activated when HPCs are removed from their natural environment. Differential gene expression analysis using next generation sequencing suggests that NAM maintains expression of several genes associated with preserving HPC function. Thus, HPCs manufactured using NAM technology are intended to preserve stem cell function, including enhanced migration, increased engraftment, ability for long-term self-renewal, and reconstitution of the immune cell repertoire.

NONCLINICAL STUDIES

PHARMACOLOGY STUDIES

Summary List of Pharmacology Studies

The following pharmacology studies were conducted to support the rationale for the administration of OMISIRGE in the proposed clinical population.

In Vitro Studies

Study Number	Study Title / Publication Citation	Report Number
1	The Effect of Nicotinamide on the Migratory Potential of $CD34^+$ Cells	NRD-007
2	Phenotype Characterization of (b) (4) Cell Cultures Treated with Cytokines Plus Nicotinamide or with Cytokines Only	NRD-013

In Vivo Studies

Study Number	Study Title / Publication Citation	Report Number
3	SCID Repopulating Potential of Non-Expanded $CD34^+$ Cells Post-Selection and their Total Progeny Following Expansion with Cytokines or Cytokine Plus Nicotinamide	NRD-006

Study Number	Study Title / Publication Citation	Report Number
4	The Effect of Nicotinamide on the Bone Marrow Homing Potential of Expanded $CD34^+$ Cells	NRD-008
5	Short-Term Engraftment Potential of Cells Cultured with Nicotinamide vs. Unmanipulated Cells	NRD-009
6	Long-Term Engraftment Potential of NAM Cultured Cells and Non-Cultured Fraction in Comparison to Unmanipulated Cells	NRD-010
7	Effect of $CD34^-$ Cells of Omidubicel Cultured Fraction on in vivo Engraftment Ability of $CD34^+$ Cells	NRD-048
8	Correlation Between the Number of Omidubicel's $CD34^+$ Cells Injected to (b) (4) Mice and Engraftment	NRD-050
9	Engraftment of Ex Vivo Expanded Human Hematopoietic Stem Cells in NOD/SCID and (b) (4) Mice	NRD-053
10	(b) (4) of Nicotinamide Cultured Cells Transplanted Along with Non-Cultured or Unmanipulated Fraction Cells	NRD-054
11	The Effect of Cryopreservation on the in vivo Engraftment Ability of Omidubicel	NRD-062

Reviewer Comments:

- *Study No. 8 (NRD-050) is briefly summarized in this review memo under 'Overview of Pharmacology Studies.' Study No. 9 (NRD-053) is not summarized in this review memo as it was a pilot study conducted to determine the appropriate irradiation dose and mouse strain for nonclinical development of the test article and does not contribute to the safety assessment. Study No. 10 (NRD-054) is not summarized in this review memo because it was a pilot study conducted to compare the (b) (4) (b) (4) of the test article to inform selection of the final formulation.*

- *Study No. 1 (NRD-007), Study No. 3 (NRD-006), Study No. 4 (NRD-008), and Study No. 5 (NRD-009) used NAM from (b) (4) at a concentration of (b) (4) The applicant reported that subsequent optimization studies (not provided) suggested the activity of (b) (4) NAM from another supplier (b) (4) was equivalent to (b) (4) NAM from (b) (4) The remainder of the nonclinical studies used (b) (4) NAM from (b) (4) which is used to manufacture the current clinical product.*

- *At different time points throughout nonclinical and clinical development, the test article was referred to as NiCord™ (b) (4) (b) (4) The current proprietary name of omidubicel-onlv is OMISIRGE. The use of these prior terms in any given study does not imply that the test article was identical to the product being reviewed for approval. With the exception of the nonclinical study titles, which were selected by the applicant, the use of "omidubicel-onlv" or "OMISIRGE" in this memo refers to the identical product being reviewed for approval.*

- *The nonclinical studies were conducted with products from different stages of development before the final manufacturing process of omidubicel-onlv was implemented. Similarly, within each clinical study, subjects received products from different stages of the manufacturing process of the test article. Product information was included in the review for each nonclinical study as provided by the applicant. In addition to differences in NAM and cytokine concentration and source, Table 1 specifies major manufacturing differences between the CF products evaluated in each nonclinical and clinical study. The only manufacturing change for the NF throughout development was the implementation of a* (b) (4) *, which was implemented during clinical trial* (b) (4)*, before initiation of P0301.*

Study No.	Report No.	(b) (4)	(b) (4)	(b) (4)	(b) (4)
Nonclinical studies					
1	NRD-007	(b) (4)			
2	NRD-013				
3	NRD-006				
4	NRD-008				
5	NRD-009				
6	NRD-010				
7	NRD-048				
8	NRD-050				
9	NRD-053				
10	NRD-054				
11	NRD-062				
12	7-RT-HAR0100				
13	7-RT-HAR0102				
14	GAM-066-AIT-BIO				
15	NRD-063				
16	NRD-064				
17	NRD-065				
18	GAM-005-EM				
Clinical studies					
	P0101	(b) (4)			
	(b) (4)				
	P0301				
	P0501				
Current clinical manufacturing process					

Table 1. Products used in nonclinical and clinical studies

Overview of Pharmacology Studies

In Vitro Studies

Study #1 (Report No. NRD-007)
The Effect of Nicotinamide on the Migratory Potential of CD34$^+$ Cells

Objective:
To assess in vitro the effect of NAM on the migratory potential of CD34$^+$ HSCs isolated from cord blood.

Methods and Key Results:
The applicant used the (b) (4) to determine cell migration potential towards (b) (4) which is a chemoattractant involved in the recruitment of HSCs to the BM after stem cell transplantation.[2]

CD34$^+$ HSCs were purified from thawed CBUs and cultured for (b) (4) in the presence of (b) (4) (b) (4)

Two experimental designs were tested:

1. In the first set of experiments, the migratory potential of the entire expanded cell population and of CD34$^+$ cells re-purified by sorting for CD34$^+$ was assessed.
2. In the second set of experiments, the migratory potential of the entire expanded cell population was assessed.

The migratory potential towards (b) (4) of CD34$^+$ cells derived from NAM$^+$ cytokine cultures was significantly higher compared to the migratory potential of CD34$^+$ cells derived from cytokine-only cultures, averaging 52.1% vs. 31%, respectively. Similarly, the migration of NAM$^+$ cytokine total cultured cells (b) (4) cells) in response to (b) (4) was higher compared to cytokine-only cultures, averaging 67.7% vs. 48.8%, respectively.

Reviewer Comment:

- *This study demonstrates that NAM could potentially improve migratory response of CD34$^+$ HSCs to* (b) (4) *however, the applicant did not provide the percentage of cells expressing* (b) (4) *Such information would help in the*

[1] P. A. Plett et al., "Treatment of circulating CD34(+) cells with SDF-1alpha or anti-CXCR4 antibody enhances migration and NOD/SCID repopulating potential," *Exp Hematol* 30, no. 9 (Sep 2002), https://doi.org/10.1016/s0301-472x(02)00880-9, https://www.ncbi.nlm.nih.gov/pubmed/12225798.
[2] A. Aiuti et al., "The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood," *J Exp Med* 185, no. 1 (Jan 6 1997), https://doi.org/10.1084/jem.185.1.111, https://www.ncbi.nlm.nih.gov/pubmed/8996247.

interpretation of the study results. It would also help to delineate whether the increase in (b) (4) *cell migration in the NAM-exposed cells was due to an increase in the percentage of* (b) (4) *cells in the* (b) *cell population or an increase in the responsiveness of cells to* (b) (4) *stimulation.*

Study #2 (Report No. NRD-013)
Phenotype Characterization of (b) (4) ***Cell Cultures Treated with Cytokines Plus Nicotinamide or with Cytokines Only***

Objective:
To compare the phenotype of (b) (4) cells cultured with NAM to (b) (4) cells cultured under the same conditions, but without NAM.

Methods and Key Results:
Purified human (b) (4) cells were cultured for (b) (4) with cytokines (b) (4) (b) (4) culture medium, and NAM (source not specified). Three different NAM concentrations were tested: (b) (4)

1. Cytokines: (b) (4) cells cultured with cytokines only
2. NAM(b) (4): (b) (4) cells cultured with cytokines (b) (4) NAM
3. NAM(b) (4) (b) (4) cells cultured with cytokines (b) (4) NAM

After (b) (4) of culture, cells were harvested, stained with lineage markers, and analyzed by (b) (4)

Results showed that the percentages of non-differentiated, early progenitor cell subsets, $CD34^+$/(b) (4) and (b) (4) were significantly higher in NAM cultures ($CD34^+$(b) (4) 1.99 ± 1.43, 7.40 ± 1.37, 8.21 ± 0.84; (b) (4) 1.14 ± 0.77, 3.74 ± 0.56, 4.15 ± 0.59 in NAM (b) (4)). The fractions of the more differentiated, lineage committed subsets expressing hematopoietic lineage specific markers (b) (4) were decreased in NAM cultures.

Reviewer Comments:

- *The data suggest that addition of NAM to cultured cells may result in expansion of early progenitor cells and inhibition of differentiation; however, without assessment of the* (b) (4) *cellular subsets before culturing and at multiple timepoints throughout the study, it is difficult to definitively interpret the data.*

- *There does not appear to be a significant difference between cells cultured with* (b) (4) *NAM and cells cultured with* (b) (4) *NAM.*

- *The source of NAM in this study is not specified. The current manufacturing process for omidubicel-only uses* (b) (4) *NAM* (b) (4)

<u>*Overview of In Vivo Studies*</u>

***In Vivo* Studies in Immunodeficient Animals**

Reviewer Comments:

- *The applicant states that some of their in vivo studies were conducted under GLP conditions. However, in these study reports, there are missing pieces of information that should be part of any GLP-compliant study. For example, in some of the study reports, the applicant did not include quality assurance (QA) statements, GLP testing facility information, and deviation reports. In addition, basic elements of a GLP report such as baseline or in-life body weights of study animals, age of study animals, information on randomization of animals to study groups, or masking of study personnel were not included.*

- *In the in vivo nonclinical study reports, the applicant uses the terminology, "engraftment," "homing efficiency," and "BM homing" interchangeably. This parameter was assessed by* (b) (4) *analysis as the percentage of human* (b) (4) *cells in mouse BM.*

- *The applicant states that successful engraftment is considered in the literature to be 0.1–1% human* (b) (4) *cells detected in mouse bone marrow.[3] They indicate that based on their experience with the SCID mice model, 0.5% human* (b) (4) *cells detected by* (b) (4) *analysis represents a distinct cell population. Therefore, a 0.5% human* (b) (4) *cell limit was set to differentiate between engrafted and non-engrafted mice, and >0.5%* (b) (4) *cells in mouse BM was considered by the applicant to be successful engraftment. However, data was not provided to confirm the validity of the cutoff limit of >0.5%, therefore the claims of "engraftment" are unsubstantiated.*

Study #3 (Report No. NRD-006)

Report Number		NRD-006
Date Report Signed		07-Nov-2020
Title		SCID Repopulating Potential of Non-Expanded CD34+ Cells Post-Selection and their Total Progeny Following Expansion with Cytokines or Cytokine Plus Nicotinamide
GLP Status		Non-GLP
Testing Facility		Gamida Cell lab
Objective(s)		To compare the engraftment of cells expanded for (b) (4) with cytokines and NAM to 1) cells expanded with cytokines only and to 2) freshly purified CD34+ cells in NOD/SCID mice
Study Animals	**Strain/Breed**	NOD/SCID
	Species	Mouse; *Mus musculus*
	Age	8 weeks old
	Body Weight	Unknown
	#/sex/group	Not specified

[3] T. Ueda et al., "Expansion of human NOD/SCID-repopulating cells by stem cell factor, Flk2/Flt3 ligand, thrombopoietin, IL-6, and soluble IL-6 receptor," *Journal of Clinical Investigation* 105, no. 7 (Apr 2000), https://doi.org/Doi 10.1172/Jci8583, <Go to ISI>://WOS:000086279900023.

Total #	69 total mice
Test Article(s)	• Freshly purified non-expanded CD34+ cells • CD34+ cells expanded for (b) (4) with cytokines only • CD34+ cells expanded for (b) (4) with cytokines and (b) (4) NAM (b) (4) o Of the test articles evaluated, this product is most similar to omidubicel-onlv. Like omidubicel-onlv, it consists of CBU-derived CD34+ cells post-(b) (4) selection following (b) (4) (b) (4) expansion with cytokines (b) (4) at (b) (4) each, in culture medium with (b) (4) and NAM. o **NOTE:** This test article was cultured with (b) (4) NAM (b) (4) while omidubicel-onlv is cultured with (b) (4) NAM (b) (4) o **NOTE:** This test article was administered as a fresh formulation without a filtration step after the (b) (4) expansion, while omidubicel-onlv is from a cryopreserved formulation with an automated cell processing step after the (b) (4) expansion.
Control Article(s)	• Vehicle control buffer (phosphate buffered saline (PBS)/ ethylenediamine tetraacetate (EDTA) • Uninfused mice
Route of Administration	Intravenous
Description of the Disease/Injury Model and Implant Procedure	NOD/SCID male mice were administered the product via tail vein injection 24 hours after a total body irradiation (TBI) dose of 375 cGy according to the study groups below.
Study Groups and Dose Levels	• **Group 1:** Non-Expanded purified CD34+ cells (n = 7 per dose level) o Dose levels: 3 x 10^3, 6 x 10^3, and 12 x 10^3 cells/mouse • **Group 2:** Cultured Cells with Cytokines (n = 7 per dose level) o Dose levels: 1.2 x 10^6, 2.4 x 10^6, and 4.8 x 10^6 cells/mouse • **Group 3:** Cultured Cells with Cytokines and NAM (n = 7 per dose level, except for 2.4 x 10^6 cells/mouse, where n = 8) o Dose levels: 1.2 x 10^6, 2.4 x 10^6, and 4.8 x 10^6 cells/mouse • **Group 4:** Control- Infused with buffer (PBS/EDTA) (n = 3) • **Group 5:** Control- Not infused (n = 2)
Dosing Regimen	Single infusion
Randomization	No
Description of Masking	Not described
Scheduled Sacrifice Time Points	4 weeks post-transplantation

Reviewer Comments:

- *This study was conducted at an early stage of test article development. Mice were infused with either non-expanded, purified CD34$^+$ cells or CD34$^+$ cells cultured with cytokines, with or without NAM. At a later stage, experiments were designed to more closely mimic the clinical setting, where the entire cell population within a CBU is infused. Therefore, in these experiments, NC cells were transplanted as total nuclear cells (TNCs) while CF cells were transplanted as the total progeny of (b) (4) cells included in the NC mononuclear cell fraction.*

- *The applicant reports that the cell doses for the CF cell groups were chosen based on data collected in pilot experiments which were not submitted with the BLA. Based on*

these prior experiments, the applicant stated that 2.4 x 10^6 cells/mouse for the cultured cells was chosen as the most effective dose level for engraftment, so 1.2 x 10^6 cells/mouse was chosen as the low dose level, and 4.8 x 10^6 cells/mouse as the high dose level to bracket this dose. In addition, 375 cGy was selected as the optimal radiation dose to maximize survival and engraftment of recipient mice (Study No. 18; GAM-005-EM).

Key Evaluations and Assessments:

- Engraftment was measured by (b) (4) as the percentage of (b) (4) human cells present in the mouse BM. Successful engraftment was defined as ≥ 0.5% human (b) (4) cells.
- The in vivo (b) (4) can measure the repopulation and differentiation capacities of human HSCs,[4] and (b) (4) frequency was calculated based on the number of non-engrafted mice at each cell concentration.

Key Results:

- For each of the 3 cell dose levels tested, mice transplanted with NAM-cultured cells showed a higher level of engraftment (i.e., human (b) (4) cells in mouse BM) relative to mice transplanted with cells cultured without NAM or mice transplanted with non-expanded, purified $CD34^+$ cells at 4 weeks post-transplantation.
- For all 3 cell dose levels tested, numbers of mice with ≥ 0.5% human (b) (4) cells in mouse BM were higher in the NAM-cultured group than in the group without NAM or the group with non-expanded $CD34^+$ cells at 4 weeks post-transplantation.
- There was a higher frequency of SRC in the NAM-cultured group relative to cells cultured without NAM and non-expanded purified $CD34^+$ cells.

Reviewer Comment:

- *The results of this study suggest that cord blood-derived HSCs cultured with NAM may increase the engraftment potential of $CD34^+$ HSCs.*

Study #4 (Report No. NRD-008)

Report Number		NRD-008
Date Report Signed		07-Nov-2020
Title		The Effect of Nicotinamide on the Bone Marrow Homing Potential of Expanded CD34+ Cells
GLP Status		Non-GLP
Testing Facility		Gamida Cell lab
Objective(s)		To assess the effect of NAM on the engraftment of CD34+ expanded cells in NOD/SCID mice
Study Animals	**Strain/Breed**	NOD/SCID
	Species	Mouse; *Mus musculus*
	Age	8 to 10 weeks old
	Body Weight	Unknown
	#/sex/group	Not specified

[4] J. C. Y. Wang et al., "High level engraftment of NOD/SCID mice by primitive normal and leukemic hematopoietic cells from patients with chronic myeloid leukemia in chronic phase," *Blood* 91, no. 7 (Apr 1 1998), https://doi.org/DOI 10.1182/blood.V91.7.2406.2406_2406_2414, <Go to ISI>://WOS:000072671900023.

Total #	Not specified
Test Article(s)	• CD34+ cells from CBUs expanded for (b) (4) with cytokines only • CD34+ cells from CBUs expanded for (b) (4) with cytokines and (b) (4) NAM (b) (4) o Of the test articles evaluated, this product is most similar to omidubicel-onlv. Like omidubicel-onlv, it consists of purified CD34+ cells derived from thawed CBUs and cultured for (b) (4) in the presence of cytokines (b) (4) (b) (4) with NAM. o **NOTE:** This test article was cultured with (b) (4) NAM (b) (4) while omidubicel-onlv is cultured with (b) (4) NAM (b) (4) o **NOTE:** This test article was administered as a fresh formulation without a filtration step after the (b) (4) expansion, while omidubicel-onlv is from a cryopreserved formulation with an automated cell processing step after the (b) (4) expansion. **NOTE:** The cultured cells were labeled with c(b) (4) (b) (4)
Control Article(s)	• Freshly purified unmanipulated CD34+ cells from thawed CBUs that were not expanded and did not undergo cell selection **NOTE:** The non-cultured cells were labeled with (b) (4) (b) (4)
Route of Administration	Intravenous
Description of the Disease/Injury Model and Implant Procedure	NOD/SCID mice were administered the product via tail vein injection 24 hours after a TBI dose of 375 cGy according to the study groups below.
Study Groups and Dose Levels	Multiple experiments were conducted consisting of the following groups: • **Experiment 1:** Groups consisted of NAM expanded CD34+ cells (n = 7 per dose level) o Dose levels: 5 x 10^6, 10 x 10^6, and 20 x 10^6 total cells/mouse • **Experiment 2:** Groups consisted of NAM expanded CD34+ cells from 3 different single CBUs and a pooled CBU group (n = 8 per single CBU and pooled CBU groups) o Dose level: 20 x 10^6 total cells/mouse • **Experiment 3:** Groups consisted of non-cultured CD34+ cells from single CBUs and a pooled CBU group (n = 8 per single CBU and pooled CBU groups) o Dose level: 20 x 10^6 total cells/mouse • **Experiment 4:** Groups consisted of a non-cultured group (dose level: 10 x 10^6 cells/mouse; n = 7), a cytokine-only expanded group (dose level: 20 x 10^6 cells/mouse; n = 7), and a NAM plus cytokine expanded group (dose level: 20 x 10^6 cells/mouse; n = 8) • **Experiment 5:** Groups consisted of a non-cultured group, a NAM cultured group, and groups where unmanipulated cells and NAM cultured cells were co-transplanted in the same mouse. Unmanipulated cells were labelled with (b) (4) and the NAM cultured group was labeled with (b) (4) o Dose level non-cultured group: 11.6 x 10^6 or 14.8 x 10^6 total cells/mouse o Dose level NAM-cultured group: 13.6 x 10^6 total cells/mouse
Dosing Regimen	Single infusion

Randomization	No
Description of Masking	Not described
Scheduled Sacrifice Time Points	24 or 48 hours post-transplantation

Key Evaluations and Assessments:

- In Experiments 1-4, engraftment of CF cells (labeled with (b) (4)) was measured by (b) (4) analysis for (b) (4)$^+$ human cells in the mouse BM.
- In Experiment 5, engraftment of CF cells (labeled with (b) (4)) and NC cells (labeled with (b) (4)) was measured by (b) (4) as "events" of (b) (4), respectively.

Key Results:

- In Experiment 1, data show significant dose-dependent engraftment of cells cultured with NAM and cytokines following infusion. The number of engrafted cells correlate with the number of cells infused (60 ± 8, 139 ± 14, and 370 ± 62 CD45$^+$ TNCs for dose levels 5 x 10^6, 10 x 10^6, and 20 x 10^6 cells/mouse, respectively).
- In Experiment 2, there was no difference in engraftment of NAM expanded CD34$^+$ cells derived from a single CBU compared to 3 pooled CBUs.
- In Experiment 3, there was no difference in engraftment of unmanipulated TNCs derived from a single CBU compared to 3 pooled CBUs.
- In Experiment 4, engraftment of NAM cultured cells was significantly higher (488 ± 72 (b) (4) TNC) than cells from the same pool of CBUs cultured with cytokines only (104 ± 20 (b) (4) TNC).
- In Experiment 5, each cell unit was transplanted separately or both the cultured and the unmanipulated cells were infused in the same mouse. Results showed that engraftment of NAM cultured cells was comparable regardless of transplantation as single group or co-transplantation with unmanipulated cells. The infusion was either with a one-day interval or on the same day. Additionally, the order of transplantation did not affect engraftment results and the overall level of engraftment of the co-transplanted groups were substantially higher than the level of engraftment of unmanipulated cells alone.

Reviewer Comments:

- *The study report indicates that engraftment is measured by (b) (4) "events" for (b) (4) human cells in the mouse BM. However, it is unclear from the study report what the term "events" means when assessing engraftment in Experiments 1-5.*

- *In Experiment 4, the dose level of NAM cultured cells and cytokine-only cultured cells was twice the dose level of the non-cultured group. Thus, comparison of the NAM cultured group or cytokine-only cultured group to the non-cultured group is not interpretable.*

- *The results suggest that NAM may increase the overall engraftment of cytokine cultured cells. Engraftment of NAM cultured cells and CD34$^+$ cells was significantly higher than engraftment of the corresponding TNC fraction before expansion. The (b) (4) (b) (4) assays (Experiments 4 and 5) suggest that the non-cultured cells do not outcompete or block engraftment when infused along with the CF of CD34$^+$ CBU cells.*

The results also suggest that the origin of CD34$^+$ cells (either from a single CBU or from a pool of CBUs) does not impact engraftment.

Study #5 (Report No. NRD-009)

Report Number		NRD-009
Date Report Signed		07-Nov-2020
Title		Short-Term Engraftment Potential of Cells Cultured with Nicotinamide vs. Unmanipulated Cells
GLP Status		Yes
Testing Facility		Gamida Cell lab
Objective(s)		To compare the 2-week engraftment potential of (b) (4) cells cultured with cytokines and NAM to unmanipulated TNCs derived from the same donor cord blood unit
Study Animals	**Strain/Breed**	NOD/SCID
	Species	Mouse; *Mus musculus*
	Age	6-8 weeks old
	Body Weight	Unknown
	#/sex/group	25/males/group **NOTE:** The applicant stated they used male mice only for this study because males are more resistant to TBI than females (Study No. 18; GAM-005-EM)
	Total #	50 mice total
Test Article(s)		• Unmanipulated TNCs from thawed CBUs that were not expanded and did not undergo cell selection • (b) (4) cells cultured with cytokines and (b) (4) NAM (b) (4) o Of the test articles evaluated, this product is most similar to omidubicel-onlv. Like omidubicel-onlv, it consists of (b) (4) cells cultured for (b) (4) with cytokines (b) (4) (b) (4) each) and NAM. o **NOTE:** This test article was cultured with (b) (4) NAM (b) (4) while omidubicel-onlv is cultured with (b) (4) NAM (b) (4) . o **NOTE:** This test article was administered as a fresh formulation without a filtration step after the (b) (4) expansion, while omidubicel-onlv is from a cryopreserved formulation with an automated cell processing step after the (b) (4) expansion.
Control Article(s)		• Vehicle control (PBS/EDTA buffer containing 0.5% human serum albumin (HSA))
Route of Administration		Intravenous (tail vein)
Description of the Disease/Injury Model and Implant Procedure		NOD/SCID male mice were administered the product via tail vein injection 24 hours after a TBI dose of 300 cGy at a volume of 200 µL according to the study groups below.
Study Groups and Dose Levels		• **Group 1:** Unmanipulated TNCs (n = 10) o Dose level: 2 x 10^6 cells/mouse • **Group 2:** Cells cultured with cytokines and (b) (4) NAM (n = 10) o Dose level: 2 x 10^6 cells/mouse • **Group 3:** Vehicle control o (PBS/EDTA buffer containing 0.5% HSA) (n = 5)
Dosing Regimen		Single infusion
Randomization		No

Description of Masking	Not described
Scheduled Sacrifice Time Points	2 weeks post-transplantation

Key Evaluations and Assessments:

- Engraftment (b) (4) human cells in the BM) was measured by (b) (4)
- This study was run in duplicate with cells originating from 2 different CBUs (25 male mice/experiment)

Key Results:

- The engraftment level as measured by human (b) (4) cells in the BM 2 weeks post-transplant was significantly higher in mice transplanted with NAM cultured cells compared to mice transplanted with unmanipulated TNCs (Experiment 1: (b) (4) (b) (4) cells 8.71 ± 1.44 vs. 1.16 ± 0.32 and Experiment 2: 26.96 ± 4.02 vs. 3.44 ± 1.37, respectively).

Reviewer Comment:

- *Although the results of the 2 CBUs are significantly different, they exhibit the same trend of increased numbers of (b) (4) cells in the BM in the cultured cell cohort compared to the unmanipulated cell cohort at 2 weeks post-transplantation.*

Study #6 (Report No. NRD-010)

Report Number		NRD-010
Date Report Signed		07-Nov-2020
Title		Long-Term Engraftment Potential of NAM Cultured Cells and Non-Cultured Fraction in Comparison to Unmanipulated Cells
GLP Status		Yes
Testing Facility		Gamida Cell lab
Objective(s)		1) To examine the engraftment ability of the CF at 6 weeks post-infusion when transplanted in combination with the NF and compare it to the equivalent unmanipulated cells and 2) to study the capacity of immune cell lineage reconstitution 6 weeks post-transplantation
Study Animals	**Strain/Breed**	NOD/SCID
	Species	Mouse; *Mus musculus*
	Age	Unknown
	Body Weight	Unknown
	#/sex/group	5 or 12 male mice/group **NOTE:** The applicant stated they used male mice only for this study because males are more resistant to TBI than females (Study No. 18; GAM-005-EM)
	Total #	29 mice total
Test Article(s)		(b) (4) cells cultured with cytokines and (b) (4) NAM (b) (4) • Like omidubicel-onlv, this test article consists of purified (b) (4) cells derived from a thawed, fractionated CBU that were cultured for (b) (4) in culture medium with (b) (4) in the presence of (b) (4) cytokines (b) (4) at (b) (4) each, and (b) (4) (b) (4) NAM.

	• **NOTE:** This test article was administered as a fresh formulation without a filtration step after the (b) (4) expansion, while omidubicel-onlv is from a cryopreserved formulation with an automated cell processing step after the (b) (4) expansion.
Control Article(s)	• Unmanipulated TNCs from thawed CBUs that were not expanded and did not undergo cell selection • Vehicle control (PBS/EDTA buffer containing 0.5% HSA)
Route of Administration	Intravenous (tail vein)
Description of the Disease/Injury Model and Implant Procedure	NOD/SCID male mice were administered the product via tail vein injection 24 hours after a TBI dose of 300 cGy at a volume of 200 µL according to the study groups below.
Study Groups and Dose Levels	• **Group 1:** Vehicle control o (PBS/EDTA buffer containing 0.5% HSA) (n = 5) • **Group 2:** (b) (4) cells cultured with cytokines and (b) (4) NAM (n = 12) o Dose level: 7.4 x 10^6 cultured fraction (CF) + 2 x 10^6 non-cultured fraction (NF) cells/mouse • **Group 3:** Unmanipulated TNCs (n = 12) o Dose level: 5 x 10^6 cells/mouse **NOTE:** The applicant states the median (b) (4) cells in the unmanipulated fraction is approximately (b) (4) and the estimated fold expansion of the cultured cells is (b) (4) Thus, they calculated (b) (4) as the ratio between the cultured cell cohort and the unmanipulated cell cohort, which reflects (b) (4) for the cultured cells and (b) (4) for the unmanipulated cells.
Dosing Regimen	Single infusion
Randomization	No
Description of Masking	Not described
Scheduled Sacrifice Time Points	6 weeks post-transplantation

Key Evaluations and Assessments:

- Engraftment (b) (4) human cells and % $CD34^+$ cells from total human (b) (4) cells) in the BM was measured by (b) (4)
- The BM (n = 3 from each group) was stained with the following human monoclonal antibodies for (b) (4) analysis: (b) (4) (b) (4), macrophages, neutrophils, megakaryocytes, platelets, erythroid, NK, B cells and the chemokine receptor specific for (b) (4)

Key Results:

- Mice transplanted with CF cells + NF cells showed increased engraftment (as measured by (b) (4) human cells in the BM) at 6 weeks post-transplantation compared to mice transplanted with unmanipulated cells with an average percentage of 33.15 ± 5.2 and 8.91 ± 1.8 of human (b) (4) cells, respectively.
- Mice transplanted with CF cells + NF cells showed increased engraftment (as measured by % $CD34^+$ cells from total human (b) (4) cells in the BM) at 6 weeks post-transplantation compared to mice transplanted with unmanipulated cells with an average percentage of 25.9 ± 1.3 and 15.6 ± 2.7 of $CD34^+$/(b) (4) cells, respectively.
- The phenotypic characterization of human (b) (4) cells in mouse BM suggest that the CF cells better retain their ability to differentiate into all blood cell lineages, including

macrophages, neutrophils, megakaryocytes, platelets, erythroid cells, NK cells, and B cells compared to unmanipulated cells.

Reviewer Comments:

- *The results suggest that the CF transplanted with the NF may have improved engraftment potential (as measured by (b) (4) human cells and % $CD34^+$ cells from total human (b) (4) cells) 6 weeks post-transplantation compared to unmanipulated cells transplanted alone.*

- *Six weeks may not be sufficient time to show long-term engraftment of BM cells. The applicant did not provide a justification for their selection of a 6-week study duration.*

Study #7 (Report No. NRD-048)

Report Number		NRD-048
Date Report Signed		10-Nov-2021
Title		Effect of CD34- Cells of Omidubicel Cultured Fraction on in vivo Engraftment Ability of CD34+ Cells
GLP Status		Yes
Testing Facility		Gamida Cell lab
Objective(s)		To evaluate the effect of expanded CD34- cells on the engraftment of expanded CD34+ cells
Study Animals	**Strain/Breed**	NOD/SCID gamma (NSG)
	Species	Mouse; *Mus musculus*
	Age	6-7 weeks old
	Body Weight	Unknown
	#/sex/group	4 female mice in control group; 10 female mice per test article group **NOTE:** Other nonclinical studies conducted by the applicant used only male mice because males are more resistant to TBI than females (Study No. 18; GAM-005-EM). The applicant does not provide a rationale for only using female mice in this study.
	Total #	64 female mice total
Test Article(s)		• CD34- cells only • CD34+ cells only • CD34+ & CD34- cells o Of the test articles evaluated, this product is most similar to omidubicel-onlv. Like omidubicel-onlv, this test article consists of purified (b) (4) cells that were cultured for (b) (4) (b) (4) in culture media consisting of (b) (4) (b) (4) o **NOTE:** After the (b) (4) culture of (b) (4) cells, CD34+ cells were re-selected using (b) (4) to obtain CD34+ and CD34- fractions. This step is not included in the manufacturing of omidubicel-onlv.
Control Article(s)		Control buffer (PBS/EDTA buffer containing 0.5% HSA)
Route of Administration		Intravenous (tail vein)
Description of the Disease/Injury Model and Implant Procedure		Female NSG mice were administered the product via tail vein injection 24 hours after a TBI dose of 250 cGy according to the study groups below.

Study Groups and Dose Levels	• **Group 1:** Vehicle control
	  ○ (PBS/EDTA buffer containing 0.5% HSA) (n = 4)
	• **Group 2:** CD34+ cells - low dose (n = 10)
	  ○ Dose level: 0.05 x 10^6 CD34+ cells/mouse
	• **Group 3:** CD34+ cells - high dose (n = 10)
	  ○ Dose level: 0.1 x 10^6 CD34+ cells/mouse
	• **Group 4:** CD34+ & CD34- cells - low dose (n = 10)
	  ○ Dose level: 0.05 x 10^6 CD34+ & ~0.28 x 10^6 CD34- cells/mouse
	• **Group 5:** CD34+ & CD34- cells - high dose (n = 10)
	  ○ Dose level: 0.1 x 10^6 CD34+ & ~0.56 x 10^6 CD34- cells/mouse
	• **Group 6:** CD34- cells - low dose (n = 10)
	  ○ Dose level: ~0.28 x 10^6 CD34- cells/mouse
	• **Group 7:** CD34- cells - high dose (n = 10)
	  ○ Dose level: ~0.56 x 10^6 CD34- cells/mouse

Dosing Regimen	Single infusion
Randomization	No
Description of Masking	Not described
Scheduled Sacrifice Time Points	3 weeks post-transplantation

Key Evaluations and Assessments:

- Engraftment (b) (4) human cells in the BM) was measured by (b) (4)

Key Results:

- The study was divided into 2 experiments:
- In Experiment 1, the average engraftment (as measured by (b) (4) human cells in the BM) in mice infused with $CD34^+$ cells was comparable to the average engraftment in mice infused with a combination of $CD34^+$ and $CD34^-$ cells (low dose level: 12.1 ± 1.7 vs. 10.5 ± 1.6 respectively; high dose level: 14.2 ± 1.4 vs. 13.8 ± 2.9 respectively). The average engraftment in mice infused with $CD34^-$ cells indicated that these mice did not engraft (0.1 ± 0.0 in both the low and high dose groups).
- In Experiment 2, the average engraftment in mice infused with a combination of $CD34^+$ and $CD34^-$ cells (8.7 ± 0.5) was slightly higher than the average engraftment in mice infused with $CD34^+$ cells only (6.1 ± 0.6). No significant differences were noted between the engraftment levels of mice infused with $CD34^+$ cells only or with a combination of $CD34^+$ and $CD34^-$ cells in the high dose groups (18.4 ± 1.8 and 20.3 ± 2.2, respectively). In the low dose group, a slight but significant (p=0.005) increase in engraftment was seen in the group infused with $CD34^+$ and $CD34^-$ cells compared to the group infused with $CD34^+$ cells only. The average percentage of engraftment in the mice infused with $CD34^-$ cells indicated that these mice did not engraft (0.1 ± 0.0 in both the low and high dose groups).

Reviewer Comment:

- *The results suggest that co-administration of $CD34^-$ cells with $CD34^+$ cells does not decrease the number of* (b) (4) *cells in the BM of mice at 3 weeks post-administration.*

- *It is not clear why the applicant used CD34$^+$ cells as opposed to* (b) (4) *cells. The applicant states that cells capable of initiating engraftment are contained within the CD34$^+$ cell fraction.[5] The CD34$^-$ cells present in the omidubicel-onlv CF graft constitute approximately 85% of the cells in the final product, while final product specifications for CD34$^+$ cells were set at ≥ 8.7%. The applicant states that the purpose of this study was to assess whether CD34$^-$ cells have an impact on the engraftment ability of CD34$^+$ cells and whether a CD34$^-$ cell fraction alone has any engraftment potential.*

Study #8 (Report No. NRD-050)
Correlation Between the Number of Omidubicel's CD34$^+$ Cells Injected to NSG Mice and Engraftment

Objective:
To understand whether a correlation exists between the number of CD34$^+$ cells infused and engraftment potential in NSG mice.

Methods and Key Results:
The applicant conducted a retrospective correlative analysis of 12 previous in vivo experiments to compare of the number of CD34$^+$ cells infused and human (b) (4) cells measured in NSG mice.

The Pearson correlation coefficient was used to measure of the linear relationship strength between the 2 variables. The correlation coefficient takes on values ranging between +1 and -1. The following guidelines for interpretation of the correlation coefficient were used:

- 0: no linear relationship
- +1: perfect positive linear relationship
- -1: a perfect negative linear relationship
- Values between 0 and 0.3 (0 and -0.3): weak positive (negative) linear relationship
- Values between 0.3 and 0.7 (-0.3 and -0.7): moderate positive (negative) linear relationship
- Values between 0.7 and 1.0 (-0.7 and -1.0): strong positive (negative) linear relationship
- The significance of the correlation was shown by calculating a p-value

Overall, the number of infused re-purified CD34$^+$ cells showed a positive correlation (correlation coefficient between 0.7 and 1.0 and p-value < 0.05) with the percentage of engrafted human (b) (4) cells in NSG mice 2 to 4 weeks after transplantation.

Reviewer Comment:

- *The retrospective statistical analysis shows a positive correlation between the number of ex vivo expanded CD34$^+$ cells transplanted, and the level of engraftment as measured by percentage of human* (b) (4) *cells in mouse BM. The significance of this as it relates to*

[5] C. J. Hogan et al., "Engraftment and development of human CD34(+)-enriched cells from umbilical cord blood in NOD/LtSz-scid/scid mice," *Blood* 90, no. 1 (Jul 1 1997), <Go to ISI>://WOS:A1997XH42800012.

product safety and activity is not clear as it is difficult to compare analysis across many studies with different protocols, study variables, manufacturing procedures, etc.

Study #11 (Report No. NRD-062)

Report Number		NRD-062
Date Report Signed		07-Nov-2020
Title		The Effect of Cryopreservation on the in vivo Engraftment Ability of Omidubicel
GLP Status		Yes
Testing Facility		Gamida Cell lab
Objective(s)		To compare the engraftment potential of cryopreserved CF cells to fresh CF cells in NOD/SCID mice
Study Animals	**Strain/Breed**	NOD/SCID
	Species	Mouse; *Mus musculus*
	Age	6-8 weeks old
	Body Weight	Unknown
	#/sex/group	5 male mice in control group; 15 male mice per test article group **NOTE:** The applicant stated they used male mice only for this study because males are more resistant to TBI than females (Study No. 18; GAM-005-EM)
	Total #	35 male mice/CF batch evaluated
Test Article(s)		Fresh CF or cryopreserved CF • Like omidubicel-onlv, these test articles consisted of (b) (4) cells that were expanded for (b) (4) with cytokines (b) (4) (b) (4) and (b) (4) NAM (b) (4) • At the end of the expansion period, the cultured cell suspension was split into 2 equal fractions. One fraction was processed as a fresh product and the second fraction was processed as a cryopreserved product • **NOTE:** The manufacturing steps of the fresh and the cryopreserved test articles in this study are the same until the (b) (4) (b) (4) • **NOTE:** The cryopreserved test article evaluated in this study does not undergo (b) (4) whereas omidubicel-onlv manufacturing includes this step.
Control Article(s)		Thawing buffer
Route of Administration		Intravenous (tail vein)
Description of the Disease/Injury Model and Implant Procedure		NOD/SCID male mice were administered the product via tail vein injection 24 hours after a TBI dose of 300 cGy at a volume of 200 µL according to the study groups below.

Study Groups and Dose Levels		Per experiment:
		• **Group 1:** Control (n=5)
		  ○ 3 mL thawing buffer (8 %w/v HSA and 6.8 %w/v Dextran 40)
		• **Group 2:** Fresh CF (n=15)
		  ○ (5×10^6 fresh cells/mouse in 0.3 mL thawing buffer)
		• **Group 3:** Cryopreserved CF (n=15)
		  ○ 5×10^6 thawed cells/mouse in 0.3 mL thawing buffer)

Dosing Regimen	Single infusion
Randomization	No
Description of Masking	Not described
Scheduled Sacrifice Time Points	3 weeks post-transplantation

Key Evaluations and Assessments:

- Number of viable cells (TNC) before and after cryopreservation of CF cells
- Number of viable cells (TNC) before and after incubation of fresh CF cells
- Engraftment (b) (4) human cells in the BM) was measured by (b) (4)
- Phenotype of fresh engrafted cells and cryopreserved engrafted cells
- Six experiments were conducted, each evaluating a different batch of CF cells

  NOTE: The applicant reported that low engraftment levels in the first 3 experiments led to inconclusive results, so only the results of the second 3 experiments were included in the study report.

Key Results:

- Human TNC recovery of both cryopreserved and fresh product after cryopreservation and incubation, respectively, was above 70% viability in the 3 experiments described. No recovery differences were observed between cryopreserved and fresh product (84% vs. 91%; 86% vs. 78%; and 107% vs. 98%, respectively).
- One experiment showed increased engraftment of the cryopreserved product compared to the fresh product (7.5 ± 1.8% to 3.6 ± 0.8%, respectively), while the other 2 experiments showed equivalent results (9.0 ± 0.9% vs. 8.5 ± 1.6% and 3.1 ± 0.7% vs. 3.1 ± 0.6% cryopreserved vs. fresh product, respectively).
- There were no significant differences in the phenotype of cryopreserved vs. fresh engrafted cells in the 3 experiments described: % CD34: 9.9 ± 1.8 vs. 11.1 ± 2.7; % (b) (4)

Reviewer Comments:

- *The cryopreservation process used for the test article in this study differs from the final manufacturing process of omidubicel-onlv as the final manufacturing process includes an* (b) (4)

- *The applicant reported that low engraftment levels in the first 3 experiments led to inconclusive results, so only the results of last 3 experiments were included in the study report. These results should have been provided as part of the study results for a GLP study.*

- *The recovery of cryopreserved product from one experiment was 107%. The applicant does not provide a discussion of how TNC recovery was greater than the baseline measurement before cryopreservation.*

SAFETY PHARMACOLOGY STUDIES

No safety pharmacology studies with omidubicel-onlv were conducted.

PHARMACOKINETIC STUDIES (Cell Distribution)

Summary List of Pharmacokinetics Studies

The following distribution studies were conducted to evaluate the distribution of the test article following administration in the NOD/SCID mouse.

In Vitro Studies

Study Number	Study Title	Report Number
12	Re-Qualification of a (b) (4) to Detect NiCord™ Blood Cells in NOD-SCID Mice	7-RT-HAR0100

NOTE: Study No. 12 (7-RT-HAR0100) is only briefly summarized in this review memo under 'Overview of Pharmacokinetics Studies.' NiCord was the previous name used for omidubicel-onlv.

In Vivo Studies

Study Number	Study Title	Report Number
13	Analysis of the Biodistribution of NiCord™ Cultured Cells + NiCord™ Negative Fraction (NF) Cells in NOD-SCID Mouse by (b) (4) (b) (4)	7-RT-HAR0102

Overview of Pharmacokinetics Studies

Study #12 (Report No. 7-RT-HAR0100)
Re-Qualification of a (b) (4) to Detect NiCord Blood Cells in NOD-SCID Mice

Objective:
To re-qualify the (b) (4) used to measure the distribution of CF + NF cells in NOD/SCID mice to provide maximum sensitivity and specificity.

Methods and Key Results:
The lower limit of detection of the assay is (b) (4) NOD-SCID mouse DNA; the lower limit of quantification is (b) (4) NOD-SCID mouse gDNA. The quantitative range of the assay is (b) (4) (b) (4)

The applicant concluded that the precision and accuracy of the assay were suitable for quantifying the amount of human gDNA contained in gDNA extracted from NOD/SCID mouse.

Study #13 (Report No. 7-RT-HAR0102)

Report Number		7-RT-HAR0102
Date Report Signed		02-Feb-2010
Title		Analysis of the Biodistribution of NiCord™ Cultured Cells + NiCord™ Negative Fraction (NF) Cells in NOD-SCID Mice by (b) (4) (b) (4)
GLP Status		Yes
Testing Facility		(b) (4)
Objective(s)		To detect and quantify the NiCord™ CF + NF in NOD/SCID mouse tissues using (b) (4)
Study Animals	**Strain/Breed**	NOD/SCID
	Species	Mouse; *Mus musculus*
	Age	7-8 weeks old
	Body Weight	Unknown
	#/sex/group	5/sex/group/time point
	Total #	90 animals total
Test Article(s)		CF cells + NF cells • Like omidubicel-onlv, this test article consisted of (b) (4) cells that were expanded for (b) (4) with cytokines (b) (4) (b) (4) and (b) (4) NAM (b) (4) • **NOTE:** The test article evaluated in this study was from a (b) (4) (b) (4) whereas omidubicel-onlv undergoes (b) (4) (b) (4)
Control Article(s)		Vehicle (b) (4)
Route of Administration		Intravenous (tail vein)
Description of the Disease/Injury Model and Implant Procedure		NOD/SCID mice were administered the product via tail vein injection 24 hours after a TBI dose of 300 cGy according to the study groups below.

Study Groups and Dose Levels		• **Group 1:** Vehicle control (n=10)
		  ○ PBS/EDTA/HSA solution
		• **Group 2:** Low dose (n=10)
		  ○ Dose level: 2×10^6 cells/mouse (CF cells) + 1×10^6 cells/mouse (NF cells)
		• **Group 3:** High dose (n=10)
		  ○ Dose level: 10×10^6 cells/mouse (CF cells) + 5×10^6 cells/mouse (NF cells)
		
		NOTE: The applicant stated that 2 x 10^6 cells/mouse was determined to be the lowest effective CF dose in prior nonclinical studies. The applicant selected the high CF dose as 5x the low dose level. The NF dose was selected as a 2:1 ratio of CF to NF based on the applicant's estimation of NF recovery after selection and freeze/thaw procedures.

Dosing Regimen		Single infusion
Randomization		No
Description of Masking		Not described
Scheduled Sacrifice Time Points		Day 1, Week 4, and Week 12 post-transplantation

Key Evaluations and Assessments:

- A (b) (4) was used to measure the distribution of the CF and NF cells in NOD/SCID mouse tissues. The following tissues were analyzed: heart, liver, lung, spleen, kidney, testes, and femur (BM) at Day 1, Week 4, and Week 12 post-transplantation.

Key Results:

- Human DNA was detected in all tissues throughout the duration of the study in most animals in both the low and high dose groups. At Week 4, there was a slight decrease in average human DNA detection across tissues compared to Day 1. At Week 12, the average number of human cell equivalents was maintained in the low dose group, increased in the high dose group, and approximately doubled from Day 1 in both groups. At Week 12, relatively high quantities of human DNA were found in femur, spleen, lung, liver, and kidney in the high dose group.
- At Day 1 and Week 4, human DNA in the testis was not quantifiable, but at Week 12, some animals in the high dose group showed relatively low levels.

Reviewer Comment:

- *Interpretability of this study is limited because 1) the study was conducted in NOD/SCID mice, which would not be expected to mimic human homing, engraftment, and maturation/differentiation and 2) the mice were not perfused prior to harvesting and preparation of the various tissues and thus the tissue samples were contaminated with circulating product.*

TOXICOLOGY STUDIES

Summary List of Toxicology Studies

The following toxicology study was conducted to evaluate the safety of the test article following administration in the NOD/SCID mouse.

Toxicology Studies:

Study Number	Study Title / Publication Citation	Report Number
14	Acute Intravenous (IV) Toxicity & Biodistribution in the Irradiated SCID Mouse	GAM-066-AIT-BIO

Toxicology Studies

Study #14 (Report No. GAM-066-AIT-BIO)

Report Number	GAM-066-AIT-BIO
Date Report Signed	11-Feb-2010
Title	Acute Intravenous (IV) Toxicity & Biodistribution in the Irradiated SCID Mouse

GLP Status		Yes
Testing Facility		(b) (4)
Objective(s)		To assess the safety of the CF + NF cells following a single intravenous infusion into irradiated NOD-SCID mice.
Study Animals	**Strain/Breed**	NOD/SCID
	Species	Mouse; *Mus musculus*
	Age	7-8 weeks old
	Body Weight	Unknown
	#/sex/group	5/sex/group/sacrifice time point
	Total #	192 animals total
Test Article(s)		CF cells + NF cells • Like omidubicel-onlv, this test article consisted of (b) (4) cells that were expanded for (b) (4) with cytokines (b) (4) (b) (4) and (b) (4) NAM (b) (4) • **NOTE:** The test article evaluated in this study was from a (b) (4) (b) (4) whereas omidubicel undergoes (b) (4) (b) (4)
Control Article(s)		PBS/EDTA/HSA (20% HSA)
Route of Administration		Intravenous (tail vein)
Description of the Disease/Injury Model and Implant Procedure		NOD/SCID mice were administered the product via tail vein injection 24 hours after a TBI dose of 300 cGy according to the study groups below.
Study Groups and Dose Levels		• **Group 1:** Vehicle control o PBS/EDTA/HSA solution at 200 uL/mouse • **Group 2:** Low dose o 2×10^6 cells/mouse (CF cells) + 1×10^6 cells/mouse (NF cells) • **Group 3:** High dose o 10×10^6 cells/mouse (CF cells) + 5×10^6 cells/mouse (NF cells)
Dosing Regimen		Single infusion
Randomization		Yes
Description of Masking		Not described
Scheduled Sacrifice Time Points		Day 1, Week 4, and Week 12 post-transplantation

Key Evaluations and Assessments:

- Clinical signs: Daily
- Body weights: Weekly
- Food consumption: Weekly
- Clinical pathology: Day 1, Week 4, Week 12
- Biodistribution: Day 1, Week 4, Week 12 (reviewed under Study No. 13; 7-RT-HAR0102)
- Necropsy and macroscopic examinations: Day 1, Week 4, Week 12

Key Results:

- Early deaths were recorded within all groups (both test article and vehicle control; both males and females), starting 10 days post-infusion and persisting until 23 days post-infusion.

  - For the males, 20/32 vehicle control early deaths were observed, while 23/32 and 19/32 early deaths were observed in the low- and high-dose test article groups, respectively.
  - For the females, 7/32 vehicle control early deaths were observed, while 9/32 and 10/32 early deaths were observed in the low- and high-dose test article groups, respectively.
- A single incident of an unscheduled death of a female was observed 44 days post-infusion in the high-dose test article group.
- No test article-related adverse reactions were observed among animals throughout the 12-week observation period.
- All observed clinical signs were consistent with irradiation-induced signs and were observed among all animals (males and females in both control and test article groups) at similar frequencies starting one day after irradiation up until Week 12.
- No statistically significant differences were observed between the survival curves for the 3 study groups for males and females.
- No gross pathological findings were evident among the vehicle control or test article groups throughout the study.
- No test article-related changes were noted in any of the animals evaluated at each termination time point. All noted lesions had relatively comparable incidence among the control and test article groups.

Reviewer Comments:

- *The incidence of unscheduled deaths in this toxicity study was higher than in each of the multiple POC studies that were conducted, beginning 10 days post-transplantation and persisting until 23 days post-transplantation. Among the males, 20/32 vehicle control mice were found dead, while 23/32 and 19/32 mice were found dead within the low and high dose test article groups, respectively. For the females, 7/32 vehicle control mice were found dead, while 9/32 and 10/32 mice were found dead within the low and high dose test article groups, respectively. No statistically significant differences were observed between the survival curves for the control group compared to the test article groups. The applicant attributes unscheduled deaths to effects from TBI. The applicant reviewed mortality data in previous experiments in NOD/SCID mice performed at the same facility in which Study No. 14 (GAM-066-AIT-BIO) was performed* (b) (4) *and found the mortality rate of NOD/SCID mice in response to TBI to be extremely variable.*

- *There does not appear to be a rationale that could explain the higher susceptibility to irradiation in this experiment compared to previous experiments. As no significant differences were observed between the survival curves for the control group compared to the test article groups, the high mortality rate observed in the study is likely due to variation in response to TBI.*

- *The study report does not include details surrounding the early death of the single female in the high-dose test article group on day 44 post-infusion. The study report states that complete autolysis was observed in most of the deceased animals that were found dead*

prior to scheduled termination. It was concluded that the autolysis process was more rapid than usual, likely induced by the overall tissue damage caused by the TBI.

- *No test article-related adverse effects were observed in body weights, food consumption, clinical pathology, or histopathology assessments. The clinical signs observed were consistent with irradiation-induced symptoms and were comparable across control vs. test article groups.*

- *The safety data suggests that a single IV infusion of the test article at either* (b) (4) *or* (b) (4) *to the* (b) (4) *NOD/SCID mouse, does not result in test article-related adverse effects in this test system.*

Developmental and Reproductive Toxicology Studies:

Per the applicant, developmental and reproductive toxicology studies were not conducted because there were no test article-related toxicity findings in reproductive organs as assessed by histopathological analyses performed on male and female mice in Study No. 14 (GAM-066-AIT-BIO). Additionally, subjects who are candidates for HSCT with omidubicel-onlv are required to undergo a conditioning regimen before infusion of omidubicel-onlv which may result in adverse, irreversible effects on reproductive function in both males and females. The applicant states that in current practice, pregnant women are normally not eligible for HSCT procedures.

Genotoxicity Studies:

Study Number	Study Title / Publication Citation	Report Number
15	A (b) (4) Molecular Cytogenic Analysis of Omidubicel	NRD-063
16	(b) (4) Cytogenetic Analysis of Omidubicel	NRD-064
17	(b) (4) of Omidubicel	NRD-065

NOTE: Study No. 15 (NRD-063) evaluated cryopreserved CF, while Study No. 16 (NRD-064) and Study No. 17 (NRD-065) evaluated fresh CF (see Table 1).

Study #15 (Report No. NRD-063)
A (b) (4) ***Molecular Cytogenic Analysis of Omidubicel***

Objective:
To screen for specific chromosome rearrangements and numerical abnormalities.

Methods and Key Results:
(b) (4) analysis was performed on CF cells and unmanipulated control cells derived from the same CBU. (b) (4) batches were analyzed, and (b) (4) were used, related to (b) (4) different hematological disorders. (b) (4) signals were obtained, and (b) (4) from each sample were scored.

Both CF cells and unmanipulated CBU showed normal (b) (4) results in most of the analyzed cells (> 95%). Sporadic abnormal cells that did not exceed 5% were observed in both CF and unmanipulated samples and were not considered abnormal for the cell source. No significant differences between the CF and unmanipulated cells were observed for each of the (b) (4) probes.

Reviewer Comment:

- *This study indicates that the CF manufacturing procedure, including NAM culture, does not appear to generate chromosomal abnormalities in the (b) (4) regions analyzed when compared to unmanipulated UCB.*

Study #16 (Report No. NRD-064)
(b) (4) Cytogenetic Analysis of Omidubicel

Objective:
To detect microscopic (b) (4) genomic abnormalities.

Methods and Key Results:

(b) (4)

Study #17 (Report No. NRD-065)
(b) (4) of Omidubicel

Objective:
To analyze the number and general structure of all 46 chromosomes after observing hypodiploidy in the (b) (4) analysis (Study No. 16; NRD-064).

Methods and Key Results:
Three batches of CF and NF cells were evaluated.

From each sample (b) (4)

No chromosomal translocations, inversions, deletions, or duplications were detected. Some of the cells presented abnormal chromosome numbers lower than 46. There was no clonal aneuploidy pattern in any of the tested samples. The CF and NF showed approximately the same rate of aneuploidy.

Reviewer Comment:

- *The results show no evidence of clonal aneuploidy and no difference in the occurrence of abnormalities between CF and NF samples. However, the applicant did not include unmanipulated cells from the same CBU as the CF and NF, so the level of background chromosomal abnormalities in unmanipulated cells is unknown. Therefore, it is difficult to interpret the results.*

Carcinogenicity/Tumorigenicity Studies:

No carcinogenicity/tumorgenicity studies were conducted with the test article and were not warranted based on the known safety profile. Genetic stability and single-dose toxicity were assessed in Study No. 10 (GAM-066-AIT-BIO) and did not reveal specific tumorigenic or oncogenic concerns.

Other Safety/Toxicology Studies:

Study Number	Study Title / Publication Citation	Report Number
18	Engraftment of Ex Vivo Expanded Human Hematopoietic Stem Cells in NOD/SCID and (b) (4) Mice	GAM-005-EM

NOTE: Study No. 18 (GAM-005-EM) is not summarized in this review memo because it was conducted to determine the appropriate (b) (4) dose and mouse strain for nonclinical development of the test article. Results showed that NOD/SCID mice were preferable over (b) (4) mice based on cell engraftment levels. (b) (4) at a dose level of (b) (4) (b) (4) was considered sub-lethal, and females appeared to be more sensitive to (b) (4) than males. Therefore, males were selected over females in some of the nonclinical studies. Of note, this same (b) (4) dose appeared to be more toxic in the mice used for the definitive GLP toxicity study (see Study No. 14; GAM-066-AIT-BIO).

APPLICANT'S PROPOSED LABEL

Subsections 8.1-8.3 of Section 8 ('Use in Specific Populations') should be revised to comply with 21 CFR 201.56(d)(1), 201.57(c)(9), and 201.57(c)(14), as applicable.

Section 13 ('Nonclinical Toxicology') should be revised to include only necessary information for the label.

CONCLUSION OF NONCLINICAL STUDIES

All nonclinical-related information has been reviewed, and no significant issues, major deficiencies, or safety concerns have been identified. The nonclinical data supports approval of the license application.

KEY WORDS/TERMS

OMISIRGE, omidubicel-onlv, NiCord, (b) (4) hematopoietic progenitor cells, CD34+ cells, cord blood unit, hematopoietic stem cell transplantation, nicotinamide, engraftment, pharmacology, distribution, toxicology, genotoxicity, NOD/SCID mice