CLINICAL STUDY PROTOCOL ALD-104

EudraCT No. 2018-001145-14

A Phase 3 Study of Lenti-D Drug Product After Myeloablative Conditioning Using Busulfan and Fludarabine in Subjects ≤ 17 Years of Age With Cerebral Adrenoleukodystrophy (CALD)

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Protocol Version: Version 8.0

Protocol Date: 30 November 2022

CCI

MEDICAL MONITOR CONTACT INFORMATION

Country-Specific 24-hour Emergency Contact Phone Numbers

Country	Phone Number
France	PPD
Germany	
Italy	
The Netherlands	
UK	
USA	

CCI

SUMMARY OF CHANGES

Substantial changes compared to Study ALD-104 Protocol v7.2 are described in the table below and consist of a revised algorithm for integration site analysis, a revised schedule of events for closer hematologic monitoring and details on central review of core needle biopsy sections, bone marrow aspirate smears and peripheral blood smears, updated clinical triggers for repeat CBCs and bone marrow evaluations, enhanced frequency and scope of bone marrow evaluations with new criteria for triggering re-evaluation, new instructions for submitting bone marrow specimens for central review and storage, and additional details with respect to assessments performed as part of integration site analyses and clinical work-up for potential malignancy. In addition, the protocol has been amended to include increased external oversight through implementation of a quarterly DMC review of hematologic and ISA data. Furthermore, the Responsible Medical Officer has been replaced and the new Responsible Medical Officer's information is provided on the cover page.

Non-substantial changes are summarized below the table.

DESCRIPTION OF EACH SUBSTANTIAL AMENDMENT

Note: added text is **bold** and deleted text shown in ~~strikeout~~. Applicable sections of the synopsis are appropriately updated.

Initial wording	Amended or New Wording	Change Reason/Justification
1.2.1. Ongoing Studies ALD-102 and LTF-304 Study ALD-102 is an international, Phase 2/3, multicenter, open-label, single-arm, single-dose study designed to evaluate the efficacy and safety of eli-cel in male subjects with CALD aged ≤ 17 years with active cerebral disease. In Study ALD-102, a myeloablative conditioning regimen of busulfan and cyclophosphamide was utilized before infusion of eli-cel. Each subject in Study ALD-102 is evaluated for 2 years after eli-cel infusion of ≥ 5.0×10^6 CD34+ cells/kg. Subjects are then followed for an additional 13 years in Study LTF-304. Safety data from ALD-102 and LTF-304 can be found in the Investigator's Brochure.	*1.2.1. ~~Ongoing~~ Studies ALD-102 and LTF-304* Study ALD-102 ~~is~~ **was** an international, Phase 2/3, multicenter, open-label, single-arm, single-dose study designed to evaluate the efficacy and safety of eli-cel in male subjects with CALD aged ≤ 17 years with active cerebral disease. In Study ALD-102, a myeloablative conditioning regimen of busulfan and cyclophosphamide was utilized before infusion of eli-cel. Each subject in Study ALD-102 ~~is~~ **was** evaluated for 2 years after eli-cel infusion of ≥ 5.0×10^6 CD34+ cells/kg. Subjects are then followed for an additional 13 years in Study LTF-304. **Study ALD-102 is now completed, while Study LTF-304 remains ongoing as of this protocol amendment.** Safety data from ALD-102 and LTF 304 can be found in the ~~Investigator's Brochure~~ **IB**.	The status of Studies ALD-102 and LTF-304 is updated as of this protocol amendment.
1.4 Potential Risks *[section contains no text]*	*1.4 Potential Risks* **Potential risks, warnings and precautions are described in the Skysona U.S. product package insert (Skysona 2022); in addition, updated safety results for bluebird bio's CALD studies can be found in the IB.**	

Initial wording	Amended or New Wording	Change Reason/Justification
1.4.1 Abnormal Proliferation of Hematopoietic Cells […] The different nature of the Lenti-D LVV should minimize, but not completely eliminate, the risk of oncogenesis by insertional mutagenesis. Unlike the γ retroviral vectors that led to leukemia, self-inactivating (SIN) LVVs, such as Lenti-D LVV, may represent a substantial improvement in terms of safety (Montini et al. 2006). SIN LVVs, in general, provide significant safety improvements over γ retroviral vectors used in earlier studies (Riviere et al. 2012). SIN LVVs lack the strong enhancer/promoter long terminal repeat (LTR) sequences of γ retroviral vectors, and, unlike γ retroviral vectors, do not preferentially integrate near gene promoter regions. Therefore, LVVs are less likely to transactivate oncogenes, and have demonstrated a significantly curtailed probability of oncogenic transformation in vitro and in vivo (Biffi et al. 2011).	*1.4.1 Abnormal Proliferation of Hematopoietic Cells* […] The different nature of the Lenti-D LVV should minimize, but not completely eliminate, the risk of oncogenesis by insertional mutagenesis. Unlike the γ-retroviral vectors that led to leukemia, self-inactivating (SIN) LVVs, such as Lenti-D LVV, may represent a substantial improvement in terms of safety (Montini et al. 2006). SIN LVVs, in general, provide significant safety improvements over γ-retroviral vectors used in earlier studies (Riviere et al. 2012). SIN LVVs lack the strong enhancer/promoter long terminal repeat (LTR) sequences of γ-retroviral vectors, and, unlike γ retroviral vectors, do not preferentially integrate near gene promoter regions. Therefore, LVVs are less likely to transactivate oncogenes, and have demonstrated a significantly curtailed probability of oncogenic transformation in vitro and in vivo (Biffi et al. 2011). **However, as described in the Skysona U.S. product package insert (Skysona 2022) and in the IB, insertional oncogenesis has been observed in the eli-cel clinical development program.**	Added note that to reflect that eli-cel has been approved by the U.S. FDA and to provide the citation for the product package insert where up to date potential risks are provided
2.2.1. Efficacy Endpoints *[…]* CCI	2.2.1. Efficacy Endpoints *[…]*	CCI

Initial wording	Amended or New Wording	Change Reason/Justification
3.1 Overall Design and Plan of the Study […] The coordinating Investigator assigned on this study will be responsible for signing off on the final ALD-104 Clinical Study Report.	*3.1 Overall Design and Plan of the Study* […] The coordinating Investigator assigned on this study will be responsible for signing off on the final ALD-104 Clinical Study Report. **Note: as of this Protocol Amendment (Version 8.0) enrollment in Study ALD-104 has been completed and patient followup remains ongoing. Further subject enrollment in this study is not anticipated.**	New final paragraph added to this section to update study recruitment status. The order of paragraphs within this section was updated to keep efficacy and safety content together (not shown as a change in this row as content was moved but not changed).
4.1 Number of Subjects Approximately 35 subjects will be infused with eli-cel after myeloablative conditioning with busulfan and fludarabine.	*4.1 Number of Subjects* Approximately 35 subjects will be infused with eli-cel after myeloablative conditioning with busulfan and fludarabine. **As of this Protocol Amendment (Version 8.0) enrollment and treatment (N=35) in Study ALD-104 have been completed; subject follow-up is ongoing. Further enrollment in this study is not expected.**	The number of subjects and the status of Studies ALD-102 and LTF-304 is updated as of this protocol amendment.
No Section 4.7	***4.7. Protocol Deviation*** **Categorization of protocol deviations into major/minor deviations will be determined prior to database lock, by a review of the protocol deviation data collected on the case report forms (CRFs).**	A new section has been added to describe characterization of protocol deviations
Impact of Force of Nature (e.g., the COVID-19 Pandemic) on Study Visits Due to force of nature (e.g., the COVID-19 pandemic), subjects may not be able to attend normal study visits. If a visit is missed due to a force of nature (e.g. unable to travel, unwilling to travel, family or subject affected by COVID-19, hospital closure, etc.), the subject may be able to complete study assessments via telemedicine or at a local facility. Sites should consult with the medical monitor and CRAs to consider alternate	*Impact of Force of Nature **or War** (e.g., the COVID-19 Pandemic**, the Russo-Ukrainian War**) on Study Visits* Due to force of nature **or war** (e.g., the COVID-19 pandemic**, the Russo-Ukrainian war**), subjects may not be able to attend normal study visits. If a visit is missed due to force of nature **or war** reasons (e.g.**,** unable to travel, unwilling to travel, family or subject affected by COVID-19, hospital closure, etc.), the subject may be able to complete study assessments via telemedicine or at a local facility. Sites should consult with the medical monitor and clinical research associates to consider alternate	Impact of force of nature text updated to include war due to the impact on subject visits of the Russo-Ukrainian War.

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arrangements, when possible, for completion of study assessments.	arrangements, when possible, for completion of study assessments.	
Section 6.1 Schedule of Events Table 3 and Table 4 provide the Schedule of Events (SOE) to be conducted during the study. Detailed descriptions of the efficacy, safety, and exploratory procedures to be conducted during this study are provided in the following sections. Additional details, including administrative information, regarding the efficacy, safety, and exploratory procedures, will be provided by the Sponsor in the SOM. *[…]* During the first year after eli-cel treatment (Month 1 to Month 12), CBCs with differential will be collected every month as shown in Table 5. Subsequently, CBCs with differential will be collected every 4 months at the study site until Month 24. Note: Unscheduled visits may be performed at any time during the study whenever necessary to assess for or to follow-up on AEs or as deemed necessary by the Investigator. Evaluations and procedures identified in the SOE, or other evaluations and procedures deemed necessary for safety, may be performed at unscheduled visits, as clinically indicated, at the Investigator's discretion in consultation with the Sponsor. *[…]* *Table 3 Schedule of Events: Screening Through Drug Product Infusion* *"Serology panel" row in Table 3 was revised*	*Section 6.1 Schedule of Events* Table 3**,** ~~and~~ Table 4 **and Table 5** provide the Schedule of Events (SOE) to be conducted during the study. Detailed descriptions of the efficacy, safety, and exploratory procedures to be conducted during this study are provided in the following sections. Additional details, including administrative information, regarding the efficacy, safety, and exploratory procedures, will be provided by the Sponsor in the SOM. *[…]* During the first year after eli-cel treatment (Month 1 ~~to~~ **through** Month 12), CBCs with differential will be collected every month as shown in **Table 5**. Subsequently, CBCs with differential will be collected every 4 months at the study site ~~until~~ **through** Month 24. **Data collection as described in this paragraph will be obtained prospectively only (that is, data should not be collected retrospectively for subjects who have passed applicable study visits as of the date of implementation of this protocol amendment).** Note: Unscheduled visits may be performed at any time during the study whenever necessary to assess for or to follow-up on AEs or **to perform enhanced CBC and BM abnormality monitoring (Section 6.5.11.1.1 and Section 6.5.11.1.2, respectively) or** as deemed necessary by the Investigator. Evaluations and procedures identified in the SOE, or other evaluations and procedures deemed necessary for safety, may be performed at unscheduled visits, as clinically indicated, at the Investigator's discretion in consultation with the Sponsor. *[…]* *Table 3 Schedule of Events: Screening Through* ***Conditioning*** ~~*Drug Product Infusion*~~ *"Serology panel" row in Table 3 was revised as follows:*	Text has been revised to increase the number/frequency of collection timepoints for CBC with differential to occur monthly during the first year after eli-cel infusion, and thereafter every 4 months until Month 24. Text also edited to note that unscheduled visits may be performed for enhanced CBC and BM abnormality monitoring. A note was added that the retrospective collection of these data is not expected for visits that had occurred prior to this protocol amendment (e.g., if a subject had already had their Month 18 Visit prior to this protocol amendment being effective at their study site, then it is not expected that such subjects would return for updated Month 18 assessments). Table 3 title updated to denote that this table includes assessments through conditioning and serology row updated as shown for clarity. Lettered footnotes are now included.

Initial wording	Amended or New Wording	Change Reason/Justification
[…]	Serology panels **I and II**. *[…]*	
Table 4 edited rows original text Bone marrow biopsy and aspirate (with ISA)	*Table 4 rows edited* Bone marrow **core needle** biopsy and aspirate (~~with~~ **including** ISA**, VCN and Storage**) *New row added under bone marrow assessments* **Additional BM evaluations may be triggered as detailed in Section 6.5.11.1.2**	Table row relating to bone marrow assessments updated to clarify that it includes core needle biopsy and aspirate sampling and that samples will be used for ISA, VCN and storage purposes.
Table 4 original footnotes: *4.* Hematology parameters include white blood cell (WBC) count with differential, hemoglobin, hematocrit, red blood cell (RBC), and platelet count. If the results from blood tests are not as expected, additional testing may need to be performed and may include a physical exam, blood tests, imaging tests, or a bone marrow biopsy.	*Table 4 updated or new footnotes:* **d** ~~4~~. Hematology parameters include white blood cell (WBC) count with differential, hemoglobin, hematocrit, red blood cell (RBC), and platelet count. If the results from blood tests are not as expected, additional testing may need to be performed and may include a physical exam, blood tests, imaging tests, or a BM biopsy **(both BM core biopsy sections and aspirate smear collection should be performed to assess morphology). See Section 6.5.11.4. for blood sample collection and storage details.**	Table 4 footnotes changed from numbered to lettered footnotes. Footnote d (formerly footnote 4) has been updated to clarify bone marrow collection and storage details.
5. A repeat performance of CBC is required within one month for any CTCAE CBC abnormality that is of Grade 2 or higher severity. Specifically, this includes abnormalities in hemoglobin levels, total white blood cell count, neutrophil count and morphology, lymphocyte count and morphology, or monocyte counts and morphology. Any two consecutive (one month apart) platelet counts of $< 100 \times 10^9$/L will trigger a bone marrow evaluation to be repeated every 4 months. See Section 6.5.11.4. In addition, a re-evaluation at the discretion of the Investigator of any new abnormal peripheral blood cell count or morphology at an interval commensurate with the severity of the change is recommended. *[…]*	**e** ~~5~~. A repeat performance of CBC is required within one month for any ~~CTCAE~~ CBC abnormality ~~that is of Grade 2 or higher severity. Specifically, this includes~~ **(including** abnormalities in hemoglobin levels, total white blood cell count, neutrophil count and morphology, lymphocyte count and morphology, or monocyte counts and morphology**)** ~~Any two consecutive (one month apart)~~ **that is of CTCAE Grade 2 or higher severity or for** platelet counts of <100 $\times 10^9$/L ~~will trigger a bone marrow evaluation to be repeated every 4 months~~. See Section 6.5.11.1.1~~6.5.11.4~~. In addition, a re-evaluation at the discretion of the Investigator of any new abnormal peripheral blood cell count or morphology at an interval commensurate with the severity of the change is recommended. **Persistent, unexpected, abnormalities of peripheral blood cell counts or morphology, including but not limited to any two**	Footnote e (formerly footnote 5) has been updated to clarify which repeat CBC and platelet values would trigger bone marrow assessment.

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	consecutive (one month apart) CTCAE Grade 2 CBC values or platelet counts of <100 x 10^9/L will trigger a BM evaluation. For frequency of subsequent BM evaluations (see Section 6.5.11.1.2). See Section 6.5.11.4 for sample collection and storage details. […] *New footnote 7 added (and subsequent footnote numbers updated accordingly)* **g. Peripheral blood smears will be created locally at the study site and submitted for central review by a single central hematopathologist. See Section 6.5.11.1.1 for further details. At the Investigator's discretion local peripheral blood smears may also be performed by the study site to direct patient care.** *New footnotes 8 and 9 added (and subsequent footnote numbers updated accordingly.* **h. Note that sites are required to collect BM aspirate post-drug product infusion per the SOE, unless discussed with the Sponsor's Medical Monitor for exceptions, such as if not clinically appropriate per local standard of care (e.g., BM aspirate would require general anesthesia, which would be considered an unacceptable risk to the subject) or not considered by the Investigator to be in the best interest of the subject to perform. For details, see Section 6.5.11.1.2.** **i. Bone marrow smear should be read locally; additional BM slides are to be created and submitted for central analyses as detailed in Section 6.5.11.1.2. See Section 6.5.11.4 for BM sample collection details. Additionally, BM aspirate (and an accompanying whole blood specimen) should be collected for storage at the time of BM sample collection to facilitate subsequent exploratory analyses that may include gene/genomic expression studies.**	New footnotes g, h and i have been added to Table 4 to note that central hematopathology review of the peripheral blood smear will be conducted.

Initial wording	Amended or New Wording	Change Reason/Justification
9. Additional blood may be collected for analysis in cell subtypes. If blood draw volume required exceeds the limit per Section 6.5.11.4, samples should be obtained over multiple days.	*[…]* **l.** ~~9.~~ Additional blood may be collected for analysis in cell subtypes, **if requested by the Sponsor**. If blood draw volume required exceeds the limit per Section 6.5.11.4, samples should be obtained over multiple days.	Former footnote 9 (now letter l) updated to note that samples will only be collected for analysis in cell subtypes if requested by the Sponsor.
Table 5 updated or new footnotes: a. Hematology parameters include white blood cell (WBC) count with differential, hemoglobin, hematocrit, red blood cell (RBC), and platelet count. If the results from blood tests are not as expected, additional testing may need to be performed and may include a physical exam, blood tests, imaging tests, or a bone marrow biopsy. b. A repeat performance of CBC is required within one month for any CTCAE CBC abnormality that is of Grade 2 or higher severity. Specifically, this includes abnormalities in hemoglobin levels, total white blood cell count, neutrophil count and morphology, lymphocyte count and morphology, or monocyte counts and morphology. Any two consecutive (one month apart) platelet counts of $< 100 \times 10^9$/L will trigger a bone marrow evaluation to be repeated every 4 months. See Section 6.5.11.4. In addition, a re-evaluation at the discretion of the Investigator of any new abnormal peripheral blood cell count or morphology at an interval commensurate with the severity of the change is recommended. c Chemistry and hematology parameters will be measured at least twice per week until neutrophil engraftment occurs.	*Table 5 updated or new footnotes:* **1** ~~a~~ Hematology parameters include white blood cell (WBC) count with differential, hemoglobin, hematocrit, red blood cell (RBC), and platelet count. If the results from blood tests are not as expected, additional testing may need to be performed and may include a physical exam, blood tests, imaging tests, or a ~~bone marrow~~ **BM** biopsy. **2** ~~b~~ A repeat performance of CBC is required within one month for any ~~CTCAE~~ CBC abnormality ~~that is of Grade 2 or higher severity. Specifically, this includes~~ **(including** abnormalities in hemoglobin levels, total white blood cell count, neutrophil count and morphology, lymphocyte count and morphology, or monocyte counts and morphology**).** ~~Any two consecutive (one month apart)~~ **that is of CTCAE Grade 2 or higher severity or for** platelet counts of <100 $\times 10^9$/L ~~will trigger a bone marrow evaluation to be repeated every 4 months~~. See Section **6.5.11.1.1** ~~6.5.11.4~~. In addition, a re-evaluation at the discretion of the Investigator of any new abnormal peripheral blood cell count or morphology at an interval commensurate with the severity of the change is recommended. **Persistent, unexpected, abnormalities of peripheral blood cell counts or morphology, including but not limited to any two consecutive (one month apart) CTCAE Grade 2 CBC values or platelet counts of <100 x 10^9/L will trigger a BM evaluation. For frequency of subsequent BM evaluations (see Section 6.5.11.1.2).** **3** ~~c~~ ~~Chemistry and h~~**H**ematology parameters will be measured at least twice per week until neutrophil engraftment occurs. **4 Peripheral blood smears will be created locally at the study site and submitted for central review by a single**	Table 5 footnotes updated to reflect current repeat CBC criteria and associated bone marrow assessment triggers. Footnote number 2 (formerly footnote b) was updated to clarify that, if needed, an unscheduled visit could provide CBC results and was updated to clarify which repeat CBC and platelet values would trigger bone marrow assessment. Specific clinical triggers for repeat CBCs, bone marrow evaluations, and ISA of bone marrow was also added. A new footnote number 4 has been added to note that central hematopathology review of peripheral blood smears will be conducted and to provide more details on requirements for collection of bone marrow samples, their submission to central review and for storage).

Initial wording	Amended or New Wording	Change Reason/Justification
	central hematopathologist. See Section 6.5.11.1.1 for further details. At the Investigator's discretion local peripheral blood smears may also be performed by the study site to direct patient care.	
6.5.11 Clinical Laboratory Tests Laboratory tests of hematology and serum chemistries will be performed as specified in the following sub-sections and in the SOE. Clinical laboratory tests are to be performed locally and reviewed by the Investigator or qualified designee (e.g., physician's assistant, nurse practitioner) appropriately listed on the Delegation of Responsibility Log for this task. All hematology and chemistry results from assessments performed at unscheduled visits should also be entered into the clinical database.	*6.5.11 Clinical Laboratory Tests* Laboratory tests of hematology and serum chemistries will be performed as specified in the following sub-sections and in the SOE. Clinical laboratory tests are to be performed locally and reviewed by the Investigator or qualified designee (e.g., physician's assistant, nurse practitioner) appropriately listed on the Delegation of Responsibility Log for this task. **In addition to the peripheral blood smears, BM core biopsy sections and BM aspirate smears required for routine local hematopathology review and per the SOE, additional peripheral blood smears (see Section 6.5.11.1), BM core biopsy sections and BM aspirate smears (see Section 6.5.11.1.2) should be prepared for study purposes and submitted to a single centralized hematopathologist. Results of the central read will be provided to the Sponsor and subsequently shared with the Investigator.** All hematology and chemistry results from assessments performed at unscheduled visits should also be entered into the clinical database. **Additional instances of clinical laboratory tests listed in this section may be performed at the Investigator's discretion as needed.** **Local tests and reads should be utilized for participant care decision making.**	Text updated to note that in addition to samples taken for routine local hematopathology review and local assessments performed per the SOE, additional peripheral blood smears, bone marrow core biopsy sections, and bone marrow aspirate smears should be prepared for study purposes and submitted to a single centralized hematopathologist for review and to note that results of the central read will be provided to the Sponsor and subsequently shared with the Investigator. Note added that additional tests may be conducted at the investigator's discretion and that local tests and reads should be utilized for participant care decision making.

Initial wording	Amended or New Wording	Change Reason/Justification
Section 6.5.11.1 Hematology, Clinical Chemistry, Liver and Adrenal Function Blood samples for hematology, clinical chemistry, liver and adrenal function are to be collected as specified in the SOE. The following clinical laboratory parameters are to be determined: *[Table of laboratory parameters]* *Hematology: CBC with differential* • White blood cell (WBC) count with differential • Platelet count • Red blood cell (RBC) count • Hemoglobin • Hematocrit *[…]* Effort should be made to obtain a platelet count 8 days after any platelet transfusion, until platelet engraftment occurs (as defined in Section 6.5.13). Additional instances of clinical laboratory tests listed in this section may be performed at the Investigator's discretion as needed.	*Section 6.5.11.1 Hematology, Clinical Chemistry, Liver and Adrenal Function* Blood samples for hematology, clinical chemistry, liver and adrenal function are to be collected as specified in the SOE. The following clinical laboratory parameters are to be determined: *[Peripheral blood smear added to table of laboratory parameters]* *Hematology: CBC with differential* • White blood cell (WBC) count with differential • Platelet count • Red blood cell (RBC) count • Hemoglobin • Hematocrit • **Peripheral blood smear** *[…]* Effort should be made to obtain a platelet count 8 days after any platelet transfusion, until platelet engraftment occurs (as defined in Section 6.5.13). Additional instances of clinical laboratory tests listed in this section may be performed at the Investigator's discretion as needed.	Peripheral blood smears added to table of laboratory parameters and removed from prior section relating to performance of additional test at the Investigator's discretion.

Initial wording	Amended or New Wording	Change Reason/Justification
[no section heading] Complete blood counts with differential will be performed per the SOE. During the first year after eli-cel treatment (Month 1 to Month 12), CBCs with differential will be collected every month as shown in Table 5. Subsequently, CBCs with differential will be collected every 4 months at the study site until Month 24. A repeat performance of CBC is required within one month for any CTCAE CBC abnormality that is of Grade 2 or higher severity. Specifically, this includes abnormalities in hemoglobin levels, total white blood cell count, neutrophil count and morphology, lymphocyte count and morphology, or monocyte counts and morphology. Any two consecutive (one month apart) platelet counts of < 100 × 10 /L will trigger a bone marrow evaluation to be repeated every 4 months. See Section 6.5.11.4. In addition, a re-evaluation at the discretion of the Investigator of any new abnormal peripheral blood cell count or morphology at an interval commensurate with the severity of the change is recommended. A hematopathology review of a peripheral blood smear to assess for dysplastic features will be conducted per the SOE. This review will be conducted by a central reader. Refer to the Study Operations Manual for further details on central expert hematopathologist review and reporting.	*[added new heading]* **6.5.11.1.1. Complete Blood Count and Peripheral Blood Smear** Complete blood counts with differential will be performed **and analyzed locally at the study site** per the SOE during the first year after eli-cel treatment (Month 1 ~~to~~ **through** Month 12)~~, CBCs with differential will be collected every month as shown in Table 5~~. Subsequently, CBCs with differential will be collected every 4 months at the study site ~~until~~ **through** Month 24. A repeat performance of CBC is required within one month for any ~~CTCAE~~ CBC abnormality ~~that is of Grade 2 or higher severity that includes~~ **(including** abnormalities in hemoglobin levels, total ~~white blood cell~~ **WBC** count, neutrophil count and morphology, lymphocyte count and morphology, or monocyte counts and morphology**) that is of CTCAE Grade 2 or higher severity or for platelet counts of <100 x 10^9/L**. ~~Any two consecutive (one month apart) platelet counts of < 100 × 10^9/L will trigger a bone marrow evaluation to be repeated every 4 months. See Section 6.5.11.4.~~ In addition, a re-evaluation at the discretion of the Investigator of any new abnormal peripheral blood cell count or morphology at an interval commensurate with the severity of the change is recommended. **Persistent, unexpected, abnormalities of peripheral blood cell counts or morphology, including but not limited to any two consecutive (one month apart) CTCAE Grade 2 CBC values or platelet counts of < 100 x 10^9/L will trigger a BM evaluation. For frequency of subsequent BM evaluations (see Section 6.5.11.1.2).** ~~A hematopathology review of a~~ **In addition,** peripheral blood smear**s should be prepared for study purposes and submitted to a single centralized hematopathologist for review** to assess for dysplastic features ~~will be conducted~~ **at Months 6, 12, 16, 20 and 24,** per the SOE. ~~This review will be conducted by a central reader.~~ **Results of the central read will be provided to the Sponsor and subsequently**	Revised collection timepoints for CBC with differential to occur at the local study site monthly during the first year after eli-cel infusion, and thereafter every 4 months until Month 24. Clarified specific clinical triggers for repeat CBCs, bone marrow evaluations, and ISA of bone marrow. Clarification is provided that peripheral blood smears should be prepared for study purposes and submitted to a single centralized hematopathologist for review to assess for dysplastic features and that, at the Investigator's discretion, local peripheral blood smears may also be performed by the study site to direct patient care.

Initial wording	Amended or New Wording	Change Reason/Justification
	shared with the Investigator (although centrally read peripheral smear slides will not be returned to the study site). At the Investigator's discretion local peripheral blood smears may also be performed by the study site to direct patient care. Refer to the ~~Study Operations Manual~~ **SOM** for further details on **preparation of additional slides for** central ~~expert hematopathologist~~ review ~~and reporting.~~	
6.5.11.4 Specialty Laboratory Sample Collection *[…]* *Bone Marrow Core Needle Biopsy and Aspiration* A bone marrow evaluation (including ISA of the bone marrow) will be performed in the event of one or more of the following specific findings: i. Persistent, unexpected, abnormalities* of peripheral blood cell counts or morphology, including but not limited to any two consecutive (one month apart) CTCAE Grade 2 CBC values or platelet counts of <100 x 10^9/L. If bone marrow evaluation is triggered by platelet counts, perform bone marrow biopsies at four month intervals until a peripheral blood platelet value of ≥125 x 10^9/L is observed. ii. Any transfusion requirement after discharge from the initial hospitalization unless the need for transfusion was related to trauma or a procedure/intervention. iii. Abnormal results from the most recent bone marrow biopsy or aspirate (see below) iv. Relative frequency of ≥ 5% for the same insertion site observed at two consecutive time points, if the insertion site is located in a gene with known biological relevance to carcinogenesis; the latter is defined as a Tier 1 gene in the Cancer Gene Census (CGC) of the Catalogue of Somatic Mutations in Cancer (COSMIC) at the time of the ISA report review	*6.1.11.4**.1.12 ~~Specialty Laboratory Sample Collection~~*** ***Bone Marrow Core Needle Biopsy and Aspiration*** **During the first year after eli-cel treatment (at Month 6 and Month 12), BM will be collected to perform routine assessments, ISA and VCN assessments (and sample created for storage). ~~A~~ Additional ~~bone marrow~~ BM** evaluation**s** will **also** be performed in the event of one or more of the following specific findings: Persistent, unexpected, abnormalities* of peripheral blood cell counts or morphology, including but not limited to any two consecutive (one month apart) CTCAE Grade 2 CBC values or platelet counts of <100 x 10^{9}~~^9~~/L. If ~~bone marrow~~ **BM** evaluation is triggered by platelet counts, perform BM biopsies at four month intervals until a peripheral blood platelet value of ≥125 x 10^{9}~~^9~~/L is observed. Any transfusion requirement after discharge from the initial hospitalization unless the need for transfusion was related to trauma or a procedure/intervention. Abnormal results from the most recent ~~bone marrow~~ **BM** biopsy or aspirate (see below) **ISA: IS in a known oncogene (i.e., relative** frequency **[RelFreq]** of ≥ 5% for the same insertion site observed at two consecutive time points, if the ~~insertion site~~ **IS** is located in a gene with known biological relevance to carcinogenesis**:**~~; the latter is~~ defined as a Tier 1 gene in the Cancer Gene Census (CGC) of the Catalogue of Somatic Mutations in Cancer (COSMIC) at the time of the ISA report review**.**	Section moved to new location (formerly was a subsection within Section 6.5.11.4) and updated to provide greater clarity on triggers for bone marrow evaluation and central reader review.

Initial wording	Amended or New Wording	Change Reason/Justification
v. Persistent oligoclonality (i.e. relative frequency of ≥ 10% for the same IS observed at two consecutive time points, or relative frequencies of ≥ 5% for the same two or more IS observed at two consecutive time points) vi. A two-fold increase in peripheral blood VCN over a 4-month period * these include abnormalities in hemoglobin levels; total white blood cell, neutrophil, lymphocyte, or monocyte counts. For new findings of moderate hypocellularity (defined for this protocol as 30-40%), marked hypocellularity (<30%), moderate dysplasia (>10%-40% of cells in one or more lineages), or marked dysplasia (>40% of cells in the pertinent lineage), one repeat evaluation is required at a 3-month interval. For new findings of moderate hypercellularity (80-90%) or marked hypercellularity (>90%), one repeat evaluation is required which may be performed at a 3 or 6-month interval, at the discretion of the Investigator. If bone marrow abnormalities on two consecutive assessments are stable or improved and CBC* is within normal limits, the bone marrow biopsy and aspirate should occur every 6 months until abnormalities are resolved. If there is worsening (e.g., change from moderate to marked) of dysplasia, decreasing hypocellularity or increasing hypercellularity, or abnormalities on CBC, bone marrow biopsies and aspirate collection frequency should remain at a 3-month interval. *hemoglobin levels, total white blood cell count, neutrophil, lymphocyte, and monocyte counts. These recommendations are not intended to preclude prior re-evaluation as warranted. Additional bone	**ISA: persistent** oligoclonality (i.e., ~~relative frequency~~ **RelFreq** of ≥ 10% for the same IS observed at two consecutive time points, or relative frequencies of ≥ 5% for the same two or more IS observed at two consecutive time points). A two-fold increase in peripheral blood VCN over a 4-month period**.** * these include abnormalities in hemoglobin levels, total ~~white blood cell~~ WBC, neutrophil, lymphocyte, ~~or~~ monocyte, **or platelet counts** and **morphology**. For new findings of moderate hypocellularity (defined for this protocol as 30-40%), marked hypocellularity (<30%), moderate dysplasia (>10%-40% of cells in one or more lineages), or marked dysplasia (>40% of cells in the pertinent lineage), one repeat evaluation is required at a 3-month interval. For new findings of moderate hypercellularity (80-90%) or marked hypercellularity (>90%), one repeat evaluation is required which may be performed at a 3 or 6-month interval, at the discretion of the Investigator. If ~~bone marrow~~ **BM** abnormalities on two consecutive assessments are stable or improved and CBC** is within normal limits, the ~~bone marrow~~ **BM** biopsy and aspirate should occur every 6 months until abnormalities are resolved. If there is worsening (e.g., change from moderate to marked) of dysplasia, decreasing hypocellularity or increasing hypercellularity, or abnormalities on CBC, ~~bone marrow~~ **BM** biopsies and aspirate collection frequency should remain at a 3-month interval. ** hemoglobin levels, total ~~white blood cell~~ WBC count, neutrophil, lymphocyte, ~~and~~ monocyte **and platelet** counts. These recommendations are not intended to preclude re-evaluation as warranted. Additional ~~bone marrow~~ **BM** evaluations should be performed, at the discretion of the Investigator and at a frequency commensurate with the integrated findings and based upon recommendations from	

Initial wording	Amended or New Wording	Change Reason/Justification
marrow evaluations should be performed at the discretion of the Investigator and at a frequency commensurate with the integrated findings and based upon recommendations from the hematopathologist and hematologist investigator (or consulting hematologist). Bone marrow evaluation will include both core needle biopsy and aspiration to assess morphology, cellularity, fibrosis, and to perform flow cytometry, conventional karyotyping, FISH, next-generation sequencing (NGS), ISA, and VCN. The BM evaluations will be performed in accordance with institutional practices under aseptic conditions. It is anticipated that institutional guidelines will specify that approximately 5 to 20 mL of BM aspirate will be collected each time. Bone marrow core biopsy and aspirate smear slides will be submitted to a single centralized expert hematopathologist after local review has been completed. Results of the central read will be provided to the Sponsor and subsequently shared with the Investigator Refer to the Study Operations Manual for further details on central expert hematopathologist review and reporting. Additionally, sites will submit bone marrow aspirate to centralized specialty laboratories for the performance of FISH (using a probe panel for common mutations in pediatric AML, MDS, and probes for MLL [11q23], MECOM, and PRDM16 rearrangement) and NGS (spanning mutations commonly observed in myeloid and lymphoid malignancies). Refer to the SOM for further details.	the hematopathologist and hematologist investigator (or consulting hematologist). ~~Bone marrow~~ **Triggered BM** evaluation will include both core needle biopsy and aspiration to assess morphology, cellularity, fibrosis, and to perform flow cytometry, **and** ~~conventional~~ karyotyping~~, FISH, next-generation sequencing (NGS), ISA, and VCN. The BM~~ **that should be carried out locally as part of normal pathology assessments, including local smear assessment. Additional aliquots of triggered BM aspirate will be collected and sent to centralized specialty laboratories for the performance of fluorescence in situ hybridization (FISH) (using a probe panel for common mutations in pediatric AML, MDS, and probes for MLL [11q23], MECOM, and PRDM16 rearrangement), next-generation sequencing (NGS) (spanning mutations commonly observed in myeloid and lymphoid malignancies), and ISA/VCN. Note that due to the time taken for central NGS and FISH results to be obtained, Investigators should initiate additional local NGS and FISH testing if results are needed more rapidly to direct participant care. An additional BM aspirate sample should be submitted to the Sponsor for storage to support potential future genetic/genomic studies.** The BM evaluations will be performed in accordance with institutional practices under aseptic conditions. It is anticipated that institutional guidelines will specify that approximately 5 to 20 mL of BM aspirate will be collected each time. **Bone marrow aspirate (and an accompanying whole blood specimen) should be collected for storage at the time of BM sample collection to facilitate subsequent exploratory analyses that may include gene/genomic expression studies.** **In addition to BM** ~~Bone marrow~~ core biopsy **sections** and aspirate smear slides ~~will~~ **created for routine local hematopathology review, additional BM core biopsy sections and aspirate smears should** be **prepared for study purposes and** submitted to a single centralized	

Initial wording	Amended or New Wording	Change Reason/Justification
	~~expert~~ hematopathologist ~~after local review has been completed~~. Results of the central read will be provided to the Sponsor and subsequently shared with the Investigator **(although centrally read slides will not be returned to the study site)**. Refer to the SOM for further details on **preparation of additional slides for submission to the single central hematopathologist and for submission of samples to centralized laboratories**. Additionally, sites will submit bone marrow aspirate to centralized specialty laboratories for the performance of FISH (using a probe panel for common mutations in pediatric AML, MDS, and probes for MLL [11q23], MECOM, and PRDM16 rearrangement) and NGS (spanning mutations commonly observed in myeloid and lymphoid malignancies). Refer to the SOM for further details.	
Inserted new Section 6.5.11.1.3	***6.5.11.1.3 Blood Sample Collection for RCL Testing, Integration Site Analysis, and VCN in Blood*** **Blood samples will be collected according to the SOE for assessments of the following:** • **RCL - If subject tested negative at all visits through M12, further samples will be archived for potential analysis in case of clinical outcomes suggesting the presence of RCL.** • **VCN in whole blood will be evaluated at every visit indicated for VCN in the SOE.** • **ISA at visits indicated (only to be performed if VCN is ≥ 0.01 vector copies per diploid genome, at the previous visit).** **Testing will be performed by central laboratories. Blood collection and processing details are included in the Laboratory Manual.** **Blood samples for ISA are to be collected according to the SOE; collection, processing and shipping details are included in the Laboratory Manual. Testing will be performed by a central laboratory. Additional blood**	New section added to provide more detail on sample collection for RCL Testing, Integration Site Analysis, and VCN in Blood

Initial wording	Amended or New Wording	Change Reason/Justification
	may be collected for additional investigations and analysis in cell subtypes, if requested by the Sponsor.	
6.5.11.2. Immunological Studies Immunological testing includes measuring levels of T cell subsets (CD4, CD8), B cells (CD19), and NK cells (CD16 or CD56). In addition, levels of immunoglobulins (IgG, IgM, and IgA) will be quantified.	*6.5.11.2. Immunological Studies* **Local i**~~I~~mmunological testing includes measuring levels of T cell subsets (CD4, CD8), B cells (CD19), and NK cells (CD16 or CD56). In addition, levels of immunoglobulins (IgG, IgM, and IgA) will be quantified.	Text updated to clarify that immunological studies should be conducted locally.
Section 6.5.11.4 Specialty Laboratory Sample Collection As this is a pediatric study, blood volume limitations will sometimes preclude the collection of all samples during a particular study visit. Table 7 enumerates the priorities for blood collection. Safety labs (see Section 6.5) have been prioritized over labs for efficacy and exploratory analyses. […] Table 7: Blood Collection: Order of Priority • ABCD1 genotype • Adrenal Function Tests • Serology Panels (infectious disease screening) • Chemistry • Hematology • VCN in Peripheral Blood Populations • Proviral Integration Site Analysis (ISA) • ALDP in Peripheral Blood Populations • RCL testing • VLCFA analysis (fasting) *Vector Copy Number by qPCR: Peripheral Blood Populations*	*Section 6.5.11.4 Specialty Laboratory Sample Collection* As this is a pediatric study, blood volume limitations will sometimes preclude the collection of all samples during a particular study visit. Table 7 enumerates the priorities for blood **and BM** collection **(BM core section slides and BM aspirates)**. Safety labs (see Section 6.5) have been prioritized over labs for efficacy and exploratory analyses. **If the results from CBC or BM tests are not as expected, additional testing may need to be performed as detailed in Section 6.5.11.1.1 and Section 6.5.11.1.2, respectively. Local tests and reads should be utilized for patient care decision making.** *[…]* **With regard to bone-marrow, both core needle biopsy and aspiration collection should be performed to assess cellularity, fibrosis, and to perform flow cytometry, karyotyping, FISH, NGS, ISA, and VCN. The BM evaluations will be performed in accordance with institutional practices under aseptic conditions. It is anticipated that institutional guidelines will specify that approximately 5 to 20 mL of BM aspirate will be collected each time. See Section 6.5.11.1.2 for further details on BM collection and for further details on criteria that would trigger additional BM assessments over and above those noted in the SOE.** **If bone-marrow assessments are warranted, both core needle biopsy and aspiration collection should be**	Table 7 updated to refine order of priority of sample collection and to include bone marrow collection details and prioritization.

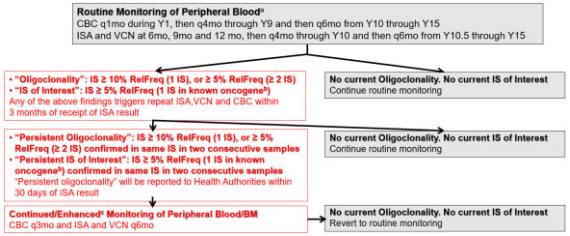
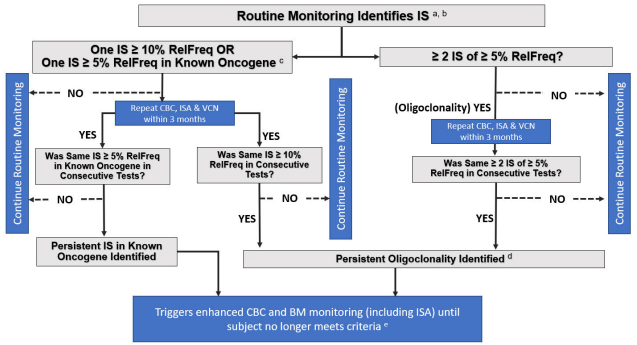
Initial wording	Amended or New Wording	Change Reason/Justification
Blood samples for VCN determination by qPCR will be collected according to the SOE. Testing will be performed by a central laboratory. Additional blood may be collected for analysis in cell subtypes. *[…]*	**performed to assess morphology, cellularity, fibrosis, and to perform flow cytometry, karyotyping, FISH, next-generation sequencing (NGS), ISA, and VCN. See Section 6.5.11.1.2 for further details on criteria that would trigger additional BM assessments over and above those noted in the SOE. Bone marrow evaluations will be performed in accordance with institutional practices under aseptic conditions. It is anticipated that institutional guidelines will specify that approximately 5 to 20 mL of BM aspirate will be collected each time.** **In addition to the BM core biopsy sections and aspirate smears read locally for routine hematopathology review or per the SOE, additional BM core biopsy sections and aspirate smears should be prepared for study purposes and submitted to a single centralized hematopathologist. Additionally, BM aspirate (and an accompanying whole blood specimen) should be collected for storage at the time of BM sample collection to facilitate subsequent exploratory analyses that may include gene/genomic expression studies. See more details below for additional sample storage details. Results of the central read will be provided to the Sponsor and subsequently shared with the Investigator.** Table 7: Blood **and Bone Marrow** Collection: Order of Priority Blood Collection Priority • *ABCD1* genotype • Adrenal Function Tests • Serology Panels (infectious disease screening) • Chemistry • Hematology **(CBC [performed locally] and peripheral blood smear [created locally and submitted to central lab])** • VCN in Peripheral Blood Populations	Table 7 priority list updated

Initial wording	Amended or New Wording	Change Reason/Justification
	• ~~Proviral~~ Integration Site Analysis (ISA) **and VCN** • **Immunological studies** • ALDP in Peripheral Blood Populations • RCL testing • VLCFA analysis (fasting) • **Whole blood specimen for storage at the time of BM assessment (to support exploratory analyses that may include gene/genomic expression)[1]** **Bone Marrow Aspirate[2] Collection Priority** • **Core needle biopsy sections and aspirate smears for morphologic assessment, including for cellularity and fibrosis** • **Local FISH (if needed more rapidly than central FISH to direct patient care)** • **Local NGS (if needed more rapidly than central NGS to direct patient care)** • **ISA and VCN** • **Karyotyping** • **FISH for central laboratories** • **NGS for central laboratories** • **Storage BM specimens (to support exploratory analyses that may include genetic and genomic studies)[1]** *[1] Whole blood collection at the time of BM sample collection should be stored to facilitate subsequent exploratory analyses* *[2] Bone marrow evaluation should also include a core needle biopsy.*	Bone marrow aspirate collection priority list provided including both local (if needed) and centralized testing details.

Initial wording	Amended or New Wording	Change Reason/Justification
	~~*Vector Copy Number by qPCR: Peripheral Blood Populations*~~ Blood samples for VCN determination by quantitative polymerase chain reaction (qPCR) will be collected according to the SOE. Testing will be performed by a central laboratory. Additional blood may be collected for analysis in cell subtypes.	Vector Copy Number by qPCR subheading deleted.
	~~*Proviral Integration Site Analysis (ISA)*~~ ~~Blood samples for ISA are to be collected according to the SOE; collection, processing and shipping details are included in the Laboratory Manual. Testing will be performed by a central laboratory. Additional blood may be collected for additional investigations and analysis in cell subtypes.~~	Deleted Proviral Integration Site Analysis subsection as these details were redundant with other content.
Storage of Samples Leftover biological samples from protocol procedures (e.g., whole blood, DNA, serum) may be stored (optional) for potential future exploratory investigations in those samples to study CALD and/or gene therapy. Bone marrow specimens should be submitted to the Sponsor for storage to support potential gene expression studies. Such samples may be stored for up to 20 years. The Sponsor will be the custodian of the samples in the repository and any unused samples will be destroyed at the Sponsor's discretion. If possible, optional blood, BM, and tissue samples also are to be collected in the event of a subject's death if an autopsy is performed. *[…]*	***Storage of Samples*** **Bone marrow specimens (accompanied by a whole blood sample) should be submitted to the Sponsor for storage to support potential future genetic and genomic studies or other exploratory studies.** Leftover biological samples from protocol procedures (e.g., whole blood~~,~~ DNA, serum) may **also** be stored (optional) for potential future exploratory investigations in those samples**,** to study CALD and/or gene therapy. ~~Bone marrow specimens should be submitted to the Sponsor for storage to support potential gene expression studies.~~ **Refer to the SOM for further details on the collection and submission of samples for storage**~~.~~ ~~Such~~ **S**amples may be stored for up to 20 years. The Sponsor will be the custodian of the samples in the repository and any unused samples will be destroyed at the Sponsor's discretion. If possible, optional blood, BM, and tissue samples also are to be collected in the event of a subject's death if an autopsy is performed. *[…]*	Added instructions for bone marrow specimens and whole blood storage to facilitate subsequent exploratory analyses that may include genetic and genomic studies

Initial wording	Amended or New Wording	Change Reason/Justification
Section 6.5.12. Assessment of Oligoclonality and/or Suspicion of Malignancy *Section 6.5.12.1. Assessment of Oligoclonality by Integration Site Analysis* Integration site analysis (ISA) will be performed to determine the insertion site (IS) profile of subjects over time, as indicated in the SOE. Figure 1 shows the algorithm that determines the frequency of monitoring by ISA to assess oligoclonality, in accordance with FDA Guidance (FDA 2020) and based on FDA consultation. While oligoclonality itself, or even monoclonality, will not a priori result in a malignancy, changes in IS relative frequency may be associated with an increase in the risk of a malignancy. Therefore, ISA monitoring is performed every 6 months throughout the study and during long-term followup. This is coupled with monitoring for hematological abnormalities via CBC with differential every 3 months during the parent study and through Year 10 during long-term follow-up and then every 6 months through Year 15 during long-term followup (see Section 6.9 for information on long-term follow-up). ISA monitoring may be repeated more frequently if there is an indication of oligoclonality or if otherwise triggered (see Section 6.5.11.4 for more details on triggered bone marrow evaluations). Such triggers include (but are not limited to) persistent unexpected CBC abnormalities (including age adjusted persistent CTCAE Grade 2 CBC values), post-discharge blood transfusion (unless due to trauma or a procedure/intervention), or a two-fold increase in peripheral blood vector copy number (PB VCN) over a 6-month period, or other abnormal results from a subject's most recent BM biopsy or aspirate or ISA findings of potential oligoclonality or IS of interest.	*Section 6.5.12 Assessment of Oligoclonality and/or Suspicion of Malignancy* *Section 6.5.12.1. Assessment of Oligoclonality by Integration Site Analysis* ~~Integration site analysis (ISA)~~ **ISA** will be performed to determine the insertion site (IS) profile of subjects over time, as indicated in the SOE. Figure 1 shows the ~~algorithm that determines the frequency~~ **set** of **ISA rules and resulting IS triggered enhanced** monitoring ~~by ISA~~ **(i.e., CBC and BM monitoring)** to assess oligoclonality, in accordance with FDA Guidance (FDA 2020) and based on **Food and Drug Administration (**FDA**)** consultation. While oligoclonality itself, or even monoclonality, will not a priori result in a malignancy, changes in IS relative frequency **(RelFreq)** may be associated with an increase in the risk of a malignancy. Therefore, ISA monitoring is performed every 6 months throughout the study and during long-term followup. This is coupled with monitoring for hematological abnormalities via CBC with differential every 3 months during the parent study and through Year 10 during long-term follow-up and then every 6 months through Year 15 during long-term followup (see Section 6.9 for information on long-term follow-up). **As discussed further below,** ISA monitoring may be repeated more frequently if there is an indication of oligoclonality or if **ISA should** otherwise **be** triggered (see Section ~~6.5.11.4~~ **6.5.11.1.2)** for more details on triggered ~~bone marrow~~ **BM** evaluations). Such triggers include (but are not limited to) persistent unexpected CBC abnormalities (including ~~age adjusted~~ persistent **[i.e., two consecutive]** CTCAE Grade 2 CBC values), post-discharge blood transfusion (unless due to trauma or a procedure/intervention), or a two-fold increase in peripheral blood vector copy number (PB VCN) over a **4 or** 6-month period **(based on the SOE)**, or other abnormal results from	Updated text to replace use of ISA algorithm terminology and replace with a figure and set of rules for retesting and enhanced monitoring that provides clear instructions for site staff. A link is provided for a more detailed IS decision tree in Appendix A. Reporting of applicable IS findings to relevant Health Authorities is clarified

Initial wording	Amended or New Wording	Change Reason/Justification
As shown in Figure 1, if an IS is detected at ≥ 10% relative frequency (RelFreq) or an IS ≥ 5% RelFreq is detected in 2 or more IS, then ISA will be repeated within 3 months of receipt of this result. If ≥ 10% or 2 or more IS ≥ 5% results are confirmed then a report of persistent oligoclonality will be submitted to the relevant Health Authorities within 30 days. This repeated observation will also trigger enhanced monitoring for hematological abnormalities, increasing the frequency of CBC with differential to every 3 months (if not already occurring at the interval) along with ISA and VCN every 6 months until the frequency no longer meets criteria. Additionally, if an IS is detected at ≥ 5% RelFreq in a known oncogene (based upon Tier 1 oncogenes in the Cancer Gene Census [CGC] in the Catalogue of Somatic Mutations in Cancer [COSMIC] at the time of the ISA report review) such an IS would be considered an IS of interest and ISA will be repeated within 3 months of receipt of this result (and would be considered a persistent IS of interest once confirmed during re-evaluation). Should the relative frequency of a persistent IS of interest reach ≥10% at two consecutive timepoints it would be considered persistent oligoclonality and a report will be submitted to the relevant Health Authorities within 30 days. Based on clinical and ISA findings, additional monitoring for malignancy may be instituted by the treating physician/Principal Investigator or Sponsor (see Section 6.5.11.4. for bone marrow evaluation recommendations and see Section 6.5.12.2 for recommended clinical work-up for potential malignancy).	a subject's most recent BM biopsy or aspirate or ISA findings of potential oligoclonality or IS ~~of interest~~ **in a known oncogene**. As shown in Figure 1, if an IS is detected at ≥ 10% ~~relative frequency (~~RelFreq~~)~~ or an IS ≥ 5% RelFreq is detected in 2 or more IS, ~~then~~ **this would be considered "*oligoclonality*" and ISA will be repeated within 3 months of receipt of this result.** If **an IS** ≥ 10% or 2 or more IS ≥ 5% ~~results~~ are confirmed**,** then a report of **"***persistent oligoclonality***"** will be submitted **by the Sponsor** to the relevant Health Authorities within 30 days **of receipt of the ISA report confirming an IS meeting the criteria for persistent oligoclonality**. This repeated observation will also trigger enhanced monitoring for hematological abnormalities, increasing the frequency of CBC with differential to every 3 months (if not already occurring at the interval) ~~along with ISA and VCN every 6 months~~ until the frequency no longer meets **oligoclonality** criteria**. Persistent oligoclonality also triggers BM assessment (for the frequency of subsequent BM assessments see [Section** 6.5.11.1.2**]). If persistent oligoclonality is not observed after initial repeat testing, CBC and other monitoring reverts to routine assessments per the SOE**. Additionally, if an IS is detected at ≥ 5% RelFreq in a known oncogene (based upon Tier 1 oncogenes in the CGC in the COSMIC at the time of the ISA report review) ~~such an IS would be considered an IS of interest~~**, then ISA (**and ~~ISA~~ **VCN) will be repeated within 3 months of receipt of this result** ~~(and would be considered~~. **If an IS in** a ~~persistent IS of interest once~~ **known oncogene is** confirmed during re-evaluation~~). Should the relative frequency of a persistent IS of interest reach ≥10% at two consecutive timepoints~~ it would be considered persistent ~~oligoclonality and a report will be submitted to the relevant Health~~	

Initial wording	Amended or New Wording	Change Reason/Justification
Figure 1: Algorithm for Frequency of ISA and CBC Monitoring  Abbrev.: BM, bone marrow; CBC, complete blood count with differential; IS, insertion site(s); ISA, integration site analysis; mo, Month; q3mo, every 3 months; q4mo, every 4 months; q6mo, every 6 months; RelFreq, relative frequency; VCN, vector copy number; Y, Year. Note that this schematic includes the assessment schedule for ISA through the subsequent long-term follow-up study. a If an IS is found at a higher frequency in a bone marrow sample (than in peripheral blood) that bone marrow frequency would be used in the algorithm. b Tier 1 oncogenes in the Cancer Gene Census (CGC) of the Catalogue of Somatic Mutations in Cancer (COSMIC) at the time of the ISA report review). c Enhanced refers to Years 10 through 15 when more frequent CBC monitoring would be triggered relative to schedule of assessment.	~~Authorities within 30 days~~. **This repeated observation will trigger enhanced monitoring for hematological abnormalities, including a BM evaluation and increasing the frequency of CBC with differential to every 3 months (if not already occurring at the interval) until the frequency no longer meets criteria. ISA and VCN will continued per the SOE.** Based on clinical and ISA findings, additional monitoring for malignancy may be instituted by the treating physician/Principal Investigator or Sponsor (see Section ~~6.5.11.4~~ **6.5.11.1.2**~~.~~ for ~~bone marrow~~ **BM** evaluation recommendations and see Section 6.5.12.2 for recommended clinical work-up for ~~potential~~ malignancy**).** **See Section 7.4.5 for definitions of current oligoclonality and current persistent oligoclonality utilized during analyses and reporting.** Figure 1: Algorithm for Frequency of ISA and CBC Monitoring **ISA Triggered Enhanced Monitoring**  Abbrev.: BM, bone marrow; CBC, complete blood count with differential; IS, insertion site(s); ISA, integration site analysis; ~~mo, Month; q3mo, every 3 months; q4mo, every 4 months; q6mo, every 6 months;~~ RelFreq, relative frequency; VCN, vector copy number; ~~Y, Year~~. Note that this schematic includes the assessment schedule for ISA through the subsequent long-term follow-up study.	Updated Figure 1 and footnotes to better reflect steps in identification of subjects with oligoclonality and persistent oligoclonality and associated enhanced monitoring. Also shows rules for repeat testing and reversion to routine monitoring as applicable. Figure footnotes are also updated as shown.

Initial wording	Amended or New Wording	Change Reason/Justification
	a ISA, VCN, CBC schedule is specified in Section 6.1 (SOE Tables). **b IS can be measured from different types of samples (peripheral blood or BM).** If an IS is found at a higher frequency in a BM sample **(e.g., triggered by persistent ISA findings)** than in **a** peripheral blood~~)~~ **sample, then** that BM frequency would be used **to assess followup** ~~in the algorithm~~. ~~b~~**c**. Tier 1 oncogenes in the Cancer Gene Census (CGC) of the Catalogue of Somatic Mutations in Cancer (COSMIC) at the time of the ISA report review~~)~~. c. Enhanced refers to Years 10 through 15 when more frequent CBC monitoring would be triggered relative to schedule of assessment. **d. "Persistent oligoclonality" will be reported (once per pertinent IS/set of IS) to applicable Health Authorities within 30 days of receipt of applicable repeat ISA result.** **e. Observation of Persistent Oligoclonality triggers increasing the frequency of CBC with differential and BM assessment to every 3 months (if not already occurring at the interval) along with ISA and VCN every 6 months until the frequency no longer meets criteria. See also Section 6.5.11.1.1 (CBC) and Section 6.5.11.1.2 (BM).**	
Section 6.5.12.2 Clinical Work-up for Potential Malignancy In the event of any suspicion of hematologic malignancy (e.g., myelodysplasia, leukemia, or lymphoma), the Medical Monitor will be notified and a work-up will be performed by the Investigator per appropriate standard of care. A suspicion of hematologic malignancy could arise in the setting of otherwise unexplained cytopenia(s), and consideration of insertional oncogenesis could arise if cytopenia(s) occurs in conjunction with a rising RelFreq of an IS in a gene of known biological relevance to carcinogenesis (i.e., oncogene or tumor suppressor gene), accompanied by a rapid increase in PB VCN. The clinical work-up may include the following:	*Section 6.5.12.2 Clinical Work-up for Potential Malignancy* In the event of any suspicion of hematologic malignancy (e.g., myelodysplasia, leukemia, or lymphoma), the Medical Monitor will be notified and a work-up will be performed by the Investigator per appropriate standard of care. A suspicion of hematologic malignancy could arise in the setting of otherwise unexplained cytopenia(s), and consideration of insertional oncogenesis could arise if cytopenia(s) occurs in conjunction with a rising RelFreq of an IS**, especially if that IS is** in a gene of known biological relevance to carcinogenesis (i.e., oncogene or tumor suppressor gene), accompanied by a rapid increase in PB VCN **(a doubling of VCN between two timepoints)**. The clinical work-up may include the following:	Updated text to clarify what is meant by rapid VCN increase and to include platelet counts as part of CBC with differential assessment

Initial wording	Amended or New Wording	Change Reason/Justification
• Physical exam • CBC with differential - including repeat performance within one month for any Grade 2 or higher severity CTCAE CBC abnormality (these include abnormalities in hemoglobin levels, total white blood cell count, neutrophil count and morphology, lymphocyte count and morphology, or monocyte counts and morphology) • Lymphocyte subsets • Imaging studies • Local and Centralized bone marrow analysis. • Cytogenetic and molecular analyses which can include SNP microarray, karyotyping, or whole-genome sequencing and must include centralized FISH and NGS (see Section 6.5.11.4 for details). *[…]* For clinical work-up after identification of persistent oligoclonality and confirmed presence of abnormal CBC, bone marrow analysis should be performed as warranted if not previously part of the clinical work-up or as triggered based upon 6.5.11.1.2 or as discussed in Section 6.5.11.4.	Physical exam CBC with differential - including repeat performance within one month for any **CTCAE** Grade 2 or higher severity ~~CTCAE~~ CBC abnormality (these include abnormalities in hemoglobin levels, total ~~white blood cell~~ **WBC** count**s**, **platelet counts [of <100 x 10^9/L],** neutrophil count**s** and morphology, lymphocyte count**s** and morphology, or monocyte counts and morphology**) (see Section 6.5.11.1.1 for details)**. Lymphocyte subsets Imaging studies Local and Centralized ~~bone marrow~~ **BM** analysis Cytogenetic and molecular analyses which can include **single nucleotide polymorphism (**SNP**)** microarray, karyotyping, or whole-genome sequencing and must include centralized FISH and NGS (see Section ~~6.5.11.4~~ **6.5.11.1.2** for details). *[…]* For clinical work-up after identification of persistent oligoclonality and confirmed presence of abnormal CBC, ~~bone marrow~~ **BM** analysis should be performed as warranted if not previously part of the clinical work-up or as triggered based upon **Section** 6.5.11.1.2 or as discussed in Section 6.5.11.4.	
7.4.1. General Methods *[…]* Tabulations will be produced for appropriate demographic, baseline, efficacy, safety, and exploratory parameters. For categorical variables, summary tabulations of the number and percentage of subjects within each category of the parameter will be presented. For continuous variables, the number of observations, mean, median, standard deviation, minimum, and maximum values will be presented, along with 1 or 2 sided exact CIs of the mean as appropriate. Time-to-event data will be summarized	*7.4.1. General Methods* *[…]* Tabulations will be produced for appropriate demographic, baseline, efficacy, safety, and exploratory parameters. For categorical variables, summary tabulations of the number and percentage of subjects within each category of the parameter will be presented. For continuous variables, the number of observations, mean, median, standard deviation, minimum, and maximum values will be presented, along with the 2-sided 95% CI of the mean, as appropriate ~~, along with 1 or 2 sided exact CIs of the mean as appropriate~~. Time-to-event data will be summarized using Kaplan-Meier	Clarified general statistical methods relating to analyses pertaining to subjects who undergo allo-HSCT.

Initial wording	Amended or New Wording	Change Reason/Justification
using Kaplan-Meier methodology. The number and percent of censored observations and events will also be presented. *[…]* In addition to the analyses described below, supportive efficacy and safety analyses will be performed on the ITT population and the NEP for some of the study endpoints, as detailed in the SAP. Note, however, that in the likely event that there is no difference in the ITT and TP populations, reference to ITT analyses will not be necessary.	methodology. The number and percent of censored observations and events will also be presented. **For proportion endpoint variables, we presented the proportion along with 2-sided exact CIs of the proportion, as appropriate.** *[…]* **If a subject treated with eli-cel undergoes an allo-HSCT in Study ALD-104, the following additional analyses may be conducted for two periods (if sample size is sufficient):** **• For safety and PD endpoints, the first period begins from day of eli-cel infusion and will end on the day before initiation of conditioning for the subsequent allo-HSCT, and the second period will begin from the day of initiation of conditioning for allo-HSCT and will end at the date of last contact. For subjects who do not undergo conditioning prior to allo-HSCT, the day before allo-HSCT will be used.** **• All the efficacy, PD, and safety endpoints will be included in the listings.**	
7.4.2. Disposition of Subjects A tabulation of the disposition of subjects will be presented, including the number enrolled, the number with any post-drug product infusion data available for analysis, and the extent of data available. The number of subjects in each analysis population will be presented, with reasons for exclusion from any specific population.	*7.4.2. Disposition of Subjects* A tabulation of the disposition of subjects will be presented, including the number enrolled, the number with any post-drug product infusion data available for analysis, and the extent of data available. The number of subjects in each analysis population will be presented, with reasons for exclusion from any specific population. A tabulation of the disposition of subjects in the Study ALD-104 ITT population will be presented overall and by investigational site, for the following: • Number and percent of subjects who initiated mobilization • Number and percent of subjects who initiated conditioning • Number and percent of subjects who were infused with eli-cel	Additional details are provided for the tabulation of disposition data.

Initial wording	Amended or New Wording	Change Reason/Justification
	• Number and percent of subjects who discontinued study, and reason for discontinuation • Number and percent of subjects who completed study, and subjects who will participate in the long-term follow-up study LTF-304 • Number and percent of subjects who are in study at the time of data cut • Descriptive statistics for the duration of follow-up for subjects who were infused with eli-cel • Subject-years of follow-up, which is the sum over all subjects' duration of follow-up • The number of subjects receiving allo-HSCT, and the reason for receiving allo-HSCT during the study.	
7.4.3. Demographic and Baseline Characteristic The following demographic and Baseline characteristic factors will be summarized: age (age at enrollment, age at CALD diagnosis), country of origin, race and ethnicity, family history, time from informed consent to eli-cel infusion, method of diagnosis of CALD, number of prior gadolinium scans, NFS and Loes at Baseline, the presence of any significant co-morbid conditions, and time from diagnosis of CALD to treatment. Subject genotype will be presented in a listing.	*7.4.3. Disposition of Subjects* The following demographic and Baseline characteristic factors will be summarized: age (age at enrollment, age at CALD diagnosis), **sex, age at eli-cel infusion, height at screening, weight at screening, body mass index (BMI) at screening**, country of origin, race and ethnicity, family history, time from informed consent to eli-cel infusion, signs and symptoms of CALD, method of diagnosis of CALD, number of prior gadolinium scans, NFS and Loes at Baseline, the presence of any significant co-morbid conditions, **availability of matched sibling donor** and time from diagnosis of CALD to treatment. Subject genotype will be presented in a listing.	Additional demographic characteristics that will be summarized are noted.

Initial wording	Amended or New Wording	Change Reason/Justification
7.4.5. Analysis of Safety Endpoints *[…]* *New subsection added*	*7.4.5. Analysis of Safety Endpoints* *[…]* ***Oligoclonality*** **Persistent oligoclonality at any time is defined as an IS ≥10% RelFreq in an initial ISA, and the IS ≥10% RelFreq results are confirmed in the subsequent ISA; or at least 2 IS ≥5% RelFreq in an initial ISA, and the same IS ≥5% RelFreq results are confirmed in the subsequent ISA.** **Current persistent oligoclonality is defined as an IS ≥10% relative frequency in an initial ISA, that is confirmed in the subsequent ISA as still being an IS ≥10% relative frequency and the ≥10% relative frequency is maintained through the last two ISA (as of a specific data cut); or at least 2 IS ≥5% relative frequency in an initial ISA and the same IS ≥5% relative frequency results are confirmed in the subsequent ISA, and the ≥5% relative frequency is maintained through the last two ISA (as of a specific data cut). Current oligoclonality is defined as an IS ≥10% relative frequency that is observed at the first time at the most recent ISA as of a specific data cut or at least 2 IS ≥5% relative frequency observed at the first time at the most recent ISA as of a specific data cut (i.e., not yet confirmed at a repeat assessment).**	A new safety analysis is added to provide the statistical definition of current oligoclonality and current persistent oligoclonality.
Section 8.6 Direct Access to Source Data *[…]* The study will be monitored by bluebird bio or its designee. Monitoring will be done by personal visits from a representative of the Sponsor (site monitor) and will include on-site review of the CRFs for completeness and clarity, cross-checking with source documents, and clarification of administrative matters. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.	*Section 8.6 Direct Access to Source Data* *[…]* The study will be monitored by bluebird bio or its designee. Monitoring will be done by ~~personal visits~~ from a representative of the Sponsor (site monitor) and will include on-site **and/or remote** review of the CRFs for completeness and clarity, cross-checking with source documents, and clarification of administrative matters. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.	Note added that on site and/or remote review of CRFs may be utilized for review.

DESCRIPTION OF EACH NON-SUBSTANTIAL AMENDMENT

- Updated text to note status of eli-cel clinical studies (including completion of Study ALD-102) and to note approval of Skysona by the U.S. FDA.
- Updated the date on the Investigator Statement page.
- Minor text edits were made to remove redundancy, improve clarity, and correct typographical errors.

CLINICAL STUDY SYNOPSIS

Protocol Title:	A Phase 3 Study of Lenti-D Drug Product After Myeloablative Conditioning Using Busulfan and Fludarabine in Subjects ≤ 17 Years of Age With Cerebral Adrenoleukodystrophy (CALD)
Protocol Number:	ALD-104
EudraCT Number:	2018-001145-14
Objective:	To evaluate the efficacy and safety of Lenti-D Drug Product (also known as elivaldogene autotemcel or Skysona, hereafter referred to as eli-cel) after myeloablative conditioning with busulfan and fludarabine in subjects with CALD
Study Design:	This will be an international, non-randomized, open-label, multi-site study in male subjects with CALD (≤ 17 years of age at enrollment). Approximately 35 subjects will be infused with eli-cel after myeloablative conditioning with busulfan and fludarabine. The study has 4 distinct phases after informed consent/assent: • Screening and Enrollment. Subjects who meet eligibility criteria based on screening assessments are considered enrolled. Patients who do not meet eligibility criteria are considered screen failures. • CD34+ Cell Collection, Transduction, Disposition of eli-cel, and Reconfirmation of Eligibility • Conditioning and Washout, followed by eli-cel Infusion on Day 1 • Maintenance (Follow-up) (Day 2 through Month 24) From Screening through when it is assessed that the subject is stably transplanted (by approximately the Month 3 Visit), visits will occur at one of a small number of sites (referred to as primary study sites). However, due to the rarity of CALD, it is likely that some subjects may have to travel far for participation at the primary study sites. Therefore, after the subject is stably transplanted, arrangements will be made wherever possible to open up a suitable site closer to the subject's home (referred to as secondary study sites) where they should attend subsequent visits. In all cases, subjects will be asked to return to their primary study site for their assessments for Month 12 and Month 24 Visits to ensure consistency in key efficacy assessments. Screening Phase tests and procedures will determine study eligibility. Subjects who are confirmed to be eligible and are enrolled in the study will undergo hematopoietic stem cell (HSC) mobilization mediated by granulocyte colony stimulating factor (G-CSF, either filgrastim or lenograstim) and plerixafor, and cells will be harvested by apheresis using institutional practice treatment guidelines. The harvested cells will be selected for the CD34+ marker to enrich for HSCs, transduced with Lenti-D lentiviral vector (LVV), stored frozen in cryopreservation solution while aliquots are being tested to ensure they meet product quality specifications. Only after the transduced cells are dispositioned for clinical use and the drug product is at the clinical site will the subject undergo myeloablation with busulfan intravenous (IV) and fludarabine IV. There should be a minimum of 48 hours of washout after conditioning before drug product infusion. Eli-cel will be administered by IV infusion through a central venous catheter.

	Back-up cells (mobilized peripheral blood mononuclear cells [PBMCs]) will also be harvested during apheresis and stored frozen in accordance with institutional guidelines. If back up cells cannot be procured from apheresis, a bone marrow (BM) harvest may be performed. All subjects will be followed for approximately 24 months post-drug product infusion under this protocol. Then, subjects are expected to be followed for an additional 13 years under a separate follow-up protocol (LTF-304).
Number of Subjects Planned:	Approximately 35 subjects will be infused with eli-cel after myeloablative conditioning with busulfan and fludarabine. As of this Protocol Amendment (Version 8.0) enrollment and treatment (N=35) in Study ALD-104 have been completed; subject follow-up is ongoing. Further enrollment in this study is not expected.
Inclusion Criteria:	1. Informed consent is obtained from a competent custodial parent or guardian with legal capacity to execute a local Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) approved consent. Informed assent will be sought from capable subjects, in accordance with the directive of the IRB/IEC and with local requirements. 2. Males aged 17 years and younger, at the time of parental/guardian consent and, where appropriate, subject assent. 3. Active CALD as defined by: a. Elevated very long chain fatty acids (VLCFA) values, and b. Active central nervous system (CNS) disease established by central radiographic review of brain magnetic resonance imaging (MRI) demonstrating i. Loes score between 0.5 and 9 (inclusive) on the 34-point scale, and ii. Gadolinium enhancement (GdE) on MRI of demyelinating lesions. 4. Neurologic Function Score (NFS) ≤ 1.
Exclusion Criteria:	1. Prior receipt of an allogeneic transplant or gene therapy. 2. Use of statins, Lorenzo's Oil, or dietary regimens used to lower VLCFA levels. Note: subjects must discontinue use of these medications at time of consent. 3. Receipt of an investigational study drug or procedure within 3 months before Screening that might confound study outcomes. Use of investigational study drugs is prohibited throughout the course of the study. 4. Any conditions that make it impossible to perform MRI studies (including allergies to anesthetics or contrast agents). 5. Hematological compromise as evidenced by: a. Peripheral blood absolute neutrophil count (ANC) < 1500 cells/mm^3, and either b. Platelet count < 100,000 cells/mm^3 or c. Hemoglobin < 10 g/dL. 6. Hepatic compromise as evidenced by: a. Aspartate transaminase (AST) value > 2.5 × upper limit of normal (ULN) b. Alanine transaminase (ALT) value > 2.5 × ULN c. Total bilirubin value > 3.0 mg/dL, except if there is a diagnosis of Gilbert's Syndrome and the subject is otherwise stable

	7. Baseline estimated glomerular filtration rate < 70 mL/min/1.73 m^2, as determined using the Chronic Kidney Disease Epidemiology Collaboration creatinine equation (see https://www.kidney.org/professionals/KDOQI/gfr_calculatorPed) 8. Cardiac compromise as evidenced by left ventricular ejection fraction < 40% 9. Immediate family member with a known or suspected Familial Cancer Syndrome (including but not limited to hereditary breast and ovarian cancer syndrome, hereditary non-polyposis colorectal cancer syndrome, and familial adenomatous polyposis). 10. Clinically significant uncontrolled, active bacterial, viral, fungal, parasitic, or prion-associated infection. 11. Positive for human immunodeficiency virus type 1 or 2 (HIV-1, HIV-2); hepatitis B virus (HBV); hepatitis C virus (HCV); human T lymphotrophic virus 1 (HTLV-1). (Note that subjects who have been vaccinated against HBV [positive for HBV surface antibodies] who are negative for other markers of prior HBV infection [e.g., negative for hepatitis B core antibody {HBVc Ab}] are eligible. Subjects with past exposure to HBV [HBc antibodies {Ab} positive and/or hepatitis B e-antigen antibody {HBeAb}-positive] are also eligible for the study provided they have a negative test for HBV deoxyribonucleic acid [DNA]. Also note that subjects who are positive for anti-hepatitis C Ab are eligible as long as they have a negative hepatitis C viral load). 12. Any clinically significant cardiovascular, hematological, or pulmonary disease, or other disease or condition that would be contraindicated for any of the other study procedures. 13. Absence of adequate contraception for fertile subjects. Male subjects and their female partners are required to use two different effective methods of contraception from Screening through at least 6 months after eli-cel infusion. If subjects are truly sexually abstinent (where true sexual abstinence is defined as being in line with the preferred and usual lifestyle of the subject), no second method is required. 14. Any contraindications to the use of G-CSF or plerixafor during the mobilization of HSCs, and any contraindications to the use of busulfan or fludarabine, including known hypersensitivity to the active substances or to any of the excipients in their formulations. 15. Known hypersensitivity to protamine sulfate.
Duration of Subject Participation:	Each subject will remain on this study for approximately 26 months from time of consent, inclusive of an approximately 24-months post-drug product infusion follow-up; subjects are expected to consent for follow-up in Study LTF-304 for another 13 years post-drug product infusion.
Test Product, Dose and Mode of Administration:	Eli-cel (autologous CD34+ cell-enriched population that contains cells transduced with LVV that encodes an ATP-binding cassette, sub-family D, member 1 (ABCD1) complementary DNA (cDNA) for human adrenoleukodystrophy protein (ALDP), suspended in a cryopreservation solution) is administered IV; dose ≥ 5.0×10^6 CD34+ cells/kg.
Reference Therapy, Dose and Mode of Administration:	Not applicable.

Data Monitoring Committee:	An independent data monitoring committee (DMC) composed of members with appropriate scientific and medical expertise to monitor the study will be convened before the study is opened. A charter describing the composition and conduct of the DMC will be drafted by the Sponsor and agreed to by all DMC members prior to the DMC's initial meeting. The DMC will meet by teleconference for scheduled meetings biannually and ad hoc as circumstances require. In addition, the DMC will review hematologic and integration site analysis (ISA) data on a quarterly basis. The DMC will have the right to recommend halting the study at any time due to concerns for the safety of the subjects.
Criteria for Evaluation – Efficacy:	The primary efficacy endpoint is: • Proportion of subjects who are alive and have none of the 6 major functional disabilities (MFDs) at Month 24 (i.e., Month 24 MFD-free survival). MFDs are: loss of communication, cortical blindness, tube feeding, total incontinence, wheelchair dependence, complete loss of voluntary movement Secondary efficacy endpoints are the following: • Proportion of subjects without GdE on MRI (i.e., GdE-) at Month 24 • Value and change in total NFS from Baseline to protocol scheduled visits • MFD-free survival over time • Overall survival • Detectable vector copy number (VCN) in peripheral blood cells by Month 6 CCI
Criteria for Evaluation – Safety:	The primary safety endpoint is: • The proportion of subjects with neutrophil engraftment after drug product infusion The secondary safety endpoints are the following: • The proportion of subjects who experience either acute (≥Grade II) or chronic graft versus host disease (GVHD) by Month 24 • Time to neutrophil engraftment post-drug product infusion • The proportion of subjects with platelet engraftment by Month 24 • Time to platelet engraftment post-drug product infusion

	• The proportion of subjects with loss of neutrophil engraftment post-drug product infusion by Month 24 • The proportion of subjects who undergo a subsequent HSC infusion by Month 24 • The proportion of subjects who experience transplant-related mortality through 100 and 365 days post-drug product infusion • Proportion of subjects with clinical ≥ Grade 3 adverse events (AEs), all drug product-related AEs, all serious adverse events (SAEs), ≥ Grade 3 infections, and clinically significant changes in laboratory parameters by Month 24 • The proportion of subjects who experience ≥ Grade II acute GVHD by Month 24 • The proportion of subjects who experience chronic GVHD by Month 24 • Number of emergency room visits (post-neutrophil engraftment) by Month 24 • Number and duration of in-patient hospitalizations (post-neutrophil engraftment) by Month 24 • Number and duration of intensive care unit (ICU) stays (post-neutrophil engraftment) by Month 24 • The number of subjects in which vector-derived replication competent lentivirus (RCL) is detected by Month 24 • The number of subjects with insertional oncogenesis (myelodysplasia, leukemia, lymphoma, etc.) by Month 24 CCI
Statistical Methods:	Three populations will be evaluated for efficacy and safety. • The **Intent-to-treat (ITT)** population will consist of subjects who initiate any study procedures, beginning with mobilization by G-CSF. • The **Transplant Population (TP)** will consist of subjects who receive eli-cel. • The **Successful Neutrophil Engraftment Population (NEP)** will be defined as subjects who 1) receive eli-cel; 2) achieve neutrophil engraftment, defined as 3 consecutive ANC laboratory values of ≥ 0.5×10^9 cells/L (after initial post-infusion nadir) obtained on different days by 42 days post-infusion of eli-cel. Statistical methods will be primarily descriptive in nature and will include point estimates and confidence limits as appropriate. The analysis of the primary efficacy endpoint, Month 24 MFD-free survival, will be based on the TP. The sample size of 35 subjects will provide a 95% two-sided exact confidence interval (CI) for the estimated MFD-free survival rate that is at most 34.6% wide (dependent on the observed MFD-free survival rate). The analyses of the secondary and CCI efficacy endpoints will be performed on the TP. The analysis of the primary safety endpoint will be the proportion of subjects with neutrophil engraftment after drug product infusion in the TP. The analyses of the secondary safety endpoints will also be performed on the TP.

	AEs, laboratory assessments, vital signs, electrocardiogram (ECG) and physical examination findings will be summarized, as appropriate, for the ITT population. The planned statistical methodology will be presented in detail in the statistical analysis plan (SAP).

LIST OF ABBREVIATIONS

Abbreviation	Definition
ABCD1	ATP-binding cassette, sub-family D, member 1
ABW	actual body weight
ADL	activities of daily living
AE	adverse event
ALD	adrenoleukodystrophy
ALDP	adrenoleukodystrophy protein
allo-HSCT	allogeneic hematopoietic stem cell transplantation
Ab	Antibodies
ALT	alanine transaminase
ANC	absolute neutrophil count
AST	aspartate transaminase
AUC	area under the curve
BAER	brain stem auditory evoked response
Bayley-III	Bayley Scales of Infant and Toddler Development, 3rd Edition
BM	bone marrow
BUN	blood urea nitrogen
C	conditioning
CALD	cerebral adrenoleukodystrophy
CBC	complete blood count
CGC	Cancer Gene Census
cDNA	complementary deoxyribonucleic acid
CI	confidence interval
CMV	cytomegalovirus
CNS	central nervous system
CRF	case report form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DMC	data monitoring committee
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid

Abbreviation	Definition
EBV	Epstein-Barr virus
ECG	electrocardiogram
eCRF	electronic case report form
eli-cel	elivaldogene autotemcel
FDA	Food and Drug Administration
FISH	Fluorescence in situ hybridization
FSIQ	full scale intelligence quotient
GCP	good clinical practice
G-CSF	granulocyte colony stimulating factor
GdE	gadolinium enhancement
GMP	good manufacturing practice
GVHD	graft versus host disease
Hb	hemoglobin
HBcAb	hepatitis B core antibody
HBeAb	hepatitis B e-antigen antibody
HBsAg	hepatitis B virus surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HEENT	head, eyes, ears, nose, and throat
HIV-1	human immunodeficiency virus type 1
HIV-2	human immunodeficiency virus type 2
HLA	human leukocyte antigen
HPC, A	hematopoietic progenitor cell, apheresis
HRQoL	health related quality of life
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplantation
HSV	herpes simplex virus
HTLV-1	human T lymphotrophic virus 1
HTLV-2	human T lymphotrophic virus 2
IB	Investigator's Brochure
IBW	ideal body weight

Abbreviation	Definition
ICF	informed consent form
ICH	International Council on Harmonisation
ICU	intensive care unit
IEC	Independent Ethics Committee
Ig	immunoglobulins (such as IgG, IgM, and IgA)
IQ	intelligence quotient
IRB	Institutional Review Board
IS	insertion site(s)
ISA	integration site analysis
ITT	Intent-to-treat
IUD	intrauterine device
IUS	intrauterine hormone-releasing system
IV	intravenous
LTR	long terminal repeat
LVV	lentiviral vector
M	mobilization
MedDRA	Medical Dictionary for Regulatory Activities
MFD	major functional disability
mL	milliliter
MRI	magnetic resonance imaging
MUD	matched unrelated donor
NCI	National Cancer Institute
NE	neutrophil engraftment
NEP	neutrophil engraftment population
NFS	neurologic function score
NGS	next-generation sequencing
PBL	peripheral blood leukocyte
PBMC	peripheral blood mononuclear cell
PB VCN	peripheral blood vector copy number
PCR	polymerase chain reaction
PCS	potentially clinically significant

Abbreviation	Definition
PD	pharmacodynamic
PedsQL	pediatric quality of life inventory
PIQ	performance intelligence quotient
PT	preferred term
qPCR	quantitative polymerase chain reaction
RBC	red blood cell
RCL	replication competent lentivirus
RelFreq	relative frequency
RNA	ribonucleic acid
SAE	serious adverse event
SAP	statistical analysis plan
SCID-X1	X-linked severe combined immunodeficiency
SES	socioeconomic status
SIN	self-inactivating
SNP	single nucleotide polymorphism
SOC	System Organ Class
SOE	Schedule of Events
SOM	Study Operations Manual
SUSAR	suspected unexpected serious adverse drug reaction
TNC	total nucleated cells
TP	Transplant Population
ULN	upper limit of normal
US	United States
VCN	vector copy number
VEP	visual evoked potential
VIQ	verbal intelligence quotient
VLCFA	very long chain fatty acids
VOD	veno-occlusive disease
VSV-G	vesicular stomatitis virus glycoprotein G
WAIS-IV	Wechsler Adult Intelligence Scale, 4th Edition
WBC	white blood cell

Abbreviation	Definition
WHO	World Health Organization
WISC-V	Wechsler Intelligence Scale for Children, 5th Edition
WPPSI-IV	Wechsler Preschool & Primary Scale of Intelligence, 4th Edition

TABLE OF CONTENTS

LIST OF TABLES IN THE TEXT

LIST OF FIGURES IN THE TEXT

1. INTRODUCTION

1.1. Cerebral Adrenoleukodystrophy

Cerebral adrenoleukodystrophy (CALD) is a rare X-linked genetic disease caused by a defect in the ATP-binding cassette, sub-family D, member 1 (*ABCD1*) gene, which encodes for adrenoleukodystrophy protein (ALDP), a peroxisomal transporter involved in the breakdown of very long chain fatty acids (VLCFA) (Moser et al. 2007). The resulting accumulation of VLCFA leads to progressive demyelination and cerebral inflammation within the brain, which (if untreated) leads to severe loss of neurological function over time, and death. In boys diagnosed with CALD, learning and behavioral problems are often observed in mid-childhood after the age of 3 years (median age 7). Most patients will die within a decade of diagnosis if they are not treated by stem cell transplantation (Moser et al. 2007).

One of the hallmarks of inflammatory disease in CALD is the presence of a compromised blood-brain-barrier behind the leading edge of demyelinating lesions, as evidenced by positive gadolinium enhancement (GdE) on brain magnetic resonance imaging (MRI), prior to treatment. Studies have shown that GdE is associated with disease progression in untreated patients as the severity of the inflammatory process appears to be correlated with the rapidity of progression.

The goal of treatment for CALD is to delay or prevent progression of the disease, thereby preventing the development of impairments which compromise the ability to function independently. There is currently no treatment approved for CALD. A beneficial effect on clinical indices of disease and long-term survival has been observed with allogeneic hematopoietic stem cell transplantation (allo-HSCT), in particular if performed early in the course of cerebral disease progression (Mahmood et al. 2007; Miller et al. 2011; Kühl et al. 2017).

Allo-HSCT is optimally performed early in the course of the disease using a human leukocyte antigen (HLA)-matched sibling hematopoietic stem cell (HSC) donor. However, a matched sibling donor is available for only ≤ 30% of patients (Miller et al. 2011), so alternative options include transplantation with cells derived from an HLA-mismatched related donor, a matched unrelated donor hematopoietic stem cell transplantation (MUD-HSCT), or transplant with cells derived from banked cord blood (umbilical cord blood transplantation).

1.2. Eli-cel

With an objective to provide an effective and safer alternative to allo-HSCT, bluebird bio is investigating the use of Lenti-D Drug Product (also known as elivaldogene autotemcel, hereafter referred to as eli-cel) in the treatment of subjects with CALD. The mode of action of eli-cel is based on treating patients with their own HSCs that have been transduced ex vivo with the Lenti-D lentiviral vector (LVV) that encodes a normal ALDP. After transplantation, these HSCs then differentiate into different cell types, including cerebral microglia, which may produce functional ALDP. The functional ALDP can then enable the peroxisomal beta-oxidation of VLCFAs, which in turn can stabilize the disease by preventing further inflammation and demyelination.

It is anticipated that transplantation of autologous CD34+ HSCs genetically modified ex vivo by the Lenti-D LVV should circumvent many of the limitations and risks associated with allo-HSCT, while providing efficacy similar to allo-HSCT. The use of a patient's own stem cells would eliminate the risks of graft rejection and acute and chronic graft versus host disease (GVHD) due to immune-incompatibility, as well as reduce complications due to long-term immunosuppression.

CCI

1.2.1. Studies ALD-102 and LTF-304

Study ALD-102 was an international, Phase 2/3, multicenter, open-label, single-arm, single-dose study designed to evaluate the efficacy and safety of eli-cel in male subjects with CALD aged ≤ 17 years with active cerebral disease. In Study ALD-102, a myeloablative conditioning regimen of busulfan and cyclophosphamide was utilized before infusion of eli-cel. Each subject in Study ALD-102 was evaluated for 2 years after eli-cel infusion of ≥ 5.0×10^6 CD34+ cells/kg. Subjects are then followed for an additional 13 years in a long-term followup (LTF) study (Study LTF-304). Study ALD-102 is completed, while Study LTF-304 remains ongoing as of this protocol amendment. Safety data from ALD-102 and LTF-304 can be found in the IB.

1.3. Rationale for Design of Study ALD-104

The objective of the current study is to evaluate the efficacy and safety of eli-cel after myeloablative conditioning with busulfan and fludarabine in subjects with CALD.

The study will enroll participants who have GdE on MRI, have a neurologic function score (NFS) ≤ 1, and a Loes score between 0.5 and 9 (inclusive) at Baseline because those with more advanced disease are unlikely to experience optimal outcomes from allo-HSCT (Miller et al. 2011) and are therefore thought to be unlikely to significantly benefit from treatment with the eli-cel.

The primary efficacy endpoint, as described in Section 2.2.1 is Month 24 major functional disability (MFD)-free survival, and the duration of 24 months follow-up was selected to allow sufficient time for potential MFDs to develop. MFDs were chosen based on their clinical significance and their impact on independent functioning, and are the following:

- loss of communication,
- cortical blindness,
- tube feeding,
- total incontinence,
- wheelchair dependence, and
- complete loss of voluntary movement.

Recent published data indicate that the use of plerixafor in pediatric patients is safe and effective for the mobilization of peripheral blood stem cells for autologous transplantation

(Teusink et al. 2016). The addition of plerixafor to granulocyte colony stimulating factor (G-CSF) in pediatric patients undergoing stem cell mobilization prior to transplantation as part of treatment of malignant tumors has also been shown to result in successful mobilization of the vast majority of patients who fail to mobilize with single agent G-CSF, as well as to produce larger yields of CD34+ cells (Maschan et al. 2015). Plerixafor is thus expected to incur minimal additional safety risks beyond the use of single agent G-CSF, to increase cell yield with reduced G-CSF exposure, and to reduce requirements for repeat apheresis attempts.

In Study ALD-102, a myeloablative conditioning regimen of busulfan intravenous (IV) and cyclophosphamide IV was utilized before infusion of eli-cel (Eichler et al. 2017). However, a conditioning regimen of busulfan and fludarabine was demonstrated to be less toxic and equally effective as a regimen of busulfan and cyclophosphamide during allo-HSCT (Bartelink et al. 2014). Specifically, literature data show that the cumulative busulfan dose determines both its efficacy and toxicity (Bartelink et al. 2012, 2014, 2016); and a cumulative busulfan exposure of between 78 mg × h/L and 101 mg × h/L, combined with the non-alkylating drug fludarabine, predicted the highest event-free survival in pediatric and young adult patients independent of indication and cell source. An increased risk of acute and chronic toxicity was noted at higher exposures, whereas an increased risk of graft rejection or disease relapse occurred at lower exposures (Bartelink et al. 2014). Pediatric patients who received busulfan in conjunction with fludarabine had lower rates of veno-occlusive disease (VOD) and chronic GVHD, as compared with pediatric patients who received busulfan in conjunction with cyclophosphamide. Additionally, the duration of neutropenia, with its associated infection risk, was shorter.

Therefore, in Study ALD-104, a myeloablative conditioning regimen of busulfan (IV) and fludarabine (IV) is recommended before infusion of eli-cel. The selected dosing regimen for busulfan and fludarabine in Study ALD-104 is consistent with the recommendations from the European Society for Immunodeficiencies in pediatric patients (Lankester 2017).

1.4. Potential Risks

Potential risks, warnings and precautions are described in the Skysona U.S. product package insert (SKYSONA 2022); in addition, updated safety results for bluebird bio's CALD studies can be found in the IB.

1.4.1. Abnormal Proliferation of Hematopoietic Cells

LVVs are retroviruses, which integrate into the chromosome of target cells upon transduction. A potential risk of this type of vector is insertional mutagenesis leading to oncogenesis.

The risk of mutagenesis for this study is limited to the hematopoietic cell compartment, since the LVV is designed not to mobilize after integration into the chromosomal deoxyribonucleic acid (cDNA) of HSCs. Gene transfer with γ-retroviral vectors has resulted in oncogenesis in the clinical studies for X-linked severe combined immunodeficiency (SCID-X1) (Hacein-Bey-Abina et al. 2008; Howe et al. 2008), chronic granulomatous disease (Stein et al. 2010), and Wiskott-Aldrich Syndrome (Boztug et al. 2010).

Due to insertional mutagenesis, 5 of 20 participants in the SCID-X1 study developed acute lymphocytic leukemia. One of the 5 participants succumbed to leukemia, while the remaining 4 were successfully treated. In the Wiskott-Aldrich study, 4 participants developed leukemia (Paruzynski et al. 2012).

The different nature of the Lenti-D LVV should minimize, but not completely eliminate, the risk of oncogenesis by insertional mutagenesis. Unlike the γ-retroviral vectors that led to leukemia, self-inactivating (SIN) LVVs, such as Lenti-D LVV, may represent a substantial improvement in terms of safety (Montini et al. 2006). SIN LVVs, in general, provide significant safety improvements over γ-retroviral vectors used in earlier studies (Rivière et al. 2012). SIN LVVs lack the strong enhancer/promoter long terminal repeat (LTR) sequences of γ-retroviral vectors, and, unlike γ -retroviral vectors, do not preferentially integrate near gene promoter regions. Therefore, LVVs are less likely to transactivate oncogenes, and have demonstrated a significantly curtailed probability of oncogenic transformation in vitro and in vivo (Biffi et al. 2011). However, as described in the Skysona U.S. product package insert (SKYSONA 2022) and in the IB, insertional oncogenesis has been observed in the eli-cel clinical development program.

Safety results for bluebird bio's CALD studies can be found in the IB.

1.4.2. Use of a Lentiviral Vector Derived from Human Immunodeficiency Virus Type 1 (HIV-1)

Because the Lenti-D LVV was derived from human immunodeficiency virus type 1 (HIV-1), a potential risk is mobilization or recombination with wild-type HIV-1. This risk is estimated to be very low for several reasons - the LVV is replication incompetent, the probability of producing a recombinant virus from a multi-plasmid transfection is very low, the transduction is ex vivo so there is no opportunity for the vector to be exposed to HIV, and a highly sensitive, validated assay is used prior to transduction to detect replication competent lentivirus (RCL).

Participants treated with eli-cel will be monitored from Baseline for 24 months under the current protocol and then for an additional 13 years under the separate, long-term follow-up protocol that will focus on long-term safety with an emphasis on detection of insertional oncogenesis and RCL. If a vector-derived RCL is detected in any participant, the Sponsor will conduct individual benefit/risk assessments, and consult with the data monitoring committee (DMC) and clinical sites, as appropriate, to determine whether enrollment should be suspended and/or any study procedures should be performed.

1.4.3. Risks of Mobilization and Transplantation

Reported warnings for G-CSF (where G-CSF is defined for this protocol to mean either filgrastim or lenograstim) can be found in the prescribing information for each product. They may include the potential for allergic reactions, splenic rupture, acute respiratory distress syndrome, and alveolar hemorrhage and hemoptysis (e.g., Neupogen [filgrastim] prescribing information; note that some additional warnings reported apply only to clinical populations with sickle cell disorders or severe chronic neutropenia and thus would not be relevant for the study population to be enrolled in Study ALD-104). Additionally, reported precautions include the potential for immunogenicity or cutaneous vasculitis (Thornley et al. 2004). Refer to the prescribing information (Neupogen PI 2016, SmPC 2015) for additional product details regarding G-CSF, including all reported adverse events (AEs) from clinical trials.

Reported warnings for plerixafor include increased circulation of leukocytes, decreased platelet counts, and potential for splenic rupture (e.g., Mozobil prescribing information; note that some additional warnings reported apply only to patients with leukemia, female patients who may become pregnant, or patients with the potential for tumor cell mobilization). The most

common AEs, reported in ≥ 10% of patients, include diarrhea, nausea, fatigue, injection site reactions, headache, arthralgia, dizziness, and vomiting. Refer to the prescribing information (Mozobil PI 2017, SmPC 2017) for additional product details regarding plerixafor, including all reported AEs from clinical trials.

Conditioning regimen-related toxicity is a frequent cause of morbidity and mortality in allo-HSCT (Clift et al. 1993; Thornley et al. 2004). Most notably, given that a goal of conditioning is immunosuppression, participants receiving such treatment are at significant risk for development of potentially fatal, opportunistic infections (e.g., cytomegalovirus [CMV], Epstein Barr Virus [EBV]). However, this risk is lower with autologous HSCT because the hematologic reconstitution is very rapid and long-term immunosuppression is not required. VOD, marked by weight gain due to ascites, hepatomegaly, and hyperbilirubinemia, may also occur (Nevill et al. 1991; Clift et al. 1993; Andersson et al. 2002). VOD can be severe, leading to multi-organ failure and ultimately death in up to 30% of cases (Richardson et al. 1998). Central nervous system (CNS) toxicities, primarily seizure, also can occur with the conditioning regimen. Although generally less significant, mucositis, skin complications, and gastrointestinal disturbances, primarily nausea, vomiting and diarrhea, commonly occur with busulfan IV and fludarabine IV conditioning (Busilvex SmPC 2017; Busulfex PI 2018; Fludarabine PI 2010). Potential effects on male infertility are noted in package inserts for both busulfan IV and fludarabine IV conditioning agents (Busilvex SmPC 2017; Busulfex PI 2018; Fludarabine PI 2010). Refer to the prescribing information regarding additional toxicities associated with busulfan IV and fludarabine IV (Busilvex SmPC 2017; Busulfex PI 2018; Fludarabine PI 2010).

Management of neutrophil engraftment failure (as defined in Section 6.5.13) is at the discretion of the Investigator. Participant will be followed as per protocol scheduled- visits (Table 3 and Table 4).

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Study Objective

- To evaluate the efficacy and safety of eli-cel after myeloablative conditioning with busulfan and fludarabine in subjects with CALD

2.2. Study Endpoints

2.2.1. Efficacy Endpoints

The primary efficacy endpoint is:

- Proportion of subjects who are alive and have none of the 6 MFDs at Month 24 (i.e. Month 24 MFD-free survival). MFDs are:
  - loss of communication
  - cortical blindness
  - tube feeding
  - total incontinence
  - wheelchair dependence
  - complete loss of voluntary movement

Secondary efficacy endpoints are the following:

- Proportion of subjects without GdE on MRI (i.e., GdE-) at Month 24
- Value and change in total NFS from Baseline to protocol scheduled visits
- MFD-free survival over time
- Overall survival
- Detectable vector copy number (VCN) in peripheral blood cells by Month 6

2.2.2. Safety Endpoints

The primary safety endpoint is:

- The proportion of subjects with neutrophil engraftment after drug product infusion

The secondary safety endpoints are the following:

- The proportion of subjects who experience either acute (≥ Grade II) or chronic GVHD by Month 24
- Time to neutrophil engraftment after drug product infusion
- The proportion of subjects with platelet engraftment by Month 24
- Time to platelet engraftment post-drug product infusion
- The proportion of subjects with loss of neutrophil engraftment post-drug product infusion by Month 24
- The proportion of subjects who undergo a subsequent HSC infusion by Month 24
- The proportion of subjects who experience transplant-related mortality through 100 and 365 days post-drug product infusion
- Proportion of subjects with clinical ≥ Grade 3 AEs, all drug product-related AEs, all serious adverse events (SAEs), ≥ Grade 3 infections, and clinically significant changes in laboratory parameters by Month 24
- The proportion of subjects who experience ≥ Grade II acute GVHD by Month 24
- The proportion of subjects who experience chronic GVHD by Month 24
- Number of emergency room visits (post-neutrophil engraftment) by Month 24
- Number and duration of in-patient hospitalizations (post-neutrophil engraftment) by Month 24
- Number and duration of intensive care unit (ICU) stays (post-neutrophil engraftment) by Month 24
- The number of subjects in which vector-derived RCL is detected by Month 24
- The number of subjects with insertional oncogenesis (myelodysplasia, leukemia, lymphoma, etc.) by Month 24

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Note: transplant-related mortality as determined by the Investigator; severity of AEs as assessed by the Investigator as described in Section 6.6.2 ; acute GVHD graded on the Acute GVHD Grading Scale (I-IV); chronic GVHD as determined by the Investigator.

3. INVESTIGATIONAL PLAN

3.1. Overall Design and Plan of the Study

This will be an international, non-randomized, open-label, multi-site study in male subjects with CALD (≤ 17 years of age at enrollment). Approximately 35 subjects will be infused with eli-cel after myeloablative conditioning with busulfan and fludarabine.

Selected subjects must meet the inclusion criteria, have none of the exclusion criteria, and have provided informed consent and assent, when applicable. The study has 4 distinct phases after informed consent:

- Screening and Enrollment. Subjects who meet eligibility criteria, based on screening assessments are considered enrolled. Patients who do not meet eligibility criteria are considered screen failures.
- CD34+ Cell Collection, Transduction, Disposition of eli-cel, and Reconfirmation of Eligibility
- Conditioning and Washout, followed by eli-cel Infusion on Day 1
- Maintenance (Follow-up) (Day 2 through Month 24)

From Screening through when it is assessed that the subject is stably transplanted (by approximately the Month 3 Visit), visits will occur at one of a small number of sites (referred to as primary study sites). However, due to the rarity of CALD, it is likely that some subjects may have to travel far for participation at the primary study sites. Therefore, after the subject is stably transplanted, arrangements will be made wherever possible to open up a suitable site closer to the subject's home (referred to as secondary study sites) where they should attend subsequent visits. In all cases, subjects will be asked to return to their primary study site for their assessments for Month 12 and Month 24 Visits to ensure consistency in key efficacy assessments.

Prior to the Screening Phase, the Investigator will identify candidates potentially meeting the study eligibility criteria, based on review of medical records and clinical test findings performed routinely as standard of care for the management of the subject's disease. The competent legal parent(s)/guardian will be informed of the option to participate in the study and all associated risks of the study procedures as well as the investigational nature of gene therapy treatment (eli-cel). Sites will follow their standard institutional practice for obtaining informed consent. (Informed assent will be sought from capable subjects, in accordance with the directive of the Institutional Review Board [IRB]/ Independent Ethics Committee [IEC] and with local requirements). The subject and his legal parent(s)/guardian should be provided with adequate time to ask questions about the study, treatment, and required procedures. A physician not associated with the study team, but knowledgeable about the gene therapy, HSCT, and CALD clinical management, must participate in the initial consent process to provide an independent perspective on the benefit-risk of study participation and other available treatment options. Written informed consent and assent (if applicable) must be obtained before the conduct of any screening tests not performed routinely in the treatment of the subject. The consent process will be performed in accordance with International Council on Harmonisation (ICH)/Good clinical practice (GCP).

Subjects who are pre-screened and considered by the Investigator to be potentially eligible and for whom written informed consent has been provided will enter the Screening Phase and undergo the tests and procedures necessary to confirm study eligibility.

Subjects who are confirmed to be eligible, based on Screening assessments, are enrolled in the study and will undergo HSC mobilization mediated by G-CSF and plerixafor and harvest by apheresis using institutional practice treatment guidelines. G-CSF is defined for this protocol to mean either filgrastim or lenograstim. The harvested cells will be selected for the CD34+ marker to enrich for HSCs, transduced with Lenti-D LVV, stored frozen in cryopreservation solution while aliquots are being tested to ensure they meet product quality specifications, and returned by IV infusion through a central venous catheter to the same subject after the subject is myeloablated with busulfan IV and fludarabine IV. The subject will only undergo myeloablation after the transduced cells are dispositioned for clinical use and the drug product is at the clinical site.

Back-up cells for rescue (mobilized peripheral blood mononuclear cells [PBMCs]) will also be harvested during apheresis and stored frozen in accordance with institutional guidelines. If back up cells cannot be procured from apheresis, a bone marrow (BM) harvest may be performed.

All subjects will be followed for 24 months post-drug product infusion under this protocol. Then, subjects are expected to be followed for an additional 13 years under a separate follow-up protocol (Study LTF-304). The 13-year follow-up study will focus on long-term safety, with an emphasis on hematologic monitoring and integration site analysis (ISA) and long-term efficacy to evaluate durability of response.

Efficacy evaluations to be performed during the study include an assessment of MFDs, as well as of functional status using NFS. Additional efficacy evaluations include determination of the extent of cerebral demyelination, as measured by Loes score, and GdE on MRI. CCI assessments will include age-appropriate Wechsler tests, PedsQL™, VLCFA levels in serum, and measurements of average VCN and fraction of cells expressing ALDP in peripheral blood.

Safety evaluations to be performed during the study include determination of survival status; documentation of AEs, including SAEs; monitoring of vital signs; physical examinations; electrocardiogram (ECG); serology panel testing; standard clinical chemistry and hematology testing; concomitant medications; and testing for integration site analysis and testing for evidence of RCL.

Periodic efficacy and safety examinations will be performed by pediatric specialists or qualified delegates, as appropriate.

The success of the transplant procedure will be assessed by time to neutrophil engraftment (as defined in Section 6.5.13). Peri-transplant and post-drug product infusion morbidity and mortality will be recorded. Each subject will be evaluated on a predetermined schedule for 24 months post-drug product infusion to assess disease progression using efficacy evaluations.

Neutrophil engraftment failure is defined in Section 6.5.13.

Subjects who experience neutrophil engraftment failure will be treated at the Investigator's discretion, and will continue to be followed for efficacy and safety assessments as detailed in the Schedule of Events (SOE) (Table 3, Table 4 and Table 5).

Platelet engraftment is defined in Section 6.5.13.

Additional details regarding study evaluations and procedures, including administrative information, will be provided by the Sponsor in the Study Operations Manual (SOM).

The coordinating Investigator assigned on this study will be responsible for signing off on the final ALD-104 Clinical Study Report (CSR).

Note: as of this Protocol Amendment (Version 8.0) enrollment in Study ALD-104 has been completed and patient followup remains ongoing. Further subject enrollment in this study is not anticipated.

3.1.1. Data Monitoring Committee

An independent DMC composed of members with appropriate scientific and medical expertise to monitor the study will be convened before the study is opened. A charter describing the composition and conduct of the DMC will be drafted by the Sponsor and agreed to by all DMC members prior to the DMC's initial meeting. The DMC will meet by teleconference for scheduled meetings biannually and ad hoc as circumstances require. In addition, the DMC will review hematologic and ISA data on a quarterly basis. The DMC will have the right to recommend halting the study at any time due to concerns for the safety of the participants. (Refer to Section 3.4.2 for the enrollment suspension criteria).

3.2. Rationale for the Study

CALD is caused by an X-linked defect in the *ABCD1* gene, which encodes for ALDP, a peroxisomal transporter involved in the breakdown of VLCFA. The result is an accumulation of VLCFA that results in significant cerebral demyelination and neurologic dysfunction in young boys (typically aged 3 to 15 years). If diagnosed early, CALD can be treated with allo-HSCT to supply hematopoietically-derived cells, such as brain microglia, with functional ALDP. However, allo-HSCT carries a significant risk of procedure-related mortality, GVHD, and graft failure (Peters et al. 2004; Miller et al. 2011). Treatment with eli-cel, which utilizes a subject's own cells, should restore ALDP function by introducing the functional gene into cells that could differentiate into microglia, without exposing the subject to most of the procedure-related morbidity and mortality risks of allogeneic transplant. The mortality risk for subjects undergoing autologous transplants using myeloablative conditioning is ~3.2% at centers with significant experience in this treatment (Ortega et al. 2003).

There is a high unmet medical need for patients diagnosed with CALD. Allo-HSCT, a therapy with reported clinical benefits, is associated with significant morbidity and mortality, with a 10% to 30% risk of failure of engraftment, life-threatening infection, and acute or chronic GVHD (Hahn et al. 2008; Miller et al. 2011), with the risk of chronic GVHD being approximately 30% (Carlens et al. 1998). In completed Study ALD-102 and ongoing Study LTF-304, as of this protocol amendment, no cases of graft failure or GVHD have been observed.

In Study ALD-102, a myeloablative conditioning regimen of busulfan and cyclophosphamide was utilized before infusion of eli-cel (Eichler et al. 2017). However, a conditioning regimen of busulfan and fludarabine was demonstrated to be less toxic and equally effective as a regimen of busulfan and cyclophosphamide during allo-HSCT (Bartelink et al. 2014). Thus, a conditioning regimen of busulfan and fludarabine is used in this study.

Therapies based on administration of autologous CD34+ HSCs transduced with an LVV, are anticipated to have no risk of GVHD, a low risk of engraftment failure, and a lower risk of death, and should have a safety advantage over allo-HSCT.

See Section 1.3 for rationale for Study Design.

3.3. Rationale for the Drug Product Dose

Eli-cel consists of an autologous CD34+ cell-enriched population that contains cells transduced with LVV that encodes an *ABCD1* cDNA for human ALDP, suspended in a cryopreservation solution in the final immediate container for the intended medical use.

Eli-cel is administered via IV infusion.

The dose for eli-cel is $\geq 5.0 \times 10^6$ CD34+ cells/kg. The minimum CD34+ dose accepted as safe practice and associated with favorable engraftment kinetics is approximately 1.5×10^6 to 3.0×10^6 cells/kg (Bender et al. 1992; Perez-Simon et al. 1998; Miyamoto et al. 2004; Jillella and Ustun 2004). However, optimal neutrophil and platelet engraftment occurs at doses around 5.0×10^6 cells/kg (Weaver et al. 1995; Hatzimichael and Tuthill 2010; Duong et al. 2014).

Given these data, Table 1 outlines the source of subject cells, usage, and dose for eli-cel to be received and for back-up cells.

Table 1: Dose specification of eli-cel and Back-up Cells for Rescue for CALD

Source	Usage	Recommended Cell Dose
Apheresis	Drug Product	$\geq 5.0 \times 10^6$ CD34+ cells/kg[a]
Apheresis	Back-up Cells	$\geq 1.5 \times 10^6$ CD34+ cells/kg
Bone Marrow Harvest	Back-up Cells	$\geq 1.5 \times 10^6$ CD34+ cells/kg OR $\geq 1.0 \times 10^8$ TNC /kg

Abbrev.: TNC, total nucleated cells

[a] If more than one drug product lot is manufactured, the total dose of the resulting multiple drug product lots must meet this criterion.

The results from each eli-cel lot's completed release testing will be used along with the number of cells and viability to determine if it is appropriate to proceed with myeloablation.

3.4. Treatment Discontinuation and Enrollment Suspension Criteria

3.4.1. Stopping Rules Prior to Conditioning

Subjects will be withdrawn from the study if they meet any of the following criteria as related to pre-conditioning assessments:

- Failure to continue to satisfy eligibility criteria
- Neurological decline (progression of cerebral disease) between Screening and Pre-conditioning Assessments as evidenced by an NFS > 1 or a Loes Score > 9.
- Failure of eli-cel to be dispositioned for clinical use. Conditioning will not begin until it is confirmed that the eli-cel has been dispositioned for clinical use.

Once myeloablation with busulfan IV and fludarabine IV has begun, there are no stopping rules for conditioning, ***except for the enrollment and infusion of drug product suspension criteria for the study as outlined in Section 3.4.2.*** In the anticipated very rare event of consent withdrawal during conditioning or the development of a new medical condition that, in the Investigator's opinion, puts the subject at risk with continued busulfan IV and fludarabine IV treatment, the Medical Monitor should be contacted immediately. In such situations in which busulfan IV and fludarabine IV conditioning has not been completed per protocol, eli-cel should not be given, and it is likely that rescue therapy with back-up cells (mobilized PBMCs), or with HSCs from an appropriately allogeneic donor if available, will be required.

3.4.2. Enrollment and Infusion of Drug Product Suspension Criteria

Enrollment in this study or infusion of drug product may be temporarily suspended at any time for safety reasons. In the event enrollment is suspended by the Sponsor, the initiation of new mobilization, conditioning, or drug product infusion of subjects will be based on the individual benefit: risk; subjects who have already been treated with eli-cel will continue in the study.

The Sponsor will inform the regulatory authorities, Investigators, each site's IRB/IEC and other appropriate institutional regulatory authorities if a decision to temporarily suspend the study is made.

Following any of the events listed below, the Sponsor will conduct individual benefit/risk assessments, and consult with the DMC and clinical sites, as appropriate, to determine whether enrollment should be suspended and/or any study procedures should be performed.

- **Death**, related to drug product or with unknown relationship to drug product
- Detection of a hematological malignancy (e.g., **myelodysplasia, leukemia, lymphoma**; see Section 6.5.12).
- Detection of **vector-derived RCL** in any subject
- **Failure** in 1 subject **to achieve detectable VCN by Month 6**
- Determination of **unexpected, clinically significant, or unacceptable risk** to subjects (e.g., development of study treatment-related Grade 3 or 4 toxicities in at least 3 subjects).

Enrollment and/or study procedures will resume only after review and recommendation from the DMC. Note that a substantial amendment will be submitted to regulatory authorities before restarting enrollment if required by local regulations.

3.5. End of Trial Definition

The end of the trial is defined as the last visit of the last subject.

4. STUDY POPULATION

Males aged 17 years and younger who have been definitively diagnosed with CALD (by finding elevated levels of VLCFA) who have an MRI Loes score between 0.5 and 9 (inclusive), an NFS ≤ 1, and GdE on MRI may be enrolled.

4.1. Number of Subjects

Approximately 35 subjects will be infused with eli-cel after myeloablative conditioning with busulfan and fludarabine.
As of this Protocol Amendment (Version 8.0) enrollment and treatment (N=35) in Study ALD-104 have been completed; subject follow-up is ongoing. Further enrollment in this study is not expected.

4.2. Subject Inclusion Criteria

Subjects must meet all of the following criteria to be eligible for inclusion in this study.

1. Informed consent is obtained from a competent custodial parent or guardian with legal capacity to execute a local IRB/IEC approved consent. (Informed assent will be sought from capable subjects, in accordance with the directive of the IRB/IEC and with local requirements.)
2. Males aged 17 years and younger, at the time of parental/guardian consent and, where appropriate, subject assent.
3. Active CALD as defined by:
    a. Elevated VLCFA values, and
    b. Active CNS disease established by central radiographic review of brain MRI demonstrating
        i. Loes score between 0.5 and 9 (inclusive) on the 34-point scale, and
        ii. GdE on MRI of demyelinating lesions.
4. NFS ≤ 1.

4.3. Subject Exclusion Criteria

Subjects meeting any of the following criteria are not eligible for inclusion in this study.

1. Prior receipt of an allogeneic transplant or gene therapy.
2. Use of statins, Lorenzo's Oil, or dietary regimens used to lower VLCFA levels.
   Note: subjects must discontinue use of these medications at time of consent.
3. Receipt of an investigational study drug or procedure within 3 months before Screening that might confound study outcomes. Use of investigational study drugs is prohibited throughout the course of the study.
4. Any conditions that make it impossible to perform MRI studies (including allergies to anesthetics or contrast agents).
5. Hematological compromise as evidenced by:
    a. Peripheral blood absolute neutrophil count (ANC) < 1500 cells/mm^3, and either

b. Platelet count < 100,000 cells/mm^3 or
c. Hemoglobin < 10 g/dL.

6. Hepatic compromise as evidenced by:
   a. Aspartate transaminase (AST) value > 2.5 × Upper limit of normal (ULN)
   b. Alanine transaminase (ALT) value > 2.5 × ULN
   c. Total bilirubin value > 3.0 mg/dL, except if there is a diagnosis of Gilbert's Syndrome and the subject is otherwise stable.
7. Baseline estimated glomerular filtration rate < 70 mL/min/1.73 m^2, as determined using the Chronic Kidney Disease Epidemiology Collaboration creatinine equation (see https://www.kidney.org/professionals/KDOQI/gfr_calculatorPed).
8. Cardiac compromise as evidenced by left ventricular ejection fraction < 40%
9. Immediate family member with a known or suspected Familial Cancer Syndrome (including but not limited to hereditary breast and ovarian cancer syndrome, hereditary non-polyposis colorectal cancer syndrome, and familial adenomatous polyposis).
10. Clinically significant uncontrolled, active bacterial, viral, fungal, parasitic, or prion-associated infection.
11. Positive for human immunodeficiency virus type 1 or 2 (HIV-1, HIV-2); hepatitis B virus (HBV); hepatitis C virus (HCV); human T lymphotrophic virus 1 (HTLV-1). Note that subjects who have been vaccinated against HBV (positive for HBV surface antibodies) who are negative for other markers of prior HBV infection (e.g., negative for HBV core Ab) are eligible. Subjects with past exposure to HBV [hepatitis B core antibody [HBcAb] -positive and/or hepatitis B e-antigen antibody [HBeAb]-positive] are also eligible for the study provided they have a negative test for HBV DNA. Also note that subjects who are positive for anti-hepatitis C Ab are eligible as long as they have a negative hepatitis C viral load).
12. Any clinically significant cardiovascular, hematological or pulmonary disease, or other disease or condition that would be contraindicated for any of the other study procedures.
13. Absence of adequate contraception for fertile subjects. Male subjects and their female partners are required to use two different effective methods of contraception from Screening through at least 6 months after eli-cel infusion. If subjects are truly sexually abstinent (where true sexual abstinence is defined as being in line with the preferred and usual lifestyle of the subject), no second method is required.
14. Any contraindications to the use of G-CSF or plerixafor during the mobilization of HSCs and any contraindications to the use of busulfan or fludarabine, including known hypersensitivity to the active substances or to any of the excipients in their formulations.
15. Known hypersensitivity to protamine sulfate.

4.4. Subject Identification and Registration

Prior to the Screening Phase, the Investigator will identify candidates potentially meeting the study eligibility criteria, based on review of medical records and clinical test findings performed routinely as standard of care for the treatment of the subject.

The legal parent(s)/ guardian of subjects who are determined by the Investigator to be potentially eligible, will be informed of the option to participate in the study and all associated risks of the study procedures as well as the investigational nature of gene therapy treatment (eli-cel). The subject's consent/assent will be obtained as described previously in Section 3.1. Once consent is obtained, the potential subject will be registered and assigned a unique 10-digit subject number. If a subject is re-screened a new number will be assigned. Once a subject number has been assigned, it cannot be reused, and the number stays with the subject even if the subject is subsequently determined to be ineligible for the study or transfers to another study center. This subject number will also be carried into the long-term follow-up study.

After provision of written informed consent and, if applicable, assent, the Investigator will further evaluate the subject for study eligibility through the screening assessments to ensure all entrance criteria are satisfied. Once eligibility to enter the study is confirmed, the subject is considered enrolled in the study. Subjects who fail to meet the eligibility criteria will be considered screen failures and therefore are not enrolled in the study.

Additional details regarding the identification and registration of subjects, including details regarding the informed consent process, are outlined in the SOM.

4.5. Subject Withdrawal from the Study

In accordance with the Declaration of Helsinki, subjects have the right to withdraw from the study at any time for any reason, without prejudice to further medical follow-up. Should a subject decide to withdraw, all efforts will be made to complete and report the observations as thoroughly as possible. Subjects who receive drug product and withdraw before study completion will be asked to complete the same assessments as specified in the SOE for Month 24 (Early Termination Visit assessments).

Although subjects have the right to withdraw from the study at any time, withdrawal after the start of conditioning and before administration of the eli-cel is strongly discouraged, as this would be considered deleterious to the subject. In such cases, the subject's stored back-up cells (mobilized PBMCs), or HSCs from an appropriately allogeneic donor, will be infused; see Section 5.2.1. The subject's reason for and date of withdrawal from the study is to be recorded on the electronic case report form (eCRF).

For subjects who withdraw for reasons other than withdrawal of consent, if withdrawal is before drug product infusion, subjects should remain on study for at least 30 days after any invasive study procedure (e.g., mobilization) before withdrawal and ongoing AEs should be followed for 30 days. In the rare case a subject undergoes myeloablation and receives back-up cells instead of eli-cel, subject should remain on the study for at least 3 months post myeloablation and AEs should be followed for the 3 month duration.

If withdrawal is after drug product infusion, subjects will be asked to complete the same assessments as specified in the SOE for Month 24 (Early Termination Visit assessments) and are expected to enroll in the long-term follow-up Study LTF-304.

4.6. Withdrawal of Subjects by Investigator or Sponsor

The Investigator and Sponsor also have the right to withdraw subjects from the study at any time due to protocol noncompliance, poor tolerance, or potential safety risks, or stopping rules (see Section 3.4 for stopping rules). Subjects who have received infusion of eli-cel and are discontinued from the study prior to study completion will be asked to complete the same assessments as specified in the SOE for Month 24 (Early Termination Visit assessments) and are expected to participate in the long-term follow-up study (LTF-304).

4.7. Protocol Deviation

Categorization of protocol deviations into major/minor deviations will be determined prior to database lock, by a review of the protocol deviation data collected on the case report forms (CRFs).

5. STUDY TREATMENTS

5.1. Description of the Lenti-D Lentiviral Vector and Eli-cel

Lenti-D Lentiviral Vector: Lenti-D LVV is a replication defective, SIN, HIV-1 based LVV pseudotyped with the vesicular stomatitis virus glycoprotein G (VSV-G) envelope protein and containing the functioning human *ABCD1* complementary DNA. Lenti-D LVV is produced by transient transfection using transfer vector pLBP100, 3 packaging plasmids, and the envelope plasmid. It is formulated in animal component free Stem Cell Growth Media.

Eli-cel: Eli-cel is defined as an autologous CD34+ cell-enriched population that contains cells transduced with LVV that encodes an *ABCD1* cDNA for human ALDP, suspended in cryopreservation solution.

5.2. Summary of Treatments to be Performed or Administered

After confirmation of eligibility, HSCs must be collected from the subject following stimulation by G-CSF and plerixafor (mobilization) (see Section 5.2.1) for 2 purposes – transduction for eli-cel and for back-up cells (to be used if the subject fails to engraft). The isolated and transduced autologous CD34+ HSCs are infused back into the subject after they have received myeloablative conditioning with busulfan IV and fludarabine IV.

In Section 3.3, Table 1 outlines the source of the subject cells, usage, and the minimum dose of eli-cel or back-up cells for rescue. Additional details are provided in the following subsections.

5.2.1. Mobilization and Apheresis Procedure

The mobilization and apheresis schedule is described in Table 2.

Table 2: Mobilization and Apheresis Schedule

Mobilization day	G-CSF	Peripheral CD34+ count[a]	Apheresis	Plerixafor (evening)
1	+			
2	+			
3	+			
4	+	+		+[b]
5	(+)	+	+	+[c]
(6)	(+)	(+)	(+)	(+)
(7)	(+)	(+)	(+)	

Abbrev.: G-CSF, granulocyte colony stimulating factor; WBC, white blood cell.
Note: Parentheses indicate optional procedure or administration of medication only if needed.
[a] Must be performed on the day prior to apheresis, as well as on the day of apheresis.
[b] Do not administer in the evening of Day 4 if the WBC is > 70 × 109 cells/L on the morning of Day 4
[c] Do not administer if CD34+ counts become available and indicate enough cells had been collected.

Up to 3 total apheresis collections may be performed as part of any one mobilization cycle. The 3 apheresis collection days may occur across 4 days after discussion with the Sponsor. Drug product will be manufactured from cells collected from either one day or over 2 consecutive days.

More than 1 mobilization cycle may be performed if needed in order to meet the required cell dose for eli-cel. If more than one mobilization cycle is required, mobilization cycles must be separated by at least 2 weeks. A BM harvest is also allowed, but only to procure back-up cells.

For details regarding traceability of the collected HSCs, see Section 5.9.

Mobilization Cycle 1

Subjects will be mobilized with G-CSF (starting dose 10 µg/kg) for 4 to 7 days. Complete blood counts (CBCs) should be performed daily during mobilization and the dosage of G-CSF should be decreased if white blood cell (WBC) count is > 70×10^9 cells/L. After the 4th G-CSF dose, plerixafor will be administered daily for up to 3 days by subcutaneous injection (0.24 mg/kg of body weight) prior to each apheresis collection. The morning after the 4th G-CSF dose, apheresis will be performed according to standard clinical procedures.

On each day of apheresis, the subject should have a physical exam, including abdominal palpation to rule out splenomegaly, and vital signs performed prior to beginning apheresis and again after completion of apheresis. Apheresis will be performed per standard clinical site practice.

Apheresis product from Mobilization Cycle 1 should be evaluated and managed as follows:

- If the Apheresis Procedure Day 1 collection is > 15×10^6 CD34+ cells/kg: Send a minimum of 12×10^6 CD34+ cells/kg to the transduction facility. The remainder (at least 1.5×10^6 CD34+ cells/kg) should be stored as back-up at the clinical site.
- If the Apheresis Procedure Day 1 collection is between 12 and 15×10^6 CD34+ cells/kg, send the entire collection to the transduction facility. The subject should return for an additional apheresis procedure on Day 2 to collect back-up cells (≥ 1.5×10^6 CD34+ cells/kg), stored at the clinical site.
- If the Apheresis Procedure Day 1 collection is < 12 × 106 CD34+ cells/kg, this collection should be held at an overnight, controlled storage facility at the clinical site and the subject should return for Apheresis Procedure Day 2. The collections from Apheresis Procedure Day 1 and Apheresis Procedure Day 2 will be sent for transduction. If the total collection over Day 1 and Day 2 is > 15 × 106 CD34+ cells/kg, back-up cells may first be removed. Otherwise, the subject should return for an additional apheresis procedure on Day 3 to collect back-up cells (≥ 1.5 × 106 CD34+ cells/kg), stored at the study site.
- If the Apheresis Procedure Day 1 collection is < 1×10^6 CD34+ cells/kg, this collection should be stored at the clinical site for back-up. The subject should return for Apheresis Procedures on Days 2-3 until the target of 12×10^6 CD34+ cells/kg is obtained for transduction.

Mobilization Cycle 2

After completion of Mobilization Cycle 1, the Sponsor will inform the clinical site of the eli-cel dose. Once all release testing has been completed, the Sponsor will inform the clinical site if the drug product has met all applicable release criteria.

If the minimum recommended cell dose has been met and the drug product has met specification, then the subject should only undergo Mobilization Cycle 2 if additional collection of autologous cells is needed for back up cells (i.e., if $< 1.5 \times 10^6$ CD34+ cells/kg have been collected and stored for rescue during Mobilization Cycle 1).

If additional autologous cells are needed, then the subject should begin Mobilization Cycle 2. Mobilization Cycle 2 should begin no sooner than 2 weeks after the completion of Mobilization Cycle 1 with the same guidelines as Mobilization Cycle 1.

If Mobilization Cycle 2 is for the procurement of additional autologous cells for eli-cel, the management of the collection from each Apheresis Procedure day will be discussed with the Medical Monitor on a case-by-case basis prior to Apheresis Procedure Day 1.

5.2.2. Bone Marrow Harvest Procedure

If sufficient back-up cells for rescue are not procured after 1 or 2 mobilization cycles, the Investigator can proceed with a BM harvest. BM harvest will be performed according to institutional practice and will occur at a minimum of 2 weeks after completion of the last mobilization cycle.

5.2.3. Transduction Process and Release Testing

All cell manipulation procedures and release testing will be performed in accordance with Good Manufacturing Practice (GMP) following process-specific standard operating procedures.

After each mobilization and apheresis procedure (Section 5.2.1), drug product manufacture will result in an independent drug product lot. Eli-cel lots which have been released for clinical use will be sent to the site with a Certificate of Analysis(es), documenting that all release testing is complete.

In some circumstances, drug product that does not meet the established specification may be administered. This requires justification of medical need as assessed by the Principal Investigator and Sponsor Responsible Medical Officer. The subject must be informed and, if applicable, a notification to the appropriate IRBs/ECs and regulatory agency(ies) must occur prior to the start of myeloablative conditioning.

5.2.4. Conditioning

Pre-conditioning assessments will be performed on Day -7 (with a window of -5 days; i.e. Day -12 through Day -7) prior to myeloablative conditioning as outlined in the SOE. If neurological decline is observed (see Section 3.4.1), as evidenced by a NFS > 1 or a Loes Score > 9, the subject will be discontinued from the study.

Conditioning will only begin once eli-cel has been dispositioned for clinical use and the drug product is at the clinical site. Myeloablative conditioning will be performed on an in-patient basis using busulfan IV and fludarabine IV.

On Days -6, -5, -4, and -3, fludarabine IV (40 mg/m^2) will be given one hour before the first daily infusion of busulfan IV, per institutional standards. Glomerular filtration rate measured during Screening and Pre-conditioning assessments is used to help calculate fludarabine exposure.

Additional information on busulfan dosing:

Investigators may choose to dose using cumulative exposure or weight-based dosing. The Investigator is to record which method they choose and must use the same method for all four days of busulfan dosing. Patients will receive busulfan on Days –6, –5, –4, and –3.

- Dosing based on cumulative exposure:
  - Busulfan dosed by cumulative exposure may be given every 6, 12 or 24 hours, with choice of dosing frequency at the investigator's discretion.
  - The Investigator is to record which dosing frequency they choose and must use the same frequency for all four days of busulfan dosing.
  - The investigator should also note which pharmacokinetic method their site uses to calculate busulfan exposure (Bartelink et al. 2012, 2016).
  - If using cumulative exposure to calculate busulfan dose, the cumulative busulfan exposure (or cumulative area under the curve [AUC] [AUC_{cum}]) will be targeted to provide an AUC 85 to 95 mg/L × h (target 90) = 85000 to 95000 ng/mL × h = 20706 to 23180 µmol*min/L with q24 hour dosing. If the Investigator uses a dosing frequency other than q24 hours, the target AUC after each individual dose will be lower though the AUC_{cum} should be the same.
  - If using the first dose to calculate busulfan exposure, the target AUC range for a single dose with a dosing frequency of every 6 hours is 1335 to 1491 µmol*min/L.
  - If the AUC for the 1st single dose falls outside the specified target range, subsequent doses can be adjusted to ensure appropriate exposure to the AUC_{cum} target range.
  - Busulfan AUC_{cum} should be measured on Day -6 and as appropriate to evaluate and adjust the cumulative exposure.
- Busulfan IV weight-based dosing will be calculated as follows:
  - For subjects ≤ 12 kg: busulfan 1.1 mg/kg/dose IV every 6 hours on Days –6, –5, –4, and –3
  - For subjects > 12 kg: busulfan 0.8 mg/kg/dose IV every 6 hours on Days –6, –5, –4, and –3

If any subjects are obese (i.e., actual body weight [ABW] is over 125% of ideal body weight [IBW]), dosing will be adjusted for IBW (see Section 10.1 for calculations).

Additional information:

After completion of the 4 day course of fludarabine and busulfan, there should be a minimum of 48 hours of washout before drug product infusion.

Prior to and during the administration of busulfan IV and fludarabine IV, prophylactic and empiric anti-convulsive, antifungal, and antibiotic treatments, and all other supportive care, including management of any complications resulting from myeloablation, will be administered by the institutional transplant team per institutional standards.

Refer to the current Package Inserts for busulfan IV and fludarabine IV, including associated AEs.

5.2.5. Infusion Procedures, Dose, and Administration

Infusion of eli-cel is to be given at least 48 hours after completion of the busulfan IV and fludarabine IV conditioning regimen.

All procedures involving eli-cel must be performed using aseptic techniques by trained personnel per institutional practice at the clinical site, including a saline line flush. Prior to administration, the eli-cel is to be thawed in a 37°C water bath or an appropriate dry bath and must be infused immediately, but no later than 4 hours after it has been thawed.

Eli-cel will be administered via IV infusion through a central venous catheter in a volume between 20 and 80 mL, according to institutional practice for infusion of hematologic stem cells at the clinical site. **Do not use an infusion filter**. The dose to be administered is $\geq 5.0 \times 10^6$ CD34+ cells/kg. The subject's weight immediately prior to the first apheresis collection will be used to calculate the final dose of eli-cel for that lot. If more than one lot of eli-cel is manufactured to achieve the minimum cell dose, infusions of each lot will occur consecutively. Consecutive infusions will also occur if a single lot is split into 2 drug product bags due to volume constraints.

There is no current evidence that eli-cel must be adjusted for obesity. If, during Screening, a subject is determined to be obese (i.e., subject ABW is over 125% of IBW), the Sponsor and the Investigator will discuss adjusting for IBW. IBW dosing that results in an ABW dose $\leq 1.5 \times 10^6$ CD34+ cells/kg would not be acceptable.

Each eli-cel bag contains 1 g dimethyl sulfoxide (DMSO). Limiting the amount of DMSO infused to no more than 1 g/kg/day is recommended (Júnior et al. 2008), and thus no subject in Study ALD-104 would exceed this amount.

Vitals signs are to be monitored concurrently during eli-cel infusion per institutional practice at the clinical site, but no less frequently than at the start, once during, and upon completion of the infusion. Infusion reactions, including anaphylaxis, will be managed according to the medical judgment of the physician overseeing the infusion.

5.3. Storage and Stability of Eli-cel

Eli-cel will be frozen and stored in cryopreservation solution in the vapor phase of liquid nitrogen at the transduction facility until release testing and dispositioning for clinical use.

Once dispositioned for clinical use, eli-cel will be stored in the vapor phase of liquid nitrogen at the clinical site until thawed for clinical use.

For details regarding traceability of eli-cel, see Section 5.9.

5.4. Storage and Administration of Back-up Cells

Mobilized back-up cells will be frozen and stored in accordance with institutional guidelines. In the event of primary neutrophil engraftment failure (as defined in Section 6.5.13), back-up cells will be administered at a dose of $\geq 1.5 \times 10^6$ CD34+ cells/kg, or $\geq 1.0 \times 10^8$ total nucleated cells (TNC)/kg (see Table 1). Back-up cells may also be administered in the event that the subject has received myeloablation and is unable to receive infusion of eli-cel for any reason (see Section 3.4). For details regarding traceability of back-up cells, see Section 5.9.

5.5. Method of Assigning Subjects to Treatment

In this open-label study, all subjects entered into the study will be assigned to active treatment.

5.6. Blinding, Packaging, and Labeling

5.6.1. Blinding and Breaking the Blind

This is an unblinded, open-label study.

5.6.2. Packaging and Labeling

Eli-cel consists of an autologous CD34+ cell-enriched population that contains cells transduced with LVV that encodes an *ABCD1* cDNA for human ALDP. Eli-cel is suspended in cryopreservation solution in the final immediate container for the intended medical use (infusion bag).

Eli-cel will be labeled by the GMP-compliant contract manufacturing site. Refer to Section 5.9 for additional details regarding product accountability.

5.7. Duration of Study Participation

Each subject will remain on this study for approximately 26 months from time of consent, inclusive of an approximately 24-months post-drug product infusion follow-up; subjects will then be expected to consent for a follow-up study for another 13 years post-drug product infusion.

5.8. Assessment of Treatment and Study Compliance

Treatment compliance will not be an issue in this study as eligible subjects receive a one-time administration of eli-cel, and will be monitored by hospital personnel.

Subject compliance with the subsequent post-drug product infusion study visits will be assessed through Month 24.

5.9. Product Accountability

Eli-cel accountability and traceability is ultimately the responsibility of the Investigator and Sponsor. However, this responsibility may be delegated to a suitably qualified Investigator who has had appropriate study-specific training and whose name has been appropriately listed on the Delegation of Responsibility Log for this task.

Detailed records will be maintained throughout the duration of the study to enable accurate accountability of the subject's autologous cells from procurement to eli-cel infusion. Procured CD34+ HSCs will be traceable back to the subject from apheresis through infusion via 2 unique identifiers and their Hematopoietic Progenitor Cell, Apheresis [HPC,A] product number (unique for each HPC,A collection). In the event that two apheresis collections are required for a subject during the same mobilization cycle, the HPC,A product numbers will act as additional traceability to the collection event. Clinical sites will enter the HPC,A product number and the 2 unique identifiers on the label of the collection bag for each subject's respective apheresis product.

Once these cells have arrived at the transduction facility, GMP procedures will be utilized throughout each step of the manufacturing process to generate a final drug product (CD34+ HSCs transduced with Lenti-D LVV) to ensure traceability. Each drug product lot is assigned a unique manufacturing lot number. Eli-cel accountability is confirmed by utilization of the same 2 unique identifiers, HPC,A product number(s), and drug product manufacturing lot number on the final drug product label. Clinical site staff will verify the 2 unique identifiers upon receipt of the drug product from the manufacturer and once again prior to infusion. The above procedure is also followed if more than one lot of eli-cel is manufactured to achieve the minimum cell dose or if a single lot is split into 2 drug product bags due to volume constraints. In this situation, the eli-cel lots or bags will be shipped together to clinical site staff. Each of these steps will be documented by the appropriate party who is handling subject cells.

These records will include details of storage and use of the eli-cel as well as storage of back-up cells. Transfer of eli-cel from the transduction facility through administration to the subject will be recorded.

The Investigator will ensure that the eli-cel is used only in accordance with this protocol. Drug accountability records indicating eli-cel inventory at the clinical site, administration to each subject, and disposal will be maintained by the clinical site. These records will adequately document that the subject was provided the eli-cel dose as specified in the protocol and should reconcile each eli-cel received by the clinical site. Accountability records will include dates, quantities, lot numbers, expiration dates (if applicable), and subject numbers. The Sponsor or its designee will review eli-cel accountability at the clinical site on an ongoing basis during monitoring visits. Additional information regarding traceability can be found in the SOM.

All material containing eli-cel will be treated and disposed of as hazardous waste in accordance with governing regulations and clinical site procedures.

In the event that drug product cannot be administered due to triggering of stopping rules or other reasons, drug product will be kept cryopreserved in the vapor phase of liquid nitrogen until further instruction by the Sponsor. The Sponsor will instruct the site staff to either destroy the drug product via their institutional procedures or return the drug product to bluebird bio.

5.10. Prior and Concomitant Medication and Therapy

To avoid confounding the results of VLCFA changes after treatment with eli-cel, medications used to lower VLCFA levels (e.g., Lorenzo's oil, statins) and other investigational therapies are to be excluded during study participation. In addition, the subject should not embark on a new low fat diet during study participation.

Subjects should not use medications with anti-retroviral activity (such as those used for HIV prophylaxis) from within 1 month of initiating mobilization until after drug product infusion.

There will be no other excluded concomitant medications. The Investigator should inquire about any intervention that may create a health hazard or other problem for study-related procedures (e.g. non-removable dentures with metal keepers that can distort brain MRI images).

After infusion of eli-cel, G-CSF is to be given as follows:

- Start G-CSF 5 days after infusion (Day 6; Day 1 is defined as day of eli-cel infusion), with dose at Investigator's discretion per usual institutional practice. G-CSF may be held to start later at the Investigator's discretion if the WBC has not fallen to nadir.
- The earliest stopping point for G-CSF is after 3 consecutive days with an ANC > 500, but G-CSF may be stopped any time after that at the Investigator's discretion.

All concomitant medications from the time informed consent is obtained through the follow-up period will be recorded in the eCRF. Medications and therapies taken specifically for the management of CALD in the 6 months prior to consent will be recorded on the eCRF.

For the purposes of this study, vaccines (e.g., COVID-19 vaccines) are considered concomitant medications. Although interactions between a vaccine and eli-cel are not expected, the protocol includes use of immunomodulatory (plerixafor, G-CSF) and immunosuppressive medication (busulfan, fludarabine). Local guidelines should be followed regarding a minimum time period between any medication to be provided as part of treatment with eli-cel and any vaccine; it is recommended that vaccines are not administered to subjects within 1 month of initiating mobilization for stem cell collection, within 1 week after receiving mobilization agents, within 1 month of initiating myeloablative conditioning, or within 6 months after drug product infusion. Revaccination post-drug product infusion should be considered per Investigator's discretion due to potential loss of immunity after myeloablative conditioning and may be administered following local guidelines.

The prescribing information of the vaccine administered should be referred to for the latest indication, contraindications and safety information as well as the latest general clinical recommendations on vaccine administration in the country or region.

All vaccinations during the study period should be documented in the CRF.

Concomitant therapies, for example, physical, or speech therapy, will be captured from the time of informed consent through the follow-up period.

6. STUDY ASSESSMENTS

6.1. Schedule of Events

Table 3, Table 4 and Table 5 provide the SOE to be conducted during the study. Detailed descriptions of the efficacy, safety, and exploratory procedures to be conducted during this study are provided in the following sections. Additional details, including administrative information, regarding the efficacy, safety, and exploratory procedures, will be provided by the Sponsor in the SOM.

Subjects who experience neutrophil engraftment failure (as defined in Section 6.5.13) will receive infusion with back-up cells or with allogeneic HSCs. These subjects will continue to be followed for efficacy and safety assessments.

Study treatments and evaluations can be considered as 4 distinct phases after informed consent:

- Screening and Enrollment. Subjects who meet eligibility criteria, based on screening assessments are considered enrolled. Patients who do not meet eligibility criteria are considered screen failures.
- CD34+ Cell Collection, Transduction, Disposition of eli-cel, and Reconfirmation of Eligibility
- Conditioning and Washout, followed by eli-cel Infusion on Day 1
- Maintenance (Follow-up) (Day 2 through Month 24)

Study Day 1 is defined as the day of infusion.

Subjects will be asked to comply with the protocol specified assessments according to the time periods enumerated in the SOE. If the timing of assessments is shifted due to scheduling conflicts (e.g., limited bed availability at the hospital, delays in screening assessments, repeat mobilization, delays in scheduling conditioning and eli-cel infusion, minimal delays in manufacturing or release testing of drug product), these will not be considered protocol deviations. In such cases, the subject will resume their schedule beginning on Day -6. If delays are greater than two weeks, the Investigator and Medical Monitor should discuss the continued eligibility of the subject for infusion.

During the first year after eli-cel treatment (Month 1 through Month 12), CBCs with differential will be collected every month as shown in Table 5. Subsequently, CBCs with differential will be collected every 4 months at the study site through Month 24. Data collection as described in this paragraph will be obtained prospectively only (that is, data should not be collected retrospectively for subjects who have passed applicable study visits as of the date of implementation of this protocol amendment).

Note: Unscheduled visits may be performed at any time during the study whenever necessary to assess for or to follow-up on AEs or to perform enhanced CBC and BM abnormality monitoring (Section 6.5.11.1.1 and Section 6.5.11.1.2, respectively) or as deemed necessary by the Investigator. Evaluations and procedures identified in the SOE, or other evaluations and procedures deemed necessary for safety, may be performed at unscheduled visits, as clinically indicated, at the Investigator's discretion in consultation with the Sponsor.

The study treatments are described in detail in Section 5.2.

Impact of Force of Nature or War (e.g., the COVID-19 Pandemic, the Russo-Ukrainian War) on Study Visits

Due to force of nature or war (e.g., the COVID-19 pandemic, the Russo-Ukrainian War), subjects may not be able to attend normal study visits. If a visit is missed due to a force of nature or war (e.g., unable to travel, unwilling to travel, family or subject affected by COVID-19, hospital closure, etc.), the subject may be able to complete study assessments via telemedicine or at a local facility. Sites should consult with the medical monitor and clinical research associates to consider alternate arrangements, when possible, for completion of study assessments.

Table 3: Schedule of Events: Screening through Conditioning

	Screening	Mobilization[a]	CD34+ Harvest (Apheresis)	Pre-conditioning Assessments	Conditioning and Monitoring
Study Day (D):	**D -60 to -45**	**D -44 to -37**	**D -40 to -37**	**D -7**	**D -6 to -1**
Visit Window (Days):	-10 to +5	-10	-	-5	-
Informed Consent[b]	+				
Search for allogeneic donor & HLA typing[c]	+				
Demographics & Medical History	+				
ABCD1 genotype[d]	(+)				
Adrenal function[e]	+				
Local lab: Blood for immunological studies	+				
Sperm / testicular tissue banking, if requested[f]	+				
Serology panels I and II	+ (I)	+ (II)			
Physical examination, Vital signs, Weight[g]	+	+[h]	+[i]	+	+[j]
Hematology[k]	+	+[l]	+[l]	+	+[m]
Clinical chemistry	+			+	+[m]
Glomerular Filtration Rate[n]	+			+	
Blood specialty labs:					
RCL	(+)[o]			(+)[o]	
ALDP (Peripheral Blood Populations)	(+)[o]			(+)[o]	
VCN (Peripheral Blood Populations)	(+)[o]			(+)[o]	
VLCFA (fasting)	+				
Neurological exam	+			+	
NFS assessment[p]	+			(+)[p]	
MFD assessment[p, q]	+			(+)[p]	
Neuropsychological tests				+	
Global assessment				+	
PedsQL				+	
Echocardiogram	+				
Electrocardiogram	+				
Brain MRI (with and without contrast)[p]	+[r]			(+)[p]	
Evoked potentials[s]	+				
Confirmation of eligibility	+			+	
G-CSF and plerixafor administration		+			
CD34+ count[t]		+	+		
Busulfan and Fludarabine administration					+
Busulfan level monitoring					+
Concomitant medication	Continuous from ICF signing				
Adverse event monitoring	Continuous from ICF signing				

[a] If more than one mobilization cycle is required, they must be separated by an interval of at least 2 weeks. See Section 5.2.1 for additional details.
[b] If a subject is below the legal age of consent and turns the legal age of consent per country regulations while on study, the subject must be re-consented at the next scheduled study visit, prior to the collection of additional study data.
[c] A preliminary search for a suitable donor will be initiated at Screening for all subjects in the event that a subject is not eligible for drug product during Pre-Conditioning Assessments, experiences engraftment failure, or cannot receive eli-cel. HLA typing does not need to be performed if historical results are available.
[d] Genotyping of *ABCD1* gene will occur in subjects for whom no historical data are available; documented *ABCD1* mutation required prior to initiating myeloablative conditioning.
[e] Adrenal function tests (cortisol and adrenocorticotropic hormone [ACTH]) are to be performed in the morning (approximately 8:00 am) during Screening before the subject has taken hydrocortisone unless subject is on steroid replacement therapy. If ACTH is significantly elevated, tests should be repeated 3 hours after taking hydrocortisone. Mineralocorticoid functions (aldosterone and plasma renin activity) are to be performed at the same time points with the subject sitting in an upright position.
[f] May occur any time before conditioning; hormonal treatment, if applicable as part of banking, should stop at least 7 days prior to conditioning.
[g]Full physical examination, including height and weight measurements, will be performed at Screening only. Vital signs will include blood pressure, pulse, respiratory rate, and temperature. During hospitalization, focused physical examinations and vitals to be performed as standard of care. AEs identified during this time will be entered into the clinical database.
[h] Focused physical examinations and vital signs will be performed prior to the first dose of G-CSF.
[i] On each day of apheresis, the subject should have a focused physical exam, including abdominal palpation to rule out splenomegaly, and vital signs performed prior to beginning apheresis and again after completion of apheresis.
[j] Focused physical examinations and vital signs will be performed each day during conditioning.
[k] Hematology parameters include white blood cell (WBC) count with differential, hemoglobin, hematocrit, red blood cell (RBC), and platelet count.
[l] Hematology will be performed each day of mobilization and apheresis.
[m] Chemistry and hematology parameters will be measured daily during conditioning; blood will be collected prior to infusion of busulfan IV and fludarabine IV.
[n] Calculated from sex, age, height, weight, and creatinine. Unit = mL/min/1.73m^2
[o] Blood for measurements of RCL, ALDP, and VCN will be drawn once, any time from Screening prior to start of conditioning.
[p] NFS assessments, MFD assessments, and brain MRIs may be repeated at any time during the study if there is evidence of clinical decline. These assessments must be repeated if more than 60 days has passed between the assessment at Screening and the start of Pre-Conditioning. However, if subject requires sedation for MRI, performing this repeat assessment is based on Investigator judgment.
[q] May be performed concurrently with NFS assessment.
[r] MRI performed within 5 days of signing of ICF can be used as the Screening MRI.
[s] The Brain Stem Auditory Evoked Response (BAER) and the Visual Evoked Potential (VEP) P100 latency will be performed at Screening.
[t] Peripheral blood CD34+ count should be performed either the day prior to or on the first planned day of apheresis.

Table 4: Schedule of Events: Drug Product Infusion through End of Study

	Eli-cel Infusion	Follow-Up Week 2	Follow-Up Month 1	Follow-Up Month 2	Follow-Up Month 3	Follow-Up Month 6	Follow-Up Month 9	Follow-Up Month 12	Follow-Up Month 16	Follow-Up Month 18	Follow-Up Month 20	Follow-Up Month 24	Early Termination
Study Day (D):	**D 1**	**D 15**	**D 30**	**D 60**	**D 90**	**D 180**	**D 270**	**D 360**	**D 480**	**D 540**	**D 600**	**D 720**	-
Visit Window (Days):	-	±7	±7	±14	±14	±14	±14	±30	±30	±60	±30	±30	NA
Eli-cel infusion[a]	+												
Physical examination, Vital signs, Weight[b]	+	+	+	+	+	+		+[c]	+		+	+[c]	+[c]
Hematology [d],[e],[f]		+	See Table 5						+		+	+	+
Hematopathology review of peripheral blood smear[g]			See Table 5						+		+	+	
Bone marrow core needle biopsy and aspirate (including ISA, VCN and Storage)[h] [i]		Additional BM evaluations may be triggered as detailed in Section 6.5.11.1.2											
Clinical chemistry[f]		+	+	+	+	+		+	+		+	+	+
Local lab: Blood for immunological studies								+				+	+
Blood specialty labs:													
RCL[j]					+	+		+				+[k]	+[k]
Integration Site Analysis[l],[m]						+	+	+	+		+	+	+
ALDP (Peripheral Blood Populations)			+	+	+	+		+	+		+	+	+
VCN (Peripheral Blood Populations) [l]			+	+	+	+	+	+	+		+	+	+
VLCFA (fasting)								+				+	+
Neurological exam			+		+	+		+		+		+	+
NFS assessment[n],[o]			+		+	+		+		+		+	+
MFD assessment[n]			+		+	+		+		+		+	+
Neuropsychological tests								+				+	+
Global assessment								+				+	+
PedsQL					+	+		+				+	+
Electrocardiogram												+	+
Brain MRI (with and without contrast)[n]						+		+		+		+	+
Evoked potentials[p]								+				+	+

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Table 4: Schedule of Events: Drug Product Infusion through End of Study

	Eli-cel Infusion	Follow -Up Week 2	Follow -Up Month 1	Follow -Up Month 2	Follow -Up Month 3	Follow -Up Month 6	Follow-Up Month 9	Follow- Up Month 12	Follow-Up Month 16	Follow -Up Month 18	Follow-Up Month 20	Follow -Up Month 24	Early Termination
Study Day (D):	**D 1**	**D 15**	**D 30**	**D 60**	**D 90**	**D 180**	**D 270**	**D 360**	**D 480**	**D 540**	**D 600**	**D 720**	**-**
Visit Window (Days):	-	±7	±7	±14	±14	±14	±14	±30	±30	±60	±30	±30	NA
Health economic data[q]		+	+	+	+	+		+	+		+	+	+
Concomitant medication	Continuous from ICF signing												
Adverse event monitoring	Continuous from ICF signing												

[a] Start G-CSF5 days after infusion (Day 6; Day 1 is defined as day of eli-cel infusion), with dose at Investigator's discretion per usual institutional practice. G-CSF may be held to start later at the Investigator's discretion if the WBC has not fallen to nadir.

[b] During hospitalization, focused physical examinations and vitals to be performed as standard of care. AEs identified during this time will be entered into the clinical database. Vital signs are to be monitored concurrently during eli-cel infusion according to institutional practice at the clinical site, but no less frequently than at the start, once during, and upon completion of the infusion.

[c] Full physical examination, including height and weight measurements, will be performed. Focused physical examinations may be performed at other visits.

[d] Hematology parameters include white blood cell (WBC) count with differential, hemoglobin, hematocrit, red blood cell (RBC), and platelet count. If the results from blood tests are not as expected, additional testing may need to be performed and may include a physical exam, blood tests, imaging tests, or a BM biopsy (both BM core biopsy sections and aspirate smear collection should be performed to assess morphology). See Section 6.5.11.4 for blood sample collection and storage details.

[e] A repeat performance of CBC is required within one month for any CBC abnormality (including abnormalities in hemoglobin levels, total white blood cell count, neutrophil count and morphology, lymphocyte count and morphology, or monocyte counts and morphology) that is of CTCAE Grade 2 or higher severity or for platelet counts of $<100 \times 10^9$/L. See Section 6.5.11.1.1. In addition, a re-evaluation at the discretion of the Investigator of any new abnormal peripheral blood cell count or morphology at an interval commensurate with the severity of the change is recommended. Persistent, unexpected, abnormalities of peripheral blood cell counts or morphology, including but not limited to any two consecutive (one month apart) CTCAE Grade 2 CBC values or platelet counts of $<100 \times 10^9$/L will trigger a BM evaluation. For frequency of subsequent BM evaluations (see Section 6.5.11.1.2). See Section 6.5.11.4 for sample collection and storage details.

[f] Chemistry and hematology parameters will be measured at least twice per week until neutrophil engraftment occurs.

[g] Peripheral blood smears will be created locally at the study site and submitted for central review by a single central hematopathologist. See Section 6.5.11.1.1 for further details. At the Investigator's discretion local peripheral blood smears may also be performed by the study site to direct patient care.

[h] Note that sites are required to collect BM aspirate post-drug product infusion per the SOE, unless discussed with the Sponsor's Medical Monitor for exceptions, such as if not clinically appropriate per local standard of care (e.g., BM aspirate would require general anesthesia, which would be considered an unacceptable risk to the subject) or not considered by the Investigator to be in the best interest of the subject to perform. For details, see Section 6.5.11.1.2.

[i] Bone marrow smear should be read locally; additional BM slides are to be created and submitted for central analyses as detailed in Section 6.5.11.1.2. See Section 6.5.11.4 for BM sample collection details. Additionally, BM aspirate (and an accompanying whole blood specimen) should be collected for storage at the time of BM sample collection to facilitate subsequent exploratory analyses that may include gene/genomic expression studies.

[j] Two samples are required, one for RCL screening test, another for potential co-culture of peripheral blood leukocytes (PBLs) if RCL screening test is positive.

[k] If a subject's previous RCL tests were all negative, this sample will be archived.

[l] Additional blood may be collected for analysis in cell subtypes, if requested by the Sponsor. If blood draw volume required exceeds the limit per Section 6.5.11.4, samples should be obtained over multiple days.
[m] ISA of BM will also be performed following specific triggers, see Section 6.5.11.4.
[n] NFS assessments, MFD assessments, and brain MRIs may be repeated at any time during the study if there is evidence of clinical decline.
[o] May be performed concurrently with NFS assessment.
[p] The BAER will be performed at Month 24. The VEP P100 latency will be performed at Month 12 and Month 24.
[q] Includes number and duration of in-patient hospitalizations (including ICU stay), clinic/doctor visits, therapy, and emergency room visits.

Table 5: Schedule of Events: Hematology Month 1 through Month 12 Visits

	Follow-Up Month 1	Follow-Up Month 2	Follow-Up Month 3	Follow-Up Month 4	Follow-Up Month 5	Follow-Up Month 6	Follow-Up Month 7	Follow-Up Month 8	Follow-Up Month 9	Follow-Up Month 10	Follow-Up Month 11	Follow- Up Month 12
Study Day (D):	**D 30**	**D 60**	**D 90**	**D 120**	**D 150**	**D 180**	**D 210**	**D 240**	**D 270**	**D 300**	**D 330**	**D 360**
Visit Window (Days):	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±30
Hematology[a b c]	+	+	+	+	+	+	+	+	+	+	+	+
Hematopathology review of peripheral blood smear[d]						+						+

[a] Hematology parameters include white blood cell (WBC) count with differential, hemoglobin, hematocrit, red blood cell (RBC), and platelet count. If the results from blood tests are not as expected, additional testing may need to be performed and may include a physical exam, blood tests, imaging tests, or a BM biopsy.
[b] A repeat performance of CBC is required within one month for any CBC abnormality (including abnormalities in hemoglobin levels, total white blood cell count, neutrophil count and morphology, lymphocyte count and morphology, or monocyte counts and morphology) that is of CTCAE Grade 2 or higher severity or for platelet counts <100 × 10^9/L. See Section 6.5.11.1.1. In addition, a re-evaluation at the discretion of the Investigator of any new abnormal peripheral blood cell count or morphology at an interval commensurate with the severity of the change is recommended. Persistent, unexpected, abnormalities of peripheral blood cell counts or morphology, including but not limited to any two consecutive (one month apart) CTCAE Grade 2 CBC values or platelet counts of <100 x 10^9/L will trigger a BM evaluation. For frequency of subsequent BM evaluations, see Section 6.5.11.1.2.
[c] Hematology parameters will be measured at least twice per week until neutrophil engraftment occurs.
[d] Peripheral blood smears will be created locally at the study site and submitted for central review by a single central hematopathologist. See Section 6.5.11.1.1 for further details. At the Investigator's discretion local peripheral blood smears may also be performed by the study site to direct patient care.

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6.2. Concurrent Human Leukocyte Antigen Search for Allogeneic Donor

During Screening, HLA typing for the subject does not need to be performed if historical results are available. A preliminary search for a suitable donor will be initiated at Screening for all subjects in the event that a subject is not eligible for drug product during Pre-Conditioning Assessments (Section 5.2.4), experiences engraftment failure, or cannot receive eli-cel.

6.3. Confirmation of Eligibility

Subject's eligibility will be confirmed prior to the start of conditioning as detailed in the SOE. If a subject is determined to be ineligible, the Medical Monitor should be contacted.

6.4. Fertility Preservation

Fertility preservation (e.g., sperm or testicular tissue banking) will be done as appropriate at the discretion of the subject, their legal guardian (as applicable), and the Investigator.

6.5. Assessments

6.5.1. Demographics and Medical History

Subject demographic data such as initials, gender, age, race, and ethnicity (if allowed by local regulation), will be obtained during Screening, and a complete medical history will be obtained during Screening and updated on Day 1 as needed. The medical history is to include all prior and current medical history, including relevant family history, and CALD disease history (including *ABCD1* genotype and imaging information).

6.5.2. Physical Examination

Complete physical examinations (including general appearance; head, eyes, ears, nose, and throat [HEENT]; cardiovascular; dermatologic, abdominal; genitourinary; lymph nodes; hepatic; musculoskeletal; respiratory; and neurological), including height measurement, will be conducted at visits indicated in the SOE. Focused physical examinations are to be conducted at all other study visits according to the SOE.

6.5.3. Vital Signs and Weight

Vital signs to be measured include systolic/diastolic blood pressure, pulse, respiration rate, and temperature. Vital signs and subject weight (in kilograms) will be measured and recorded according to the SOE; measurement details are included in the SOM.

See Section 6.5.11.4 for guidance on weight-based blood draws.

6.5.4. Electrocardiogram/Echocardiogram

A 12-lead ECG will be obtained as per the SOE and read locally.

A standard 2D Doppler echocardiogram will be performed at Screening and read by the site cardiologist. Ejection fraction and overall clinical interpretation will be captured.

6.5.5. Neurologic Examination

The neurologic examination is to include ophthalmologic and audiologic examinations administered by a pediatric neurologist or other appropriately trained and qualified physician according to the SOE. Visual acuity will be assessed using either a wall chart or a pocket eye chart. Visual fields will be assessed by perimetry testing or by visual confrontation by neurological examination. Examination details are included in the SOM.

6.5.6. Neurologic Functioning Score (NFS) Assessment

Assessment of subject status using NFS is to be performed by a pediatric neurologist or other appropriately trained and qualified physician, when the pediatric neurologist is unavailable, according to the SOE. The presence or absence of disabilities that prevent independent functioning will be recorded during these examinations using the definitions described in Section 10.3. As noted in the SOE, if the screening through pre-conditioning period exceeds 60 days or there is clinical suspicion of neurological decline, the NFS assessment should be repeated ahead of myeloablative conditioning (see Section 3.4.1).

As indicated in Table 9 of Section 10.3, a score of "0" denotes absence of clinical signs of cerebral disease. Maximal signs within a domain score the total of all grades within that domain. For example, score of a subject with cortical blindness is 3, which is sum of the scores of the vision impairment/field cut and cortical blindness.

Study-specific training will be administered to the assessors and documented prior to study start and throughout the study to ensure standardization of administration of the NFS. Refer to the SOM for additional details regarding the administration of the NFS. AE and SAE reporting is described in Section 6.6.1.

6.5.7. Major Functional Disabilities (MFDs)

MFDs include loss of communication, cortical blindness, tube feeding, total incontinence, wheelchair dependence, and complete loss of voluntary movement, which are defined in Table 8 of Section 10.2. MFDs, as assessed by a pediatric neurologist or other appropriately trained and qualified physician, must be immediately reported as SAEs (See Section 6.6.4).

6.5.8. Neuropsychological Tests

The goal of neuropsychological testing is to assess the subject in key areas for signs of dysfunction due to CALD. A series of neuropsychological tests are recommended based on the subject's age at the time of the assessment. If possible, it is recommended that the same assessor administer the tests throughout the course of the study and that the tests be administered in the same order each time. Details regarding the sequence of testing administration and scoring are included in the SOM. The same test should be administered throughout the study even if the subject ages out of a particular test (e.g., if WPPSI-IV was administered during Pre-Conditioning assessments, WPPSI-IV should be administered at all subsequent visits). If an assessment cannot be administered or completed due to subject non-compliance or a language barrier, that will not be considered a protocol deviation.

Table 6: Neuropsychological Tests to be Performed

Domain	Neuropsychological Test	Age Range at Screening (years: months)	Approximate Administration Time[a]
General Intelligence	Bayley Scales of Infant and Toddler Development, 3rd ed. (Bayley-III) (Connolly et al. 2012)	< 2:6	25 minutes
	Wechsler Preschool & Primary Scale of Intelligence[b], 4th ed. (WPPSI-IV) (https://www.pearsonclinical.com/psychology.html)	2:6 to 7:7	30 minutes
	Wechsler Intelligence Scale for Children[b, c], 5th ed. (WISC-V) (https://www.pearsonclinical.com/psychology.html)	6:0 to 16:11	65 minutes
	Wechsler Adult Intelligence Scale[d], 4th ed. (WAIS-IV) (Wisdom et al. 2012)	≥ 16	60 minutes
Fine Motor	Purdue Pegboard (Gardner and Broman 1979; Wilson et al. 1982; Yeudall et al. 1986)	≥ 4	5 minutes
Health Related Quality of Life	Peds Quality of Life[e] (PedsQL) (Varni et al. 2001)		
	Parent-reported	2 to 18	45 minutes
	Subject-reported	≥ 5	15 minutes
Socioeconomic Status	SES test derived from Hollingshead and Redlich (Hollingshead and Redlich 1954, 2007; Hollingshead 2011)	All ages	5 minutes

[a] Approximate total administration time will range from: 1 to 2 hours for subjects aged 6 to 16 years and 1 hour for subjects aged 3 to 5 years; however this administration time will vary between subjects depending on reaction time, frustration thresholds, and the need for breaks.
[b] Substitute Picture Completion for Picture Concepts on Wechsler Preschool and Primary Scale of Intelligence and Wechsler Intelligence Scale for Children.
[c] Include the Visual Spatial Domain, Verbal Domain, Processing Speed, Fluid Reasoning, and Working Memory subtests of the WISC-V. For non-English speakers, only the Visual Spatial Domain, Processing Speed, and Fluid Reasoning should be included
[d] Substitute Picture Completion for Visual Puzzles on Wechsler Adult Intelligence Scale
[e] For subjects < 5 years of age, parents will complete the PedsQL. For subjects ≥ 5 years of age, both the parents and the subject will complete the appropriate PedsQL. Parent administered measures will require approximately 45 minutes and will be completed while the subject is being evaluated

Global Assessment:

Behavioral assessments will be conducted at each neuropsychological assessment per SOE. This assessment is usually done at the beginning of each session and takes approximately 2 minutes. Information about the subject's current academic status relative to his peers, in addition to qualitative assessment of the subject's attention, motivation, word retrieval, visual and auditory

processing, and ability to participate in the evaluation will be conducted. Additional details are provided in the SOM.

***General Intelligence:* Bayley Scales of Infant and Toddler Development, Wechsler Preschool and Primary Scale of Intelligence, Wechsler Intelligence Scale for Children, and Wechsler Adult Intelligence Scale**

The Bayley Scales of Infant and Toddler Development, 3rd Edition (Bayley-III) has been validated for subjects aged less than 2.6 years and takes approximately 25 minutes to administer (Connolly et al. 2012). The test was designed to identify young children with development delays by measuring performance in 5 different domains including cognition, language, social-emotional, motor and adaptive behavior.

Performance intelligence quotient (PIQ), verbal intelligence quotient (VIQ), and full scale intelligence quotient (FSIQ) will be measured using the age-appropriate Wechsler Intelligence Scales. The Wechsler Preschool and Primary Scale of Intelligence 4th Edition (WPPSI-IV) is administered to preschool and early elementary-aged children to determine their intelligence quotient (IQ) (https://www.pearsonclinical.com/psychology.html). The test is validated for children aged 2:6 to 7:7 years. The version for children ages 2:6 to 3:11 includes 4 subtests that will be used to determine composite scores for VIQ, PIQ and FSIQ. The exam takes approximately 30 minutes to administer.

The Wechsler Intelligence Scale for Children, 5th Edition (WISC-V) is an individually administered intelligence test that takes approximately 65 minutes to administer and has been validated in children aged 6 to 16:11 years (https://www.pearsonclinical.com/psychology.html).

The test can be completed without reading or writing and generates an intelligence quotient which represents a child's general cognitive ability. Validation testing was performed in children with diverse cognitive abilities ranging from gifted children, to those on the autism spectrum. WISC-V has also been validated with measures of achievement, memory, adaptive behaviour, emotional intelligence, and giftedness. Only the Nonverbal Domain, Verbal Domain, Processing Speed, Fluid Reasoning, and Working Memory subtests will be used.

The Wechsler Adult Intelligence Scale, 4th Edition (WAIS-IV) has been validated for subjects aged 16 and older and takes approximately 60 minutes to administer (Wisdom et al. 2012). The WAIS was designed to measure intelligence and includes 10 subtests that will be used to determine composite scores for Verbal Comprehension, Perceptual Reasoning, Working Memory, Processing Speed and Full-Scale IQ.

***Fine Motor:* Purdue Pegboard**

The Purdue Pegboard is a timed physical test that measures manual dexterity and brain function and is validated in children aged 4 years and older (Gardner and Broman 1979; Wilson et al. 1982; Yeudall et al. 1986). The test takes approximately 5 minutes to administer.

***Health Related Quality of Life Assessment*: Peds Quality of Life**

In the absence of a validated tool to assess Health Related Quality of Life Assessment (HRQoL) in CALD, HRQoL will be assessed using tools developed for other diseases. The PedsQL™ Generic Core Scales (Varni et al. 2001) will be administered to the caregiver and/or subject by a qualified staff member, as appropriate to the age of the subject. This test can be administered in children aged 2 to 18 years and takes approximately 15 minutes to administer. Once the subject

reaches 5 years of age, both the parent-reported and the subject-reported PedsQL questionnaires should be completed. Positive treatment effects on PedsQL will provide supporting evidence of treatment benefit. PedsQL information collected in this study will also guide development of approaches to assess HRQoL in subjects with CALD.

Socioeconomic Status

Socioeconomic status (SES) will be assessed by obtaining maternal and paternal occupations and years of education based on a formula developed by Hollingshead and Redlich (Hollingshead and Redlich 1954, 2007). The test is validated for all ages and takes approximately 5 minutes to administer. It will only be done during Pre-Conditioning assessments.

All of the neuropsychological examinations will be administered by a qualified healthcare professional according to the SOE. Study-specific training will be administered to the assessors and documented prior to study start and throughout the study to ensure standardization of administration of these tests. Refer to the SOM for additional details regarding the administration of the neuropsychological tests.

6.5.9. Evoked Potential (Electrophysiology)

Electrophysiological assessment to be performed according to the SOE, when feasible, and with consideration for the subject's age. This will include the Visual Evoked Potential (VEP) P100 latency and Brain Stem Auditory Evoked Response (BAER). VEP provides objective information about the visual system; BAER tests auditory brainstem function.

6.5.10. Brain Magnetic Resonance Imaging (Loes Score and Pattern)

Brain MRI, with and without contrast, will be performed according to the SOE; image acquisition details and shipping details are included in the Site Procedure Manual. Additional advanced imaging techniques may be performed on an exploratory basis to better characterize the degree of cerebral involvement; refer to Site Procedure Manual for additional details.

All MRIs will be assessed by a central reviewer, using the 34-point Loes scoring scale, which is widely used to diagnose and follow subjects with CALD (Loes et al. 2003). The Independent Review Manual will be used by the central reviewer. This charter describes the procedure for the image reading and scoring.

MRIs will also be used to determine white matter lesion progression using volumetric analysis.

As noted in the SOE, brain MRIs may be repeated at any time during the study if there is evidence of clinical decline. If more than 60 days has passed since the screening MRI was performed, or there is clinical suspicion of neurological decline, the MRI should be repeated ahead of myeloablative conditioning (see Section 3.4.1). However, if subject requires sedation for MRI, performing this repeat assessment is based on Investigator judgment.

6.5.11. Clinical Laboratory Tests

Laboratory tests of hematology and serum chemistries will be performed as specified in the following sub-sections and in the SOE.

Clinical laboratory tests are to be performed locally and reviewed by the Investigator or qualified designee (e.g., physician's assistant, nurse practitioner) appropriately listed on the Delegation of Responsibility Log for this task.

In addition to the peripheral blood smears, BM core biopsy sections and BM aspirate smears required for routine local hematopathology review and per the SOE, additional peripheral blood smears (see Section 6.5.11.1), BM core biopsy sections and BM aspirate smears (see Section 6.5.11.1.2) should be prepared for study purposes and submitted to a single centralized hematopathologist. Results of the central read will be provided to the Sponsor and subsequently shared with the Investigator.

Specialty laboratory evaluations including RCL testing, ISA, and VCN assessments are also to be performed. See Section 6.5.11.4 and Section 6.5.12 for further details.

All hematology and chemistry results from assessments performed at unscheduled visits should also be entered into the clinical database.

Additional instances of clinical laboratory tests listed in this section may be performed at the Investigator's discretion as needed.

Local tests and reads should be utilized for participant care decision making.

6.5.11.1. Hematology, Clinical Chemistry, Liver and Adrenal Function

Blood samples for hematology, clinical chemistry, liver and adrenal function are to be collected as specified in the SOE.

The following clinical laboratory parameters are to be determined:

Hematology: CBC with differential	
• White blood cell (WBC) count with differential • Platelet count • Red blood cell (RBC) count	• Hemoglobin • Hematocrit • Peripheral blood smear
Clinical chemistry (including Liver Function Tests)	
• Sodium (Na) • Potassium (K) • Chloride (Cl) • Magnesium (Mg) • Phosphorus (P) • Alanine aminotransferase (ALT) • Bilirubin (total and direct[a])	• Blood urea nitrogen (BUN) • Creatinine • Glucose • Calcium (Ca) • Aspartate aminotransferase (AST) • Alkaline phosphatase
Adrenal Function Tests	
• Cortisol • Aldosterone (subject in upright position)	• Adrenocorticotropic hormone (ACTH) • Plasma renin activity (subject in upright position)

a Direct bilirubin only required if total bilirubin is abnormal.

Effort should be made to obtain a platelet count 8 days after any platelet transfusion, until platelet engraftment occurs (as defined in Section 6.5.13).

6.5.11.1.1. Complete Blood Count and Peripheral Blood Smear

Complete blood counts with differential will be performed and analyzed locally at the study site per the SOE, during the first year after eli-cel treatment (Month 1 through Month 12). Subsequently, CBCs with differential will be collected every 4 months at the study site through Month 24.

A repeat performance of CBC is required within one month for any CBC abnormality (including abnormalities in hemoglobin levels, total WBC count, neutrophil count and morphology, lymphocyte count and morphology, or monocyte counts and morphology) that is of CTCAE Grade 2 or higher severity or for platelet counts of $<100 \times 10^9$/L. In addition, a re-evaluation at the discretion of the Investigator of any new abnormal peripheral blood cell count or morphology at an interval commensurate with the severity of the change is recommended. Persistent, unexpected, abnormalities of peripheral blood cell counts or morphology, including but not limited to any two consecutive (one month apart) CTCAE Grade 2 CBC values or platelet counts of $< 100 \times 10^9$/L will trigger a BM evaluation. For frequency of subsequent BM evaluations (see Section 6.5.11.1.2).

In addition, peripheral blood smears should be prepared for study purposes and submitted to a single centralized hematopathologist for review to assess for dysplastic features at Months 6, 12, 16, 20 and 24, per the SOE. Results of the central read will be provided to the Sponsor and subsequently shared with the Investigator (although centrally read peripheral smear slides will not be returned to the study site). At the investigator's discretion local peripheral blood smears may also be performed by the study site to direct patient care. Refer to the SOM for further details on preparation of additional slides for submission for central review.

6.5.11.1.2. Bone Marrow Core Needle Biopsy and Aspiration

During the first year after eli-cel treatment (at Month 6 and Month 12), BM will be collected to perform routine assessments, ISA and VCN assessments (and sample created for storage). Additional BM evaluations will also be performed in the event of one or more of the following specific findings:

i. Persistent, unexpected, abnormalities* of peripheral blood cell counts or morphology, including but not limited to any two consecutive (one month apart) CTCAE Grade 2 CBC values or platelet counts of $<100 \times 10^9$/L. If BM evaluation is triggered by platelet counts, perform BM biopsies at four month intervals until a peripheral blood platelet value of $\geq 125 \times 10^9$/L is observed.
ii. Any transfusion requirement after discharge from the initial hospitalization unless the need for transfusion was related to trauma or a procedure/intervention.
iii. Abnormal results from the most recent BM biopsy or aspirate (see below)
iv. ISA: IS in a known oncogene (i.e., relative frequency [RelFreq] of ≥ 5% for the same IS observed at two consecutive time points, if the IS is located in a gene with known biological relevance to carcinogenesis: defined as a Tier 1 gene in the Cancer Gene Census (CGC) of the Catalogue of Somatic Mutations in Cancer (COSMIC) at the time of the ISA report review.

v. ISA: persistent oligoclonality (i.e., RelFreq of ≥ 10% for the same IS observed at two consecutive time points, or relative frequencies of ≥ 5% for the same two or more IS observed at two consecutive time points).
vi. A two-fold increase in peripheral blood VCN over a 4-month period.

[*] these include abnormalities in hemoglobin levels, total WBC, neutrophil, lymphocyte, monocyte, or platelet counts and morphology.

For new findings of moderate hypocellularity (defined for this protocol as 30-40%), marked hypocellularity (<30%), moderate dysplasia (>10%-40% of cells in one or more lineages), or marked dysplasia (>40% of cells in the pertinent lineage), one repeat evaluation is required at a 3-month interval.

For new findings of moderate hypercellularity (80-90%) or marked hypercellularity (>90%), one repeat evaluation is required which may be performed at a 3 or 6-month interval, at the discretion of the Investigator.

If BM abnormalities on two consecutive assessments are stable or improved and CBC** is within normal limits, the BM biopsy and aspirate should occur every 6 months until abnormalities are resolved. If there is worsening (e.g., change from moderate to marked) of dysplasia, decreasing hypocellularity or increasing hypercellularity, or abnormalities on CBC, BM biopsies and aspirate collection frequency should remain at a 3-month interval.

[**] hemoglobin levels, total WBC count, neutrophil, lymphocyte, monocyte and platelet counts.

These recommendations are not intended to preclude re-evaluation as warranted. Additional BM evaluations should be performed, at the discretion of the Investigator and at a frequency commensurate with the integrated findings and based upon recommendations from the hematopathologist and hematologist investigator (or consulting hematologist).

Triggered BM evaluation will include both core needle biopsy and aspiration to assess morphology, cellularity, fibrosis, and to perform flow cytometry and karyotyping that should be carried out locally as part of normal pathology assessments, including local smear assessment. Additional aliquots of triggered BM aspirate will be collected and sent to centralized specialty laboratories for the performance of fluorescence in situ hybridization (FISH) (using a probe panel for common mutations in pediatric AML, MDS, and probes for MLL [11q23], MECOM, and PRDM16 rearrangement), next-generation sequencing (NGS) (spanning mutations commonly observed in myeloid and lymphoid malignancies), and ISA/VCN. Note that due to the time taken for central NGS and FISH results to be obtained, Investigators should initiate additional local NGS and FISH testing if results are needed more rapidly to direct participant care. An additional BM aspirate sample should be submitted to the Sponsor for storage to support potential future genetic/genomic studies. The BM evaluations will be performed in accordance with institutional practices under aseptic conditions. It is anticipated that institutional guidelines will specify that approximately 5 to 20 mL of BM aspirate will be collected each time. Bone marrow aspirate (and an accompanying whole blood specimen) should be collected for storage at the time of BM sample collection to facilitate subsequent exploratory analyses that may include gene/genomic expression studies.

In addition to BM core biopsy sections and aspirate smear slides created for routine local hematopathology review, additional BM core biopsy sections and aspirate smears should be prepared for study purposes and submitted to a single centralized hematopathologist. Results of the central read will be provided to the Sponsor and subsequently shared with the Investigator (although centrally read slides will not be returned to the study site).

Refer to the SOM for further details on preparation of additional slides for submission to the single central hematopathologist and for submission of samples to centralized laboratories.

6.5.11.1.3. Blood Sample Collection for RCL Testing, Integration Site Analysis, and VCN in Blood

Blood samples will be collected according to the SOE for assessments of the following:

- RCL - If subject tested negative at all visits through M12, further samples will be archived for potential analysis in case of clinical outcomes suggesting the presence of RCL.
- VCN in whole blood will be evaluated at every visit indicated for VCN in the SOE.
- ISA at visits indicated (only to be performed if VCN is ≥ 0.01 vector copies per diploid genome, at the previous visit).

Testing will be performed by central laboratories. Blood collection and processing details are included in the Laboratory Manual.

Blood samples for ISA are to be collected according to the SOE; collection, processing and shipping details are included in the Laboratory Manual. Testing will be performed by a central laboratory. Additional blood may be collected for additional investigations and analysis in cell subtypes, if requested by the Sponsor.

6.5.11.2. Immunological Studies

Local immunological testing includes measuring levels of T cell subsets (CD4, CD8), B cells (CD19), and NK cells (CD16 or CD56). In addition, levels of immunoglobulins (IgG, IgM, and IgA) will be quantified.

6.5.11.3. Serology Panel (Infectious Disease Testing)

Screening: Serology Panel I

Screening serology will be evaluated using standard methods. The serology panel for eligibility includes HIV-1 and HIV-2; hepatitis B virus surface antigen (HBsAg); anti-HBs and HBcAb; HCV Ab; and HTLV-1. Any other serology required by the contract drug product manufacturing organization or local guidelines or based on subject's risk factors or clinical evidence of infection with other communicable disease agents or disease before mobilization or infusion of drug product (including for example testing for CMV, EBV, herpes simplex virus (HSV), HCV core antigen, VZV IgG, human T lymphotrophic virus 2 (HTLV-2), syphilis, toxoplasmosis, tuberculosis, Trypanosoma cruzi, and West Nile Virus) are permitted; if any of these tests are performed and a clinically relevant result is obtained, results must be discussed with the Medical Monitor to determine eligibility.

On Day 1 of Mobilization: Serology Panel II

Blood samples will be collected according to country-specific and institutional guidelines. If screening serology (Panel I) was completed within the timeframe before HSC collection or transplant allowed by institutional and country-specific guidelines, those specific tests need not be repeated for Panel II. In case apheresis begins later than 30 days after screening, then infectious disease labs should be drawn again.

6.5.11.4. Specialty Laboratory Sample Collection

As this is a pediatric study, blood volume limitations will sometimes preclude the collection of all samples during a particular study visit. Table 7 enumerates the priorities for blood and BM collection (BM core section slides and BM aspirates). Safety labs (see Section 6.5) have been prioritized over labs for efficacy and exploratory analyses. If the results from CBC or BM tests are not as expected, additional testing may need to be performed as detailed in Section 6.5.11.1.1 and Section 6.5.11.1.2, respectively. Local tests and reads should be utilized for patient care decision making.

Per European recommendation on clinical trials conducted in the pediatric population, trial-related blood loss per individual, should not exceed 3% of the total blood volume during a period of four weeks and should not exceed 1% at any single time (*https://ec.europa.eu/health/sites/default/files/files/eudralex/vol-10/2017_09_18_ethical_consid_ct_with_minors.pdf*). A CRF is included to calculate the maximum weight-based blood draw at each study visit. If blood draw volume required exceeds this limit, samples should be obtained over multiple days. Any deviations to these recommendations should be justified. United States (US) sites may follow European recommendations or may adhere to local institutional practices.

Examples of guidelines for blood draw limits in pediatrics can be found at: *http://www.who.int/bulletin/volumes/89/1/BLT-10-080010-table-T2.html*

With regard to bone-marrow, both core needle biopsy and aspiration collection should be performed to assess cellularity, fibrosis, and to perform flow cytometry, karyotyping, FISH, NGS, ISA, and VCN. The BM evaluations will be performed in accordance with institutional practices under aseptic conditions. It is anticipated that institutional guidelines will specify that approximately 5 to 20 mL of BM aspirate will be collected each time. See Section 6.5.11.1.2 for further details on BM collection and for further details on criteria that would trigger additional BM assessments over and above those noted in the SOE.

In addition to the BM core biopsy sections and aspirate smears read locally for routine hematopathology review or per the SOE, additional BM core biopsy sections and aspirate smears should be prepared for study purposes and submitted to a single centralized hematopathologist. Additionally, BM aspirate (and an accompanying whole blood specimen) should be collected for storage at the time of BM sample collection to facilitate subsequent exploratory analyses that may include gene/genomic expression studies. See more details below for additional sample storage details. Results of the central read will be provided to the Sponsor and subsequently shared with the Investigator.

Table 7: Blood and Bone Marrow Collection: Order of Priority

Blood Collection Priority • *ABCD1* genotype • Adrenal Function Tests • Serology Panels (infectious disease screening) • Chemistry • Hematology (CBC [performed locally] and peripheral blood smear [created locally and submitted to central lab]) • Integration Site Analysis (ISA) and VCN • Immunological studies • ALDP in Peripheral Blood Populations • RCL testing • VLCFA analysis (fasting) • Whole blood specimen for storage at the time of BM assessment (to support exploratory analyses that may include gene/genomic expression)[a]
Bone Marrow Aspirate[b] Collection Priority • Core needle biopsy sections and aspirate smears for morphologic assessment, including for cellularity and fibrosis • Local FISH (if needed more rapidly than central FISH to direct patient care) • Local NGS (if needed more rapidly than central NGS to direct patient care) • ISA and VCN for central laboratories • Local karyotyping • FISH for central laboratories • NGS for central laboratories • Storage BM specimens (to support exploratory analyses that may include genetic and genomic studies)[a]

Abbrev.: BM, bone marrow; CBC
[a] Whole blood collection at the time of BM sample collection should be stored to facilitate subsequent exploratory analyses.
[b] Bone marrow evaluation should also include a core needle biopsy

Blood samples for VCN determination by quantitative polymerase chain reaction (qPCR) will be collected according to the SOE. Testing will be performed by a central laboratory. Additional blood may be collected for analysis in cell subtypes.

Replication Competent Lentivirus Testing

Blood samples for RCL testing will be collected and tested using a RCL screening assay as well as a confirmatory assay if the screening assay is positive. Testing will be performed by a central laboratory. If RCL is confirmed, the site may be requested to perform local HIV testing to determine if the RCL is drug product-related or a result of a natural HIV infection. Once available, the results of these tests must be shared with the Sponsor immediately.

ABCD1 Genotype

Blood samples for mutational analysis of the *ABCD1* gene will be collected according to the SOE if a historical result is not available. The results of the mutational analysis are required before commencing conditioning.

ALDP Expression: Peripheral Blood Populations

Blood samples for determination of ALDP expression will be collected according to the SOE. Testing will be performed by a central laboratory.

VLCFA Analysis

Blood samples for measurement of VLCFA levels in serum are to be collected in the fasting state according to the SOE to determine if VLCFA levels decline following therapy compared to Baseline levels. Testing will be performed by a central laboratory.

Storage of Samples

Bone marrow specimens (accompanied by a whole blood sample) should be submitted to the Sponsor for storage to support potential future genetic and genomic studies or other exploratory studies. Leftover biological samples from protocol procedures (e.g., whole blood DNA, serum) may also be stored (optional) for potential future exploratory investigations in those samples, to study CALD and/or gene therapy. Refer to the SOM for further details on the collection and submission of samples for storage.

Samples may be stored for up to 20 years. The Sponsor will be the custodian of the samples in the repository and any unused samples will be destroyed at the Sponsor's discretion. If possible, optional blood, BM, and tissue samples also are to be collected in the event of a subject's death if an autopsy is performed.

Collection and storage of the optional samples described above will be subject to discretionary approval from each center's IRB/IEC and the subject's specific written consent. Samples will be labeled with a unique identification number that includes no subject identifying information.

Note that apheresis product collected as part of the manufacture of the drug product may be used to study the manufacturing process. In particular, extra apheresis product may be used to understand how the process may be improved or made more robust. These potential studies are not optional.

Other potential uses of the apheresis product are for non-manufacturing improvement research, such as biomarker analyses of proteins, DNA, ribonucleic acid (RNA), and other molecules to study CALD and/or gene therapy, are optional.

6.5.12. Assessment of Oligoclonality and/or Suspicion of Malignancy

6.5.12.1. Assessment of Oligoclonality by Integration Site Analysis

ISA will be performed to determine the insertion site (IS) profile of subjects over time, as indicated in the SOE. Figure 1 shows the set of ISA rules and resulting IS triggered enhanced monitoring (i.e., CBC and BM monitoring) to assess oligoclonality, in accordance with Food and Drug Administration (FDA) Guidance (FDA 2020) and based on FDA consultation. While oligoclonality itself, or even monoclonality, will not a priori result in a malignancy, changes in IS RelFreq may be associated with an increase in the risk of a malignancy.

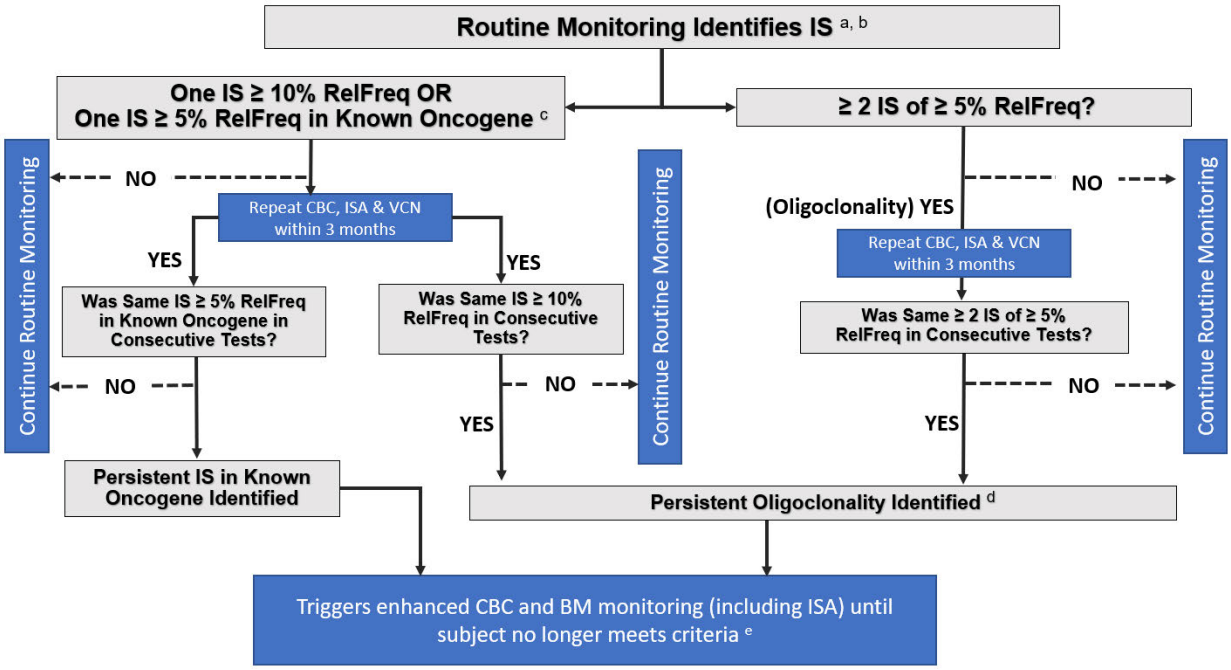
As discussed further below, ISA monitoring may be repeated more frequently if there is an indication of oligoclonality or if ISA should otherwise be triggered (see Section 6.5.11.1.2 for more details on triggered BM evaluations). Such triggers include (but are not limited to) persistent unexpected CBC abnormalities (including persistent [i.e., two consecutive] CTCAE Grade 2 CBC values), post-discharge blood transfusion (unless due to trauma or a procedure/intervention), or a two-fold increase in peripheral blood vector copy number (PB VCN) over a 4 or 6-month period (based on the SOE), or other abnormal results from a subject's most recent BM biopsy or aspirate or ISA findings of potential oligoclonality or IS in a known oncogene.

As shown in Figure 1, if an IS is detected at ≥ 10% RelFreq or an IS ≥ 5% RelFreq is detected in 2 or more IS, this would be considered "*oligoclonality*" and ISA will be repeated within 3 months of receipt of this result. If an IS ≥ 10% or 2 or more IS ≥ 5% are confirmed, then a report of "*persistent oligoclonality*" will be submitted by the Sponsor to the relevant Health Authorities within 30 days of receipt of the ISA report confirming an IS meeting the criteria for persistent oligoclonality. This repeated observation will also trigger enhanced monitoring for hematological abnormalities, increasing the frequency of CBC with differential to every 3 months (if not already occurring at the interval) until the frequency no longer meets oligoclonality criteria. Persistent oligoclonality also triggers BM assessment (for the frequency of subsequent BM assessments see [Section 6.5.11.1.2]). If persistent oligoclonality is not observed after initial repeat testing, CBC and other monitoring reverts to routine assessments per the SOE.

Additionally, if an IS is detected at ≥ 5% RelFreq in a known oncogene (based upon Tier 1 oncogenes in the CGC in the COSMIC at the time of the ISA report review), then ISA (and VCN) will be repeated within 3 months of receipt of this result. If an IS in a known oncogene is confirmed during re-evaluation it would be considered persistent. This repeated observation will trigger enhanced monitoring for hematological abnormalities, including a BM evaluation and increasing the frequency of CBC with differential to every 3 months (if not already occurring at the interval) until the frequency no longer meets criteria. ISA and VCN will continued per the SOE.

Based on clinical and ISA findings, additional monitoring for malignancy may be instituted by the treating physician/Principal Investigator or Sponsor (see Section 6.5.11.1.2 for BM evaluation recommendations and see Section 6.5.12.2 for recommended clinical work-up for malignancy). See Section 7.4.5 for definitions of current oligoclonality and current persistent oligoclonality utilized during analyses and reporting.

Figure 1: ISA Triggered Enhanced Monitoring



Abbrev.: BM, bone marrow; CBC, complete blood count with differential; IS, insertion site(s); ISA, integration site analysis; RelFreq, relative frequency; VCN, vector copy number.
a ISA, VCN, CBC schedule is specified in Section 6.1 (SOE Tables).
b IS can be measured from different types of samples (peripheral blood or BM). If an IS is found at a higher frequency in a BM sample (e.g., triggered by persistent ISA findings) than in a peripheral blood sample, then that BM frequency would be used to assess followup.
c Tier 1 oncogenes in the Cancer Gene Census of the Catalogue of Somatic Mutations in Cancer at the time of the ISA report review.
d "Persistent oligoclonality" will be reported (once per pertinent IS/set of IS) to applicable Health Authorities within 30 days of receipt of applicable repeat ISA result.
e Observation of Persistent Oligoclonality triggers increasing the frequency of CBC with differential and BM assessment to every 3 months (if not already occurring at the interval) along with ISA and VCN every 6 months until the frequency no longer meets criteria. See also Section 6.5.11.1.1 (CBC) and Section 6.5.11.1.2 (BM).

6.5.12.2. Clinical Work-up for Potential Malignancy

In the event of any suspicion of hematologic malignancy (e.g., myelodysplasia, leukemia, or lymphoma), the Medical Monitor will be notified and a work-up will be performed by the Investigator per appropriate standard of care. A suspicion of hematologic malignancy could arise in the setting of otherwise unexplained cytopenia(s), and consideration of insertional oncogenesis could arise if cytopenia(s) occurs in conjunction with a rising RelFreq of an IS, especially if that IS is in a gene of known biological relevance to carcinogenesis (i.e., oncogene or tumor suppressor gene), accompanied by a rapid increase in PB VCN (a doubling of VCN between two timepoints).

The clinical work-up may include the following:

- Physical exam

- CBC with differential - including repeat performance within one month for any CTCAE Grade 2 or higher severity CBC abnormality (these include abnormalities in hemoglobin levels, total WBC counts, platelet counts [of <100 x 10^9/L], neutrophil counts and morphology, lymphocyte counts and morphology, or monocyte counts and morphology) (see Section 6.5.11.1.1 for details).
- Lymphocyte subsets
- Imaging studies
- Local and centralized BM analysis
- Cytogenetic and molecular analyses which can include single nucleotide polymorphism (SNP) microarray, karyotyping, or whole-genome sequencing and must include centralized FISH and NGS (see Section 6.5.11.1.2 for details).

In addition, clinical work-up to rule out infectious cause or autoimmune disease may be considered. If the clinical work-up indicates a diagnosis of hematologic malignancy, the Sponsor will convene an urgent safety review meeting. Further analyses will be determined by the Sponsor, in consultation with the independent DMC. All efforts should be made to confirm the source of malignancy. It should be noted that it may not be possible to distinguish the source of malignancy (e.g., arising from underlying pathophysiology of the disease, transplant-related medications or procedures, or from expansion of gene-modified cells due to insertional oncogenesis). In cases of vector-containing hematologic malignancy, single BM colony in combination with IS-specific polymerase chain reaction (PCR) should be used to confirm the specific IS present in the same clone.

For clinical work-up after identification of persistent oligoclonality and confirmed presence of abnormal CBC, BM analysis should be performed as warranted if not previously part of the clinical work-up or as triggered based upon Section 6.5.11.1.2 or as discussed in Section 6.5.11.4.

6.5.13. Engraftment and Engraftment Failure

For the purposes of this protocol, successful neutrophil engraftment is defined as 3 consecutive ANC laboratory values of $\geq 0.5 \times 10^9$cells/L (after initial post-infusion nadir) obtained on different days by Day 43. Platelet engraftment is defined as achieving 3 consecutive unsupported platelet counts of $\geq 20 \times 10^9$ cells/L (after initial post-infusion nadir) obtained on different days while no platelet transfusions are administered for 7 days immediately preceding and during the evaluation period.

Neutrophil engraftment failure will be defined as follows:

- Primary neutrophil engraftment failure: failure to achieve 3 consecutive ANC laboratory values of $\geq 0.5 \times 10^9$cells/L (after initial post-infusion nadir) obtained on different days by 42 days post-infusion of eli-cel
- Secondary neutrophil engraftment failure: i) achievement of neutrophil engraftment followed by ii) sustained decline in ANC to $< 0.5 \times 10^9$ cells/L for 3 consecutive measurements on different days after 42 days post-infusion of eli-cel, without alternate etiology

6.6. Adverse Events

Monitoring of AEs will be conducted from the signing of informed consent. AEs for all subjects (excluding screen failures) will be recorded in the CRFs starting from the time informed consent is signed through the Month 24 Visit. All SAEs (including screen failures) will be reported from the signing of informed consent/assent on the SAE report form.

All AEs should be monitored until they are resolved or are clearly determined to be due to a subject's stable or chronic condition or inter-current illness(es). For subjects who withdraw for reasons other than withdrawal of consent, any SAEs open at the time of discontinuation should be followed-up until resolution or are determined to be a stable or chronic condition.

Note that at the completion of Study ALD-104, subjects are expected to enroll into a long-term follow-up study (LTF-304), that will monitor the safety of subjects (including drug product-related AEs) through a total of 15 years after eli-cel infusion.

6.6.1. Adverse Events Definitions

6.6.1.1. Adverse Events

An AE is any untoward medical occurrence associated with the use of a drug in subjects, whether or not considered drug related. An AE may include a change in physical signs, symptoms, and/or clinically significant laboratory change occurring in any phase of a clinical study. This definition includes intercurrent illnesses or injuries, and exacerbation of pre-existing conditions. A pre-existing condition is a clinical condition (including a condition being treated) that is diagnosed before the subject signs the Informed Consent Form (ICF) and is documented as part of the subject's medical history.

6.6.1.2. Unexpected Adverse Events

An AE is considered unexpected with eli-cel if it is not consistent in nature or severity with the eli-cel reference safety information which is contained in the current Lenti-D Drug Product IB.

6.6.1.3. Conditioning-related Adverse Events

Busulfan and fludarabine are cytotoxic drugs that cause profound myelosuppression. Accordingly, subjects will experience intended hematologic events (e.g., neutropenia, thrombocytopenia, anemia) and expected non-hematologic events (e.g., mucositis [stomatitis], nausea, vomiting, alopecia, pyrexia) as a result of receiving busulfan IV and fludarabine IV. For the purposes of this protocol, the prescribing information for busulfan and fludarabine should be consulted for full information on these side effects.

The intended profound myelosuppression (manifested by neutropenia, thrombocytopenia, and/or anemia) and expected events that occur after the initiation of busulfan IV and fludarabine IV conditioning are considered to be the direct consequence of the conditioning regimen and are to be reported as AEs, but should be attributed to conditioning on the AE eCRF, as applicable.

6.6.1.4. Serious Adverse Events

An SAE is any AE that:

- Results in death.
- Is life-threatening. i.e., the subject was at immediate risk of death at the time of the event. It does not include an AE that, had it occurred in a more severe form, might have caused death.
- Requires in-patient hospitalization or prolongation of existing hospitalization. Hospitalization admissions occurring during the study period that are for procedures *planned prior to study entry* do not meet this criterion, unless there is a complication resulting from procedure that prolongs hospitalization.
- Results in persistent or significant disability/incapacity; or a substantial disruption of a subject's ability to conduct normal life functions.
- Is a congenital anomaly/birth defect.
- Is an important medical event.
  - An important medical event is an AE that may not result in death, be life-threatening, or require hospitalization but may be considered serious when, based upon appropriate medical judgment, it may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs.
  - For the purposes of this study, any new malignancy or new diagnosis of a neurologic, rheumatologic, or hematologic disorder that, in the Investigator's opinion, is clinically significant and requires medical intervention will be considered medically important and therefore serious.

The following should be reported as SAEs for the purposes of this study (see Section 6.6.4 for SAE reporting):

- MFDs, as defined in Section 10.2
- Engraftment failure, as defined in Section 6.5.13

6.6.2. Adverse Event Assessment of Severity and Relationship

For all AEs, the Investigator must determine the severity of the event and the relationship to eli-cel.

For immediate reporting of SAEs, the Investigator must provide assessments of relationship and serious criteria at the time of SAE report submission to the Sponsor.

Severity will be assessed by the Investigator using the following criteria per the NCI CTCAE, Version 4.03, including for AEs that are a result of a laboratory abnormality.

- **Grade 1**: Mild, asymptomatic, or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- **Grade 2**: Moderate; minimal, local, or noninvasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)
- **Grade 3**: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL.
- **Grade 4**: Life-threatening consequences; urgent intervention indicated.
- **Grade 5**: Death related to AE.

Relationship: The Investigator is required to provide an assessment of the relationship of eli-cel to all AEs. The following is a guideline for determining the relationship of eli-cel to an AE:

- **Not Related:** Exposure to the study treatment did not occur, or the occurrence of the AE is not reasonably related in time, or the AE is considered not related to eli-cel.
- **Unlikely Related:** The study treatment and the AE were not closely related in time, and/or the AE could be explained more consistently by causes other than exposure to eli-cel.
- **Possibly Related:** The study treatment and the AE were reasonably related in time, and the AE could be explained equally well by causes other than exposure to eli-cel.
- **Related:** The study treatment and the AE were reasonably related in time, and the AE was more likely explained by exposure to eli-cel than by other causes, or the study treatment was the most likely cause of the AE.

For the purpose of safety reporting and analyses, all AEs that are classified as “possibly related” or “related” will be considered eli-cel treatment-related events.

6.6.3. Procedures for AE and SAE Collection and Reporting

Each subject must be carefully monitored for the development of any AEs. This information should be obtained in the form of non-leading questions (e.g., “How are you feeling?”) and from signs and symptoms detected during each examination, laboratory assessments, observations of study personnel, and spontaneous reports from subjects.

AEs for all eligible subjects (i.e., excluding screen failures) will be recorded in the CRF. Any clinically significant laboratory abnormality or other clinically significant finding is considered an AE and the AE must be recorded on the appropriate pages of the CRF. The diagnosis/underlying etiology rather than the signs/symptoms should be reported as the AE, when possible. If no diagnosis is available, report only the signs and symptoms that met AE criteria as individual AE terms.

6.6.4. Immediate Reporting of SAEs

All SAEs for all subjects, including screen failures, must be immediately reported on the SAE report form to the Sponsor (or designee) within 24 hours of the Investigator (or designee) becoming aware of the SAE. All SAEs must be reported whether or not they are considered causally related to eli-cel. The SAE report form completion guidelines can be found in the SOM. All follow-up information on SAEs must also be immediately reported to the Sponsor or designee (i.e., within 24 hours).

Copies of all SAE reports and associated documentation submitted to the Sponsor or designee will be kept in the Investigator's study site file.

Please refer to the SAE report form and associated guidelines for information on how to immediately submit SAE reports to the Sponsor or designee.

6.6.5. Safety Reporting to Regulatory Authorities, Ethics Committees, and Investigators

If there are suspected unexpected serious adverse reactions (SUSARs) associated with the use of eli-cel, the Sponsor or designee will notify the appropriate regulatory agency(ies), central ethics committees, and all participating Investigators in accordance with applicable regulations.

The Investigator or Sponsor will notify the IRB/EC and other appropriate institutional regulatory bodies of any SUSARs or unanticipated problems, in accordance with local requirements.

6.7. Pregnancy and Contraception

Pregnancy of female partners is neither an AE nor an SAE, unless a complication relating to the pregnancy occurs (e.g., spontaneous abortion, which requires reporting as an SAE). However, all pregnancies of female partners occurring during this study are to be reported in the same time frame as SAEs using the Pregnancy Form. SAEs experienced by a female partner of a male subject during the course of the pregnancy are required to be immediately reported (i.e., within 24 hours) on the SAE report form.

The course of all pregnancies, including perinatal and neonatal outcome, regardless of whether the subject has discontinued participation in the study, will be followed until outcome, including follow-up of the health status of the newborn at 6 weeks of age and annually thereafter for 2 years. SAEs experienced by the newborn within 6 weeks of age are required to be immediately reported (i.e., within 24 hours) on the SAE report form. In cases of a male study subject, pregnancies resulting from sperm banking prior to the receipt of drug product will not be followed.

Busulfan has been shown in animal studies to be teratogenic (see package insert for additional details). Male subjects and their female partners are required to use two different effective methods of contraception from Screening through at least 6 months after drug product infusion. Birth control methods considered to be highly effective include hormonal contraception associated with inhibition of ovulation, intrauterine device (IUD), intrauterine hormone-releasing system (IUS), bilateral tubal occlusion, vasectomized partner, and sexual abstinence. Acceptable birth control methods which may not be considered as highly effective include progestogen-only oral hormonal contraception where inhibition of ovulation is not the primary mode of action,

male or female condom with or without spermicide, and cap, diaphragm, or sponge with spermicide (http://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf). If subjects are truly sexually abstinent (where true sexual abstinence should be defined as being in line with the preferred and usual lifestyle of the subject), no second method is required. (Periodic abstinence [calendar, symptothermal, post-ovulation methods], withdrawal [coitus interruptus], spermicides only, and lactational amenorrhoea method are not acceptable methods of contraception). Beyond 6 months, subjects should discuss with their physician prior to resuming unprotected intercourse.

6.8. Unscheduled Visits

Unscheduled visits may be performed at any time during the study whenever necessary to assess for or to follow-up on AEs or as deemed necessary by the Investigator. Evaluations and procedures to be performed at unscheduled visits will be at the Investigator's discretion in consultation with the Sponsor and may be based on those listed in the SOE. Unscheduled visits, including any unscheduled assessments or procedures performed at the visit, should be promptly entered into the CRF.

6.9. Long-term Follow-Up Protocol

All subjects will be followed for 24 months after drug product infusion under this protocol unless they withdraw consent. For subjects with 24 months follow-up, if appropriate consent (or assent if applicable) is obtained, subjects will be followed for an additional 13 years under a separate long-term follow-up protocol (LTF-304) which will focus on long-term safety and efficacy.

7. STATISTICAL PROCEDURES

Details of the statistical analysis will be provided in a separate document (the Statistical Analysis Plan [SAP]). This section provides a general overview of these plans.

7.1. Sample Size Estimation

The number of subjects planned to be infused with eli-cel is approximately 35.

The sample size of 35 subjects will provide a 95% two-sided exact confidence interval (CI) for the estimated MFD-free survival rate that is at most 34.6% wide (dependent on the observed MFD-free survival rate). If 17 out of 35 subjects are MFD-free, the exact 95% CI will be (31.4%, 66.0%) with a width of 34.6%; if 26 out of 35 subjects are MFD-free, the exact 95% CI will be (56.7%, 87.5%) with a width of 30.8%. This sample size is appropriate for nominal comparisons with other CALD studies. In study ALD-101, the allo-HSCT treated cohort meeting similar eligibility criteria as that of ALD-104, that excluded matched siblings had an MFD-free survival rate of 76% with exact 95% CI of 50.1% to 90.4%; while the untreated cohort had a MFD-free survival rate of 21% with exact 95% CI of 6.1% to 45.6%.

7.2. Populations for Analysis

Three populations will be evaluated for efficacy and safety.

The **Intent-to-treat (ITT)** population will consist of those subjects who initiate any study procedures, beginning with mobilization by G-CSF.

The **Transplant Population (TP)** will consist of subjects who receive eli-cel.

The **Successful Neutrophil Engraftment Population (NEP)** will be defined as those subjects who 1) receive eli-cel; 2) engraft (as defined in Section 6.5.13)

7.3. Planned Analyses

7.3.1. Interim Analysis

Interim analyses are planned in support of regulatory submissions. The timing of these analyses and the number of subjects included in each analysis will take into account specific requests from regulatory agencies and applicable regulatory guidance. The rationale for each analysis will be documented.

7.3.2. Final Analysis

A final analysis will be performed when all subjects treated with eli-cel complete the study.

7.3.3. Additional Data Review

Analyses of study data may also be performed for the purposes of internal data review, preparing for regulatory meetings, and updating the scientific community.

Safety data are reviewed on an ongoing basis for signal detection and to support preparation of regulatory submission documents.

7.3.4. Impact of the COVID-19 Pandemic

A review will be performed to determine which assessments are likely to have been affected by the COVID-19 pandemic, and if applicable, analyses will be performed to measure the effect of disruptions due to the pandemic on these assessments.

7.4. Statistical Methods

7.4.1. General Methods

Statistical analyses are planned to support the study objective of evaluating the efficacy and safety of eli-cel after myeloablative conditioning with busulfan and fludarabine in subjects with CALD. This study is a single arm, non-randomized, open-label study due to the rarity and severity of the disease. Therefore, statistical methods will be primarily descriptive in nature, and will include point estimates and confidence limits as appropriate.

Tabulations will be produced for appropriate demographic, baseline, efficacy, safety, and exploratory parameters. For categorical variables, summary tabulations of the number and percentage of subjects within each category of the parameter will be presented. For continuous variables, the number of observations, mean, median, standard deviation, minimum, and maximum values will be presented, along with the 2-sided 95% CI of the mean, as appropriate. Time-to-event data will be summarized using Kaplan-Meier methodology. The number and percent of censored observations and events will also be presented. For proportion endpoint variables, we presented the proportion along with 2-sided exact CIs of the proportion, as appropriate.

All study data will be included in subject level listings. For primary and secondary efficacy endpoints, Loes score and pattern, and the neuropsychological and quality-of-life endpoints, Baseline is defined as the closest timepoint that is prior to conditioning. For safety endpoints and all laboratory data, Baseline is defined as the closest timepoint that is prior to mobilization.

In addition to the analyses described below, supportive efficacy and safety analyses will be performed on the ITT population and the NEP for some of the study endpoints, as detailed in the SAP. Note, however, that in the likely event that there is no difference in the ITT and TP populations, reference to ITT analyses will not be necessary.

If a subject treated with eli-cel undergoes an allo-HSCT in Study ALD-104, the following additional analyses may be conducted for two periods (if sample size is sufficient):

- For safety and PD endpoints, the first period begins from day of eli-cel infusion and will end on the day before initiation of conditioning for the subsequent allo-HSCT, and the second period will begin from the day of initiation of conditioning for allo-HSCT and will end at the date of last contact. For subjects who do not undergo conditioning prior to allo-HSCT, the day before allo-HSCT will be used.
- All the efficacy, PD, and safety endpoints will be included in the listings.

7.4.2. Disposition of Subjects

A tabulation of the disposition of subjects will be presented, including the number enrolled, the number with any post-drug product infusion data available for analysis, and the extent of data available. The number of subjects in each analysis population will be presented, with reasons for

exclusion from any specific population. A tabulation of the disposition of subjects in the study ALD-104 ITT population will be presented overall and by investigational site, for the following:

- Number and percent of subjects who initiated mobilization
- Number and percent of subjects who initiated conditioning
- Number and percent of subjects who were infused with eli-cel
- Number and percent of subjects who discontinued study, and reason for discontinuation
- Number and percent of subjects who completed study, and subjects who will participate in the long-term follow-up study LTF-304
- Number and percent of subjects who are in study at the time of data cut
- Descriptive statistics for the duration of follow-up for subjects who were infused with eli-cel
- Subject-years of follow-up, which is the sum over all subjects' duration of follow-up
- The number of subjects receiving allo-HSCT, and the reason for receiving allo-HSCT during the study.

7.4.3. Demographic and Baseline Characteristics

The following demographic and Baseline characteristic factors will be summarized: age (age at enrollment, age at CALD diagnosis), sex, age at eli-cel infusion, height at screening, weight at screening, body mass index (BMI) at screening, country of origin, race and ethnicity, family history, time from informed consent to eli-cel infusion, signs and symptoms of CALD, method of diagnosis of CALD, number of prior gadolinium scans, NFS and Loes at Baseline, the presence of any significant co-morbid conditions, availability of matched sibling donor and time from diagnosis of CALD to treatment. Subject genotype will be presented in a listing.

7.4.4. Analysis of Efficacy Endpoints

7.4.4.1. Analysis of Primary Efficacy Endpoint

Population to be analyzed

The primary analysis of the primary efficacy endpoint, Month 24 MFD-free survival, will be performed on the TP. Analyses will also be performed on the ITT (if the ITT is different from the TP) and NEP, which will be considered as supportive (i.e., as sensitivity analyses) of the results in the TP.

Analysis

In order to be considered a success for the primary endpoint, a subject must be alive at or after the Month 24 Visit, and must not develop an MFD (i.e., subjects who do not have an MFD evaluation at Month 24, but are MFD-free after the Month 24 Visit window, will be considered a success for the primary endpoint). Subjects who (i) die or develop an MFD on or before their Month 24 Visit, (ii) withdraw or are lost to follow-up before their Month 24 Visit, (iii) require (and undergo) rescue cell administration or a second transplant before their Month 24 Visit, will be considered treatment failures in this primary efficacy analysis.

7.4.4.2. Analysis of Secondary and CCI Efficacy Endpoints

Populations to be analyzed

The analyses of the secondary and exploratory efficacy endpoints (Section 2.2.1) will be performed on the TP unless otherwise specified.

Analyses

The following time-to-event endpoints will be analyzed using the Kaplan-Meier method:

- MFD-free survival over time.

  Time from drug product infusion to either a rescue cell administration or second transplant, MFD, or death due to any cause, whichever occurs first, will be analyzed. Subjects who do not experience a rescue cell administration or second transplant, MFD, or death, and subjects who discontinue the study prematurely, will be censored at the time of the last observation at which they were MFD-free.

- Overall survival

All other secondary and exploratory efficacy endpoints will be descriptively summarized.

In addition, the change over time in individual efficacy parameters will be described by presentation of summary statistics at each protocol scheduled evaluation time. Box-plots and by subject plots by visit may be presented as appropriate.

7.4.5. Analysis of Safety Endpoints

The subject counts, percentages, and 95% CI (as appropriate) will be presented for following endpoints in the TP:

- The proportion of subjects with neutrophil engraftment after drug product infusion (primary safety endpoint)
- The proportion of subjects who experience either acute (≥ Grade II) or chronic GVHD by Month 24
- The proportion of subjects with platelet engraftment by Month 24
- The proportion of subjects with loss of neutrophil engraftment post-drug product infusion by Month 24
- The proportion of subjects who undergo a subsequent HSC infusion by Month 24
- The proportion of subjects who experience transplant-related mortality through 100 and 365 days post-drug product infusion
- Proportion of subjects with clinical ≥ Grade 3 AEs, all drug product-related AEs, all SAEs, ≥ Grade 3 infections, and clinically significant changes in laboratory parameters by Month 24
- The proportion of subjects who experience ≥ Grade II acute GVHD by Month 24
- The proportion of subjects who experience chronic GVHD by Month 24
- The number of subjects in which vector-derived RCL is detected by Month 24
- The number of subjects with insertional oncogenesis (myelodysplasia, leukemia, lymphoma, etc.) by Month 24
- The number of subjects with persistent oligoclonality by Month 24

The following endpoints will be descriptively summarized for the TP:

- Time to neutrophil engraftment post-drug product infusion
- Time to platelet engraftment post-drug product infusion
- Number of emergency room visits (post-neutrophil engraftment) by Month 24
- Number and duration of in-patient hospitalizations (post-neutrophil engraftment) by Month 24
- Number and duration of ICU stays (post-neutrophil engraftment) by Month 24

In addition, the general safety profile of treatment with eli-cel will be summarized in the ITT through the evaluation of all AEs, laboratory assessments, vital signs, ECG and physical examination findings, as appropriate, during the study.

Transplant-related Mortality

Transplant-related mortality will be summarized for 2 intervals: from Study Day 1 through 100 days post-drug product infusion, and from Study Day 1 through 365 days post-drug product infusion. The number and percent of transplant-related deaths as well as the exact 95% CIs will be presented for the TP.

Adverse Events

All subjects receiving any part of at least 1 dose of the mobilizing agent (G-CSF) prior to eli-cel infusion will be evaluated for safety (the ITT population). The safety analyses will include evaluation of the incidence of treatment emergent AEs (i.e., AEs that occur at or after the initiation of eli-cel infusion) by preferred term and body system coded using the Medical Dictionary for Regulatory Activities (MedDRA). AEs will be summarized for those events that occur:

1. from signing the informed consent up to the start of mobilization;
2. from the start of mobilization up to the start of conditioning;
3. from the start of conditioning until the day before neutrophil engraftment;
4. from the day of neutrophil engraftment through 12 months post-drug product infusion;
5. from the day after 12 months post-drug product infusion through the entire 24-month study period.
6. from the start of eli-cel infusion through the entire 24-month study period.

See the SAP for a comprehensive list of study periods and additional details.

All AEs will be tabulated by System Organ Class (SOC) and Preferred Term (PT) for each of the study periods defined above. The following AEs will also be tabulated in the same manner: SAEs, CTCAE ≥ Grade 3 AEs, all drug product-related AEs, all CTCAE ≥ Grade 3 infections, AEs leading to early termination, and AEs leading to death.

All AEs occurring on study will be provided in data listings. Additionally, deaths will also be listed.

Laboratory Data

Baseline for laboratory data will be the value closest to but prior to mobilization.

The actual value and change from baseline to each study Visit and to the last on-study assessment will be summarized for each clinical laboratory parameter (hematology, clinical chemistry, and liver function tests) using the ITT population.

Potentially clinically significant (PCS) values will be defined and analyzed in the SAP.

Oligoclonality

Persistent oligoclonality at any time is defined as an IS ≥10% RelFreq in an initial ISA, and the IS ≥10% RelFreq results are confirmed in the subsequent ISA; or at least 2 IS ≥5% RelFreq in an initial ISA, and the same IS ≥5% RelFreq results are confirmed in the subsequent ISA.

Current persistent oligoclonality is defined as an IS ≥10% relative frequency in an initial ISA, that is confirmed in the subsequent ISA as still being an IS ≥10% relative frequency and the

≥10% relative frequency is maintained through the last two ISA (as of a specific data cut); or at least 2 IS ≥5% relative frequency in an initial ISA and the same IS ≥5% relative frequency results are confirmed in the subsequent ISA, and the ≥5% relative frequency is maintained through the last two ISA (as of a specific data cut). Current oligoclonality is defined as an IS ≥10% relative frequency that is observed at the first time at the most recent ISA as of a specific data cut or at least 2 IS ≥5% relative frequency observed at the first time at the most recent ISA as of a specific data cut (i.e., not yet confirmed at a repeat assessment).

Vital Signs and Physical/Neurological Examinations

The change from Baseline to each on-study evaluation will be summarized for each parameter of vital signs. A data listing of vital signs will be provided.

Physical/neurological examination results, including status (normal/abnormal), will be presented in a subject level listing.

Echo- and electro-cardiograms

Echo- and electro-cardiogram data for each subject will be provided in a data listing.

Concomitant Medications

Concomitant medications will be coded using the World Health Organization (WHO) Drug Dictionary (the version of the WHO Drug Dictionary will be determined at the time of analysis). Results will be tabulated by anatomic therapeutic class and preferred term. Medications will be tabulated by interval, including ICF to < mobilization (M), M to < conditioning (C), C to < neutrophil engraftment (NE), NE to Month 12, >M12 to M24, D1 to Month 24.

8. ADMINISTRATIVE AND REGULATORY REQUIREMENTS

8.1. Good Clinical Practice (GCP)

The study will be conducted in accordance with the ICH Guideline for GCP and all other applicable local regulatory requirement(s). The consent (or assent if applicable) process will be performed in accordance with local regulations and site specific institutional practice, if applicable. The Investigator will be thoroughly familiar with the appropriate use of the eli-cel as described in the protocol and IB. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Site Master Files should be established at the beginning of the study, maintained for the duration of the study, and retained according to the appropriate regulations.

8.2. Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB/IEC will review all appropriate study documentation in order to safeguard the rights, safety, and well-being of the subjects. The study will only be conducted at sites where IRB/IEC approval has been obtained. The Protocol, IB, ICF, advertisements (if applicable), written information given to the subjects, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC.

8.3. Subject Information and Informed Consent

After the study has been fully explained, written informed consent will be obtained from the subject's parent, guardian, or legal representative prior to study participation. For subjects who have already received eli-cel, an ICF addendum may be used to share protocol updates or new information with the subject. If a subject is below the legal age of consent at the time of ICF signing and turns the legal age of consent per country regulations while on study, the subject must be re-consented at the next scheduled study visit, prior to the collection of additional study data. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s). In addition, if consistent with local regulations, the Investigator will seek assent from the subject if he is at least 7 years of age. Sites will follow their standard institutional practice for obtaining informed consent. See Section 3.1. Consent to this study indicates consent to 15 years of follow up, with the first 2 years in this Study ALD-104 and 13 additional years in long-term follow-up Study LTF-304.

8.4. Subject Confidentiality

In order to maintain subject privacy, all CRFs, eli-cel accountability records, study reports, and communications will identify the subject using initials (in compliance with local regulation) and the assigned subject number. The Investigator will grant monitor(s) and auditor(s) from bluebird bio or its designee and regulatory authority(ies) access to the subject's original medical records for verification of data gathered on the CRFs and to audit the data collection process. The subject's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations.

8.5. Protocol Compliance

The Investigator will conduct the study in compliance with the protocol provided by bluebird bio, and given approval/favorable opinion by the IRB/IEC and the appropriate regulatory authority(ies). Modifications to the protocol should not be made without agreement of both the Investigator and bluebird bio. Changes to the protocol will require written IRB/IEC approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to subjects. The IRB/IEC may provide, if applicable regulatory authority(ies) permit, expedited review and approval/favorable opinion for minor change(s) in ongoing studies. bluebird bio will submit all protocol modifications to the regulatory authority(ies) in accordance with the governing regulations.

When immediate deviation from the protocol is required to eliminate an immediate hazard(s) to subjects, the Investigator will contact bluebird bio, if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be fully described in the CRF and source documentation.

8.6. Direct Access to Source Data

Monitoring and auditing procedures developed by bluebird bio will be followed in compliance with GCP guidelines.

The study will be monitored by bluebird bio or its designee. Monitoring will be done by visits from a representative of the Sponsor (site monitor) and will include on-site and/or remote review of the CRFs for completeness and clarity, cross-checking with source documents, and clarification of administrative matters. The review of medical records will be performed in a manner to ensure that subject confidentiality is maintained.

The site monitor will ensure that the investigation is conducted according to protocol design and regulatory requirements by frequent communications (letter, e-mail, telephone, and fax).

Regulatory authorities, the IEC/IRB, and/or bluebird bio's clinical quality assurance group may request access to all source documents, CRFs, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the Investigator, who must provide support at all times for these activities.

8.7. Electronic Case Report Form Completion

bluebird bio, Inc. will provide the study sites with an eCRF for each subject. Required study data will be captured on eCRFs via electronic data capture (EDC) unless otherwise specified in this document. Except for data points for which the protocol or SOM indicate that the eCRF may serve as source documentation, data are to be obtained from the subject's source documents and then entered into the eCRF by authorized site personnel. Clinical data that are not recorded on the eCRF will be electronically captured and transferred to the Sponsor or its designee through a secure external data transfer process.

It is the Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the subject's eCRF. Source documentation supporting the eCRF data should indicate the subject's participation in the study and should document the dates and details of study procedures, AEs, and subject status.

The Investigator, or designated representative, should complete the eCRF as soon as possible after information is collected, preferably on the same day that a study subject is seen for an examination, treatment, or any other study procedure. Any outstanding entries must be completed immediately after the final examination. An explanation should be given for all missing data.

The Investigator must sign and date the Investigator's Statement at the end of the eCRF to endorse the recorded data.

bluebird bio will retain the originals of all CRFs. The Investigator will retain a copy of all completed eCRFs.

8.8. Record Retention

The Investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s). Records will be retained for at least 2 years after the last marketing application approval or 2 years after formal discontinuation of the clinical development of the investigational product or according to applicable regulatory requirement(s). If the Investigator withdraws from the responsibility of keeping the study records, custody must be transferred to a person willing to accept the responsibility. bluebird bio, Inc. must be notified in writing if a custodial change occurs.

The Sponsor has full rights over any invention, discovery, or innovation, patentable or not, that may occur when performing the study.

8.9. Liability and Insurance

bluebird bio, Inc. has subscribed to an insurance policy covering, in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards.

8.10. Publication and Presentation of Study Findings and Use of Information

All information regarding eli-cel supplied by bluebird bio to the Investigator is privileged and confidential information. The Investigator agrees to use this information to accomplish the study and will not use it for other purposes without consent from bluebird bio. It is understood that there is an obligation to provide bluebird bio with complete data obtained during the study. The information obtained from the clinical study will be used towards the development of eli-cel and may be disclosed to regulatory authority(ies), other Investigators, corporate partners, or consultants as required.

It is anticipated that the results of this study will be presented at scientific meetings and/or published in a peer reviewed scientific or medical journal. A Publications Committee comprised of Investigators participating in the study and representatives from bluebird bio, as appropriate, will be formed to oversee the publication of the study results, which will reflect the experience of all participating study centers. Subsequently, individual Investigators may publish results from the study in compliance with their agreement with bluebird bio.

A pre-publication manuscript is to be provided to bluebird bio at least 30 days prior to the submission of the manuscript to a publisher. Similarly, bluebird bio will provide any company prepared manuscript to the Investigators for review at least 30 days prior to submission to a publisher.

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10. APPENDICES

10.1. Dose Adjustment Formulas for Ideal Body Weight

Ideal Body Weight Formulas (Subjects 1 to 18 Years of Age):

Height (ht) less than 60 inches (less than 152 cm)

IBW = [(ht^2) × 1.65] ÷ 1000, where ht = cm, IBW = kg

Height (ht) greater than 60 inches (greater than 152 cm)

Males IBW = 39 + [2.27 × (ht – 60)], where ht = inches, IBW = kg

Adjusted Ideal Body Weight Formula:

AIBW = IBW + [(0.25) × (ABW- IBW)], where ABW= actual body weight

10.2. Major Functional Disabilities

The 6 disabilities considered MFDs in this study are defined in Table 8, and these were chosen based on their clinical significance and their impact on independent functioning.

Table 8: Major Functional Disabilities

MFD	Definition
Loss of communication	Individual should meet one of the following criteria (psychogenic syndromes, such as catatonia, should be ruled out): (1) With normal consciousness and ability to perform movements, individual does not follow command and/or permanently fails to perform verbal or nonverbal simple task on neurologic evaluation, or (2) Individual is permanently mute and unable to communicate by verbal or non-verbal ways.
Cortical blindness	Individual fails to visually track, find objects, or count fingers. Individual has permanent and complete vision loss affecting bilateral vision. Pupils may react to light.
Tube feeding	Individual is not able to swallow safely by mouth to maintain nutrition and hydration. Alternative method of feeding required.
Wheelchair dependence	Individual is unable to take more than a few steps, restricted to wheelchair; may need aid to transfer; wheels himself, but may require motorized chair for full day's activities.
Complete loss of voluntary movement	Individual is unable to effectively use his upper and lower extremities to perform simple or one-step activities. The criteria may still be met if there are singular apparently random movements of the arms.
Total incontinence	In an individual who was previously continent, the permanent and continuous loss of urinary and/or fecal control.

10.3. Neurologic Function Score for ALD

The NFS is a 25-point composite scale that assesses functional disabilities (Moser et al. 2000). It was designed by Dr. Gerald Raymond and colleagues specifically for the consistent and reproducible clinical evaluation of patients with CALD. It assesses 15 functional domains affected by the disease and is the most common clinical evaluation tool used by clinical specialists caring for these patients (Moser et al. 2000; Miller et al. 2011).

Assessment of subject status using NFS is to be performed by a pediatric neurologist or other appropriately trained and qualified physician as described in Section 6.5.6 .

Table 9: Neurologic Function Score for CALD

Symptom / Neuroexam	Definition	Score
Hearing / auditory processing problems	Individual with previously normal hearing develops permanent auditory processing difficulties and impairment of comprehension to verbal sounds on neurologic evaluation.	1
Aphasia / apraxia	Individual should meet one of the following two criteria: (1) Individual with previously age appropriate speech and language development has impaired fluency or naming or repetition or content or comprehension or motor speech on the clinical examination; patient may have partial or incomplete aphasia or motor speech disorder of the speech, or (2) Individual with newly developed apraxia. Apraxia can be defined as 'loss of the ability to execute or carry out any complicated learned and purposeful movements, despite having the desire and the physical ability to perform the movement', examples of apraxia include, but are not limited to, limb-kinetic apraxia, ideomotor apraxia, conceptual apraxia, speech apraxia etc.	1
Loss of communication	Individual should meet one of the following criteria (psychogenic syndromes, such as catatonia, should be ruled out): (1) With normal consciousness and ability to perform movements, individual does not follow command and/or permanently fails to perform verbal or nonverbal simple task on neurologic evaluation, or (2) Individual is permanently mute and unable to communicate by verbal or non-verbal ways.	3
Vision impairment /field cut	An individual with previously normal (corrected) vision develops visual field defect affecting one or both eyes, and/or maximal visual acuity (corrected) worse than 20/30 using bedside pocket vision screening card.	1
Cortical blindness	Individual fails to visually track, find objects, or count fingers. Individual has permanent and complete vision loss affecting bilateral vision. Pupils may react to light.	2
Swallowing / other CNS dysfunctions	Swallowing is safe; however individual requires minimal cueing to use compensatory strategies. The individual may occasionally self-cue. All nutrition and hydration needs are met by mouth at mealtime.	2
Tube feeding	Individual is not able to swallow safely by mouth to maintain nutrition and hydration. Alternative method of feeding required.	2
Running difficulties / hyperreflexia	An individual with previously normal gait develops minimal but permanent difficulties during running. He may be fully ambulatory without aid, or may have some limitation of full activity or requires minimal assistance.	1
Walking difficulties / spasticity / spastic gait (no assistance)	Individual develops walking difficulties but is ambulatory without aid; disability severe enough to preclude full daily activities.	1

Table 9: Neurologic Function Score for CALD

Symptom / Neuroexam	Definition	Score
Spastic gait (needs assistance)	Individual requires constant bilateral assistance (canes, crutches, braces).	2
Wheelchair dependence	Individual is unable to take more than a few steps, restricted to wheelchair; may need aid to transfer; wheels himself, but may require motorized chair for full day's activities.	2
Complete loss of voluntary movement	Individual is unable to effectively use his upper and lower extremities to perform simple or one-step activities. The criteria may still be met if there are singular apparently random movements of the arms.	3
Episodes of incontinence	Individual who was previously continent for at least 6 months develops permanent and frequent episodes of hesitance, urgency, retention of bowel or bladder, or urinary incontinence during daytime and nighttime (diurnal and nocturnal enuresis).	1
Total incontinence	In an individual who was previously continent, the permanent and continuous loss of urinary and/or fecal control.	2
Nonfebrile seizures	Individual who develops non-febrile seizure.	1

Protocol Title:	A Phase 3 Study of Lenti-D Drug Product After Myeloablative Conditioning Using Busulfan and Fludarabine in Subjects ≤ 17 Years of Age With Cerebral Adrenoleukodystrophy (CALD)
Protocol Number:	ALD-104 Version 8.0, 30 November 2022

INVESTIGATOR STATEMENT

I have read, understood, and agree to abide by all the conditions and instructions contained in this protocol.

I understand that all documentation provided to me by bluebird bio or its designated representative(s) concerning this study that has not been published previously will be kept in the strictest confidence. This documentation includes the study protocol, Investigator's Brochure, case report forms, and other scientific data.

I agree to personally conduct or supervise the described investigation(s).

I agree to inform any subjects, or any persons used as controls, that the drug product is being used for investigational purposes and I will ensure that the requirements relating to obtaining informed consent, as per local regulations and under Good Clinical Practice (GCP), are met.

I agree to report to the Sponsor adverse events that occur in the course of the investigation(s) in accordance with this protocol and as required by local regulations and under GCP.

I have read and understand the information in the Investigator's Brochure, including the potential risks and side effects of the drug product.

I agree to maintain adequate and accurate records and to make those records available for inspection in accordance with local regulations and under GCP.

I will ensure that an ethics committee that complies with all local regulations and GCP requirements will be responsible for the initial and continuing review and approval of the clinical investigation.

I also agree to promptly report to the ethics committee all changes in the research activity and all unanticipated problems involving risks to human subjects or others.

I agree that this study will not commence without the prior approval of the appropriate national health authorities together with a properly constituted ethics committee. I agree that no changes will be made to the study protocol without the prior written approval of bluebird bio and the aforementioned regulatory bodies, as applicable in the relevant laws and regulations.

I agree to ensure that all associates, colleagues, and employees assisting in the conduct of the study(ies) are informed about their obligations in meeting the above commitments.

Investigator Name	Investigator Signature	Date

Investigational site or name of institution and location (printed)