CLINICAL STUDY PROTOCOL ALD-102

EudraCT No. 2011-001953-10

A Phase 2/3 Study of the Efficacy and Safety of Hematopoietic Stem Cells Transduced With Lenti-D Lentiviral Vector for the Treatment of Cerebral Adrenoleukodystrophy (CALD)

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Protocol Version: Version 10.0

Protocol Date: 23 September 2020

CONFIDENTIAL

This study will be conducted according to the protocol and in compliance with Good Clinical Practice, the ethical principles stated in the Declaration of Helsinki, and other applicable regulatory requirements.

MEDICAL OFFICER CONTACT INFORMATION

Country-Specific 24-hour Emergency Contact Phone Numbers

Country	Phone Number
Argentina	0 800 345 5462
Australia	1 800 270 922
France	0 800 90 95 95 CCI [redacted]
Germany	0 800 100 2341
UK	0 800 069 8015
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SUMMARY OF CHANGES

Substantial changes implemented in version 10.0 (23 September 2020) compared to version 9.0 (24 August 2018) of the protocol are described in the table below and include a revised algorithm for assessing clonal predominance, a clarification of safety endpoints, a notification for subjects of the potential for further investigations following unexpected laboratory results, instructions for sites on vaccine administration, new guidelines around the impact of the COVID-19 pandemic, and a revised guideline around the follow-up of newborns.

Non-substantial changes implemented are also described in the following list. In addition, several minor changes were made to correct typographical errors and to rephrase some sentences to improve clarity.

DESCRIPTION OF EACH SUBSTANTIAL AMENDMENT

Note: added text is **bold** and deleted text shown in ~~strikeout~~.

Initial wording	Amended or New Wording	Reason/Justification for change
Section 2.2.2 Safety Endpoints The secondary safety endpoints are the following: *Unedited endpoints are not listed* • Incidence of insertional mutagenesis leading to clonal dominance or leukemia by Month 24	**Section 2.2.2** Safety Endpoints The secondary safety endpoints are the following: *Unedited endpoints are not listed* • ~~Incidence of insertional mutagenesis leading to clonal dominance or leukemia by Month 24~~ • **The number of subjects with insertional oncogenesis (myelodysplasia, leukemia, lymphoma, etc.) by Month 24** **The exploratory safety endpoint is:** • [redacted]	[redacted]
Section 2.2.3 Other Exploratory Endpoints Exploratory endpoints include : [redacted]	**Section 2.2.3** Other Exploratory Endpoints Exploratory endpoints include : [redacted]	Revised efficacy endpoints to provide individual outputs rather than change over time, as absolute values are a more informative parameter for analysis.

Initial wording	Amended or New Wording	Reason/Justification for change
[redacted]	[redacted]	
Section 6.1 Schedule of Events	**Section 6.1** Schedule of Events **<u>Impact of the COVID-19 Pandemic on Study Visits</u>** **Due to the COVID-19 pandemic, subjects may not be able to attend normal study visits. If a visit is missed due to COVID-19 reasons (e.g. unable to fly, unwilling to travel, family or subject affected by COVID-19, hospital closure, etc.), the subject may be able to complete study assessments at a facility that is local to his home.**	Added text to provide guidelines around study procedures and assessments impacted by the COVID-19 pandemic.

Initial wording	Amended or New Wording	Reason/Justification for change
Section 6.1 Schedule of Events **Table 3** Schedule of Events: Drug Product Infusion through End of Study Hematology[2] [2] Hematology parameters include white blood cell (WBC) count with differential, hemoglobin, hematocrit, red blood cell (RBC), and platelet count.	**Section 6.1** Schedule of Events **Table 3** Schedule of Events: Drug Product Infusion through End of Study Hematology[2] [2] 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 to allow for further investigation of stem cells.**	Allows for further investigation when additional follow-up is required.
Section 6.5.11.4 Specialty Laboratory Sample Collection	**Section 6.5.11.4** Specialty Laboratory Sample Collection **Clinical work-up for unexpected blood test results:** **If the results from blood tests are not as expected, additional testing may need to be performed to allow for further investigation of stem cells and may include.** • **Physical exam** • **Blood tests** • **Imaging tests** • **Bone marrow biopsy**	

Initial wording	Amended or New Wording	Reason/Justification for change
Section 6.5.12 Assessment of Clonal Dominance and/or Suspicion of Leukemia/Lymphoma **Section 6.5.12.1** Assessment of Clonal Dominance An integration site analysis (ISA) will be performed on whole blood according to the SOE. The frequency of the ISA should be increased for subjects with VCN of ≥0.3 if the ISA demonstrates that a mappable insertion site (IS) contributes >30% to the total number of retrieved IS as follows (see also schematic in Figure 1), or at any time at the discretion of the Investigator and Sponsor: • If ISA detects an IS contributing >30% to the total IS, ISA should be repeated twice, each test approximately 3 months apart. • If result is ≤30% clonal contribution at either repeat ISA, monitoring of the subject returns to the protocol-defined schedule. • If result is >30% and ≤90% clonal contribution at the first repeat, and ≤50% clonal contribution at the second repeat, monitoring of the subject also returns to the protocol-defined schedule. • If result is >30% and ≤90% clonal contribution at the first repeat, and >50% clonal contribution at the second repeat, clonal dominance criteria are met and clinical work-up for malignancy should be initiated. • If ISA result is >90% clonal contribution at any time, clonal dominance criteria are met and clinical work-up for malignancy should be initiated.	**Section 6.5.12** Assessment of Clonal **Predominance** ~~Dominance~~ and/or ~~Suspicion of Leukemia/Lymphoma~~ **Insertional Oncogenesis (Malignancy**) **Section 6.5.12.1** Assessment of Clonal **Predominance** ~~Dominance~~ ~~An integration site analysis (ISA) will be performed on whole blood according to the SOE. The frequency of the ISA should be increased for subjects with VCN of ≥0.3 if the ISA demonstrates that a mappable insertion site (IS) contributes >30% to the total number of retrieved IS as follows (see also schematic in Figure 1), or at any time at the discretion of the Investigator and Sponsor:~~ ~~• If ISA detects an IS contributing >30% to the total IS, ISA should be repeated twice, each test approximately 3 months apart.~~ ~~• If result is ≤30% clonal contribution at either repeat ISA, monitoring of the subject returns to the protocol-defined schedule.~~ ~~• If result is >30% and ≤90% clonal contribution at the first repeat, and ≤50% clonal contribution at the second repeat, monitoring of the subject also returns to the protocol-defined schedule.~~ ~~• If result is >30% and ≤90% clonal contribution at the first repeat, and >50% clonal contribution at the second repeat, clonal dominance criteria are met and clinical work-up for malignancy should be initiated.~~ ~~• If ISA result is >90% clonal contribution at any time, clonal dominance criteria are met~~	The assessment of clonal predominance will now be assessed based on frequency of clones with LVV insertions rather than based on frequency of individual LVV insertion sites. Based on clinical experience, the previous algorithm, which depended on Relative IS-Frequencies to identify clones that may have expanded to become predominant within a subject, was unable to account for multiple integration sites (IS) in a single cell. Our evolving understanding of the strengths and weaknesses of the ISA methods suggest that clonal contribution as determined by IS-specific qPCR normalized against human genomic genes is the most suitable current method available to accurately determine the relative predominance of each given clone independent of how many unique IS may be present within that clone.

Initial wording	Amended or New Wording	Reason/Justification for change
Note that for the purposes of this protocol, the term "oligoclonality" is used to describe situations in which the above clonal dominance criteria apply. Therefore, if a subject meets the above criteria and further work-up is required, a regulatory submission will be performed as per FDA Guidance for Industry: Gene Therapy Clinical Trials - Observing Subjects for Delayed Adverse Events (November 2006).	~~and clinical work-up for malignancy should be initiated.~~ ~~Note that for the purposes of this protocol, the term "oligoclonality" is used to describe situations in which the above clonal dominance criteria apply. Therefore, if a subject meets the above criteria and further work-up is required, a regulatory submission will be performed as per FDA Guidance for Industry: Gene Therapy Clinical Trials - Observing Subjects for Delayed Adverse Events (November 2006).~~ **Figure 1 outlines the updated algorithm for assessment of clonal predominance. Screening integration site (IS) analysis (ISA) will be performed as indicated in the schedule of events using high-throughput, semi-quantitative methods which identify IS based on vector sequence primers. IS identified in the screening assay are considered as being of interest when the overall peripheral blood VCN is > 0.3 c/dg AND either any relative IS frequency is > 30% OR multiple IS are apparently in the same clone and add up to > 30%. Multiple IS apparently in the same clone is defined as more than one relative frequency where values are within 20% of each other (e.g. 5% ± 1%, 10% ± 2%, 15% ± 3%, etc.), as well as any additional cases identified through bluebird bio internal review of ISA reports. When multiple IS are apparently in the same clone, it will be recommended to confirm that those IS are in a single clone (e.g. bone marrow or peripheral blood colony-forming unit assay). IS of interest will be interrogated, from the timepoint of interest and**	

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	available previous timepoints, using a quantitative assay (e.g. qPCR) designed to detect the specific IS and determine an IS-specific VCN to estimate clonal contribution. **If results of the quantitative, IS-specific follow-up assay reveal an IS-specific VCN ≤ 0.5 c/dg, estimating ≤ 50% clonal contribution, repeat ISA screening will continue at the regularly scheduled timepoints. However, according to scientific judgement or interest, investigative follow-up may be initiated by bluebird bio in collaboration with an investigator and additional interval, unscheduled ISA testing may be performed.** **If results of the quantitative, IS-specific follow-up assay reveal an IS-specific VCN > 0.5 c/dg, estimating > 50% clonal contribution, criteria will be met to consider the subject as having a predominant clone. This threshold also applies to individual lineage evaluations (myeloid, lymphoid, etc.) when performed. Clinical work-up will be recommended for a predominant clone (see next sections). A report to relevant regulatory authorities will be required when a persistent, predominant clone is identified (2 or more timepoints), and the report will be made within 30 days of receipt of IS-specific VCN results from the second timepoint when the persistent, predominant clone is identified.** ***Figure 1 was updated to reflect the updated ISA algorithm***	

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Section 6.5.12.3 Clinical Work-up for Malignancy If any of the above criteria is met, the Medical Monitor will be notified, and a work-up will be performed that may include the following: • Physical exam • CBC with differential lymphocyte subsets • Studies to rule out infectious cause • Studies to rule out autoimmune disease • Imaging studies, as appropriate • Bone marrow analysis **Bone Marrow Aspiration** The subject will undergo BM aspiration if clonal dominance is observed. The procedure may also be done at the Investigator's discretion, if there is concern about clonal expansion. The BM aspiration 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 will be collected each time.	**Section 6.5.12.3** Clinical Work-up for Malignancy If any of the above criteria is met, the Medical Monitor will be notified, and a work-up will be performed **that may occur in stages** and may include some of the following **at each stage**: • Physical exam • CBC with differential ~~and lymphocyte subsets~~ • **Lymphocyte subsets** • Studies to rule out infectious cause • Studies to rule out autoimmune disease • Imaging studies, as appropriate • Bone marrow analysis **Bone Marrow Aspiration** The subject ~~will~~ **may** undergo BM aspiration if clonal **pre**dominance is observed. The procedure may also be done at the Investigator's discretion, if there is concern about clonal expansion. The BM aspiration 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 will be collected each time.	

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Section 6.7 Pregnancies and Contraception 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 to 6 weeks of age. SAEs experienced by newborn within 6 weeks of age are required to be immediately reported (i.e. within 24 hours) on the SAE report form.	**Section 6.7** Pregnancies and Contraception 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~~ **resolution. Information will be requested on the status of the mother and infant at 6 weeks of age an annually thereafter for 2 years,** ~~including follow-up of the health status of the newborn to 6 weeks of age~~. 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.**	Following regulatory guidance, added text to extend the time period for follow-up of newborns.
Section 7.3 Planned Analyses	**Section 7.3** Planned Analyses **Section 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 analyses will be performed to measure the effect of disruptions due to the pandemic on these assessments.**	Added text to provide guidelines around study procedures and assessments impacted by the COVID-19 pandemic.

DESCRIPTION OF EACH NON-SUBSTANTIAL AMENDMENT

- Updated the Title page to reflect a change in Responsible Medical Officer.
- Added information page for country-specific 24-hour emergency contact phone numbers to reach the Responsible Medical Officer.
- Replaced "Lenti-D Drug Product" in the synopsis and body text with "elivaldogene autotemcel" or the abbreviation "eli-cel", which is the designated INN/USAN.
- Replaced "clonal dominance" with "clonal predominance" in the synopsis and body text to reflect a change in nomenclature.
- Removed detail of point estimate needed for meeting success criterion from synopsis
- Updated language and references in Section 1.5.1 to more accurately account for the number of subjects treated in gene therapy studies since the previous amendment as per the most recent Investigator's Brochure v10.0.
- Clarified in Section 4.5 that only subjects who receive drug product will be asked to complete Early Termination Visit assessments if they withdraw before study completion.
- Added text to Section 5.10 instructing sites to follow institutional guidelines when administering vaccines.
- Removed text in Section 6.5.11.4 that indicated ISA would only be performed if VCN is ≥ 0.01 c/dg, since ISA is run in parallel with VCN and RCL, therefore a preliminary VCN check in not performed.
- Corrected a statement in Section 6.6 to reflect that SAEs, not AEs, will continue to be monitored following subject discontinuation from the study.
- Amended language in Section 6.6.1 to direct Investigators to reference the prescribing information for conditioning agents for conditioning-related AEs and that that conditioning-related AEs should be attributed to conditioning in the eCRF rather than a "conditioning-related event" which is not an available category.
- Clarified that AEs are graded by the general guidelines per the CTCAE rather than the specific criteria for individual AE terms in Section 6.6.2.
- Details have been added to Section 7.3 clarifying how interim and final data analyses will be performed and how safety data will be reviewed.
- Clarified the definition of treatment emergent adverse event in Section 7.4.5 as occurring on or after initiation of eli-cel infusion rather than on the day of eli-cel infusion to align with language in the Statistical Analysis Plan.
- Added statement in Section 7.4.5 to see the Statistical Analysis Plan for a comprehensive list of study periods and additional details.

CLINICAL STUDY SYNOPSIS

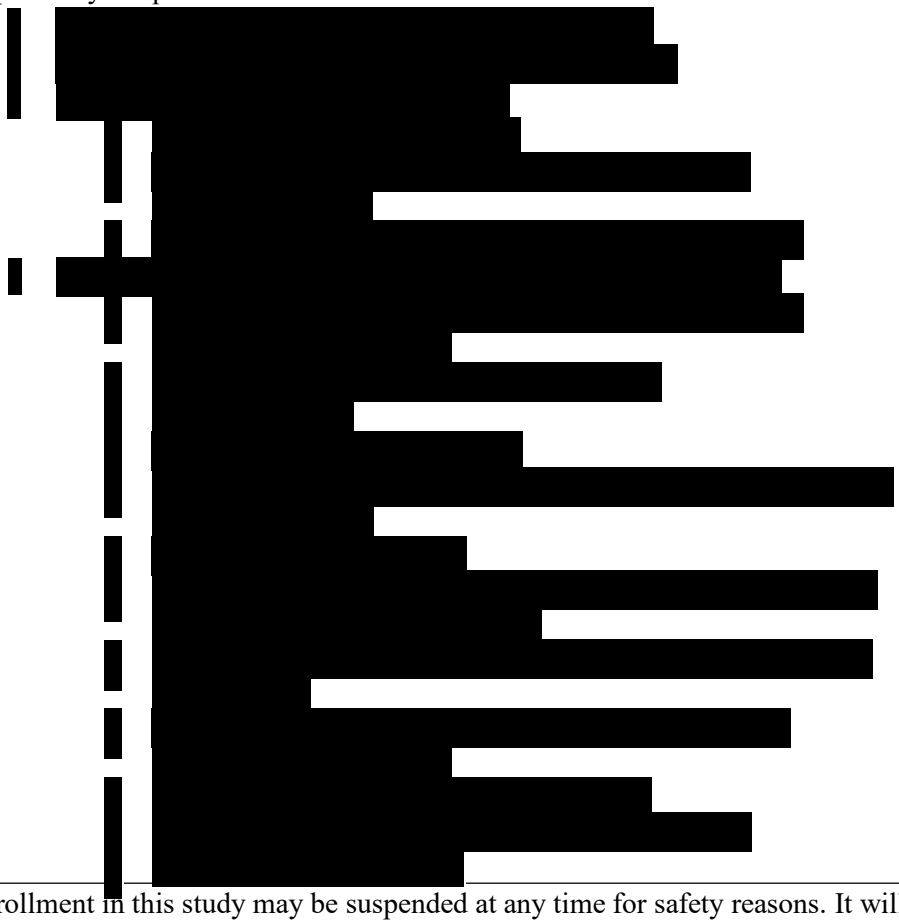
Protocol Title:	A phase 2/3 study of the efficacy and safety of hematopoietic stem cells transduced with Lenti-D lentiviral vector for the treatment of cerebral adrenoleukodystrophy (CALD)
Protocol Number:	ALD-102
EudraCT Number:	2011-001953-10
Objectives:	The study objectives are to: • Evaluate the efficacy of Lenti-D Drug Product (also known as elivaldogene autotemcel, hereafter referred to as eli-cel) in subjects with CALD • Evaluate the safety of eli-cel in subjects with CALD
Study Design:	This will be an international, non-randomized, open-label, multi-site, single-dose study in male subjects with CALD (≤ 17 years of age at enrollment). Approximately 30 subjects will be infused with eli-cel. The study will begin with a staggered infusion schedule. The data monitoring committee (DMC) will review safety and neutrophil engraftment data on Subject 1 prior to approving infusion of Subject 2. Following a review of safety and neutrophil engraftment data from the first 2 subjects, and with the recommendation of the DMC, parallel infusions will be allowed. Selected subjects must meet the inclusion criteria, have none of the exclusion criteria, and have provided informed consent. The study has 4 distinct phases after informed consent: • Screening • CD34+ Cell Collection, Transduction, Disposition of eli-cel, and Re-confirmation of Eligibility • Conditioning and Washout followed by eli-cel Infusion (transplant) on Day 0 • Maintenance (Follow-up) (Day 1 through 24 ± 1 month [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 may be made to open up a suitable site closer to the subject's home (referred to as secondary study sites) where they could 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 treatment of the subject. The competent 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). In addition, if consistent with local regulation, 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. 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, hematopoietic stem cell transplantation (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 Conference of Harmonization (ICH)/Good clinical practice (GCP), local regulations, and site-specific institutional practice. Subjects who are pre-screened and considered by the Investigator to be 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, will undergo G-CSF-mediated, and potentially plerixafor-mediated, hematopoietic stem cell (HSC) mobilization 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 HSC, transduced with Lenti-D lentiviral vector, stored frozen in cryopreservative 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 cyclophosphamide IV and busulfan 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 (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 harvest may be performed. All subjects will be followed for 24 months post-drug product infusion under this protocol. Then, if appropriate consent is obtained, subjects will be followed for an additional 13 years under a separate follow-up protocol (LTF-304).
Number of Subjects Planned:	Approximately 30 subjects will be infused with eli-cel.
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 cerebral ALD 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 on MRI of demyelinating lesions. 4. Neurologic Function Score (NFS) ≤ 1.
Exclusion Criteria:	1. Receipt of an allogeneic transplant or gene therapy. 2. Availability of a willing 10/10 human leukocyte antigen (HLA)-matched sibling donor (excluding female heterozygotes). 3. 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. 4. 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. 5. Any conditions that make it impossible to perform MRI studies (including allergies to anesthetics or contrast agents). 6. Hematological compromise as evidenced by: o Peripheral blood absolute neutrophil count (ANC) < 1500 cells/mm^3, o Platelet count < 100,000 cells/mm^3, or o Hemoglobin < 10 g/dL. o Uncorrected bleeding disorder. 7. Hepatic compromise as evidenced by: o Aspartate transaminase (AST) value > 2.5 × ULN o Alanine transaminase (ALT) value > 2.5 × ULN o Total bilirubin value > 3.0 mg/dL, except if there is a diagnosis of Gilbert's Syndrome and the subject is otherwise stable 8. Renal compromise as evidenced by abnormal renal function (actual or calculated creatinine clearance < 50 mL/min) 9. Cardiac compromise as evidenced by left ventricular ejection fraction < 40% 10. 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). 11. Clinically significant active bacterial, viral, fungal, parasitic, or prion-associated infection 12. Positive for human immunodeficiency virus type 1 or 2 (HIV-1, HIV-2); hepatitis B; hepatitis C; human T lymphotropic virus 1 (HTLV-1). (Note that subjects who have been vaccinated against hepatitis B [hepatitis B surface antibody-positive] who are negative for other markers of prior hepatitis B infection [e.g., negative for hepatitis B core Ab] are eligible. Subjects with past exposure to HBV [HBcAb positive and/or 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). 13. Any clinically significant cardiovascular or pulmonary disease, or other disease or condition that would be contraindicated for any of the other study procedures. 14. 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 drug product 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. 15. Any contraindications to the use of G-CSF during the mobilization of HSCs, and any contraindications to the use of busulfan or cyclophosphamide, including known hypersensitivity to the active substances or to any of the excipients in their formulations.
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 will then be asked to consent for a follow-up study for another 13 years post-drug product infusion.
Duration of Study:	From enrollment of the first subject to post-drug product infusion observation of the last subject enrolled, the study duration is expected to be 56 months.
Test Product, Dose and Mode of Administration:	Eli-cel (autologous CD34+ cell-enriched population that contains cells transduced with lentiviral vector encoding human adrenoleukodystrophy protein, suspended in a cryopreservative solution) is administered intravenously; dose $\geq 5.0 \times 10^6$ 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 review safety and neutrophil engraftment data on Subject 1 prior to proceeding with the transplant of Subject 2; and on Subject 2 prior to allowing the transplant of subsequent subjects. Following a review of safety and neutrophil engraftment data from the first 2 subjects and, with the recommendation of the DMC, parallel treatment (transplant) will be allowed. The DMC will meet by teleconference at regular intervals, approximately once every 6 months, or more frequently if needed, and depending on speed of subject enrollment and amount of new data generated. The DMC will be charged with review of all unexpected eli-cel treatment-related SAEs following notification by the Sponsor. 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 include the following: • Proportion of subjects who demonstrate resolution of gadolinium positivity on MRI (i.e., GdE-) at Month 24 • Time to sustained resolution of gadolinium positivity on MRI (i.e., GdE-). Sustained is defined as gadolinium resolution without a

	subsequent evaluation indicating gadolinium positivity • Change in total NFS from Baseline to Month 24 • MFD-free survival over time • Overall survival [redacted]
Criteria for Evaluation – Safety:	The primary safety endpoint is: • The proportion of subjects who experience either acute (≥ Grade II) or chronic graft versus host disease (GVHD) by Month 24. The secondary safety endpoints are the following: • Proportion of subjects with neutrophil engraftment by 42 days post-drug product infusion • Time to neutrophil engraftment post-drug product infusion • Proportion of subjects with platelet engraftment by Month 24 • Time to platelet engraftment post-drug product infusion • Proportion of subjects with loss of engraftment post-drug product infusion by Month 24 • Proportion of subjects who undergo a subsequent HSC infusion by Month 24 • Proportion of subjects with transplant-related mortality through 100 and 365 days post-drug product infusion • Proportion of subjects with and severity of clinical ≥ Grade 3 AEs, all drug-product related AEs, all SAEs, ≥ Grade 3 infections, and changes in laboratory parameters by Month 24 • Proportion of subjects with ≥ Grade II acute GVHD by Month 24 • Proportion of subjects with 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 ICU stays (post-neutrophil engraftment) by Month 24 • Incidence of vector-derived RCL at Month 24 • The number of subjects with insertional oncogenesis (myelodysplasia, leukemia, lymphoma, etc.) by Month 24 The exploratory safety endpoint is: • [redacted]

Criteria for Evaluation – Exploratory	Exploratory endpoints include: 
Enrollment Suspension Criteria:	Enrollment in this study may be suspended at any time for safety reasons. It will be the responsibility of the DMC to make a recommendation to the Sponsor if they believe there is reasonable cause for suspending enrollment. The Sponsor will inform the regulatory authorities and the Investigators, and each site's Institutional Review Board (IRB)/Independent Ethics Committee (IEC) and other appropriate institutional regulatory bodies will be promptly notified, if a decision to suspend enrollment is made. In the event enrollment is suspended, no new mobilization, conditioning, or drug product infusion of subjects will be initiated, but subjects who have already been treated with eli-cel will continue in the study. If mobilization has been initiated, cell collection will be completed at Investigator's discretion. Likewise, if the study is halted while a subject is undergoing conditioning, conditioning will be completed at Investigator's discretion, and every effort will be made to restart the study prior to their scheduled infusion. However, a subject may be infused with their back-up cells following conditioning if the study cannot be restarted in time. Enrollment and treatment with drug product will be temporarily suspended for any of the following reasons pending review and recommendations from the DMC and the appropriate communication with the relevant regulatory agency(ies): • Death, until the cause of the death is determined • Detection of leukemia/lymphoma due to vector-mediated insertional oncogenesis

	• Detection of vector-derived RCL in any subject • Failure in 1 subject to achieve reconstitution with transduced cells, requiring use of back-up cells or HSCs from an appropriately allogeneic donor. Engraftment failure is defined as a 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 (transplant) of eli-cel. • 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).
Statistical Methods:	Three populations will be evaluated for safety, efficacy, and exploratory analyses. • The Intent-to-treat population (ITT) will consist of those subjects who initiate any study procedures, beginning with stimulation by G-CSF. This population will be used for the analyses of some of the safety endpoints and for the supportive analysis of the primary efficacy endpoint, if it is different from the Transplant Population (TP). • The Transplant population (TP) will consist of subjects who receive eli-cel. This population will be used for the analyses of all efficacy endpoints and some of the safety endpoints. • The Successful Neutrophil Engraftment Population (NEP) will consist of subjects who received eli-cel and achieved neutrophil engraftment defined as having 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. The NEP will be used for the supportive analysis of the primary efficacy endpoint as well as for some of the other efficacy and safety endpoints, if it is different from the TP. It is recognized that formal, confirmatory statistical hypotheses are extremely difficult to formulate in the context of this subject population, where few historical data are available regarding the efficacy endpoints. Further, it is not possible to have a truly randomized comparison of eli-cel to other potentially curative modes of treatment, such as allogeneic HSCT, due to the rarity of the disease. Therefore, 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 performed on the TP. The lower bound of the 2-sided 95% exact confidence interval of the Month 24 MFD-free survival must be > 50% in order for the primary endpoint to be met. The analyses of the secondary and exploratory efficacy endpoints will be performed on the TP. The analysis of the primary safety endpoint will be to compare the proportion of subjects who have experienced either acute (≥ Grade II) or chronic GVHD at Month 24 in the TP population from Study ALD-102 to the rate seen in the allo-HSCT-treated population in Study ALD-103. The general safety profile of treatment with eli-cel will be summarized through the longitudinal evaluation of AEs, laboratory assessments, vital signs, ECG, and physical examination findings. Safety parameters will be summarized across each time point. The planned statistical methodology will be presented in detail in the 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
ALT	alanine transaminase
ANC	absolute neutrophil count
AST	aspartate transaminase
AUC	area under the curve
BAER	brain stem auditory evoked response
CCI	CCI
BM	bone marrow
CCI	CCI
BUN	blood urea nitrogen
CBC	complete blood count
CALD	Cerebral Adrenoleukodystrophy
CI	confidence interval
CMV	Cytomegalovirus
CNS	central nervous system
CRE	conditioning-related event
CRF	case report form
CSR	clinical study report
CTCAE	Common Terminology Criteria for Adverse Events
DMC	Data Monitoring Committee
DMSO	dimethyl sulfoxide
EBV	Epstein-Barr virus
ECG	electrocardiogram
eCRF	electronic case report form
eli-cel	elivaldogene autotemcel
EU	European Union
FDA	Food and Drug Administration
FSIQ	Full Scale Intelligence Quotient
GCP	Good Clinical Practice

Abbreviation	Definition
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
HBsAb	hepatitis B surface antibody
HBsAg	hepatitis B surface antigen
HBV	hepatitis B virus
HCV	hepatitis C virus
HEENT	head, ears, eyes, nose, and throat
HIV	human immunodeficiency virus
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 Assessment
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplant(ation)
HSV	herpes simplex virus
HTLV-1	human T lymphotropic virus 1
HTLV-2	human T lymphotropic virus 2
ICF	informed consent form
ICH	International Conference on Harmonization
IBW	ideal body weight
IEC	Independent Ethics Committee
IgG	immunoglobulin G
IQ	intelligence quotient
IRB	Institutional Review Board
IS	integration site(s)
ISA	integration site analysis
ITT	the intent-to-treat population
IV	intravenous
LLN	lower limit of normal
LTR	long terminal repeat
LVV	lentiviral vector

Abbreviation	Definition
MedDRA	Medical Dictionary for Regulatory Activities
MFD	major functional disability
MMP	matrix metalloproteinase(s)
MRI	magnetic resonance imaging
MUD	matched unrelated donor
NCI	National Cancer Institute
NCS	Nerve Conduction Study
NE	neutrophil engraftment
NEP	Neutrophil Engraftment Population
NFS	Neurologic Function Score
PBL	peripheral blood leukocyte
PBMC	peripheral blood mononuclear cell
PCR	polymerase chain reaction
CCI	CCI
PIQ	Performance Intelligence Quotient
qPCR	quantitative polymerase chain reaction
RBC	red blood cell
RCL	replication competent lentivirus
RNA	ribonucleic acid
RT-PCR	reverse transcriptase polymerase chain reaction
SAE	serious adverse event
SAP	statistical analysis plan
SCGM	stem cell growth media
SCID-X1	X-linked severe combined immunodeficiency
SES	socioeconomic status
SIN	self-inactivating
SOE	Schedule of Events
SOM	Study Operations Manual
SSEP	somatosensory evoked potential
SUSAR	suspected unexpected serious adverse drug reaction
TMT	Trail Making Test
TP	Transplant Population
TPE	ALD-102-Eligible Transplant Population
TPES	Strictly ALD-102-Eligible Transplant Population
TPG	GdE+ Transplant Population
ULN	upper limit of normal
UK	United Kingdom

Abbreviation	Definition
US	United States
CCI [redacted]	CCI [redacted]
VCN	vector copy number
VEP	Visual Evoked Potential
VIQ	Verbal Intelligence Quotient
VLCFA	very long chain fatty acids
VOD	veno-occlusive disease
VMI	Developmental Test of Visual-Motor Integration, 6th ed
VSV-G	vesicular stomatitis virus G
CCI [redacted]	CCI [redacted]
WBC	white blood cell
CCI [redacted]	[redacted]
[redacted]	[redacted]

TABLE OF CONTENTS

LIST OF TABLES IN THE TEXT

LIST OF FIGURES IN THE TEXT

1. INTRODUCTION

1.1. Cerebral X-Linked Adrenoleukodystrophy (CALD)

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 gadolinium enhancement (GdE+) on brain magnetic resonance imaging (MRI) (Engelen et al. 2012). Studies have shown that GdE+ is a predictive biomarker of disease progression as the severity of the inflammatory process appears to be correlated with the rapidity of progression (Powers et al. 1992; Melhem et al. 1996, 2000; Engelen et al. 2012).

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. The current standard of care for treatment of boys with CALD is allogeneic hematopoietic stem cell transplantation (allo-HSCT), which can improve overall survival and functional outcomes (Baumann et al. 2003; Peters et al. 2004; Mahmood et al. 2007; Miller et al. 2011).

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 who does not carry the mutation. 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 (MUD)-HSCT, or transplant with cells derived from banked cord blood (umbilical cord blood transplantation).

1.2. Retrospective Study ALD-101

bluebird bio, Inc. (bluebird bio) has completed a multi-institutional, retrospective case review and data collection study of 137 boys with CALD, including 72 untreated cases and 65 cases who underwent allo-HSCT in or after 2001 (Study ALD-101). Data were collected from 5 centers including 4 in the US and 1 in France.

Review of the literature and findings from Study ALD-101 have shown that while there are a wide number of cognitive, behavioral, functional, and radiological modalities utilized to assess patients with CALD, only 2 are utilized widely: 1) the Neurologic Function Score (NFS), a relatively simple 25-point scale that assesses functional domains typically compromised by CALD (Moser et al. 2000); and 2) brain MRI (with or without gadolinium), with MRI findings

scored using a system developed expressly for CALD, the Loes score (a 34-point scale that roughly describes disease burden throughout the brain (Loes et al. 1994).

In Study ALD-101, Baseline (defined as the value closest to the date of CALD diagnosis) MRI findings, as assessed by Loes score, and NFS were major predictors of survival, with longer median survival in the Untreated Cohort observed in patients with lower Baseline scores than in those with higher scores (clinical study report [CSR] ALD-101, v2.0). Estimated 2- and 5-year survival rates were 74% and 55%, respectively for the entire Untreated Cohort with a median survival time of 91.9 months. Improved 2- and 5-year survival rates were observed in the Untreated Cohort for boys with a Baseline NFS ≤1, 92% and 74%, respectively, than for boys with an NFS >1, 52% and 25%, respectively. Higher 2- and 5-year survival rates also were observed for patients with Loes ≤ 5 than for those with Loes > 5.

Consistent with the literature (e.g., (Melhem et al. 2000)) data from Study ALD-101 show that GdE+ is highly predictive of the likelihood of rapid progression (CSR ALD-101). Most (18/21) GdE+ untreated subjects had increased NFS scores, whereas most (7/9) subjects without gadolinium enhancement (i.e., negative for gadolinium enhancement; GdE-) had stable low NFS scores, for at least 18 months after initial GdE- MRI.

Findings from Study ALD-101 also support the efficacy of allogeneic HSCT, as evidenced by stabilization of NFS and Loes score. After a period of decline in the post-allogeneic HSCT period, the majority of NFS and Loes scores stabilized post-allogeneic HSCT Unfortunately, allogeneic HSCT is unlikely to be of benefit to patients with an NFS > 1 or Loes score > 10 (Peters et al. 2004; Miller et al. 2011) (CSR ALD-101).

Safety findings from Study ALD-101 were consistent with the CALD literature. Previous studies reported a 10% to 30% risk of failure of engraftment, life-threatening infection, and acute or chronic graft-versus-host disease (GVHD) (Hahn et al. 2008; Miller et al. 2011), with the risk of chronic GVHD being approximately 30% (Carlens et al. 1998). Findings from Study ALD-101 included an overall incidence of serious adverse events (SAEs) of 51%, including a 29% incidence of infections as the SAE; an 18% incidence of graft failure; a 45% incidence of acute GVHD; and a 21% incidence of chronic GVHD. The risk of life-threatening adverse events (AEs) associated with allogeneic HSCT highlights the unmet need for safe treatment of CALD, particularly for patients without a willing matched sibling donor.

1.3. 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. Eli-cel's mode of action 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 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. The use of a patient's own stem cells would eliminate the risks of graft rejection

and acute and chronic GVHD due to immune-incompatibility, and also reduce complications due to long-term immunosuppression.

Nonclinical data indicate that in vitro transduction of human HSCs and fibroblasts with Lenti-D LVV results in expression of ALDP, and both in vitro and in vivo nonclinical studies suggest that eli-cel can be used safely in clinical studies for the treatment of CALD (see nonclinical section of the Investigator's Brochure).

Long-term follow-up of 4 subjects from clinical Study TG04.06.01, that utilized a LVV that is closely related to Lenti-D LVV (CG1711hALD) to transduce autologous HSCs, suggest that this approach is well-tolerated and can arrest disease progression. The results of the first 2 treated subjects have been published (Cartier et al. 2009), and after follow-up of 6 and 10 years post-transplantation in May 2017 for 2 subjects, and 4 and 6 years post-transplantation in March 2013 for the other 2 subjects (who did not give consent for follow up studies after this date), no AE considered by the Investigator to be related to drug product has been observed, and specifically no development of human immunodeficiency virus (HIV) positivity, vector-derived replication competent lentivirus (RCL), clonal predominance, leukemia, or lymphoma has been observed. Additionally, 3 of the 4 subjects treated with drug product had stabilization of Loes and NFS and no major functional disabilities.

1.4. Rationale for Study Design

[redacted]

1.5. Potential Risks

1.5.1. 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 DNA 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 subjects in the SCID-X1 study developed acute lymphocytic leukemia. One of the 5 subjects succumbed to leukemia, while the remaining 4 were successfully treated. In the Wiskott-Aldrich study, 4 subjects 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 (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).

To date, more than 200 subjects have been treated in gene therapy studies involving lentiviral vectors and HSCs, with follow-up times up to 10 years in some studies, with no published cases of LVV therapy-related leukemia or lymphoma in any patient (e.g., (Cartier et al. 2009; Cavazzana-Calvo et al. 2010; Aiuti et al. 2013; Biffi et al. 2013; Hacein-Bey Abina et al. 2015; Ribeil et al. 2017; Eichler et al. 2017; Thompson et al. 2018) (data on file). Safety results for bluebird bio's CALD studies can be found in the Investigator's Brochure.

1.5.2. Use of a Lentiviral Vector Derived from Human Immunodeficiency Virus Type 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 RCL.

Subjects 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 (LTF-304; EudraCT No: 2015-002805-13; NCT02698579) 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 subject, enrollment in the study will be suspended until a full investigation by the Data Monitoring Committee (DMC) occurs with notification of the appropriate regulatory authorities.

1.5.3. Risks of Mobilization and Transplantation

Reported warnings for granulocyte colony stimulating factor (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 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 the ALD-102 study). Additionally, reported precautions include the potential for immunogenicity or cutaneous vasculitis (Thornley et al. 2004). Refer to the prescribing information (package insert) for additional product details regarding G-CSF, including all reported 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 (package insert) for additional product details regarding plerixafor, including all reported AEs from clinical trials.

Toxicity associated with the busulfan IV plus cyclophosphamide IV conditioning regimen (i.e., conditioning regimen-related toxicity) is a frequent cause of morbidity and mortality in allogeneic HSCT (Clift et al. 1993; Thornley et al. 2004). Most notably, given that the goal of conditioning is immunosuppression, subjects receiving such treatment are at significant risk for development of potentially fatal, opportunistic infections (e.g., cytomegalovirus [CMV], Epstein Barr Virus [EBV]). In fact, this risk is low with autologous HSCT because the hematologic reconstitution is very rapid and long-term immunosuppression is not required. Veno-occlusive disease (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 toxicities, primarily seizure, also can occur with the conditioning regimen. Although generally less significant, mucositis, skin complications, including hyperpigmentation and desquamation, and gastrointestinal disturbances, primarily nausea, vomiting and diarrhea, commonly occur with cyclophosphamide IV and busulfan IV conditioning (Richardson et al. 1998; Tran et al. 2000; Andersson et al. 2002). Male infertility (i.e., reduced testosterone levels) has also been associated with use of the busulfan IV plus cyclophosphamide IV conditioning regimen (Grigg et al. 2000; Meistrich 2009). Refer to the prescribing information (package insert) regarding additional toxicities associated with busulfan IV and cyclophosphamide IV.

Engraftment failure is defined as a failure to achieve 3 consecutive absolute neutrophil count (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 (transplant) of eli-cel. Management of neutrophil engraftment failure is at the discretion of the Investigator. Subject will be followed as per protocol-scheduled visits (Table 2 and Table 3).

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Study Objectives

- Evaluate the efficacy of eli-cel in subjects with CALD
- Evaluate the safety of eli-cel 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 who demonstrate resolution of gadolinium positivity on MRI (i.e., GdE-) at Month 24
- Time to sustained resolution of gadolinium positivity on MRI (i.e., GdE-). Sustained is defined as gadolinium resolution without a subsequent evaluation indicating gadolinium positivity
- Change in total NFS from Baseline to Month 24
- MFD-free survival over time
- Overall survival

[redacted]

2.2.2. Safety Endpoints

The primary safety endpoint is:

- The proportion of subjects who experience either acute (≥ Grade II) or chronic GVHD by Month 24.

The secondary safety endpoints are the following:

- Proportion of subjects with neutrophil engraftment by 42 days post-drug product infusion
- Time to neutrophil engraftment post-drug product infusion
- Proportion of subjects with platelet engraftment by Month 24
- Time to platelet engraftment post-drug product infusion
- Proportion of subjects with loss of engraftment post-drug product infusion by Month 24
- Proportion of subjects who undergo a subsequent HSC infusion by Month 24
- Proportion of subjects with transplant-related mortality through 100 and 365 days post-drug product infusion
- Proportion of subjects with and severity of clinical ≥ Grade 3 AEs, all drug-product related AEs, all SAEs, ≥ Grade 3 infections, and changes in laboratory parameters by Month 24
- Proportion of subjects with ≥ Grade II acute GVHD by Month 24
- Proportion of subjects with 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 ICU stays (post-neutrophil engraftment) by Month 24
- Incidence of vector-derived RCL at Month 24
- The number of subjects with insertional oncogenesis (myelodysplasia, leukemia, lymphoma, etc.) by Month 24

The exploratory safety endpoint is:

[redacted]

2.2.3. Other Exploratory Endpoints

Exploratory endpoints include:

3. INVESTIGATIONAL PLAN

3.1. Overall Design and Plan of the Study

This will be an international, non-randomized, open-label, multi-site, single-dose study in male subjects with CALD (≤ 17 years of age at enrollment). Approximately 30 subjects will be infused with eli-cel. The study will begin with a staggered infusion schedule. The DMC will review safety and neutrophil engraftment data on Subject 1 prior to approving infusion of Subject 2. Following a review of safety and neutrophil engraftment data from the first 2 subjects, and with the recommendation of the DMC, parallel infusions will be allowed.

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

- Screening
- CD34+ Cell Collection, Transduction, Disposition of eli-cel, and Re-confirmation of Eligibility
- Conditioning and Washout, followed by eli-cel Infusion (transplant) on Day 0
- Maintenance (Follow-up) (Day 1 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 may be made to open up a suitable site closer to the subject's home (referred to as secondary study sites) where they could 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 treatment of the subject. The competent 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). In addition, if consistent with local regulation, 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. 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 Conference of Harmonization (ICH)/Good clinical practice (GCP), local regulations, and site-specific institutional practice.

Subjects who are pre-screened and considered by the Investigator to be 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, will undergo G-CSF-mediated, and potentially plerixafor-mediated, HSC mobilization 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 HSC, transduced with Lenti-D LVV, stored frozen in cryopreservative 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 cyclophosphamide IV and busulfan 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, if appropriate consent is obtained, subjects will 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 integration site analysis (ISA), and long-term efficacy to evaluate durability of response.

Efficacy evaluations to be performed during the study include assessment of functional status using NFS for an overall score and for determination of MFDs. Additional efficacy evaluations include determination of effects of cerebral demyelination, as measured by Loes score, and gadolinium enhancement on MRI. CCI [redacted] Based on treatment experience with allogeneic HSC transplant in CALD subjects (Miller et al. 2011) and the post-drug product infusion follow-up of 4 subjects treated in Study TG04.06.01 in France, it is expected that it will take at least 12 months to observe evidence of disease stabilization.

The success of the transplant procedure will be assessed by time to neutrophil engraftment defined as achieving 3 consecutive ANC laboratory values of $\geq 0.5\times10^9$ cells/L (after initial post-infusion nadir) obtained on different days by 42 days post-infusion (transplant) of eli-cel. 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 will be defined as follows:

- Primary neutrophil engraftment failure: failure to achieve neutrophil engraftment (as defined above).
- Secondary neutrophil engraftment failure: i) achieved neutrophil engraftment followed by ii) sustained decline in ANC to $< 0.5\times10^9$ cells/L for 3 consecutive measurements on different days after primary neutrophil engraftment

Subjects who experience neutrophil engraftment failure will be treated at the Investigator's discretion, and will continue to be followed for safety and efficacy assessments as detailed in the schedule of events (SOE) (Table 2 and Table 3).

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 proviral insertion sites and evidence of insertional mutagenesis.

Periodic efficacy and safety examinations will be performed by a specialist in pediatric transplant medicine, and a pediatric neurologist or other appropriately trained and qualified healthcare provider with expertise in CALD.

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-102 CSR.

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 review safety and neutrophil engraftment data on Subject 1 prior to proceeding with the transplant of Subject 2; and on Subject 2 prior to allowing the transplant of subsequent subjects. Following a review of safety and neutrophil engraftment data from the first 2 subjects and, with the recommendation of the DMC, parallel treatment (transplant) will be allowed. The DMC will meet by teleconference at regular intervals, approximately once every 6 months, or more frequently if needed, and depending on speed of subject enrollment and amount of new data generated. The DMC will be charged with review of all unexpected eli-cel treatment-related SAEs following notification by the Sponsor. The DMC will have the right to recommend halting the study at any time due to concerns for the safety of the subjects. (Refer to Section 3.4.2 for the enrollment suspension criteria).

3.2. Rationale for the Study

[redacted]

[redacted]

3.3. Rationale for the Dose Selected

[redacted]

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 Day -11 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 cyclophosphamide IV has begun on Day -10, there are no stopping rules for conditioning, ***except for the enrollment 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 cyclophosphamide IV treatment, the Medical Monitor should be contacted immediately. In such situations in which busulfan IV and cyclophosphamide 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 appropriate allogeneic donor if available, will be required.

3.4.2. Enrollment Suspension Criteria

Enrollment in this study may be suspended at any time for safety reasons. It will be the responsibility of the DMC to make a recommendation to the Sponsor if they believe there is reasonable cause for suspending enrollment. The Sponsor will inform the regulatory authorities and the Investigators, and each site's Institutional Review Board (IRB)/Independent Ethics Committee (IEC) and other appropriate institutional regulatory bodies will be promptly notified, if a decision to suspend enrollment is made. In the event enrollment is suspended, no new mobilization, conditioning, or drug product infusion of subjects will be initiated, but subjects who have already been treated with eli-cel will continue in the study. If mobilization has been initiated, cell collection will be completed at Investigator's discretion. Likewise, if the study is halted while a subject is undergoing conditioning, conditioning will be completed at Investigator's discretion, and every effort will be made to restart the study prior to their scheduled infusion. However, a subject may be infused with their back-up cells following conditioning if the study cannot be restarted in time.

Enrollment and treatment with drug product will be temporarily suspended for any of the following reasons pending review and recommendations from the DMC and the appropriate communication with the relevant regulatory agency(ies):

- **Death**, until the cause of the death is determined*
- Detection of **leukemia/lymphoma** due to vector-mediated insertional oncogenesis** (see Section 6.5.12).
- Detection of **vector-derived RCL** in any subject

- **Failure** in 1 subject **to achieve reconstitution** with transduced cells, requiring use of back-up cells or HSCs from an appropriately allogeneic donor. Engraftment failure is defined as a 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 (transplant) of eli-cel.
- 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).

*The death of a subject on Study ALD-102 after receiving eli-cel will result in a hold of further enrollment and treatment with drug product until an investigation into the cause of death is performed. If it is determined that the death was not related to the drug product, then enrollment/treatment with drug product may resume. If the relationship between the drug product and the death is not clear, or it appears that the death may be related to the drug product, enrollment and treatment with drug product will be held until the DMC assessment and recommendations as described above.
**If a subject is diagnosed with leukemia or lymphoma after receiving eli-cel, enrollment will be held until determination is made as to whether the malignancy was related to a vector-mediated insertion. Once this assessment occurs, if the malignancy is not related to a vector-mediated insertion, enrollment may resume. If the relationship between the malignancy and an insertion is not clear or it appears they may be related, enrollment will be held until the DMC assessment and recommendations as described above.

(For assessment of clonal predominance see Section 6.5.12).

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 a MRI Loes score between 0.5 and 9 (inclusive), an NFS ≤ 1, and gadolinium enhancement on MRI may be enrolled.

4.1. Number of Subjects

Approximately 30 subjects will be infused with eli-cel.

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 cerebral ALD as defined by:
    a. Elevated VLCFA values, and
    b. Active central nervous system (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. Gadolinium enhancement (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. Receipt of an allogeneic transplant or gene therapy.
2. Availability of a willing 10/10 HLA-matched sibling donor (excluding female heterozygotes).
3. 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.
4. 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.
5. Any conditions that make it impossible to perform MRI studies (including allergies to anesthetics or contrast agents).

6. Hematological compromise as evidenced by:
    a. Peripheral blood ANC count < 1500 cells/mm^3,
    b. Platelet count < 100,000 cells/mm^3, or
    c. Hemoglobin (Hb) < 10 g/dL.
    d. Uncorrected bleeding disorder.
7. 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
8. Renal compromise as evidenced by abnormal renal function (actual or calculated creatinine clearance < 50 mL/min)
9. Cardiac compromise as evidenced by left ventricular ejection fraction < 40%
10. 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).
11. Clinically significant active bacterial, viral, fungal, parasitic, or prion-associated infection
12. Positive for human immunodeficiency virus type 1 or 2 (HIV-1, HIV-2); hepatitis B virus (HBV); hepatitis C virus (HCV); human T lymphotropic 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 [HBcAb positive and/or 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).
13. Any clinically significant cardiovascular or pulmonary disease, or other disease or condition that would be contraindicated for any of the other study procedures.
14. 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 drug product 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.
15. Any contraindications to the use of G-CSF during the mobilization of HSCs, and any contraindications to the use of busulfan or cyclophosphamide, including known hypersensitivity to the active substances or to any of the excipients in their formulations.

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. As confirmation, bluebird bio will provide the Investigator with written verification of each subject's registration. 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.

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 have received infusion of eli-cel 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 appropriate 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).

4.6. Investigator or Sponsor Termination of Subject Participation

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 will be asked to participate in the long-term follow-up study (LTF-304), except subjects who have withdrawn their consent.

5. STUDY TREATMENTS

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

Lenti-D Lentiviral Vector: Lenti-D LVV is a replication defective, SIN, HIV-1 based LVV pseudotyped with the vesicular stomatitis virus 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 (SCGM).

Eli-cel: Eli-cel is defined as an autologous CD34+ cell-enriched population that contains cells transduced with LVV encoding human adrenoleukodystrophy protein, suspended in cryopreservative solution in the final immediate container for the intended medical use.

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 (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 (transplanted) back into the subject after they have received a myeloablative conditioning with busulfan IV and cyclophosphamide IV.

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

More than one mobilization cycle may be performed if needed in order to meet the required dose for eli-cel. Each mobilization cycle may include up to 3 apheresis collection days. Drug product will be manufactured from cells collected from either one day or over 2 consecutive days. Additional apheresis collections should be used to obtain back-up cells for rescue.

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 6 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. The morning after the 4th G-CSF dose, apheresis will be performed according to standard clinical procedures. If peripheral blood CD34+ count on that morning is < 50 cells/µL, a 5th dose of G-CSF will be administered and plerixafor will be administered by subcutaneous injection at 0.24 mg/kg of body weight approximately 10 hours prior to the next day's collection. Plerixafor can be given daily for up to 4 days.

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. Up to 3 total collections may be performed as part of any one mobilization cycle.

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: Remove back-up cells to be stored at the clinical site and send a minimum of 12×10^6 CD34+ cells/kg to the transduction facility.
- 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 ($\geq 1.5 \times 10^6$ CD34+ cells/kg), stored at the clinical site.
- If the Apheresis Procedure Day 1 collection is < 12×10^6 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 > 15×10^6 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 ($\geq 1.5 \times 10^6$ 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 cell dose. Once all release testing has been completed, the Sponsor will inform the clinical site if the drug product has met specification or not.

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 < 1.5×10^6 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), each drug product manufacture will result in an independent drug product lot. Eli-cel lots which have been dispositioned for clinical use will be sent to the site with a Certificate of Analysis(es), documenting that all release testing is complete.

If eli-cel does not meet pre-defined specifications, it will not be dispositioned for clinical use. In such cases, the Investigator may either withdraw the subject from the study or discuss further mobilization options with the subject.

5.2.4. Conditioning

Pre-conditioning assessments will be performed on Day -11 (with a window of -3 days; i.e., Day -14 through Day -11) 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 cyclophosphamide IV.

Weight-based dosing of busulfan IV will be administered on Days -10, -9, -8, and -7 and weight-based dosing of cyclophosphamide IV will be administered on Days -5, -4, -3, and -2.

Additional information on busulfan dosing:

- Busulfan IV will be administered as a 2-hour IV infusion in 16 single doses on 4 consecutive days
- 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 17,000 to 21,000 µmol*min/L.
- If using the first dose to calculate busulfan exposure, the target AUC range for a single dose is 1190 to 1310 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. Subjects should be monitored for AUC_{cum} as necessary.
- 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 –10, –9, –8, and –7
- For subjects >12 kg: busulfan 0.8 mg/kg/dose IV every 6 hours on Days –10, –9, –8, and –7

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 on cyclophosphamide dosing:

Cyclophosphamide 50 mg/kg/day will be administered as a 2-hour IV infusion with hydration as per institutional protocol.

Cyclophosphamide dose adjustments for IBW are recommended but not required. If any subjects are obese (i.e., subject ABW is over 125% of IBW), dosing will be adjusted for IBW (see Section 10.1 for calculations).

Prior to and during the administration of busulfan IV and cyclophosphamide 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 cyclophosphamide IV, including associated AEs.

5.2.5. Infusion (Transplant) Procedures, Dose, and Administration

Infusion of eli-cel is to be given approximately 48 hours (see Section 5.2.4) after completion of the busulfan IV and cyclophosphamide 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 and must be infused immediately, but no later than 4 hours after it has been thawed.

Eli-cel will be administered via IV infusion (transplant) 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 ≥ 5.0×10^6 CD34+ cells/kg. 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. 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.

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 ≤ 1.5×10^6 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 (Junior et al. 2008), and thus no subject in Study ALD-102 would exceed this amount.

Vitals signs are to be monitored concurrently during eli-cel infusion (transplant) per institutional practice at the clinical site, but no less frequently than at the start, once during, and upon completion of the infusion (transplant). 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 cryopreservative 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 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), back-up cells will be administered at a dose of $\geq 1.5 \times 10^6$ CD34+ cells/kg, or $\geq 1.0 \times 10^8$ 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 (transplant) 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 encoding human adrenoleukodystrophy protein. Eli-cel is suspended in cryopreservative solution in the final immediate container for the intended medical use (infusion bag).

Eli-cel will be labeled by the transduction facility according to GMP. Refer to Section 5.9 for additional details regarding product accountability.

5.7. 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 will then be asked 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 are ultimately the responsibility of the Investigator and Sponsor. However, this responsibility may be delegated to a suitably qualified Investigator listed on Food and Drug Administration (FDA) Form 1572 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

[redacted]

6. STUDY ASSESSMENTS

6.1. Schedule of Events

Table 2 and Table 3 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.

Subjects who experience neutrophil engraftment failure (defined as 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 [transplant] of eli-cel) will receive infusion with back-up cells (mobilized PBMCs) or with allogeneic HSCs. These subjects will continue to be followed for safety and efficacy.

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

- Screening
- CD34+ Cell Collection, Transduction, Disposition of eli-cel, and Re-confirmation of Eligibility
- Conditioning and Washout followed by eli-cel Infusion (transplant) on Day 0
- Maintenance (Follow-up) (Day 1 through Month 24)

Study Day 0 is defined as the day of infusion (transplant).

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), these will not be considered protocol deviations. In such cases, the subject will resume their schedule beginning on Day -10.

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 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 the COVID-19 Pandemic on Study Visits

Due to the COVID-19 pandemic, subjects may not be able to attend normal study visits. If a visit is missed due to COVID-19 reasons (e.g. unable to fly, unwilling to travel, family or subject affected by COVID-19, hospital closure, etc.), the subject may be able to complete study assessments at a facility that is local to his home.

Table 2: Schedule of Events: Screening through Drug Product Infusion

	Screening	Mobilization[1]	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 -11**	**D -10 to -1**
Visit Window (Days):	-10 to +5	-10	-	-3	-
Informed Consent	+				
Search for allogeneic donor & HLA typing[2]	+				
Demographics & Medical History	+				
ABCD1 genotype[3]	(+)				
Adrenal function[4]	+				
Local lab: Blood for immunological studies	+				
CCI					
Sperm/testicular tissue banking, if requested[6]	+				
Serology panel	+ (I)	+ (II)			
Physical examination, Vital signs[7]	+	+[8]	+[9]	+	+[10]
Hematology[11]	+	+[12]	+[12]	+	+[13]

[1] 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.

[2] 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 at Day -11, experiences engraftment failure, or cannot receive Lenti-D Drug Product (e.g., drug product does not meet specifications). HLA typing does not need to be performed if historical results are available.

[3] Genotyping of *ABCD1* gene will occur in subjects for whom no historical data is available; documented *ABCD1* mutation required prior to initiating myeloablative conditioning (Day -10)

[4] 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.

[5] CCI

[6] May occur any time before conditioning; hormonal treatment, if applicable as part of banking, should stop at least 7 days prior to conditioning.

[7] Physical examinations will include measurement of weight at all visits and height at Screening only. Full physical exam to be performed at Screening only. During hospitalization, focused physical examinations will be performed twice per week until discharge; Vital signs will include blood pressure, pulse, respiratory rate, and temperature.

[8] Focused physical examinations and vital signs will be performed prior to the first dose of G-CSF.

[9] 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.

[10] Focused physical examinations and vital signs will be performed each day during conditioning.

[11] Hematology parameters to be determined include white blood cell (WBC) count with differential, hemoglobin, hematocrit, red blood cell (RBC), and platelet count

[12] Hematology will be performed each day of mobilization and apheresis.

	Screening	Mobilization[1]	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 -11**	**D -10 to -1**
Visit Window (Days):	-10 to +5	-10	-	-3	-
Clinical chemistry	+			+	+[13]
Blood specialty labs: • Dried blood spot collection	+				
CCI [redacted]					
Neurological exam	+			+	
NFS assessment[16]	+			(+)[16]	
MFD assessment[16, 17]	+			(+)[16]	
CCI [redacted]					
Echocardiogram	+				
Electrocardiogram	+				
Brain MRI (with and without contrast)[16]	+			(+)[16]	
Evoked potentials[19]	+				
Confirmation of eligibility	+			+	
G-CSF (and plerixafor if required) administration		+			

[13] Chemistry and hematology parameters will be measured daily during conditioning; blood will be collected prior to infusion of busulfan IV and cyclophosphamide IV

CCI [redacted]

[16] 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 since the NFS and MRI at Screening and the start of Pre-Conditioning assessments. However, if subject requires sedation for MRI, performing this repeat assessment is based on Investigator judgment.

[17] May be performed concurrently with NFS assessment.

[18] Only the Socioeconomic Status test derived from Hollingshead and Redlich will be done at Screening.

[19] Evoked potentials to be performed may include BAER, visual evoked potential (VEP), nerve conduction studies (NCS), and somatosensory evoked potential (SSEP) from all 4 limbs, depending on subject age and ability to participate in the assessments. NCS may be performed in the upper and lower limbs (sural, peroneal, tibial, and median nerves). The VEP will be performed at Screening, Month 12 and Month 24. The BAER, SSEP, and NCS will be performed at Screening and Month 24. It is at PI's discretion whether to perform SSEP and NCS.

	Screening	Mobilization [1]	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 **-11**	**D -10 to -1**
Visit Window (Days):	-10 to +5	-10	-	-3	-
$CD34^+$ count [20]		+	+		
Busulfan and Cyclophosphamide administration					+
Busulfan level monitoring					+
Concomitant medication	Continuous from ICF signing				
Adverse event monitoring	Continuous from ICF signing				

[20] Peripheral blood CD34+ count should be performed either the day prior to or on the first planned day of apheresis.

Table 3: Schedule of Events: Drug Product Infusion through End of Study

	Lenti-D Drug Product 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 15	Follow Up Month 18	Follow Up Month 21	Follow Up Month 24	Early Termination
Study Day (D):	**D 0**	**D 15**	**D 30**	**D 60**	**D 90**	**D 180**	**D 270**	**D 360**	**D 450**	**D 540**	**D 630**	**D 720**	-
Visit Window (Days):	-	±7	±7	±14	±14	±14	±14	±30	±30	±30	±30	±30	NA
Drug Product infusion	+												
Physical examination, Vital signs[1]	+	+	+	+	+	+	+	+	+	+	+	+	+
Hematology[2]		+[3]	+[3]	+	+	+	+	+	+	+	+	+	+
Clinical chemistry		+[3]	+[3]	+	+	+	+	+	+	+	+	+	+
Local lab: Blood for immunological studies					+	+		+				+	+
CCI [redacted]													
Blood specialty labs:													
• Dried blood spot collection								+[5]				+[5]	+[5]
• RCL[6]					+	+		+				+[7]	+[7]
• Integration site analysis						+		+		+		+	+
CCI [redacted]													
• Exploratory biomarkers[8]			+	+		+	+	+	+	+	+	+	+

[1] Physical examinations will include measurement of weight at all visits. During hospitalization, focused physical examinations will be performed twice per week until discharge. Vital signs will include blood pressure, pulse, respiratory rate, and temperature. Vital signs are to be monitored concurrently during Lenti-D Drug Product 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. Following infusion, vital signs will be performed daily during hospitalization and at least twice per week after discharge until neutrophil engraftment occurs.

[2] Hematology parameters to be determined 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 to allow for further investigation of stem cells.

CCI [redacted]

[6] Two samples are required, one for RCL screening test, another for potential co-culture of PBLs if RCL screening test is positive.

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

[8] Blood for analyses of chitotriosidase (central laboratory) and for storage for potential analysis of antibodies against the transgene.

	Lenti-D Drug Product 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 15	Follow Up Month 18	Follow Up Month 21	Follow Up Month 24	Early Termination
Study Day (D):	**D 0**	**D 15**	**D 30**	**D 60**	**D 90**	**D 180**	**D 270**	**D 360**	**D 450**	**D 540**	**D 630**	**D 720**	-
Visit Window (Days):	-	±7	±7	±14	±14	±14	±14	±30	±30	±30	±30	±30	NA
• Optional: blood for storage						+		+				+	+
CCI [redacted]													
Neuropsychological tests								+				+	+
Global assessment								+				+	+
CCI [redacted]													
Electrocardiogram												+	+
Brain MRI (with and without contrast)[10]			+			+		+		+		+	+
Evoked potentials[12]								+				+	+
Health economic data[13]		+	+	+	+	+	+	+	+	+	+	+	+
Concomitant medication	Continuous from ICF signing												
Adverse event monitoring	Continuous from ICF signing												

[9] (+) To be performed only if peripheral blood shows a single clone with integrated lentiviral vector sequences persistently representing >10% of total PBLs and concurrent presence of leukocytosis (WBC count >30,000 cells/µL/mm3) or at the Investigator's discretion.

C [redacted]

C [redacted]

[12] Evoked potentials to be performed may include BAER, visual evoked potential (VEP), nerve conduction studies (NCS), and somatosensory evoked potential (SSEP) from all 4 limbs, depending on subject age and ability to participate in the assessments. NCS may be performed in the upper and lower limbs (sural, peroneal, tibial, and median nerves). The VEP will be performed at Screening, Month 12 and Month 24. The BAER, SSEP, and NCS will be performed at Screening and Month 24. It is at PI's discretion whether to perform SSEP and NCS.

[13] Includes number and duration of in-patient hospitalizations (including ICU stay), clinic/doctor visits, therapy, and emergency room visits.

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 at Day -11, experiences engraftment failure, or cannot receive eli-cel (e.g., drug product does not meet specifications).

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 gender, age, race, and ethnicity, will be obtained during Screening, and a complete medical history will be obtained during Screening and updated on Day 0 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) and focused physical examinations are to be conducted 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 kgs) will be measured and recorded according to the SOE; measurement details are included in the SOM.

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 fields will be assessed by perimetry testing or by visual confrontation by neurological examination. Examination details are included in the SOM. Refer to Section 6.6 for the reporting of CALD-related changes as AEs.

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 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 7 of Section 10.2, 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.

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 6 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).

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 version should be administered throughout the study even if the subject ages out of a particular test. 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.

At Screening, only the socioeconomic status (SES) test derived from Hollingshead and Redlich is to be administered.

Age-appropriate neuropsychological tests will be administered on other visits according to the SOE.

CCI

6.5.9. Evoked Potential (Electrophysiology)

Electrophysiological assessment to be performed, when feasible, and with consideration for the subjects age. This will include the Visual Evoked Potential (VEP), Brain Stem Auditory Evoked Response (BAER), and Somatosensory Evoked Potential (SSEP) from all 4 limbs and of Nerve Conduction Study (NCS) of the upper and lower limbs (the sural, peroneal [fibular], tibial, and median nerves). These should be performed according to the SOE.

VEP provides objective information about the visual system; BAER tests auditory brainstem function; SSEPs are recorded by stimulating peripheral nerves, most commonly the tibial, median, or ulnar nerves, typically with an electrical stimulus, and are used to assess the function of a subject's spinal cord; and NCS is commonly used to evaluate the function, especially the ability of electrical conduction, of the motor and sensory nerves of the human body.

It is at the Principal Investigator's discretion to administer SSEP and NCS.

6.5.10. Brain Magnetic Resonance Imaging (Loes Score)

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 who is blinded to the subject identification and time point, using the 34-point Loes scoring scale, which is widely used to diagnose and follow subjects with CALD. The Independent Review Manual will be used by the central reviewer. This charter describes the procedure for the image blinding, reading, and scoring.

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).

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

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

[a] Direct bilirubin only required if total bilirubin is abnormal.

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

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 (immunoglobulin G [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; HBV surface antigen (HBsAg) and HBV core Ab (HBcAb); hepatitis C virus (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, HSV, HCV core antigen, VZV IgG, 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 5 enumerates the priorities for blood collection. Safety labs (see Section 6.5) have been prioritized over labs for efficacy and exploratory analyses.

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 (European-Commission 2002). Any deviations to these recommendations should be justified.

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

Table 5: Blood Collection: Order of Priority

- Adrenal Function Tests
- Serology Panels (infectious disease screening)
- Chemistry
- Hematology
- [redacted]
- Proviral Integration Site Analysis in PBLs
- RCL test
- *ABCD1* genotype
- [redacted]

Clinical work-up for unexpected blood test results:

If the results from blood tests are not as expected, additional testing may need to be performed to allow for further investigation of stem cells and may include.

- Physical exam
- Blood tests
- Imaging tests
- Bone marrow biopsy

Dried Blood Spot Collection

Given the rarity of this disease, a blood sample will be collected on filter paper according to the SOE for measurement of lyso-PC in the dried blood spot. This sample is intended to facilitate the development of a newborn screening assay for ALD. Refer to the Laboratory Manual for further details regarding the collection, processing, and storage of this sample.

CCI [redacted]

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 should be determined before commencing conditioning.

Replication Competent Lentivirus (RCL) Testing

Blood samples for RCL testing will be collected and tested using an RCL screening assay. If the RCL screening assay is positive, the site may be requested to perform local HIV-1 screening assay, and if positive, follow-up with an HIV-1 Western blot, at the subject's next scheduled visit, or earlier at the discretion of the Investigator. Once available, the results of these tests must be shared with the Sponsor immediately. Additionally, a confirmatory test to assess the presence of RCL in PBLs will be performed.

Confirmation of RCL would lead to suspension of the enrollment of new subjects (see Section 3.4.2). Further, presence of RCL will be considered an SAE and will be handled and reported as described in Section 6.6.2.

Proviral Integration Site Analysis

Blood samples for proviral ISA are to be collected according to the SOE; collection, processing and shipping details are included in the Laboratory Manual.

Exploratory Biomarkers: MMP, Chitotriosidase, Antibodies Against Transgenic Product

[redacted]

Optional Blood for Storage

Optional blood and tissue samples will be collected per the SOE for future research. CCI [redacted] Such samples may be stored until repository is discontinued. 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. Leftover samples from protocol procedures (e.g., blood draw for ISA or BM collection) will also be stored for potential optional future analyses as described above.

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 CCI [redacted] are optional.

6.5.12. Assessment of Clonal Predominance and/or Suspicion of Insertional Oncogenesis (Malignancy)

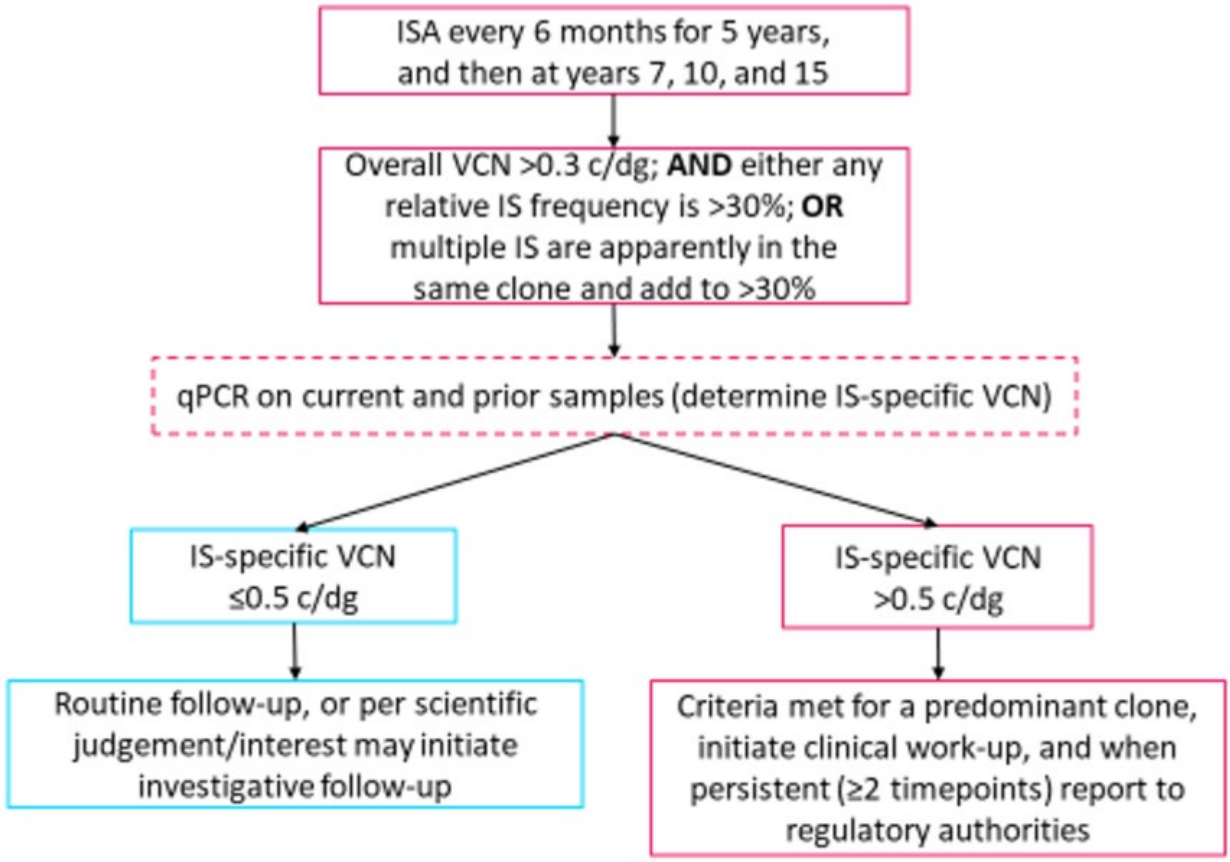
6.5.12.1. Assessment of Clonal Predominance

Figure 1 outlines the updated algorithm for assessment of clonal predominance. Screening integration site (IS) analysis (ISA) will be performed as indicated in the Schedule of Events using high-throughput, semi-quantitative methods which identify IS based on vector sequence primers. IS identified in the screening assay are considered as being of interest when the overall peripheral blood VCN is > 0.3 c/dg AND either any relative IS frequency is > 30% OR multiple IS are apparently in the same clone and add up to > 30%. Multiple IS apparently in the same clone is defined as more than one relative frequency where values are within 20% of each other (e.g. 5% ± 1%, 10% ± 2%, 15% ± 3%, etc.), as well as any additional cases identified through bluebird bio internal review of ISA reports. When multiple IS are apparently in the same clone, it will be recommended to confirm that those IS are in a single clone (e.g. bone marrow or peripheral blood colony-forming unit assay). IS of interest will be interrogated, from the timepoint of interest and available previous timepoints, using a quantitative assay (e.g. qPCR) designed to detect the specific IS and determine an IS-specific VCN to estimate clonal contribution.

If results of the quantitative, IS-specific follow-up assay reveal an IS-specific VCN ≤ 0.5 c/dg, estimating ≤ 50% clonal contribution, repeat ISA screening will continue at the regularly scheduled timepoints. However, according to scientific judgement or interest, investigative follow-up may be initiated by bluebird bio in collaboration with an investigator and additional interval, unscheduled ISA testing may be performed.

If results of the quantitative, IS-specific follow-up assay reveal an IS-specific VCN > 0.5 c/dg, estimating > 50% clonal contribution, criteria will be met to consider the subject as having a predominant clone. This threshold also applies to individual lineage evaluations (myeloid, lymphoid, etc.) when performed. Clinical work-up will be recommended for a predominant clone (see next sections). A report to relevant regulatory authorities will be required when a persistent, predominant clone is identified (2 or more timepoints), and the report will be made within 30 days of receipt of IS-specific VCN results from the second timepoint when the persistent, predominant clone is identified.

Figure 1: Schematic for Assessment of Clonal Predominance



Abbrev.: c/dg, copies per diploid genome; IS, integration site; ISA, integration site analysis; qPCR, quantitative polymerase chain reaction; VCN, vector copy number;

6.5.12.2. Other Criteria that can Trigger Clinical Work-up for Malignancy

- Any clinical suspicion of malignancy including leukemia or lymphoma
- Unexplained WBC count > 30,000 (cells/µL) on two consecutive measurements
- After achievement of a WBC count within the normal range post-drug product infusion and engraftment of gene-modified cells, the development of a WBC < 1000 (cells/µL) on two consecutive measurements

6.5.12.3. Clinical Work-up for Malignancy

If any of the above criteria is met, the Medical Monitor will be notified, and a work-up will be performed that may occur in stages and may include some of the following at each stage:

- Physical exam
- CBC with differential
- Lymphocyte subsets

- Studies to rule out infectious cause
- Studies to rule out autoimmune disease
- Imaging studies, as appropriate
- Bone marrow analysis

If clinical results indicate a diagnosis of a malignancy or myelodysplasia, enrollment into this study will be suspended, and further analyses will be determined by the Sponsor, in consultation with the DMC (see also Section 3.1.1). It should be noted it may not be possible to distinguish the source of malignancy, e.g. arising from transplant-related medications or procedures, or from expansion of gene-modified cells due to insertional mutagenesis, and all efforts should be made to confirm the source of malignancy before determining to halt or alternatively to resume the study.

If there is no evidence of malignancy or myelodysplasia, subject will continue to be monitored as per the protocol-defined SOE, or more frequently at discretion of the Investigator and Sponsor.

Bone Marrow Aspiration

The subject may undergo BM aspiration if clonal predominance is observed. The procedure may also be done at the Investigator's discretion, if there is concern about clonal expansion. The BM aspiration 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 will be collected each time.

Vector Copy Number by qPCR: Bone Marrow $CD34_+$

If clonal predominance is observed, BM samples are to be used for determination of VCN in BM by qPCR, as indicated in the SOE. Testing will be performed by a central laboratory.

6.6. Adverse Events

Monitoring of AEs will be conducted from informed consent through the duration of the study. AEs, as defined in Section 6.6.1, will be monitored and recorded in the case report forms (CRFs) from the time informed consent is signed as follows:

- All SAEs, as defined in Section 6.6.1, through Month 24
- All drug product-related AEs (related or possibly related, as defined in Section 6.6.2) through Month 24
- CALD-related ≥ Grade 2 AEs, through Month 24
- All ≥ Grade 2 AEs, through Month 12
- All ≥ Grade 1 AEs, through 30 days after drug product infusion

All AEs will be monitored until they are completely resolved or determined to be a stable or chronic condition. 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.

6.6.1. Definitions, Documentation, and Reporting

Adverse Event: 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 concurrent 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. Any worsening of CALD that qualify as a ≥ Grade 2 AE should be reported (see Section 6.5.6).

Unexpected Adverse Events

An AE is considered unexpected with eli-cel if it is not consistent in nature or severity with eli-cel reference safety information which is contained in the current eli-cel Investigator's Brochure.

Conditioning-related Events

Busulfan and cyclophosphamide 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 cyclophosphamide IV. For the purposes of this protocol, the prescribing information for busulfan and cyclophosphamide 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 cyclophosphamide 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 CRF, as applicable.

Serious Adverse Events

An SAE is any AE, occurring at any dose and regardless of causality that:

- Results in death.
- Is life-threatening. Life-threatening means that the subject was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires subject hospitalization or prolongation of existing hospitalization. Hospitalization admissions and/or surgical operations scheduled to occur during the study period but planned prior to study entry are not considered AEs if the illness or disease existed before the subject was enrolled in the study, provided that it did not deteriorate in an unexpected manner during the study (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a subject's ability to conduct normal life functions (e.g. MFDs as defined in Section 10.2 are required to be reported as an SAE; see Section 6.6.2 for SAE reporting).

- Is a congenital anomaly/birth defect.
- Is an important medical event.
    - An important medical event is an event that may not result in death, be life threatening, or require hospitalization but may be considered an SAE 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.
    - For the purposes of this protocol, while the subject remains hospitalized for study treatment, Grade 3 and Grade 4 lab values (per CTCAE criteria) that are related to myeloablative conditioning (i.e. busulfan and cyclophosphamide) will not be reported as an SAE unless they meet the requirement of being immediately life threatening.

6.6.2. Procedures for AE and SAE 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, observations of study personnel, and spontaneous reports from subjects.

All AEs (serious and non-serious) spontaneously reported by the subject and/or in response to an open question from study personnel or revealed by observation, physical examination or other diagnostic procedures will be recorded on the appropriate page of the CRF. Any clinically relevant deterioration in laboratory assessments or other clinical findings are considered an AE and the AE must be recorded on the appropriate pages of the CRF. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event.

All unexpected, related Grade 3 to 4 AEs must be reported to the Sponsor, and IRB/IEC, within 5 days (unless an SAE then must be reported within 24 hours - see below). All AEs reported to the Sponsor will be included in the Annual Reports, and reports to the DMC at periodic reporting intervals.

All SAEs that occur during the course of the study must be promptly reported by the Investigator to the Sponsor (or designee) within 24 hours from the point in time when the Investigator (or designee) becomes aware of the SAE. All SAEs must be reported whether or not they are considered causally related to eli-cel. SAE forms, created specifically by bluebird bio, will be provided to each clinical site. If the SAE report is supplied as a narrative, it will include, at minimum, the protocol number, Investigator name and site number, subject number, a description of the event, an assessment by the Investigator regarding the severity of the SAE, the relatedness of the SAE to eli-cel, date of onset, and time and amount of study treatment infusion (i.e., total number of transduced CD34+ HSCs that were transplanted). A sample of the SAE

form can be found in the SOM. Follow-up information on the SAE may be requested by bluebird bio.

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

If there are suspected unexpected serious adverse drug 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/IEC and other appropriate institutional regulatory bodies of all SUSARs or unanticipated problems, in accordance with local regulations.

For both serious and non-serious AEs, the Investigator must determine the severity of the event, and the relationship of the event to study treatment.

Severity will be assessed by the Investigator using the following criteria per the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03 including for AEs that are a result of a laboratory abnormality. The CTCAE displays Grades 1 through 5 with unique clinical descriptions of severity for each AE based on the general guideline below.

- **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.

If the severity (grade) changes within a day, the maximum severity (grade) should be recorded. If the severity (grade) changes over a longer period of time, the changes should be recorded as separate events (having separate onset and stop dates for each grade). Decreases in AE severity should not be captured. The AE should remain open at the highest grade until the AE resolves.

Relationship (i.e. 'Causality assessment'): The Investigator is required to provide their assessment of the relationship of eli-cel to all AEs. Relationship to other study related procedures other than eli-cel (i.e. mobilization, apheresis, conditioning, or study procedure) may also be required. The following is a guideline for determining the relationship of eli-cel to an AE:

- **Not Related:** Exposure to the defined study treatment did not occur, or the occurrence of the AE is not reasonably related in time.
- **Unlikely Related:** The AE occurred in a reasonable time after the defined study treatment and is doubtfully related to the investigational agent/procedure.

- **Possibly Related:** The defined study treatment and the AE were reasonably related in time, and the AE could be explained equally well by causes other than exposure to the defined study treatment.
- **Related:** The defined study treatment and the AE were reasonably related in time, and the AE was more likely explained by exposure to the defined study treatment than by other causes, or the defined 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.

6.7. 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 resolution. Information will be requested on the status of the mother and infant 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 drug used). 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 amenorrhea 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.

6.9. Long-term Follow-Up Protocol

All subjects will be followed for 24 months post-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 the ALD-102 study Statistical analysis Plan (SAP) and the associated inter-study 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 30.

The sample size for this study was not determined by formal statistical methods. The rarity, severity, and rapidly progressive nature of CALD significantly constrain enrollment. For example, from 2009 through 2013, there were only approximately 20 to 24 allo-HSCTs per year performed on subjects with ALD in the US (*http://www.cibmtr.org*).

7.2. Populations for Analysis

Three populations will be evaluated for safety, efficacy, and exploratory analyses.

The **Intent-to-treat population (ITT)** will consist of those subjects who initiate any study procedures, beginning with stimulation by G-CSF. This population will be used for the analyses of some of the safety endpoints and for the supportive analysis of the primary efficacy endpoint, if it is different from the Transplant Population (TP).

The **Transplant population (TP)** will consist of subjects who receive eli-cel. This population will be used for the analyses of all efficacy endpoints and some of the safety endpoints.

The **Successful Neutrophil Engraftment Population (NEP)** will consist of subjects who received eli-cel and achieved neutrophil engraftment defined as having 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. The NEP will be used for the supportive analysis of the primary efficacy endpoint as well as for some of the other efficacy and safety endpoints, if it is different from the TP.

7.2.1. Populations for Comparison in Inter-study Analysis:

The **Strictly ALD-102-Eligible Transplant Population (TPES)** consists of subjects in Studies ALD-101 and ALD-103 who received allo-HSCT and satisfied the following criteria: i) NFS ≤ 1 at Baseline, ii) Loes score ≥ 0.5 and ≤ 9 at Baseline, iii) GdE+ at Baseline.

The **ALD-102-Eligible Transplant Population (TPE)** consists of subjects in Studies ALD-101 and ALD-103 who received allo-HSCT and satisfied the following criteria: i) NFS≤ 1 at Baseline, ii) Loes score ≤ 9 at Baseline, iii) GdE+ at Baseline or Loes ≥ 0.5)

GdE+ Transplant Population (TPG) consists of subjects in Studies ALD-101 and ALD-103, who received allo-HSCT and were GdE+ at Baseline.

7.3. Planned Analyses

The initial analysis is planned after the first cohort of 17 subjects treated with eli-cel complete Study ALD-102. This initial analysis reflects the analysis that was planned at the time of protocol inception, and results of the initial analysis will be the basis for determining the success or failure of the study. To gather experience at EU manufacturing site, a second cohort of subjects was opened. A final analysis will be performed when all subjects in the second cohort complete the study and will be considered supportive.

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

Safety data are reviewed on an ongoing basis for signal detection and to support preparation of regulatory submission documents. Analyses of study data may also be performed for the purposes of internal data review, preparing for regulatory meetings, and updating the scientific community.

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 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

It is recognized that formal, confirmatory statistical hypotheses are extremely difficult to formulate in the context of this subject population, where few historical data are available regarding the efficacy endpoints. Further, it is not possible to have a truly randomized comparison of eli-cel to other potentially curative modes of treatment, such as allo-HSCT, due to the rarity 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, and safety 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 CIs of the mean as appropriate. Time to event data will be summarized using Kaplan-Meier methodology using 25th, 50th (median), and 75th percentiles with associated two-sided 95% CIs, as well as percent of censored observations and events.

In addition to the analyses described below, additional supportive efficacy and safety analyses will be performed on the ITT population and the NEP, as outlined 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.

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, as described in the SAP.

Subject disposition will also be presented by investigational site.

The number of subjects in each analysis population will be presented, with reasons for exclusion from any specific population.

7.4.3. Demographic and Baseline Characteristics

The following demographic and Baseline characteristic factors will be summarized: age at enrollment, age at CALD diagnosis, gender, country of origin, race and ethnicity, family history, method of diagnosis of CALD, signs and symptoms of CALD, NFS at Baseline, Loes score and pattern at Baseline, time from CALD diagnosis to eli-cel infusion (months), time from informed consent to eli-cel infusion (days), and the presence of any significant co-morbid conditions (defined as any ongoing medical history). 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 analysis of the primary efficacy endpoint, Month 24 MFD-free survival, will be performed on the TP. Analyses will also be produced on the NEP and ITT if different from the TP, but these will be considered as supportive (i.e., as sensitivity analyses) of the results in the TP.

Additionally, supportive analysis of comparison of Month 24 MFD-free survival will be made between the TP and the TPE, TPES, and TPG populations of Study ALD-103.

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 NFS 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.

The lower bound of the 2-sided 95% exact CI of the Month 24 MFD-free survival must be > 50% in order for the primary endpoint to be met. The clinically meaningful benchmark of 50% is supported by data from published literature and the ALD-101 retrospective observational study.

In Study ALD-101, the MFD-free survival rate in untreated GdE+ subjects within 2 years of their first GdE+ MRI is 21%, with an exact 95% CI of 6.1% to 45.6%. Therefore, the defined clinically meaningful benchmark of 50% is above the upper 95% CI of the Month 24 MFD-free survival in untreated GdE+ subjects in Study ALD-101.

Furthermore, the threshold value of 50% is also consistent with ALD-101 data in the "ALD-102 eligible" allo-HSCT treated cohort that excluded matched siblings: the lower 95% CI of the mean MFD-free survival rate at 24 months is 50.1% (mean 76% with exact 95% CIs of 50.1% to 93.2%). These findings are supported by Month 24 MFD-free survival rates published in the literature, which ranged between 50% and 90% (Baumann et al. 2003; Peters et al. 2004; Beam et al. 2007; Miller et al. 2011).

This success criterion of the lower bound of the 2-sided 95% exact CI of the Month 24 MFD-free survival having to be > 50% would be met with a point estimate of 76.5% (13 of 17 subjects) in the initial analysis, and with a point estimate of 70.0% (21 of 30 subjects) in the final analysis.

7.4.4.2. Analysis of Secondary and Exploratory Efficacy Endpoints

Populations to be analyzed

The analyses of the secondary and [redacted] efficacy endpoints (Section 2.2.1) will be performed on the TP. Analyses may also be produced on the NEP, but these will be considered as supportive of the results in the TP.

Analyses

The following endpoints will be presented with 2-sided 95% exact CIs:

- Proportion of subjects who demonstrate resolution of gadolinium positivity on MRI (i.e., GdE-) at Month 24
- Proportion of subjects who maintain a Loes score ≤ 9 or do not increase their Loes score by ≥ 6 points from Baseline
- CCI [redacted]

CCI [redacted]

In addition, the change over time in individual efficacy parameters will be described by presentation of summary statistics at each evaluation time. For each subject, figures that display NFS and Loes Score will be presented.

The following time-to-event endpoints will be evaluated longitudinally over time, using Kaplan-Meier statistics:

- MFD-free survival over time. Time from drug product infusion to either second transplant, MFD, or death due to any cause, whichever occurs first, will be analyzed.

- Subjects who do not experience 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.
- Time to resolution of gadolinium positivity on MRI (i.e., GdE-)
- Overall survival

For primary and secondary efficacy endpoints and CCI [redacted], Baseline is defined as the timepoint closest, but prior to conditioning.

Comparison to Efficacy Data from Studies ALD-101 and ALD-103

Comparison of selected ALD-102 efficacy endpoints in an inter-study analysis will be performed on the TPE, TPES, and TPG of ALD-101 and ALD-103.

Additionally, efficacy comparisons will be performed between Study ALD-102 subjects and untreated subjects in Study ALD-101 who are similar to those enrolled in Study ALD-102.

Analyses to be performed will be detailed in the inter-study SAP.

7.4.5. Analysis of Safety Endpoints

For safety endpoints, all laboratory data CCI [redacted] levels), vital signs, echo- and electro-cardiograms, Baseline is defined to be the assessment closest to but prior to mobilization.

The general safety profile of treatment with eli-cel will be summarized through the longitudinal evaluation of AEs, laboratory assessments, vital signs, ECG, and physical examination findings. Safety parameters will be summarized across each time point.

The primary safety endpoint will be the proportion of subjects who experience either acute (≥ Grade II) or chronic GVHD by Month 24. This analysis will be to compare the rates of ≥ Grade II and chronic GVHD in the TP population from Study ALD-102 to the rates seen in the allo-HSCT-treated population in Study ALD-103, using exact methods for binomial data.

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 on 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. after signing the informed consent to before start of mobilization;
2. from the start of mobilization up to before start of conditioning;
3. from the start of conditioning until the day before neutrophil engraftment (for subjects who do not achieve neutrophil engraftment, the study period is 42 days post-drug product infusion);

4. from neutrophil engraftment through Month 24 (for subjects who do not achieve neutrophil engraftment, the beginning of this study period is 42 days post-drug product infusion);
5. from the start of eli-cel infusion through Month 12;
6. from Month 12 through Month 24;
7. from the start of eli-cel infusion through Month 24; and
8. from signing of the informed consent through Month 24

Summaries will be provided for all AEs, serious AEs, Grade 3, 4, and 5 AEs (as assessed using NCI CTCAE Version 4.03), drug-product related AEs, and AEs leading to early termination.

See the Statistical Analysis Plan for a comprehensive list of study periods and additional details.

All AEs occurring on study will be provided in data listings, and in addition, by-subject listings will be provided for subject deaths, SAEs, subjects requiring hospitalization due to an adverse effect of therapy, drug product-related AEs, and AEs leading to withdrawal (defined as the inability to receive the therapy).

Transplant-related mortality through 100 and 365 days post-drug product infusion will be summarized for 2 intervals: from Study Day 0 through 100 days post-drug product infusion, and from Study Day 0 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.

Blood will be tested for replication-competent lentivirus at Months 3, 6, 12, and 24. Results will be listed.

Laboratory Data

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

The actual value and change from baseline to each on-study evaluation 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.

Vital Signs and Physical/Neurological Examinations

The change from Baseline to each on-study evaluation will be summarized for vital signs. Vital sign measurements will be presented for each subject in a data listing.

Physical/neurological examination results will be summarized to indicate change in status (normal/abnormal) from Baseline to each post-Baseline assessment. All physical/neurological examination findings will be presented in a data listing.

Echo- and electro-cardiograms

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

Concomitant Medications

[redacted]

Comparison to Safety Data from Studies ALD-101 and ALD-103

Additional comparison of ALD-102 safety endpoints in the inter-study analysis will be performed on the overall Transplant Population that were treated with allo-HSCT in Studies ALD-101 and ALD-103.

Analyses to be performed will be detailed in the SAP, and include incidence of SAEs, ≥ Grade 3 AEs, ≥ Grade 3 infections, successful engraftment, transplant-related mortality, and acute and chronic GVHD.

The AE analysis period starts at the day of HSC infusion for the cross-study analysis, per study ALD-103 reporting requirements.

7.4.6. Analysis of Other Exploratory Endpoints

[redacted]

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 Investigator's Brochure. 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, Investigator's Brochure, 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. 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.

8.4. Subject Confidentiality

In order to maintain subject privacy, all CRFs, eli-cel accountability records, study reports, and communications will identify the subject by initials 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 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.

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 (IBW) Formulas (Subjects 1 to 18 Years of Age):

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

IBW = [(ht^2) x 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 (AIBW) 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 6 , and these were chosen based on their clinical significance and their impact on independent functioning.

Table 6: Major Functional Disabilities (MFD)

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 Neurologic Function Scale (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 7: Neurologic Function Score (NFS) 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
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

Table 7: Neurologic Function Score (NFS) for CALD

Symptom / Neuroexam	Definition	Score
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

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Protocol Title:	A phase 2/3 study of the efficacy and safety of hematopoietic stem cells transduced with Lenti-D lentiviral vector for the treatment of cerebral adrenoleukodystrophy (CALD)
Protocol Number:	ALD-102 Version 10.0, 23 September 2020

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)