CLINICAL STUDY PROTOCOL HGB-206

A Phase 1/2 Study Evaluating Gene Therapy by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with the LentiGlobin BB305 Lentiviral Vector in Subjects with Severe Sickle Cell Disease

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SUMMARY OF CHANGES

Version and date of the previous version: Version 12.0 (01 June 2022)
Version and date of the new version: Version 13.0 (17 January 2023)

Substantial changes, defined as changes that are likely to have a significant impact on the safety or physical or mental integrity of the clinical study participants, or the scientific value of the study, compared to Protocol HGB-206 Version 12.0 are described in the table below. These changes include adding ISA assessments on bone marrow at Month 12 and Month 24 (Early Termination Visits Assessments); updating the definition of oligoclonality within the ISA algorithm; and providing a detailed explanation for assessments used for risk and clinical work-up for hematologic malignancy.

Substantial and non-substantial are described below. In addition, several minor changes were also made to reorganize content for clarity, correct typographical errors, and align the use of terminology and formatting with current standards.

DESCRIPTION OF EACH SUBSTANTIAL CHANGE

Note: in the following table, added text is in ***bold italics*** and deleted text is in ~~strikethrough~~.

<table>
<tr><th>Section of current document</th><th>Changes in New Document
(Version 13.0 as compared to Version 12.0)</th><th>Rationale</th></tr>
<tr><td>Section(s) concerned:
Section 6.1 Schedule of Events, Table 4

<table><tr><th>Procedures</th><th>D360
M12
(±30)</th><th>D720
M24
(±30)</th></tr><tr><td>Central lab:
Bone marrow for NGS[14], storage[19], and exploratory PD</td><td>X</td><td>X</td></tr></table></td><td>Section(s) concerned:
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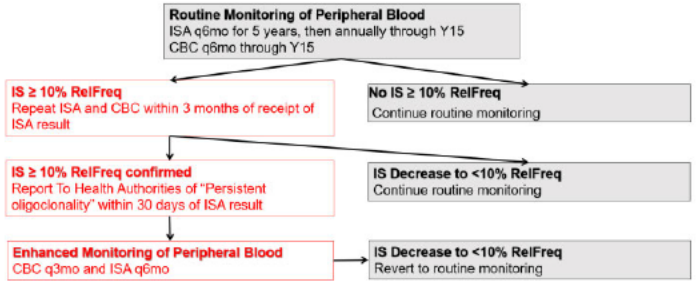
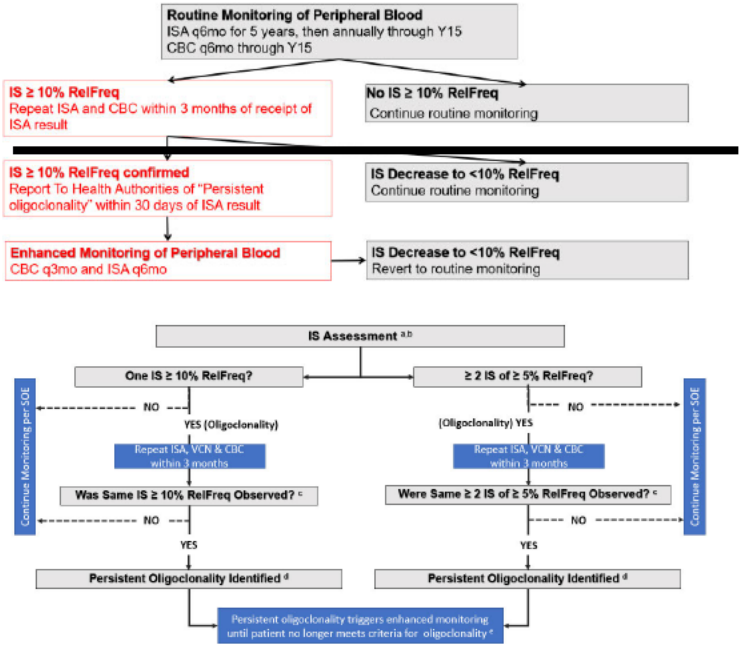
Bone marrow for NGS[14], ISA, storage[19], and exploratory PD</td><td>X</td><td>X</td></tr></table></td><td>Added integration site analysis (ISA) assessment on bone marrow (BM) at M12 and M24 (Early Termination Visit Assessments) to enable exploratory comparison of ISA results on BM and peripheral blood (PB).</td></tr>
<tr><td>Section(s) concerned:
Section 6.2.28.1 Assessment of Oligoclonality by Integration Site Analysis

[…]

ISA monitoring may be repeated more frequently if there is an indication of oligoclonality. As shown in Figure 4 if an IS is detected at ≥ 10% relative frequency (RelFreq), ISA will be repeated within 3 months of receipt of this result. If the ≥ 10% RelFreq result is confirmed, the IS will be considered as of interest, and a report of persistent oligoclonality will be submitted to the relevant Health Authorities within 30 days. This repeated observation will</td><td>Section(s) concerned:
Section 6.2.28~~.1~~ Assessment of Oligoclonality by Integration Site Analysis

[…]

ISA monitoring may be repeated more frequently if there is an indication of oligoclonality. As shown in Figure 4 if an IS is detected at ≥ 10% relative frequency (RelFreq) for 1 IS, or ≥ 5% RelFreq for ≥ 2 IS, ISA will be repeated within 3 months of receipt of this result. If the ~~≥ 10% RelFreq~~ result is confirmed~~, the IS will be considered as of interest, and~~ a report of persistent oligoclonality will be submitted to the relevant Health Authorities within 30 days. This repeated observation</td><td>Out of an abundance of caution, and to help inform future understanding of clonal frequencies and the impact of clonal dynamics on potential risk for malignancy, the ISA algorithm was updated to redefine oligoclonality to include ≥ 5%</td></tr>
</table>

Section of current document	Changes in New Document (Version 13.0 as compared to Version 12.0)	Rationale
also trigger enhanced monitoring for hematological abnormalities, increasing the frequency of CBC with differential to every 3 months along with ISA every 6 months until the frequency of the IS of interest decreases below 10% RelFreq (1 IS) or below 5% RelFreq (multiple IS). […] Figure 4: Algorithm for Frequency of ISA Monitoring  Abbrev.: CBC, complete blood count; IS, integration site(s); ISA, integration site analysis; q3mo, every 3 months; q6mo, every 6 months; RelFreq, relative frequency; Y, Year. Note that this schematic includes the assessment schedule for ISA through the subsequent long-term follow-up study. […]	will also trigger enhanced monitoring for hematological abnormalities, increasing the frequency of CBC with differential to every 3 months along with ISA ***and VCN*** every 6 months until the ***criteria for oligoclonality is no longer met***~~frequency of the IS decreases below 10% RelFreq~~. […] Figure 4: Algorithm for Frequency of ISA Monitoring  Abbrev.: ~~CBC, complete blood count;~~ IS, ~~integration~~ ***insertion*** site(s); ISA, integration site analysis; ~~q3mo, every 3 months; q6mo, every 6 months;~~ RelFreq, relative frequency~~; Y, Year.~~ ~~Note that this schematic includes the assessment schedule for ISA through the subsequent long-term follow-up study.~~	RelFreq for ≥ 2 IS each. Clarification is provided that enhanced monitoring includes VCN, and a new ISA algorithm figure is included for clarity.

Section of current document	Changes in New Document (Version 13.0 as compared to Version 12.0)	Rationale
	[a] ISA is assessed per the schedule of events; CBC with differential and VCN are to be carried out whenever ISA is performed (Table 4). ***[b] If ISA is assessed concurrently from PB and BM, the assessment with higher RelFreq values should be used.*** ***[c] Samples obtained one month or more apart can be considered consecutive measurements.*** ***[d] Persistent oligoclonality will be reported to the relevant Health Authorities within 30 days.*** ***[e] At a minimum, enhanced monitoring includes CBC with differential every 3 months and ISA every 6 months.Additional monitoring for malignancy may be instituted by the treating physician/Principal Investigator (see Section 6.2.29).*** […]	
Section(s) concerned: Section 6.2.28.2 Clinical Work-up for Potential Malignancy In the event of any suspicion of hematological malignancy (i.e., myelodysplasia, leukemia, or lymphoma), the Medical Monitor will be notified and a work-up will be performed by the Investigator per appropriate standard of care. A suspicion of malignancy could arise in the setting of otherwise unexplained cytopenia(s), and consideration of insertional oncogenesis could arise if cytopenia(s) occurs in conjunction with a rising RelFreq of an IS in a gene of known biological relevance to carcinogenesis (i.e., oncogene or tumor suppressor gene), accompanied by a rapid increase in PB VCN. The clinical work-up may include the following: • Physical exam • CBC with differential • Lymphocyte subsets	Section(s) concerned: Section 6.2.~~28.2~~29 ***Assessments for Risk of Hematologic Malignancy and*** Clinical Work-up~~ for Potential Malignancy~~ ~~In the event of any suspicion of hematological malignancy (i.e., myelodysplasia, leukemia, or lymphoma), the Medical Monitor will be notified and a work-up will be performed by the Investigator per appropriate standard of care. A suspicion of malignancy could arise in the setting of otherwise unexplained cytopenia(s), and consideration of insertional oncogenesis could arise if cytopenia(s) occurs in conjunction with a rising RelFreq of an IS in a gene of known biological relevance to carcinogenesis (i.e., oncogene or tumor suppressor gene), accompanied by a rapid increase in PB VCN.~~ ~~The clinical work-up may include the following:~~ ~~• Physical exam~~ ~~• CBC with differential~~ ~~• Lymphocyte subsets~~	Per FDA recommendation, a detailed explanation is included for how primary assessments are intended to inform the potential decision for clinical work-up for potential hematologic malignancy. Additionally, specific recommendations are providing around the use of reflexive assessments during clinical

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• Imaging studies • Bone marrow analysis • Cytogenetic and molecular analyses which can include fluorescence in situ hybridization (FISH), SNP microarray, karyotyping, or whole-genome sequencing […]	• ~~Imaging studies~~ • ~~Bone marrow analysis~~ • ~~Cytogenetic and molecular analyses which can include fluorescence in situ hybridization (FISH), SNP microarray, karyotyping, or whole-genome sequencing~~ ***Routine assessments carried out per the SOE (Table 4) for risk of potential hematologic malignancy are presented in Figure 5 . Clinical presentation and CBC with differential are considered standard assessments, whereas BM biopsy, ISA, NGS, and karyotyping are considered supportive assessments in the context of gene therapy. In the event of suspicion of hematologic malignancy (i.e., myelodysplasia, leukemia, or lymphoma), discussion with the Medical Monitor is required and should include an evaluation of local versus central testing. Clinical work-up is to be performed by the Investigator per appropriate standard of care and in alignment with WHO and NCCN guidelines (Arber et al. 2016; Greenberg et al. 2022).***	work-up for potential hematologic malignancy. Notably, if BM biopsy is performed as part of clinical work-up, ISA on BM should be included.

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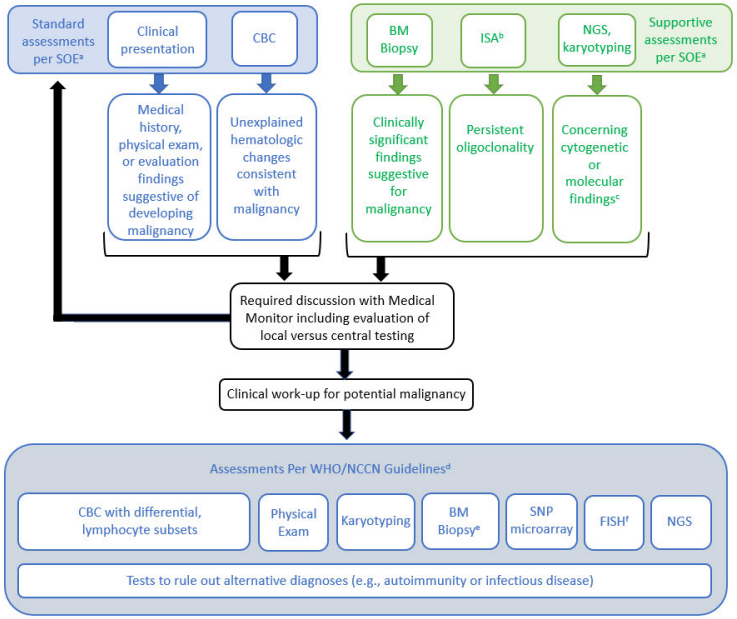
For clinical work-up after identification of persistent oligoclonality and confirmed presence of abnormal CBC, bone marrow analysis is recommended if not previously performed as part of the clinical work-up.	***Figure 5: Assessments for Risk of Hematologic Malignancy per Schedule of Events and Clinical Work-up***  ***Abbrev.: AML, acute myeloid leukemia; BM, bone marrow; CBC, complete blood count; FISH, fluorescence in situ hybridization; IS, insertion site; ISA, integration site analysis; MDS, myelodysplastic syndrome; NCCN, National Comprehensive Cancer Network; NGS, next generation sequencing; RNA-seq, RNA sequencing; SNP, single nucleotide polymorphism; VCN, vector copy number; WHO, World Health Organization*** ***[a] Routine monitoring include assessments per SOE (Table 4) and ongoing patient education on risk of malignancy.*** ***[b] Turnaround times for ISA results can vary in duration, typically taking approximately 10 weeks from sample collection to data delivery. ISA may be done on PB or BM per the SOE (Table 4).***	

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	[c] In addition to NGS and karyotype, other genetic testing results that are suggestive of potential risk for malignancy may be taken into account. *[d] If a subject has persistent oligoclonality (see Section 6.2.29) and other cause for clinical work-up, additional assessments such as BM biopsy, RNA-seq, lineage-specific ISA (to be performed on BM only if as available), or IS-specific VCN can be considered; (Arber et al. 2016; Greenberg et al. 2022).* *[e] ISA should be done on BM if taken as part of clinical work-up* *[f] In the context of clinical work-up for hematologic malignancy, FISH assessments should be local, according to guidelines, as local laboratories are certified in this context. Central FISH may be performed retrospectively on stored samples if results from local work-up are unclear.* *Under specific circumstances, after discussion with the Medical Monitor, BM biopsy, RNA-seq/RT-PCR, lineage-specific ISA, and IS-specific VCN may be considered appropriate assessments during clinical work-up, specifically if there is persistent oligoclonality as identified with ISA AND at least one of the following:* • *Medical History, physical exam, or evaluation findings suggestive of developing malignancy OR* • *Unexplained hematologic changes consistent with malignancy OR* • *Clinically significant findings suggestive for malignancy on BM pathology OR* • *Concerning cytogenetic or molecular findings on karyotype, NGS, or other genetic testing results that are suggestive of potential risk for malignancy* *Note at any time, regardless of clonality measurements, any additional clinical work-up may be performed at the Investigator's discretion if there is suspicion of malignancy. In the event of unclear diagnosis, additional supportive assessments (e.g., whole genome sequencing or whole exome sequencing) can be considered in individual, specific cases.*	

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In addition, clinical work-up to rule out infectious cause or autoimmune disease may be considered. If the clinical work-up indicates no evidence of myelodysplasia, leukemia, or lymphoma, the subject will continue to be monitored as per the protocol-defined SOE, or more frequently at discretion of the treating physician/Principal Investigator. If the clinical work-up indicates a diagnosis of myelodysplasia, leukemia, or lymphoma, the Sponsor will convene an urgent safety review meeting. Further analyses will be determined by the Sponsor, in consultation with the DMC. All efforts should be made to confirm the source of malignancy. It should be noted that it may not be possible to distinguish the source of malignancy (e.g., arising from underlying pathophysiology of the disease, transplant-related medications or procedures, or from expansion of gene-modified cells due to insertional oncogenesis). For clinical work-up after identification of persistent oligoclonality and confirmed presence of abnormal CBC, bone marrow analysis is recommended if not previously performed as part of the clinical work-up.	~~In addition, clinical work-up to rule out infectious cause or autoimmune disease may be considered.~~ If the clinical work-up indicates no evidence of myelodysplasia, leukemia, or lymphoma, the subject will continue to be monitored as per the protocol-defined SOE, or more frequently at discretion of the treating physician/Principal Investigator. If the clinical work-up indicates a diagnosis of myelodysplasia, leukemia, or lymphoma, the Sponsor will convene an urgent safety review meeting. Further analyses will be determined by the Sponsor, in consultation with the DMC. All efforts should be made to confirm the source of malignancy. It should be noted that it may not be possible to distinguish the source of malignancy (e.g., arising from underlying pathophysiology of the disease, transplant-related medications or procedures, or from expansion of gene-modified cells due to insertional oncogenesis). ~~For clinical work-up after identification of persistent oligoclonality and confirmed presence of abnormal CBC, bone marrow analysis is recommended if not previously performed as part of the clinical work-up.~~	

DESCRIPTION OF EACH NON-SUBSTANTIAL CHANGE

- Added clarifying footnotes to the schedule of events (Table 4) to specify that CBC with differential and VCN are to be carried out whenever ISA is performed.
- Section 6.2.28.1, Assessment of Oligoclonality by Integration Site Analysis, was redesignated as Section 6.2.28. Section 6.2.28.2, Clinical Work-up for Potential Malignancy, was redesignated as Section 6.2.29 and renamed for clarity to align to new content (Assessments for Risk of Hematologic Malignancy and Clinical Work-up).

CLINICAL STUDY PROTOCOL SYNOPSIS

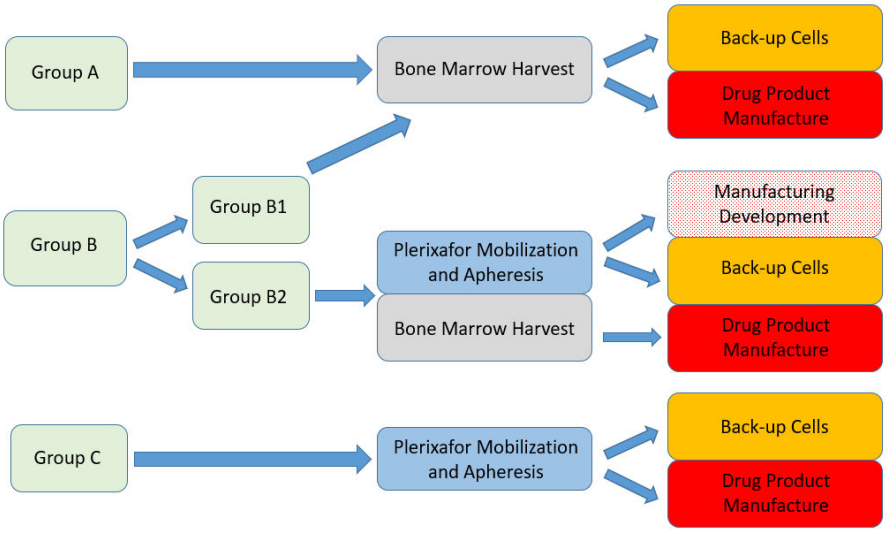
Protocol Title:	A Phase 1/2 Study Evaluating Gene Therapy by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with the LentiGlobin BB305 Lentiviral Vector in Subjects with Severe Sickle Cell Disease
Protocol Number:	HGB-206
Objectives:	**Primary objective** Evaluate the efficacy of treatment with bb1111 (lovotibeglogene autotemcel, also known as LentiGlobin BB305 Drug Product for Sickle Cell Disease) in subjects with severe sickle cell disease (SCD). **Secondary objective** Evaluate the safety of treatment with bb1111 in subjects with severe SCD.
Study Design:	This is a non-randomized, open label, multi-site, single dose, Phase 1/2 study in approximately 50 adults and adolescents with severe SCD. The study will evaluate hematopoietic stem cell (HSC) transplantation (HSCT) using bb1111 (lovotibeglogene autotemcel, also known as LentiGlobin BB305 Drug Product for SCD), an autologous CD34+ cell-enriched population from patients with sickle cell disease that contains hematopoietic stem cells transduced with BB305 lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene, suspended in cryopreservation solution. Treatment is divided into 4 stages, as follows. <u>Stage 1- Screening and eligibility assessment</u> Subjects will be screened and undergo clinical, imaging, and laboratory assessments for eligibility determination. <u>Stage 2 - Stem cell harvest, drug product manufacture and disposition</u> If subjects are taking hydroxyurea (HU), it must be discontinued at least 30 days prior to stem cell harvest. It may be restarted between completion of the stem cell harvest(s) and admission to the hospital for myeloablation but should be stopped again at least 2 days before initiating myeloablation. For at least 60 days prior to stem cell harvest and continuing through until the start of conditioning, subjects will undergo a transfusion regimen (exchange or simple, as available or needed) to reach a target hemoglobin (Hb) of 10 g/dL prior to mobilization (and 8 to 10 g/dL prior to conditioning; not to exceed 12 g/dL prior to either mobilization or conditioning) and a pre-transfusion target hemoglobin S (HbS) proportion in the blood of < 30% to reduce the risk of SCD-related complications. The last exchange transfusion must occur within 4 days of start of mobilization, and the HbS proportion in the blood must be < 20% after the last exchange transfusion. If a subject experiences an SCD-related crisis within 1 month of the start of mobilization, the transfusion regimen will be adjusted, and mobilization will be postponed until the subject is crisis-free for a period of 1 month. In case of iron overload, subjects should be adequately chelated; however, iron chelation must be discontinued at least 7 days prior to stem cell harvest. It may be restarted between completion of the stem cell harvest(s) and admission to the hospital for myeloablation but should be stopped again at least 7 days before initiating myeloablation.

Subjects who are not documented to have met these parameters or who have experienced any significant new sickle-related complications within 1 month of the start of mobilization will require additional approval from the Medical Monitor before undergoing mobilization/apheresis.

Subjects will undergo stem cell harvest via mobilization with plerixafor (0.24 mg/kg) and subsequent apheresis to collect HSCs for both drug product manufacture and cryopreservation of back-up cells for rescue. Apheresis should begin approximately 4-6 hours after plerixafor administration. If more than 1 apheresis day is required, platelet counts must be confirmed to be $\geq 75 \times 10^9$/L within 24 hours of subsequent apheresis sessions, prior to administration of plerixafor on that day. If platelet counts do not meet these criteria, mobilization and apheresis should be deferred until platelet counts recover to $\geq 75 \times 10^9$/L. At least 1.5×10^6 $CD34^+$ cells/kg will be cryopreserved for rescue. Following transduction of isolated CD34+ HSCs with BB305 lentiviral vector, the bb1111 cell dose will be $\geq 3.0 \times 10^6$ $CD34^+$ cells/kg for each subject.

Subjects for whom adequate HSCs are collected to achieve the minimum cell dose but not for rescue, and who do not tolerate plerixafor or do not effectively mobilize with plerixafor may be offered the option of bone marrow harvest for collection of back-up cells only, provided that bb1111 manufactured from mobilized cells has met criteria for dispositioning for clinical use. 1.0×10^8 total nucleated cells (TNC)/kg derived from bone marrow will be collected for rescue.

Subjects were divided into 3 groups, depending on how stem cells were harvested, and their use for either rescue (back-up cells) or drug product manufacture, as shown in the following schematic:



In Groups A and B1 (no further enrollment), both drug product and back-up cells were derived from bone marrow.

In Group B2 (no further enrollment), plerixafor mobilization and apheresis were used for collection of back-up cells and exploratory manufacturing development, and it was planned for subjects in Group B2 to then undergo bone marrow harvest for drug product manufacture.

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	Subjects in Group B2 who tolerated and successfully mobilized with plerixafor for back-up cells, and who had not yet had drug product manufactured with bone marrow harvest, were given the option to delay collection of cells for drug product manufacture until Group C opened, and they could undergo drug product manufacture from cells obtained by a subsequent cycle of plerixafor mobilization. Group C was opened upon (1) confirmation of the safety and tolerability of plerixafor mobilization in Group B2, and (2) regulatory authority approval of the drug product manufacturing process with plerixafor-mobilized HSCs. In Group C, it is planned that all subjects will undergo mobilization with plerixafor and subsequent apheresis to collect HSCs both for drug product manufacture and for cryopreservation of back-up cells for rescue. Subjects who do not tolerate plerixafor or do not effectively mobilize with plerixafor may be offered the option of bone marrow harvest for collection of back-up cells only, provided that drug product manufactured from mobilized cells has met criteria for dispositioning for clinical use. As of Version 8.0 of this protocol, Group C will include adolescent subjects ≥ 12 and < 18 years of age in addition to subjects ≥ 18 and ≤ 50 years of age. It should also be noted that while all Group A subjects and some Group B subjects received drug product manufactured using "Process 1", all current and future Group C subjects will receive drug product manufactured using an optimized process termed "Process 2". Treatment with drug product manufactured using Process 2 (versus Process 1) has resulted in increased drug product vector copy number (VCN), peripheral blood VCN, and hemoglobin A containing $\beta^{A\text{-}T87Q}$-globin (HbA^{T87Q}) levels across multiple bluebird bio-sponsored clinical studies. bb1111 must be release tested and dispositioned for clinical use and stored at the study site before the subject can begin myeloablative conditioning with busulfan. <u>Stage 3 –Myeloablative conditioning and infusion of bb1111</u> The subject's suitability for transplant will be reconfirmed with clinical laboratory tests (complete blood count [CBC], serum chemistry, liver function tests, and pregnancy test), physical examination, performance status, cytogenetics, and other tests based on institutional requirements. The results of these tests will be reviewed, and availability of the drug product on site and the availability of back-up cells will be confirmed before the site can proceed with conditioning. As described above, a transfusion regimen (exchange or simple, as available and needed) will be continued until the start of conditioning to reach a target Hb (prior to conditioning) of 8 to 10 g/dL (not to exceed 12 g/dL) and a pre-transfusion target HbS proportion in the blood of < 30% to reduce the risk of SCD-related complications; last exchange transfusion must occur within 1 week of start of the conditioning regimen. In the event of pulmonary complications (e.g., acute chest syndrome (ACS), pneumonia) between Screening and the start of conditioning, a pulmonary function test (PFT) must be performed. After resolution of pulmonary complications, and prior to conditioning, PFT results must meet the following requirements:

	○ Baseline oxygen saturation ≥ 90% without supplemental oxygen (excluding periods of SCD crisis, severe anemia or infection). ○ Baseline carbon monoxide diffusing capacity (DL_{CO}) ≥ 50% (corrected for Hb) in the absence of infection. If DL_{CO} cannot be assessed due to age or cognition-related restrictions, there must be a normal respiratory exam, chest radiograph without pulmonary infiltrates, and oxygen saturation by pulse oximetry ≥ 90% on room air. Busulfan (Busulfex® preferred, if available) will be administered intravenously (IV) at a starting dose of 3.2 mg/kg/day or 0.8 mg/kg every 6 hours (q6h) for 4 consecutive days (for subjects weighing < 35 kg, a q6h dose of 0.8 mg/kg is preferred; for subjects weighing ≥ 35 kg, either once daily (qd) or q6h dosing can be used, at the discretion of the Investigator). The dose of busulfan will be adjusted based upon busulfan pharmacokinetics (PK) in order to maintain appropriate levels for myeloablation (area under the curve [AUC] goal of 1250 [range 1100 to 1350] µM*min for a q6h dosing regimen, or 5000 [range 4400 to 5400] µM*min for a qd dosing regimen). The dosage should be calculated on the basis of the lower of the ideal versus actual body weight. The dose may be adjusted appropriately based upon actual plasma busulfan levels observed. Clinical sites must be able to measure first and third day busulfan PK. Based on busulfan PK results for day 1 and day 3 of busulfan dosing, busulfan dose adjustments should be made for subsequent dosing. If feasible, daily busulfan PK measurement is recommended. Anti-seizure prophylaxis must begin at least 12 hours before initiating busulfan and must continue for at least 24 hours after completion of the 4 days of busulfan administration. All drugs other than phenytoin are allowed for anti-seizure prophylaxis. After completion of the 4-day course of busulfan, there must be a minimum of 48 hours before bb1111. Busulfan levels will be measured 48 and 72 hours after final dose of busulfan for retrospective confirmation of adequate wash-out. On Day 1, bb1111 will be administered after thawing via IV infusion. <u>Stage 4 – Follow-up for approximately 24 months after drug product infusion</u> Subjects will be followed in the hospital for adverse events (AEs), and laboratory parameters will be followed to monitor engraftment. The subject may be discharged after neutrophil engraftment occurs (≥ 0.5×10^9 absolute neutrophil count (ANC)/L for 3 consecutive measurements on different days after the initial post-infusion nadir) and the subject is considered medically stable per institutional guidelines. From drug product infusion through hospital discharge after neutrophil engraftment, subjects are to be medically managed with the goal of maintaining the following hematologic targets: • Hb 8 to 10 g/dL (not to exceed 12 g/dL), HbS < 30% and • platelet count ≥ 50×10^9/L After discharge, management of transfusions for subjects with SCD will follow the institutional standard of care at the clinical site to achieve total Hb

	and HbS proportions appropriate for each subject's clinical status. To achieve this, the transfusion program may be progressively reduced as the contribution of Hb containing $\beta^{A\text{-}T87Q}$-globin rises. Continued transfusion needs > 6 months post-treatment or any alternative plans should be discussed with the Medical Monitor. Subjects will be followed in this protocol through the Month 24 Visit. Thereafter, subjects are expected to enroll in a separate long-term follow-up protocol that will assess safety and efficacy beyond Month 24 for a total of 15 years after drug product infusion.
Number of Subjects Planned:	A total of approximately 50 subjects will be treated with bb1111 in Study HGB-206. Seven subjects have already been treated in Group A, and 2 subjects have been treated in Group B. A total of approximately 41 subjects in Group C (including subjects consented under Versions 7.0, 8.0, and 9.0 of this protocol) will be treated with bb1111, approximately 35 of which must meet the severe vaso-occlusive event (VOE) criteria as set forth in Inclusion Criterion #3.1. Subjects who withdraw prior to drug product infusion may be replaced.
Inclusion Criteria:	Subjects must: 1. Be ≥ 12 and ≤ 50 years of age at time of consent. 2. Have a diagnosis of SCD, with either β^S/β^S or β^S/β^0 or β^S/β^+ genotype. 3. Previous Inclusion Criterion #3 is no longer applicable and has been replaced with Criterion #3.1. 3.1. In the setting of appropriate supportive care measures (e.g., pain management plan) have experienced **at least 4 severe VOEs in the 24 months prior to informed consent as defined below.** For the purposes of this study, a severe VOE is defined as an event with no medically determined cause other than a vaso-occlusion, requiring a ≥ 24-hour hospital or emergency room (ER) observation unit visit or at least 2 visits to a day unit or ER over 72 hours with both visits requiring intravenous treatment. Exception: priapism does not require hospital admission but does require a medical facility visit; 4 priapism episodes that require a visit to a medical facility (without inpatient admission) are sufficient to meet criterion. Severe VOEs include: a. an episode of acute pain with no medically determined cause other than a VOE b. Acute chest syndrome (ACS), defined by an acute event with pneumonia-like symptoms (e.g., chest pain, fever [> 38.5°C], tachypnea, wheezing or cough, or findings upon lung auscultation) and the presence of a new pulmonary infiltrate consistent with ACS and requiring oxygen treatment and/or blood transfusion. c. Acute hepatic sequestration, defined by a sudden increase in liver size associated with pain in the right upper quadrant, abnormal results of liver-function test not due to biliary tract

	disease, and reduction in Hb concentration by at least 2 g/dL below the baseline value d. Acute splenic sequestration, defined as sudden enlargement of the spleen and reduction in Hb concentration by at least 2 g/dL below the baseline value. e. Acute priapism: defined as a sustained, unwanted painful erection lasting more than 2 hours and requiring care at a medical facility (with or without hospitalization) 4. Previous Inclusion Criterion #4 is no longer applicable as of Protocol Amendment 8.0. 5. Have a Karnofsky performance status of ≥ 60 (≥ 16 years of age) or a Lansky performance status of ≥ 60 (< 16 years of age). 6. Previous Inclusion Criterion #6 is no longer applicable as of Protocol Amendment 8.0. 7. Have either experienced HU failure at any point in the past (defined as > 1 VOE or ≥ 1 ACS after HU has been prescribed for at least 6 months) or must have intolerance to HU (intolerance is defined as the patient being unable to continue to take HU per PI judgment). 8. Be treated and followed for at least the past 24 months prior to Informed Consent in medical center(s) that maintained detailed records on sickle cell disease history, including incidence of VOEs and severe VOEs, aplastic crises, infectious complications, SCD-related chronic complications, SCD-related surgery, neurovascular evaluation (including MRI/A and TCD), pRBC transfusions (including indications, volume and units of pRBCs, associated pre-transfusion HbS and total Hb values and post-transfusion adverse events [including allo-immunization]), SCD-specific treatment history (e.g., HU, L-glutamine, iron overload, and chelation history), and use of pain medication.
Exclusion Criteria:	Subjects are excluded if they meet any of the following criteria: 1. Positive for presence of human immunodeficiency virus type 1 or 2 (HIV-1 or HIV-2), hepatitis B, hepatitis C, human T-lymphotrophic virus-1 (HTLV-1) or -2 (HTLV-2), active syphilis. 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 antibody] are eligible. Subjects with past exposure to HBV [HBc Ab positive and/or HBe Ab positive] are also eligible for the study provided they are negative for HBV DNA. Subjects who are positive for anti-hepatitis C antibody are eligible as long as they have an undetectable hepatitis C viral load. Where clinically and/or regionally indicated, other tests may be performed, in which case relevant positive results suggesting active infection would exclude the subject from participating, depending on regional guidelines: for example, malaria, tuberculosis, active toxoplasmosis, Trypanosoma cruzi, or West Nile Virus.

	2. Clinically significant, active bacterial, viral, fungal, or parasitic infection, as determined by the Investigator, e.g., active relapsing malaria. 3. Inadequate bone marrow function, as defined by an absolute neutrophil count of $< 1 \times 10^9$/L ($< 0.5 \times 10^9$/L for subjects on hydroxyurea treatment) or a platelet count $< 100 \times 10^9$/L 4. Previous Exclusion Criterion #4 is no longer applicable as of Protocol Amendment 8.0. 4.1 Severe cerebral vasculopathy, defined by any history of: overt ischemic or hemorrhagic stroke, abnormal transcranial Doppler (> 200 cm/sec) requiring chronic transfusion, occlusion or stenosis in the circle of Willis, or presence of Moyamoya disease. Subjects with radiologic evidence of silent infarction in the absence of any of the above criteria would still be eligible. 5. Previous Exclusion Criterion #5 is no longer applicable as of Protocol Amendment 8.0. 6. Previous Exclusion Criterion #6 is no longer applicable as of Protocol Amendment 8.0. 7. Previous Exclusion Criterion #7 is no longer applicable as of Protocol Amendment 8.0. 8. Baseline oxygen saturation < 90% without supplemental oxygen (excluding periods of SCD crisis, severe anemia or infection). 9. Baseline carbon monoxide diffusing capacity (DL_{CO}) < 50% (corrected for Hb) in the absence of infection. If DLco cannot be assessed due to age or cognition-related restrictions, there must be a normal respiratory exam, chest radiograph without pulmonary infiltrates, and oxygen saturation by pulse oximetry ≥ 90% on room air. 10. Baseline left ventricular ejection fraction (LVEF) < 45% measured by cardiac echography. 11. Clinically significant pulmonary hypertension at baseline, as defined by the requirement for ongoing pharmacologic treatment or the consistent or intermittent use of supplemental home oxygen. 12. Baseline estimated glomerular filtration rate (eGFR) < 70 mL/min/1.73 m^2, as determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (see http://www.kidney.org/professionals/kdoqi/gfr_calculator.cfm). 13. Advanced liver disease, defined as: a. Persistent aspartate transaminase, alanine transaminase, or direct bilirubin value > 3× the upper limit of normal (ULN), **or** b. Baseline prothrombin time or partial thromboplastin time > 1.5× ULN, suspected of arising from liver disease, **or** c. Magnetic Resonance Imaging (MRI) of the liver demonstrating clear evidence of cirrhosis, **or** d. MRI findings suggestive of active hepatitis, significant fibrosis, inconclusive evidence of cirrhosis, or liver iron

	concentration ≥ 15 mg/g require follow-up liver biopsy in subjects ≥ 18 years of age. In subjects < 18 years of age, these MRI findings are exclusionary, unless in the opinion of the Investigator, a liver biopsy could provide additional data to confirm eligibility and would be safe to perform. If a liver biopsy is performed based on MRI findings, any evidence of cirrhosis, bridging fibrosis, or significant active hepatitis will be exclusionary. 14. For subjects who have history of iron overload or serum ferritin levels > 1000 ng/mL, a cardiac MRI is required. Cardiac T2* < 10 ms results in exclusion. 15. Contraindication to anesthesia. 16. Any contraindications to the use of plerixafor during the mobilization of hematopoietic stem cells and any contraindications to the use of busulfan and any other medicinal products required during the myeloablative conditioning, including hypersensitivity to the active substances or to any of the excipients. 17. Any prior or current malignancy or immunodeficiency disorder, except previously treated, non-life threatening, cured tumors such as squamous cell carcinoma of the skin. 18. Prior receipt of an allogeneic transplant. 19. 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). 20. Diagnosis of significant psychiatric disorder of the subject that, in the Investigator's judgment, could seriously impede the ability to participate in the study. 21. Pregnancy, or breastfeeding in a postpartum female, or absence of adequate contraception for fertile subjects. Females of childbearing potential must agree to use a medically acceptable method of birth control such as oral contraceptive, intrauterine device, barrier and spermicide, or contraceptive implant/injection from time of consent through at least 6 months after drug product infusion. Male subjects must agree to use effective contraception (including condoms) from Conditioning through at least 6 months after drug product infusion. 22. Participation in another clinical study with an investigational drug within 30 days of Screening. 23. Previous Exclusion Criterion #23 is no longer applicable as of Protocol Amendment 8.0. 24. Prior receipt of gene therapy. 25. Previous Exclusion Criterion #25 is no longer applicable as of Protocol Amendment 9.0. 26. An assessment by the Investigator that the subject or parents/caregivers (as required) will not be able to comply with the study procedures outlined in the study protocol.

	27. Patients needing therapeutic anticoagulation treatment during the period of conditioning through platelet engraftment (patients on prophylactic doses of anticoagulants not excluded per this criteria). 28. Unable to receive RBC transfusion. 29. Any other condition that would render the subject ineligible for HSCT, as determined by the attending transplant physician. 30. Applicable to subjects < 18 years of age only: Availability of a willing, matched HLA-identical sibling hematopoietic cell donor.
Concomitant Medications/Therapies:	Subjects will be permitted to take their usual medications during the study, with the following exceptions: erythropoietin, which should be discontinued 60 days prior to stem cell collection; HU, which should be discontinued 30 days prior to HSC mobilizations and collections; and iron chelation therapy, which should be discontinued at least 7 days prior to conditioning. Hydroxyurea may be restarted between completion of the stem cell harvest(s) and admission to the hospital for myeloablation but should be stopped again at least 2 days before initiating myeloablation. In case of iron overload, subjects should be adequately chelated; however, iron chelation must be discontinued at least 7 days prior to stem cell harvest. Phlebotomy or iron chelation therapy may be restarted no sooner than 3 months after transplant. Subjects should not use medications with anti-retroviral activity (such as those used for HIV prophylaxis) from within 1 month of initiating mobilization until after completion of stem cell collection for drug product manufacture. Blood products will be filtered and irradiated, as needed. Management of post-transplant transfusions for subjects with SCD will follow the institutional standard of care at the clinical site to achieve total Hb and HbS proportions appropriate for each subject's clinical status. To achieve this, the transfusion program may be progressively reduced as the contribution of hemoglobin containing $\beta^{A\text{-}T87Q}$-globin rises. Continued transfusion needs > 6 months post-treatment or any alternative plans should be discussed with the Medical Monitor. Following HSC transplant, medications other than blood transfusions to treat sickle cell disease or anemia (e.g., HU, erythropoietin, L-glutamine, crizanlizumab, voxelotor) are not permitted without prior discussion with and explicit approval of the Sponsor's Medical Monitor.
Duration of Subject Participation:	Time between Screening and drug product infusion will be variable and is estimated generally to be between 3 to 5 months (e.g., up to 3 months between Screening and Mobilization, followed by approximately 2 months before drug product infusion). Thereafter the subject is planned to remain on study for approximately 24 months. Eligible subjects are expected to enroll in a separate long-term follow-up study until approximately 15 years post-drug product infusion.
Test Product, Dose and Mode of Administration:	bb1111 is an autologous CD34+ cell-enriched population from patients with sickle cell disease that contains hematopoietic stem cells transduced with BB305 lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene, suspended in cryopreservation solution. All subjects are to receive $\geq 3.0 \times 10^6$ $CD34^+$ cells/kg of bb1111 on Day 1 via intravenous infusion.

Efficacy Endpoints:	**Primary Efficacy Endpoint:** VOE-CR, defined as complete resolution of VOEs, between 6 months and 18 months after drug product infusion
	Key Secondary Efficacy Endpoints: • sVOE-CR, defined as complete resolution of severe VOEs, between 6 months and 18 months after drug product infusion • Globin Response, defined as meeting the following criteria for a continuous period of at least 6 months after drug product infusion: a. Weighted average HbA^{T87Q} percentage of non-transfused total Hb[1] ≥ 30% AND b. Weighted average non-transfused total Hb[1] increase of ≥ 3 g/dL compared to baseline total Hb[2] OR weighted average non-transfused total Hb[1] ≥ 10 g/dL
	Additional Secondary Efficacy Endpoints: **Clinical and Disease Evaluation Endpoints:** • Change in the annualized number of VOEs in the 24 months after drug product infusion compared to the 24 months prior to Informed Consent • Change in the annualized number of severe VOEs in the 24 months after drug product infusion compared to the 24 months prior to Informed Consent • VOE-CR24, defined as complete resolution of VOEs, between 6 months and 24 months after drug product infusion • sVOE-CR24, defined as complete resolution of severe VOEs between 6 months and 24 months after drug product infusion • sVOE-75, defined as at least a 75% reduction in annualized severe VOEs in the 24 months after drug product infusion compared to the 24 months prior to Informed Consent **Characterization of Globin Response:** • Proportion of subjects who meet the definition of Globin Response at Month 24 • Duration of Globin Response **Hematologic Endpoints:** • Weighted average for the following at Month 6, 12, 18, and 24: – non-transfused total Hb[1] – HbS percentage of non-transfused total Hb[1] – HbS percentage of non-transfused total Hb[1] ≤ 70%, ≤ 60%, ≤ 50% – HbA^{T87Q} percentage of non-transfused total Hb[1] – non-HbS[3] percentage of non-transfused total Hb[1] • Assessment of the following over time:

	– non-transfused total Hb[1] – HbS percentage of non-transfused total Hb[1] – HbA^{T87Q} percentage of non-transfused total Hb[1] – non-HbS^{3} percentage of non-transfused total Hb[1] • Change from baseline in hemolysis markers, including absolute reticulocyte count, % reticulocytes/erythrocytes, total bilirubin, haptoglobin, and lactate dehydrogenase • Change from baseline in markers of iron stores including ferritin, liver iron content, and if assessed at baseline, cardiac iron content • Change from baseline in annualized frequency and volume of packed red blood cell (pRBC) transfusions between 6 months and 24 months after drug product infusion • Change from baseline in markers of stress erythropoiesis, including erythropoietin and serum transferrin receptor **SCD Burden and Chronic Complications Assessments:** • Change from baseline in renal function as measured by eGFR • Change from baseline in cardiac-pulmonary function via echocardiogram (tricuspid regurgitant jet velocity [TRJV], LVEF) and pulmonary function tests • Change from baseline in meters walked during 6-minute walk test **Hospitalizations and Quality of Life:** • Change from baseline in annualized VOE-related hospital admissions and days • Change from baseline in patient-reported quality of life, as measured by Patient Reported Outcomes Measurement Information System (PROMIS)
	Exploratory Efficacy Endpoints: • Change from baseline in cerebral vasculature and prior brain parenchymal injury evaluation at Month 12 and Month 24 (as measured by cerebral magnetic resonance angiography [MRA]/magnetic resonance imaging [MRI] in all subjects, and transcranial Doppler [TCD] for subjects ≤ 16 years old at Informed Consent) • Change from baseline in bone mineral density (BMD) evaluation using dual x-ray absorptiometry (DXA) at Month 24 • Change from baseline in brain natriuretic peptide • Change from baseline in Patient reported Outcome (PRO) measures including: – Overall health: EuroQol-5D (EQ-5D-3L or EQ-5D-Y) – Work productivity, as measured by the Work Productivity and Activity Impairment Questionnaire-General Health (WPAI-GH or Caregiver WPAI-GH) – Cognitive function as measured by PROMIS Short Form 6a • Evaluation of chronic pain using AAPT

	• Change from baseline in pain medication use • Exploratory assays to assess change from baseline in sickle cell characteristics and bone marrow pathophysiology
Pharmacodynamic Endpoints:	• Vector copy number (VCN) in peripheral blood over time • Expression of $\beta^{A\text{-}T87Q}$-globin, β^{S}-globin, and other β-like-globins in peripheral blood over time Additional methods may be used to evaluate pharmacodynamics.
Safety Endpoints:	Safety will be evaluated by the following: • Safety and tolerability of plerixafor for mobilization • Success and kinetics of HSC engraftment • Transplant-related mortality through 100 days post-drug product infusion and through 365 days post-drug product infusion • Detection of vector-derived replication competent lentivirus (RCL) in any subject • The number of subjects with insertional oncogenesis (myelodysplasia, leukemia, lymphoma, etc.) • The number of subjects with persistent oligoclonality • Frequency and severity of AEs/SAEs • Laboratory parameters over time, including the following immunology tests: Screening for irregular antibodies, lymphocyte subpopulation evaluation (CD3, CD4, CD8, CD19, CD16/CD56) • Incidence of acute and/or chronic graft-versus-host disease (GVHD) • Other safety labs over time: chemistries, LFTs (AST, ALT, ALP, GGT, bilirubin [total and direct]), hematology, coagulation parameters • Hormonal testing over time: estradiol (females only); total testosterone (males only) TSH, free T3, free T4, cortisol, ACTH, FSH, LH (all subjects) • Change from baseline in methemoglobin concentration at Month 12 and Month 24 • Number of subjects with presence of a chromosomal abnormality or genetic mutation associated with hematologic malignancies over time
Statistical Methods:	<u>Sample Size Estimation</u> The sample sizes for Group A and B were not determined by formal statistical methods. Approximately 41 subjects will be enrolled in Group C, approximately 35 of whom must meet the severe VOE criteria as set forth in Inclusion Criterion #3.1. Assuming 80% of subjects in Group C who have at least 4 VOEs in the 24 months prior to Informed Consent will meet the primary efficacy endpoint VOE-CR, 35 subjects will provide more than 99% power to reject the null hypothesis of 40% at 1-sided alpha of 0.025, using the Exact Test per EAST® (Version 6). The success criterion is 60% (21 out of 35) of subjects meeting the primary efficacy endpoint, if exactly 35 subjects with at

	least 4 VOEs in the 24 months prior to Informed Consent are enrolled and received drug product in Group C. Power calculations were also performed for the key secondary efficacy endpoints of sVOE-CR and Globin Response. Assuming 85% of subjects in Group C with at least 4 VOEs in the 24 months prior to Informed Consent will meet sVOE-CR, 35 subjects will provide more than 99% power to reject the null hypothesis of 50% at 1-sided alpha of 0.025, using the Exact Test per EAST® (Version 6); the success criterion is 69% (24 out of 35) of subjects meeting sVOE-CR, if exactly 35 subjects with at least 4 VOEs in the 24 months prior to Informed Consent are enrolled and receive drug product in Group C. Assuming 70% of subjects will meet Globin Response, 41 subjects will provide approximately 96% power to reject the null hypothesis of 40% at 1-sided alpha of 0.025, using the Exact Test per EAST® (Version 6); the success criterion is 59% (24 out of 41) of subjects meeting Globin Response, if exactly 41 subjects are enrolled and received drug product in Group C. Populations for analysis The following subject populations will be evaluated and used for presentation and analysis of the data: • Intent-to-Treat (ITT) Population: All subjects who initiate any study procedures, beginning with stem cell collection procedures (mobilization/apheresis or bone marrow harvest). • Transplant Population (TP): All subjects who receive drug product. • Transplant Population for VOE (TPVOE): Subset of TP subjects with at least 4 VOEs in the 24 months prior to Informed Consent. • Successful Engraftment Population (SEP): A subset of TP subjects who, following busulfan myeloablation and drug product infusion, successfully engraft with drug product, defined as 3 consecutive absolute neutrophil count (ANC) $\geq 0.5 \times 10^9$/L laboratory values obtained on different days after the initial post-infusion nadir by Day 43. The ITT is the primary population for the analysis of safety, the TPVOE is the primary population for VOE-related endpoints including the primary efficacy endpoint of VOE-CR and the key secondary endpoint of sVOE-CR, the TP is the primary population for the key secondary efficacy endpoint of Globin response, and the other secondary efficacy endpoints and pharmacodynamic endpoints. The SEP will be used to provide supportive data for subjects who engraft. Analysis of the primary efficacy endpoint in Group C The primary endpoint of VOE-CR will be tested against the null hypothesis of 40% using a 1-sided exact binomial test. The number and percentage of subjects reaching the primary endpoint will be presented. The associated 2-sided exact 95% confidence interval will be provided. The primary endpoint will be analyzed in the TPVOE population in Group C subjects. Analysis of the key secondary efficacy endpoints in Group C The key secondary endpoint of sVOE-CR will be tested against the null hypothesis of 50% using a 1-sided exact binomial test. The number and

	percentage of subjects reaching the endpoint will be presented. The associated 2-sided exact 95% confidence interval will be provided. The endpoint will be analyzed in the TPVOE population in Group C subjects. The key secondary endpoint of Globin Response will be tested against the null hypothesis of 40% using a 1-sided exact binomial test. The number and percentage of subjects reaching the endpoint will be presented. The associated 2-sided exact 95% confidence interval will be provided. The endpoint will be analyzed in the TP in Group C subjects. The planned statistical methodology details will be presented in the Statistical Analysis Plan (SAP).

[1] Non-transfused total Hb is the total g/dL of HbS + HbF + HbA_2 + HbA^{T87Q}. For subjects with a β^+ allele, HbA will also be included in the calculation of "non-transfused total Hb" only for samples taken ≥ 60 days after last pRBC transfusion.

[2] Baseline total Hb is defined as follows:

The average of the 2 most recent Hb assessments made at or prior to the Screening evaluation, which meet the following criteria:

(i) Assessments must be separated by at least 1 month from each other.

(ii) Assessments must have been drawn no earlier than 24 months prior to Informed Consent and may include the Hb result from Screening.

(iii) The subject will not have received a pRBC transfusion within 3 months prior to each Hb assessment.

For subjects who are on chronic, recurrent transfusions, and do not have 2 Hb assessments which meet criteria (i), (ii), and (iii), the following criteria can be used: 2 Hb values which meet criteria (i) and (iii) that are found within 24 months prior to the start of a regular transfusion program.

[3] Non-HbS is the total g/dL of HbF + HbA_2 + HbA^{T87Q}. For subjects with a β^+ allele, HbA will also be included in the calculation of "non-HbS" only for samples taken ≥ 60 days after last pRBC transfusion.

LIST OF ABBREVIATIONS

Abbreviation	Definition
6MWD	the distance that a patient can quickly walk on a flat, hard surface in a period of 6 minutes
6MWT	six-minute walk test
ACS	acute chest syndrome
AE	adverse event
allo-HSCT	allogeneic hematopoietic stem cell transplantation
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AML	acute myeloid leukemia
ANC	absolute neutrophil count
aPTT	activated partial thromboplastin time
AST	aspartate aminotransferase
AUC	area under the curve
BMD	bone mineral density
BNP	brain natriuretic peptide
CBC	complete blood count
CKD-EPI	Chronic Kidney Disease Epidemiology Collaboration
CMV	cytomegalovirus
CRE	conditioning-related events
CRF	case report form
CRP	C-reactive protein
DL_{CO}	carbon monoxide diffusing capacity
DLT	dose limiting toxicities
DMC	Data Monitoring Committee
DNA	deoxyribonucleic acid
DXA	dual x-ray absorptiometry
EBV	Epstein-Barr virus
EC	Ethics Committee
EFS	event-free survival
EU	European Union
eGFR	estimated glomerular filtration rate
FDA	Food and Drug Administration

Abbreviation	Definition
FEV_1	forced expiratory volume in 1 second
FISH	fluorescence in situ hybridization
FSH	follicle stimulating hormone
FVC	forced vital capacity
GCP	Good Clinical Practice
G-CSF	granulocyte colony stimulating factor
GFR	glomerular filtration rate
GGT	gamma-glutamyl transferase
GVHD	graft-versus-host-disease
Hb	hemoglobin
HbA	hemoglobin A, that contains β-globin
HbA_2	hemoglobin A_2, that contains δ-globin
HbA^{T87Q}	HbA, that contains $\beta^{A\text{-}T87Q}$-globin
HBcAb	hepatitis B core antibody
HbF	hemoglobin F, that contains γ-globin
HbS	hemoglobin S, that contains β^S-globin
HBsAb	hepatitis B surface antibody
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
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 cells obtained by apheresis
HPLC	high-performance liquid chromatography
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplantation
HSV	herpes simplex virus
HTLV-1	human T lymphotrophic virus 1
HTLV-2	human T lymphotrophic virus 2
HU	hydroxyurea
ICF	informed consent form
ICH	International Council for Harmonisation

Abbreviation	Definition
IEC	Independent Ethics Committee
INN	international nonproprietary name
IRB	Institutional Review Board
IS	insertion site
ISA	integration site analysis
ITT	intent-to-treat
IV	intravenous
LCR	locus control region
LDH	lactate dehydrogenase
LH	luteinizing hormone
LVEF	left ventricular ejection fraction
LVV	lentiviral vector
MDS	myelodysplastic syndrome
MedDRA	Medical Dictionary for Regulatory Activities
MRA	magnetic resonance angiography
MRI	magnetic resonance imaging
MSD	matched-sibling donor
NGS	next generation sequencing
OS	overall survival
PBLs	peripheral blood leukocytes
PCR	polymerase chain reaction
PD	pharmacodynamics
PFTs	pulmonary function tests
PI	Principal Investigator
PK	pharmacokinetics
pRBC(s)	packed red blood cell(s)
PRO	Patient-reported Outcome
PROMIS	Patient-reported Outcomes Measurement Information System
PT	prothrombin time
PTH	parathyroid hormone
q6h	every 6 hours
qd	once daily
qPCR	quantitative polymerase chain reaction

Abbreviation	Definition
RBC	red blood cell
RCL	replication-competent lentivirus
RNA	ribonucleic acid
RV	respiratory volume
SAE	serious adverse event
SAP	statistical analysis plan
SCD	sickle cell disease
SEP	successful engraftment population
SOE	Schedule of Events
SOM	Study Operations Manual
SUSAR	suspected unexpected serious adverse reaction
T3	3,5,3'triiodothyronine
T4	thyroxine
TCD	transcranial Doppler
TNC	total nucleated cells
TP	transplant population
TPVOE	transplant population for VOE
TRJV	tricuspid regurgitant jet velocity
TSH	thyroid stimulating hormone
ULN	upper limit of normal
US	United States
VCN	vector copy number
VOC	vaso-occlusive crisis
VOE	vaso-occlusive event
VZV	varicella zoster virus
WBC	white blood cell (count)
WPAI-GH	Work Productivity and Activity Impairment Questionnaire – General Health

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1. INTRODUCTION

1.1. Sickle Cell Disease

Sickle cell disease (SCD) is a rare hereditary blood disorder caused by a point mutation within codon 6 of the β-globin gene that results in the production of an abnormal β^{E6V}-globin (β^S-globin) (INGRAM 1957). The most frequent and severe SCD phenotype, also known as sickle cell anemia, results when 2 copies of the β^S-globin gene (β^S) are present (homozygous β^S genotype, β^S/β^S). Compound heterozygosity with certain other mutations in the β-globin gene (e.g., β^S/β^C (SC genotype) or β^S/β^0 genotypes, where a β^0 mutation does not produce a β-globin [Sβ-thalassemia genotype]), can produce an SCD spectrum that may be as phenotypically severe as that typically associated with β^S/β^S SCD.

Worldwide, neonatal screening indicates that the number of newborns with SCD is approximately 400,000 annually, with 312,000 being of the β^S/β^S genotype, mostly in sub-Saharan Africa and India (Piel et al. 2013). The US Centers for Disease Control and Prevention estimates SCD to affect 90,000 to 100,000 persons in the US (Centers for Disease Control and Prevention, http://www.cdc.gov/ncbddd/sicklecell/data.html). While SCD is relatively rare in the US and Europe, it represents a significant public health burden, given the intensive management needed to improve survival and care for affected patients. From 1989 to 1993, an average of 75,000 hospitalizations due to SCD occurred in the US (Centers for Disease Control and Prevention).

Sickle cell disease is associated with significant morbidity and mortality, particularly in developing countries where it is most prevalent. Mortality in Africa is high, with the most vulnerable period being under 5 years of age (Makani et al. 2011), mainly due to bacterial infections associated with the early loss of splenic function resulting from splenic damage from sickle cells. In the US and Europe, neonatal diagnosis, early penicillin prophylaxis, extended vaccinations, and comprehensive care have improved health outcomes and survival during childhood (Quinn et al. 2010; Colombatti et al. 2016).

β^S-globin causes hemoglobin S (HbS) to form rigid polymers upon deoxygenation or other stress, e.g., dehydration, fever, etc. In turn, the intracellular formation of HbS polymers results in deformation of the red blood cell (RBC) into the characteristic sickle shape that decreases its flexibility and is responsible for chronic hemolytic anemia and acute microvascular vaso-occlusions or infarctions, resulting in acute life-threatening conditions, chronic multiple organ damage and premature death (Madigan and Malik 2006). Moreover, systemic endothelial dysfunction develops related to a decrease in nitric oxide bioavailability due to hemolysis, reactive oxygen species production, and intravascular inflammation (Morris et al. 2000, 2005; Aslan et al. 2001; Reiter et al. 2002; Gladwin et al. 2003; Reiter and Gladwin 2003; Dubert et al. 2017).The individual baseline rate of hemolysis is stable over time (Taylor et al. 2008). While a single point mutation leading to the production of HbS is the primary source of clinical complications in SCD, the presentation of the disease is heterogeneous. Severely affected patients may experience diverse complications such as severe anemia, repeated acute painful vaso-occlusive events (VOEs) due to small-vessel obstruction (vaso-occlusive crises [VOCs]; sickle cell crises), acute chest syndrome (ACS; acute event with pneumonia-like symptoms), cerebral vasculopathy, and chronic organ damage that may involve the bones, kidneys, heart, liver, and lungs or result in severe infectious complications related to functional hyposplenism.

The need for intensive therapies, including transfusions, and frequent hospitalizations greatly impairs activities of daily living and quality of life. Most patients experience painful, chronic symptoms due to recurrent underlying crises and potentially chronic complications such as bone and joint damage and ulcers. The burden of the disease increases with age and is associated with early mortality.

Cerebro-vascular accidents are a common cause of neurological injury. The chance of having a cerebro-vascular accident by 20 years of age was estimated at 11% for β^S/β^S SCD patients (Ohene-Frempong et al. 1998). Although early transcranial Doppler (TCD) screening and intensification therapy allowed the reduction of stroke risk by age 18 to 1.9% in a recent study, the cumulative cerebral risk (including stroke, abnormal TCD, stenosis, and silent strokes) remains high at approximately 50% by age 14 (Bernaudin et al. 2011). Both silent cerebral infarcts and overt stroke are associated with lower cognitive functioning (Armstrong et al. 1996). Although stroke is the major cause of morbidity in patients with SCD, ACS is the major cause of mortality in these patients. In the cooperative study of SCD (CSSCD), patients older than 20 years of age with an episode of ACS had lower survival compared to SCD patients without an episode of ACS (Castro et al. 1994).

Two overlapping subphenotypes of SCD have been proposed (Kato et al. 2007): the "hyperviscosity" subphenotype with higher hemoglobin (Hb) levels and a higher prevalence of vaso-occlusive pain crises, ACS, and osteonecrosis, and the "hyperhemolysis-endothelial dysfunction" subphenotype, in which patients have a pattern of low Hb levels and higher levels of hemolysis markers, such as reticulocyte count, serum lactate dehydrogenase (LDH), and bilirubin, as well as a higher prevalence of pulmonary hypertension, stroke, leg ulcers and priapism.

Management of SCD entails a number of approaches, including prevention and treatment of infection, pain management and transfusion therapy. Specific treatment for SCD is limited to chronic blood transfusions, Endari (L-glutamine), Droxia or Siklos (hydroxyurea; referred to as HU), Adakveo (crizanlizumab-tmca), Oxbryta (voxelotor), and allogeneic hematopoietic stem cell (HSC) transplantation (HSCT), which to date has been the only curative treatment.

Chronic blood transfusion therapy to maintain HbS below 30% significantly reduces the risk of recurrent stroke and decreases the incidence of associated co-morbidities but leads to allo-immunization or delayed hemolysis transfusion reactions in approximately 30% of transfused SCD patients compared to 2 to 5% of all transfusion recipients. Thus, continued transfusions are problematic and may lead to life-threatening complications (Friedman et al. 1996; Meunier et al. 2008; Campbell-Lee and Kittles 2014). Iron overload is often a major issue in SCD patients on chronic transfusion therapy, with ensuing long-term liver complications and the need for iron chelation therapy (de Montalembert et al. 2017). In addition, chronic active hepatitis and resultant hepatic failure is a significant complication of transfusion therapy. As many as 11% to 20% of patients with SCD on chronic transfusion regimens are infected with hepatitis C virus (HCV) (DeVault et al. 1994; Arlet et al. 2016).

L-glutamine oral powder (Endari) is approved in the US (Endari PI, 2017) for use in reducing the acute complications of SCD in adults and pediatric patients ≥ 5 years of age. In May 2019, the Committee of Medicinal Products for Human Use (CHMP) in the European Union (EU) issued a negative opinion for Xyndari (brand name in EU) stating that the data in the marketing

authorization application did not show that the drug was effective at reducing the number of SCD crises or hospital visits.

Hydroxyurea (HU) has been helpful in a subset of patients only, with response rates of approximately 40% to 50% (Ma et al. 2007). Patients who respond show a decrease in the rate of pain and ACS events (Charache et al. 1995; Steinberg et al. 1997), and beneficial findings in other organs have also been reported (Hankins et al. 2008). Hydroxyurea is a well-tolerated medication with few significant short-term toxicities; e.g., transient and reversible myelotoxicity, occasional gastrointestinal discomfort, nail or skin hyperpigmentation, and skin ulceration. However, HU is a chemotherapeutic drug; and given its primary mechanism of action and in vitro reports as a clastogen, mutagen, teratogen and carcinogen, the long-term safety profile of HU treatment in SCD remains an ongoing concern (McGann and Ware 2011). Although most studies to date have confirmed the beneficial role of HU in preventing VOEs and ACS in all age groups, the inferiority of HU in patients with stroke and chronic transfusions has been established (Ware and Helms 2012). Importantly, HU is a palliative, but not a curative, treatment modality, and studies assessing the utilization of HU in patients with SCD have identified patient compliance as a barrier to its use and efficacy (Brandow and Panepinto 2010). While there is some non-randomized evidence that patients treated with HU may live longer than those not treated with HU, patients treated with HU still experience SCD complications and, unfortunately, still die of their disease (Bakanay et al. 2005; Voskaridou et al. 2010).

Adakveo (crizanlizumab) is a humanized IgG2 kappa monoclonal antibody against P-selectin that blocks interactions between endothelial cells, platelets, red blood cells, and leukocytes. It is indicated to reduce the frequency of VOCs in adults and pediatric patients aged 16 years and older with SCD (Adakveo PI, 2019). Adakveo does not impact that pathophysiological basis of the disease but is designed to reduce a specific symptom of the disease, namely pain due to vaso-occlusive disease. Efficacy was evaluated in the pivotal study and Adakveo demonstrated a lower median annual rate of VOC compared to placebo (1.63 versus 2.98); however, VOC often persisted despite treatment. Thus, it could not be considered a potentially curative therapy and symptoms often persist despite treatment.

Oxbryta (voxelotor) is a hemoglobin S polymerization inhibitor indicated for the treatment of SCD in adults and pediatric patients 12 years of age and older. In clinical trials, Oxbryta treatment led to an increase in total hemoglobin of approximately 1 g/dL (Oxbryta PI, 2019). However, subjects who were receiving chronic packed red blood cell (pRBC) transfusions were excluded from these studies. Importantly, treatment with Oxbryta did not demonstrate any difference in annualized vaso-occlusive events nor did it show any improvement in patient-reported outcomes. Thus, severely affected patients who can experience acute, painful and quantifiable VOEs may not benefit from this treatment given no notable reductions in VOC/ACS events were observed in Oxbryta clinical trials.

Allogeneic hematopoietic stem cell transplantation is currently the only available curative treatment for patients with SCD, and is indicated for pediatric patients with an HLA-matched sibling donor who have severe clinical symptoms such as clinically overt stroke, frequent VOEs, and ACS while on HU (Fitzhugh et al. 2014), where organ damage has been documented and reduced lifespan is expected. Unfortunately, only approximately 14% of patients have a suitable, sibling, human leukocyte antigen (HLA)-matched donor, and identifying a matched donor is a major barrier to allogeneic HSCT (Vermylen 2003). Furthermore, allogeneic HSCT is associated

with 5 to 20% mortality, in large part due to complications from graft-versus-host-disease (GVHD) (Lucarelli et al. 1993; Locatelli et al. 2003; Vermylen 2003).

Allogeneic hematopoietic stem cell transplantation involves a conditioning regimen to eradicate existing HSCs to allow for successful engraftment of subsequently infused allo-HSCs. Specific inclusion and exclusion criteria to maximize success rates and minimize risks of the procedure are now well established (Bernaudin et al. 2007; Walters et al. 2010). Allogeneic hematopoietic stem cell transplantation outcomes are generally better when performed earlier in life (Kamani et al. 2011). In a recent study of 1000 patients with SCD who received allo-HSCT from an HLA-matched sibling donor, 5-year probabilities of overall survival (OS) and event-free survival (EFS) were 92.9% and 91.4%, respectively. The 5-year OS was 95% and 81% for patients younger than 16 years and those aged 16 years or older respectively; the corresponding EFS was 93% and 81% (Gluckman et al. 2017).

The use of intra-familial, partially matched donors or matched, unrelated donors presents a safety risk to patients, and is infrequently employed for SCD. Additional donor sources and modifications to allo-HSCT are being investigated but are currently limited to experienced centers and clinical trials (Bolaños-Meade et al. 2012; Hsieh et al. 2014; Shenoy et al. 2016; Allen et al. 2017). In the context of SCD, an international expert panel has recommended that allo-HSCT should only be offered using a myeloablative conditioning regimen and only to young symptomatic patients with available matched-sibling donors (MSDs) (Angelucci et al. 2014). The absence of suitable donors, the significant risks of transplantation, and the requirement for post-transplant immunosuppression indicate unmet medical needs, even with allogeneic HSCT, for SCD patients.

1.2. bb1111

bb1111 (lovotibeglogene autotemcel, also known as LentiGlobin BB305 Drug Product for SCD) is an autologous CD34+ cell-enriched population from patients with sickle cell disease that contains HSCs transduced with BB305 lentiviral vector encoding the $\beta^{A\text{-}T87Q}$-globin gene. Expression of $\beta^{A\text{-}T87Q}$-globin is driven by the erythroid lineage-specific globin locus control region (LCR). $\beta^{A\text{-}T87Q}$-globin is a single amino acid variant of β-globin that conserves the protein's function while allowing for quantification relative to other globin species. Additionally, $\beta^{A\text{-}T87Q}$-globin has anti-sickling properties because the amino acid change from threonine to glutamine at position 87 sterically inhibits the polymerization of Hb chains.

After undergoing myeloablation, subjects receive bb1111 via intravenous (IV) infusion, leading to peripheral reconstitution with corrected erythrocytes. As bb1111 consists of genetically modified autologous cells, there is no risk of GVHD.

Extensive nonclinical studies have demonstrated that transfer of the human $\beta^{A\text{-}T87Q}$-globin gene under the control of the β-globin promoter and enhancer elements of the β-globin locus control region (LCR) into HSCs by means of LVVs reliably results in long-term correction of SCD in relevant mouse models (Pawliuk et al. 2001). Similarly, effective ex vivo $\beta^{A\text{-}T87Q}$-globin gene transfer into human CD34+ HSCs was observed (Imren et al. 2004) (see the Investigator's Brochure).

As described in the protocol synopsis, Study HGB-206 includes 3 cohorts (Groups A, B, and C). In Group A, drug product was manufactured using stem cells collected via bone marrow harvest

and using a manufacturing process termed Process 1, a process which has since been discontinued. In Group B, bone marrow harvest was also used to collect stem cells for drug product manufacture; however, a new drug manufacturing process termed Process 2 (currently in use) was implemented. Of the 2 subjects in Group B, 1 subject received drug product manufactured entirely via Process 2, and the other subject (Subject 1313) received a drug product lot manufactured via Process 1 and a drug product lot manufactured via Process 2. In Group C, drug product was manufactured from stem cells collected via plerixafor mobilization followed by apheresis, and all Group C subjects have received drug product manufactured using Process 2. All subjects enrolled into Group C are planned to receive drug product manufactured via Process 2 using stem cells collected by plerixafor mobilization followed by apheresis.

Through the improvements introduced into Study HGB-206 with Process 2, we have shown that production of hemoglobin A containing $\beta^{A\text{-}T87Q}$-globin (HbA^{T87Q}) is dependent on VCN, such that increased VCN leads to sustained HbA^{T87Q} expression and HbS decrease. With the introduction of Process 2, subjects have achieved robust anti-sickling Hb levels in the absence of exogenous hemoglobin A (HbA) from pRBC transfusions and experienced reductions in VOC/ACS events post-treatment. These data suggest that HbA^{T87Q} production may improve the clinical status of patients with SCD.

The safety profile post bb1111 gene therapy is generally consistent with that of autologous stem cell transplant, myeloablative single-agent busulfan conditioning, and underlying SCD. See the Investigator's Brochure for details and safety/efficacy updates.

1.3. Rationale

1.3.1. Rationale for the Study

Severe SCD is a devastating monogenic disease with a clear unmet need. While SCD varies substantially from patient to patient, at a population level there are broad trends in presentation of the disease by age. Infants with SCD, who have hemoglobin F (HbF) levels that are preventative of HbS polymerization, are largely asymptomatic. When HbF production decreases, and HbS production increases, the disease can present with a number of hallmark acute symptoms including dactylitis, anemia, mild jaundice or an enlarged spleen. Acute attacks from splenic sequestration can occur as early as 3 months of age but, more typically, before 2 years of age (Serjeant 2013). The most frequent clinical complications in pediatric SCD are pain, infection, acute splenic sequestration, ACS, and stroke (Kanter and Kruse-Jarres 2013). Pediatric SCD patients are also at a substantially elevated risk for overt and silent strokes that can negatively impact cognition. One recent study demonstrated that approximately 50% of patients have some form of cerebral vasculopathy by age 14 (Bernaudin et al. 2011). In recent decades, interventions such as neonatal diagnosis, penicillin prophylaxis, extended vaccination, and comprehensive care have significantly reduced childhood mortality for people with SCD in developed countries (Lanzkron et al. 2013). However, the accumulated burden of SCD-associated morbidity increases as patients enter adulthood, resulting in progressive organ damage (Buchanan et al. 2010), more frequent emergency department visits and hospitalizations (Brousseau et al. 2010), reduced quality of life (Dampier et al. 2011), and ultimately shortened survival. Approximately 50% of people with SCD do not survive to the fifth decade of life, and nearly half of those who do will have some form of irreversible organ damage (Powars et al. 2005). The most common causes of death among adult SCD patients include ACS,

VOE complications, multiorgan or renal failure, stroke, and heart failure (Platt et al. 1994; Kanter and Kruse-Jarres 2013). Although stroke is the major cause of morbidity in adult patients with SCD, ACS is the major cause of mortality in these patients.

In the US and Europe, the life expectancy of adults with SCD is estimated to be 20 to 30 years lower than that for unaffected individuals, with a median age at death for patients with SCD of approximately 39 years of age (Hassell 2010) to 45 years of age, and not exceeding the 5th decade of life since the 1990s (Platt et al. 1994; Hassell 2010; Lanzkron et al. 2013; Ngo et al. 2014; Payne et al. 2017). Between 1979 and 2005, pediatric mortality has decreased by 3% per year, while adult mortality has increased by 1% per year (Lanzkron et al. 2013). Several studies have reported that the risk of death is particularly high during the transition time from pediatric to adult care, potentially due to a lack of adult health care for these patients, or to the fact that these patients didn't survive before HU and transfusion therapy (Quinn et al. 2010; Hamideh and Alvarez 2013).

Allo-HSCT from an MSD is the only potentially curative treatment available. However, unfortunately, only approximately 14% of patients have a suitable, sibling, human leukocyte antigen (HLA)-matched donor, and identifying a matched donor is a major barrier to allo-HSCT (Vermylen 2003). Furthermore, allo-HSCT is associated with 5% to 20% mortality, in large part due to complications from graft-versus-host disease (GVHD) (Lucarelli et al. 1993; Platt et al. 1994; Locatelli et al. 2003; Vermylen 2003). Engraftment failure and rejection are also significant risks of such therapy. The use of intra-familial, partially matched donors or matched, unrelated donors is infrequently employed as it is not an option for most patients with SCD given the higher risk associated with these transplants. The absence of suitable donors, the significant risks of transplantation, and the requirement for post-transplant immunosuppression therapy indicate an unmet medical need, even with allo-HSCT, for patients with SCD.

In order to provide a potentially curative treatment to a larger number of patients with SCD, the Sponsor is developing bb1111, an autologous CD34+ cell-enriched population from patients with SCD that contains HSCs transduced with BB305 LVV encoding the $\beta^{A\text{-}T87Q}$-globin gene.

Treatment of the subject's own HSCs with BB305 LVV through transduction should eliminate the risk of GVHD. Furthermore, clinical benefit should be obtainable without all HSCs being transduced. A number of clinical studies have demonstrated that after allo-HSCT (using myeloablative or non-myeloablative conditioning regimens), stable mixed chimerism is sufficient to reverse SCD-related symptoms (Walters et al. 2001; Andreani et al. 2011; Hsieh et al. 2014; King et al. 2015; Saraf et al. 2016). It has been recently demonstrated that a minimum of 20% donor myeloid chimerism is required to reverse the SCD phenotype in subjects undergoing nonmyeloablative HSCT from HLA-matched sibling or haploidentical donors (Fitzhugh et al. 2017). These findings suggest that low levels of donor engraftment can result in significant donor-derived erythrocyte levels and functional improvement of hemoglobinopathies (Andreani et al. 2011). This can be explained by the large differences between donor and recipient RBC survival times, and the progressive advantage of the selection of donor cells during erythroid differentiation, contrary to what is usually observed in cases of mixed chimerism after allograft for a malignant hematologic disease (Wu et al. 2005).

This Phase 1/2 study will assess the efficacy and safety of transplantation with bb1111 in adults and adolescents with severe SCD. Efficacy endpoints that evaluate complete resolution of severe VOEs, production of HbA^{T87Q}, and changes in total Hb levels, along with secondary endpoints

assessing HbS percentage of total Hb, reduction in frequency of VOEs, hemolysis, and other biochemistry and physiological parameters, will allow for an evaluation of whether transplantation with bb1111 will provide clinical benefit. In combination with the safety endpoints, these data should allow for a determination of benefit: risk of this treatment modality in patients with severe SCD and be the basis for subsequent larger efficacy studies.

1.3.2. Rationale for Selection of Primary and Key Secondary Efficacy Endpoints

The primary endpoint of VOE-CR (see Section 2.2.1.1) assesses for complete resolution of VOEs between 6 months and 18 months after drug product infusion.

VOEs are associated with declining quality of life, increased risk of acute and chronic morbidities, cumulative disease progression and an increased risk of sudden death (Ballas et al. 2012; Novelli and Gladwin 2016; Kato et al. 2018; Payne et al. 2020). These traumatic events happen frequently, with estimates that vaso-occlusive crises result in 90% of SCD-related hospital admissions (Jang et al. 2021), and 88% of patients report contending with VOE pain at home (Thompson and Eriator 2014). VOEs are also frequently underreported, with only 10% of patients seeking treatment for pain events and many opting to delay medical treatment (Smith et al. 2008; Linton et al. 2020). Complete resolution of VOEs therefore represents a clinically meaningful outcome of drug product treatment.

The key secondary endpoint of sVOE-CR (see Section 2.2.1.2) assesses for complete resolution of severe VOEs between 6 months and 18 months after drug product infusion. These events are VOEs that require significant medical intervention and treatment (see Section 6.2.5). Severely affected patients can experience VOEs that require intravenous (IV) opioids and hospitalization, and resolution of these sVOEs also represents a clinically meaningful outcome of drug product treatment.

The key secondary endpoint of Globin Response (see Section 2.2.1.2) is a composite endpoint for evaluation of production of HbA^{T87Q} and total (non-transfused) Hb. Assessment of Hb production was chosen for the following reasons: chronic anemia induces hypoxia, which is a crucial factor in the onset of sickle cell-related events; a correlation is expected between resolution of anemia and correction of other manifestations of SCD. In addition, by measuring the increase in total Hb, we can also assess subjects with hemolysis (these subjects may not experience frequent VOEs, and therefore a reduction in these events cannot be measured). Production of HbA^{T87Q} is being evaluated because the $\beta^{A\text{-}T87Q}$-globin gene encodes a β-globin variant that has been shown to exhibit anti-sickling properties while otherwise maintaining the same functionality as wild-type β-globin.

1.3.3. Rationale for the β-globin Gene Used

The β-globin transgene used in this study encodes the wild-type human β-globin with a single modification at amino acid position 87 [β^{A87} Thr:Gln ($\beta^{A\text{-}T87Q}$)]. This $\beta^{A\text{-}T87Q}$-globin has an advantage over normal β-globin for gene therapy of SCD, as $\beta^{A\text{-}T87Q}$-globin has been shown in vitro and in vivo to have anti-sickling properties similar to those of human γ-globin (fetal Hb) and thus should be more active in preventing sickling in subjects with SCD than the wild-type β-globin, which does not actively inhibit sickling (Pawliuk et al. 2001). In addition, the in vivo protein level expressed by this variant can be quantified by high-performance liquid

chromatography (HPLC) and distinguished from that of wild-type β-globin from transfused RBCs (Cavazzana-Calvo et al. 2010).

1.3.4. Rationale for Plerixafor Mobilization

Transduction of CD34+ cells after their mobilization and apheresis using a combination of granulocyte colony stimulating factor (G-CSF) and plerixafor has led to successful engraftment of transduced cells with high levels of therapeutic transgene in subjects with β-thalassemia (Thompson et al. 2018). Although G-CSF is generally well-tolerated for stem cell mobilization, severe complications, including multi-organ failure, ACS and vaso-occlusive episodes have been reported with its use in patients with SCD, presumed due to the hyperleukocytosis caused by G-CSF (Fitzhugh et al. 2009). Plerixafor, which reversibly inhibits the CXCR4–SDF1 interaction within the bone marrow microenvironment resulting in rapid mobilization, has been proposed as an alternative to G-CSF due to its different mode of action and its emerging safety profile (Yannaki et al. 2012, 2013). Single-agent use of plerixafor has been shown to be safe and effective for the rapid mobilization of CD34+ cells in both healthy subjects (Liles et al. 2003) and patients with multiple myeloma and non-Hodgkin's lymphoma (Devine et al. 2004). More recently, in thalassemic patients, mobilization with plerixafor alone resulted in CD34+ cell yields similar to those obtained using G-CSF mobilization (Yannaki et al. 2012). Plerixafor mobilization in these subjects produced only mild adverse events (AEs), e.g., nausea, diarrhea and injection site erythema. Most relevant to the SCD population, who are often functionally asplenic, single-agent plerixafor did not cause the same dose-limiting leukocytosis observed in splenectomized thalassemic patients receiving G-CSF.

A recent Phase 1/2 study has shown that plerixafor can be safely used to mobilize HSCs in sickle cell patients; a single injection of plerixafor resulted in rapid mobilization of large numbers of CD34+ cells that engrafted very efficiently in immunodeficient mice, and no AEs were observed (Lagresle-Peyrou et al. 2018). The interim results of another clinical trial of plerixafor mobilization in SCD were also recently published (Boulad et al. 2018).This study is a Phase 1 dose escalation study to support the safety and efficacy of single-agent plerixafor use in 15 subjects with severe SCD. Relatively high numbers of CD34+ cells were mobilized after plerixafor administration at dose levels ranging from 0.08 mg/kg to 0.24 mg/kg. Although increases in white cell counts and absolute neutrophil counts were observed, the levels seen were assessed as acceptable for the safety of subjects with SCD. There was a low rate of serious adverse events (SAEs); 2 subjects developed vaso-occlusive crises at plerixafor doses of 0.08 mg/kg and 0.24 mg/kg, respectively.

These data suggested that the risk of plerixafor provoking severe SCD complications should be substantially less than that of G-CSF. In addition, a recent multicenter Phase 1/2 study has provided evidence that plerixafor mobilization/apheresis is safer than bone marrow harvest as a method for collection of stem cells from patients with SCD (Tisdale et al. 2020).

As of 17 May 2017, 3 subjects in Study HGB-206 had undergone mobilization with plerixafor followed by subsequent apheresis, without dose-limiting toxicity. All 3 subjects received a single dose of 0.24 mg/kg of plerixafor, followed approximately 4 to 6 hours later by apheresis, during which between 5.6×10^6 and 15.3×10^6 CD34+ cells/kg were collected. After cells were removed for rescue, the remaining CD34+ cells were sent to bluebird bio for exploratory manufacturing

development. The CD34+ cells procured from plerixafor-mobilized apheresis were of sufficient quantity and quality for the manufacture of bb1111 based on process recovery and drug product characterization data. Based on these data, and after adjudication by the DMC, the decision was made to move forward with plerixafor mobilization and apheresis for the collection of CD34+ cells for drug product manufacture for all enrolled subjects.

Safety evaluation of subjects in Study HGB-206 has demonstrated the general safety and tolerability of plerixafor at a dose of 0.24 mg/kg, and none of the life-threatening sequelae associated with G-CSF administration in this population has been observed. Based upon these data, dose-limiting toxicities (DLTs) are no longer collected, and the safety of plerixafor is now monitored in a standard manner.

1.3.5. Rationale for the Dose Selected

The bb1111 cell dose will be $\geq 3.0 \times 10^6$ $CD34^+$ cells/kg, with drug product manufactured using cells collected by apheresis after plerixafor mobilization for each subject.

The dose of CD34+ cells to be administered is based on accepted safe practice to achieve rapid and robust hematopoietic reconstitution with long-term engraftment after autologous transplantation. Although the theoretical objective is to provide as many CD34+ cells as possible with doses up to 15×10^6 cells/kg (Jillella and Ustun 2004), this dose is rarely achievable. A minimum dose of CD34+ cells to be administered is not clearly defined in the literature, as doses of 1.0×10^6 CD34+ cells/kg or lower result in engraftment, but with delays in neutrophil and platelet recovery (Miyamoto et al. 2004). There is a consensus that the minimum CD34+ dose associated with favorable engraftment kinetics is approximately 1.5×10^6 to 3.0×10^6 cells/kg (Bender et al. 1992; Perez-Simon et al. 1998; Miyamoto et al. 2004; Jillella and Ustun 2004). Table 1 summarizes the minimum dose of bb1111 and back-up cells to be given.

Table 1: Dose of bb1111 or Back-up Cells for Severe SCD in Study HGB-206

Usage	Dose
Drug Product (cells obtained by apheresis)	$\geq 3.0 \times 10^6$ $CD34^+$ cells/kg[a]
Back-up Cells (cells obtained by bone marrow harvest)	$\geq 1.0 \times 10^8$ TNC/kg
Back-up Cells (cells obtained by apheresis)	$\geq 1.5 \times 10^6$ $CD34^+$ cells/kg

Abbrev.: TNC, total nucleated cells

[a] Note that this dose represents the cell count after transduction. If more than 1 transduction is performed, the total dose of the drug products together must meet this criterion

2. STUDY OBJECTIVES AND ENDPOINTS

2.1. Study Objectives

The primary study objective is to:

- Evaluate the efficacy of treatment with bb1111 in subjects with severe SCD.

The secondary study objective is to:

- Evaluate the safety of treatment with bb1111 in subjects with severe SCD.

2.2. Study Endpoints

2.2.1. Efficacy Endpoints

2.2.1.1. Primary Efficacy Endpoint

VOE-CR, defined as complete resolution of VOEs, between 6 months and 18 months after drug product infusion

2.2.1.2. Key Secondary Efficacy Endpoints

- sVOE-CR, defined as complete resolution of severe VOEs, between 6 months and 18 months after drug product infusion
- Globin Response, defined as meeting the following criteria for a continuous period of at least 6 months after drug product infusion:

  a. Weighted average HbA^{T87Q} percentage of non-transfused total Hb[1] $\geq$ 30%

  AND

  b. Weighted average non-transfused total Hb[1] increase of $\geq$ 3 g/dL compared to baseline total Hb[2] OR weighted average non-transfused total Hb[1] $\geq$ 10 g/dL

[1] Non-transfused total Hb is the total g/dL of HbS + HbF + HbA_2 + HbA^{T87Q}. For subjects with a β^+ allele, HbA will also be included in the calculation of "non-transfused total Hb" only for samples taken $\geq$ 60 days after last pRBC transfusion.
[2] Baseline total Hb is defined as follows:
The average of the 2 most recent Hb assessments made at or prior to the Screening evaluation, which meet the following criteria:
(i) Assessments must be separated by at least 1 month from each other.
(ii) Assessments must have been drawn no earlier than 24 months prior to Informed Consent and may include the Hb result from Screening.
(iii) The subject will not have received a pRBC transfusion within 3 months prior to each Hb assessment.
For subjects who are on chronic, recurrent transfusions, and do not have 2 Hb assessments which meet criteria (i), (ii), and (iii), the following criteria can be used: 2 Hb values which meet criteria (i) and (iii) that are found within 24 months prior to the start of a regular transfusion program.

2.2.1.3. Additional Secondary Efficacy Endpoints

Clinical and Disease Evaluation Endpoints:

- Change in the annualized number of VOEs in the 24 months after drug product infusion compared to the 24 months prior to Informed Consent

- Change in the annualized number of severe VOEs in the 24 months after drug product infusion compared to the 24 months prior to Informed Consent
- VOE-CR24, defined as complete resolution of VOEs between 6 months and 24 months after drug product infusion
- sVOE-CR24, defined as complete resolution of severe VOEs between 6 months and 24 months after drug product infusion
- sVOE-75, defined as at least a 75% reduction in annualized severe VOEs in the 24 months after drug product infusion compared to the 24 months prior to Informed Consent

Characterization of Globin Response:

- Proportion of subjects who meet the definition of Globin Response at Month 24
- Duration of Globin Response

Hematologic Endpoints:

- Weighted average for the following at Month 6, 12, 18, and 24:
  - non-transfused total Hb[1]
  - HbS percentage of non-transfused total Hb[1]
  - HbS percentage of non-transfused total Hb[1] ≤ 70%, ≤ 60%, ≤ 50%
  - HbA^{T87Q} percentage of non-transfused total Hb[1]
  - non-HbS[3] percentage of non-transfused total Hb[1]
- Assessment of the following over time:
  - non-transfused total Hb[1]
  - HbS percentage of non-transfused total Hb[1]
  - HbA^{T87Q} percentage of non-transfused total Hb[1]
  - non-HbS[3] percentage of non-transfused total Hb[1]

[1] Non-transfused total Hb is the total g/dL of HbS + HbF + HbA_2 + HbA^{T87Q}. For subjects with a β^+ allele, HbA will also be included in the calculation of "non-transfused total Hb" only for samples taken ≥ 60 days after last pRBC transfusion.
[3]Non-HbS is the total g/dL of HbF + HbA_2 + HbA^{T87Q}. For subjects with a β^+ allele, HbA will also be included in the calculation of "non-HbS" only for samples taken ≥ 60 days after last pRBC transfusion.

- Change from baseline in hemolysis markers, including absolute reticulocyte count, % reticulocytes/erythrocytes, total bilirubin, haptoglobin, and lactate dehydrogenase
- Change from baseline in markers of iron stores including ferritin, liver iron content, and if assessed at baseline, cardiac iron content
- Change from baseline in annualized frequency and volume of packed red blood cell (pRBC) transfusions between 6 months and 24 months after drug product infusion

- Change from baseline in markers of stress erythropoiesis, including erythropoietin and serum transferrin receptor

SCD Burden and Chronic Complications Assessments:

- Change from baseline in renal function as measured by eGFR
- Change from baseline in cardiac-pulmonary function via echocardiogram (tricuspid regurgitant jet velocity [TRJV], LVEF) and pulmonary function tests
- Change from baseline in meters walked during 6-minute walk test

Hospitalizations and Quality of Life:

- Change from baseline in annualized VOE-related hospital admissions and days
- Change from baseline in patient-reported quality of life, as measured by Patient Reported Outcomes Measurement Information System (PROMIS)

2.2.1.4. Exploratory Efficacy Endpoints

- Change from baseline in cerebral vasculature and prior brain parenchymal injury evaluation at Month 12 and Month 24 (as measured by cerebral magnetic resonance angiography [MRA]/magnetic resonance imaging [MRI] in all subjects, and transcranial Doppler [TCD] for subjects ≤ 16 years old at Informed Consent)
- Change from baseline in bone mineral density (BMD) evaluation using dual x-ray absorptiometry (DXA) at Month 24
- Change from baseline in brain natriuretic peptide
- Change from baseline in Patient reported Outcome (PRO) measures including:
  - Overall health: EuroQol-5D (EQ-5D-3L or EQ-5D-Y)
  - Work productivity, as measured by the Work Productivity and Activity Impairment Questionnaire-General Health (WPAI-GH or Caregiver WPAI-GH)
  - Cognitive function as measured by PROMIS Short Form 6a
- Evaluation of chronic pain using AAPT
- Change from baseline in pain medication use
- Exploratory assays to assess change from baseline in sickle cell characteristics and bone marrow pathophysiology

2.2.2. Pharmacodynamic Endpoints

- Vector copy number (VCN) in peripheral blood over time
- Expression of $\beta^{A\text{-}T87Q}$-globin, β^{S}-globin, and other β-like globins in peripheral blood over time

Additional methods may be used to evaluate pharmacodynamics.

2.2.3. Safety Endpoints

Safety will be evaluated by the following:

- Safety and tolerability of plerixafor for mobilization
- Success and kinetics of HSC engraftment
- Transplant-related mortality through 100 days post-drug product infusion and through 365 days post-drug product infusion
- Detection of vector-derived replication competent lentivirus (RCL) in any subject
- The number of subjects with insertional oncogenesis (myelodysplasia, leukemia, lymphoma, etc.)
- The number of subjects with persistent oligoclonality
- Frequency and severity of AEs/SAEs
- Laboratory parameters over time, including the following immunology tests: Screening for irregular antibodies, lymphocyte subpopulation evaluation (CD3, CD4, CD8, CD19, CD16/CD56)
- Incidence of acute and/or chronic graft-versus-host disease (GVHD)
- Other safety labs over time: chemistries, LFTs (AST, ALT, ALP, GGT, bilirubin [total and direct]), hematology, coagulation parameters
- Hormonal testing over time: estradiol (females only); total testosterone (males only) TSH, free T3, free T4, cortisol, ACTH, FSH, LH (all subjects)
- Change from baseline in methemoglobin concentration at Month 12 and Month 24
- Number of subjects with presence of a chromosomal abnormality or genetic mutation associated with hematologic malignancies over time

3. INVESTIGATIONAL PLAN

3.1. Overall Design and Plan of the Study

This is a non-randomized, open label, multi-site, single dose, Phase 1/2 study in approximately 50 adults and adolescents with severe SCD. The study will evaluate HSCT using bb1111, an autologous CD34+ cell-enriched population from patients with SCD that contains HSCs transduced with BB305 LVV encoding the $\beta^{A\text{-}T87Q}$-globin gene.

Treatment is divided into 4 stages as follows.

Stage 1 – Screening and eligibility-assessment

Subjects will be screened and undergo clinical, imaging, and laboratory assessments for eligibility determination.

Stage 2 – Stem cell harvest, drug product manufacture and disposition

If subjects are taking HU, it must be discontinued at least 30 days prior to stem cell harvest. It may be restarted between completion of the stem cell harvest(s) and admission to the hospital for myeloablation but should be stopped again at least 2 days before initiating myeloablation.

For at least 60 days prior to stem cell harvest and continuing through until the start of conditioning, subjects will undergo a transfusion regimen (exchange or simple, as available or needed) to reach a target Hb of 10 g/dL prior to mobilization (and 8 to 10 g/dL prior to conditioning; not to exceed 12 g/dL prior to either mobilization or conditioning) and a pre-transfusion target HbS proportion in the blood of < 30% to reduce the risk of SCD-related complications. The last exchange transfusion must occur within 4 days of start of mobilization, and the HbS proportion in the blood must be < 20% after the last exchange transfusion. If a subject experiences an SCD-related crisis within 1 month of the start of mobilization, the transfusion regimen will be adjusted, and mobilization will be postponed until the subject is crisis-free for a period of 1 month. In case of iron overload, subjects should be adequately chelated; however, iron chelation must be discontinued at least 7 days prior to stem cell harvest. It may be restarted between completion of the stem cell harvest(s) and admission to the hospital for myeloablation but should be stopped again at least 7 days before initiating myeloablation.

Subjects who are not documented to have met these parameters or who have experienced any significant new SCD-related complications within 1 month of the start of mobilization will require additional approval from the Medical Monitor before undergoing mobilization/apheresis.

Subjects will undergo stem cell harvest via mobilization with plerixafor (0.24 mg/kg) and subsequent apheresis to collect HSCs for both drug product manufacture and cryopreservation of back-up cells for rescue. Apheresis should begin approximately 4-6 hours after plerixafor mobilization. If more than 1 apheresis day is required, platelet counts must be confirmed to be $\geq 75 \times 10^9$/L within 24 hours of subsequent apheresis sessions, prior to administration of plerixafor on that day. If platelet counts do not meet these criteria, mobilization and apheresis should be deferred until platelet counts recover to $\geq 75 \times 10^9$/L. At least 1.5×10^6 $CD34^+$ cells/kg will be cryopreserved for rescue. Following transduction of isolated CD34+ HSCs with BB305 lentiviral vector, the bb1111 cell dose will be $\geq 3.0 \times 10^6$ $CD34^+$ cells/kg for each subject.

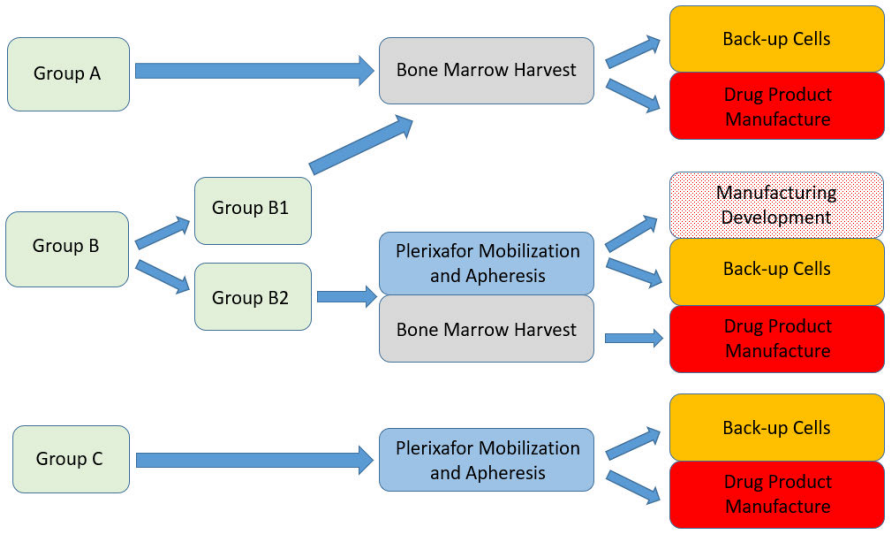
Subjects for whom adequate HSCs are collected to achieve the minimum cell dose but not for rescue, and who do not tolerate plerixafor or do not effectively mobilize with plerixafor may be offered the option of bone marrow harvest for collection of back-up cells only, provided that drug product manufactured from mobilized cells has met criteria for dispositioning for clinical use. 1.0×10^8 total nucleated cells (TNC)/kg derived from bone marrow will be collected for rescue.

Subjects were divided into 3 groups, depending on how stem cells were harvested, and their use for either rescue or drug product manufacture, as shown in the schematic below (see Figure 1).

In Group A, both drug product and back-up cells were derived from bone marrow. Treatment was staggered between the first and second subject in Group A: the second subject began myeloablative conditioning only after the first subject engrafted (defined as 3 consecutive ANC laboratory values $\geq 0.5 \times 10^9$/L obtained on different days) and the DMC reviewed the initial safety data from the first subject.

In Group B1, both drug product and back-up cells were derived from bone marrow.

Figure 1: Schematic for Study Design



In Group B2, plerixafor mobilization and apheresis were used for collection of back-up cells and exploratory manufacturing development, and it was planned for subjects in Group B2 to then undergo bone marrow harvest for drug product manufacture. At least 3 subjects in Group B2 were required for evaluation of DLT at each dose of plerixafor. Plerixafor dosing of initial subjects in Group B2 was staggered. Data Monitoring Committee members reviewed the AE profile during plerixafor treatment in mobilization cycle 1 for the first subject treated in Group B2 and planned to adjudicate on any issues of potential safety significance that could affect determination of DLT before dosing of the second subject with plerixafor. A similar procedure was followed after dosing of the second subject in order to permit dosing of the third subject. The DMC reviewed aggregate safety data in Group B2 after 3 subjects had been dosed and confirmed that there are no safety concerns precluding dosing of subjects in Group C with plerixafor.

Group C was opened upon (1) confirmation of the safety and tolerability of plerixafor mobilization in Group B2, and (2) regulatory authority approval of the drug product manufacturing process with plerixafor-mobilized HSCs. In Group C, it is planned that all subjects will undergo mobilization with plerixafor and subsequent apheresis to collect HSCs both for drug product manufacture and for cryopreservation of back-up cells for rescue.

The first 2 subjects in Group C received drug product infusion in a staggered fashion: neutrophil engraftment must occur in the first subject treated in Group C before initiating busulfan conditioning for the second subject.

Subjects who were consented under a prior version of the protocol (and who may or may not have back-up cells collected and/or inadequate drug product manufactured under a prior version of the protocol), may be allowed to be reconsented under the current version of the protocol. Depending on the disposition of previously collected/manufactured cells, subjects may be excluded from the overall non-safety analyses of the Group in which they participated.

Subjects in Group B2 who tolerated and effectively mobilized with plerixafor were given the option to postpone drug product manufacture until Group C opened, to allow drug product manufacture using plerixafor mobilized cells. In Group C, subjects for whom adequate HSCs are collected to achieve the minimum cell dose but not for rescue, and who do not tolerate plerixafor or do not effectively mobilize with plerixafor may be offered the option of bone marrow harvest for collection of back-up cells only, provided that drug product manufactured from mobilized cells has met criteria for dispositioning for clinical use.

As of Version 8.0 of this protocol, Group C will include adolescent subjects ≥ 12 and < 18 years of age in addition to subjects ≥ 18 and ≤ 50 years of age. It should also be noted that while all Group A subjects and some Group B subjects received drug product manufactured using "Process 1", all current and future Group C subjects will receive drug product manufactured using an optimized process termed "Process 2". Treatment with drug product manufactured using Process 2 (versus Process 1) has resulted in increased drug product VCN, peripheral blood VCN, and HbA^{T87Q} levels across multiple bluebird bio-sponsored clinical studies.

The drug product must be release tested and dispositioned for clinical use and stored at the study site before the subject can begin myeloablative conditioning with busulfan.

Stage 3 –Myeloablative conditioning and infusion of bb1111

The subject's suitability for transplant will be reconfirmed with clinical laboratory tests (complete blood count [CBC], serum chemistry, liver function tests, and pregnancy test), physical examination, performance status, cytogenetics, and other tests based on institutional requirements. The results of these tests will be reviewed, and availability of the drug product on site and the availability of back-up cells will be confirmed before the site can proceed with conditioning.

As described above, a transfusion regimen (exchange or simple, as available and needed) will be continued until the start of conditioning to reach a target Hb (prior to conditioning) of 8 to 10 g/dL (not to exceed 12 g/dL) and a pre-transfusion target HbS proportion in the blood of < 30% to reduce the risk of SCD-related complications; last exchange transfusion must occur within 1 week of start of the conditioning regimen. In the event of pulmonary complications (e.g., ACS, pneumonia) between Screening and the start of conditioning, a PFT must be

performed. After resolution of pulmonary complications, and prior to conditioning, PFT results must meet the following requirements:

- Baseline oxygen saturation ≥ 90% without supplemental oxygen (excluding periods of SCD crisis, severe anemia or infection).
- Baseline carbon monoxide diffusing capacity (DL_{CO}) ≥ 50% (corrected for Hb) in the absence of infection. If DL_{CO} cannot be assessed due to age or cognition-related restrictions, there must be a normal respiratory exam, chest radiograph without pulmonary infiltrates, and oxygen saturation by pulse oximetry ≥ 90% on room air.

Busulfan (Busulfex® preferred, if available) will be administered intravenously (IV) at a starting dose of 3.2 mg/kg/day or 0.8 mg/kg every 6 hours (q6h) for 4 consecutive days; for subjects weighing < 35 kg, a q6h dose of 0.8 mg/kg is preferred; for subjects weighing ≥ 35 kg, either once daily (qd) or q6h dosing can be used, at the discretion of the Investigator. The dose of busulfan will be adjusted based upon busulfan pharmacokinetics (PK) in order to maintain appropriate levels for myeloablation (area under the curve [AUC] goal of 1250 [range 1100 to1350] µM*min for a q6h dosing regimen, or 5000 [range 4400 to 5400] µM*min for a qd dosing regimen). The dosage should be calculated on the basis of the lower of the ideal versus actual body weight. The dose may be adjusted appropriately based upon actual plasma busulfan levels observed. Clinical sites must be able to measure first and third day busulfan PK. Based on busulfan PK results for day 1 and day 3 of busulfan dosing, busulfan dose adjustments should be made for subsequent dosing. If feasible, daily busulfan PK measurement is recommended.

Anti-seizure prophylaxis must begin at least 12 hours before initiating busulfan and must continue for at least 24 hours after completion of the 4 days of busulfan administration. All drugs other than phenytoin are allowed for anti-seizure prophylaxis.

After completion of the 4-day course of busulfan, there must be a minimum of 48 hours before drug product infusion. Busulfan levels will be measured 48 and 72 hours after final dose of busulfan for retrospective confirmation of adequate wash-out.

On Day 1, bb1111 will be administered after thawing via IV infusion.

Stage 4 – Follow-up, for approximately 24 months after drug product infusion

Subjects will be followed in the hospital for AEs, and laboratory parameters will be followed to monitor engraftment. The subject may be discharged after neutrophil engraftment occurs ($\geq 0.5 \times 10^9$ ANC/L for 3 consecutive measurements on different days after the initial post-infusion nadir) and the subject is considered medically stable per institutional guidelines.

From drug product infusion through hospital discharge after neutrophil engraftment, subjects are to be medically managed with the goal of maintaining the following hematologic targets:

- Hb 8 to 10 g/dL (not to exceed 12 g/dL), HbS < 30% and
- platelet count ≥ 50×10^9/L.

After discharge, management of transfusions for subjects with SCD will follow the institutional standard of care at the clinical site to achieve total Hb and HbS proportions appropriate for each subject's clinical status. To achieve this, the transfusion program may be progressively reduced

as the contribution of Hb containing $\beta^{A\text{-}T87Q}$-globin rises. Continued transfusion needs > 6 months post-treatment or any alternative plans should be discussed with the Medical Monitor.

Subjects will be followed in this protocol through the Month 24 Visit. Thereafter, subjects are expected to enroll in a separate, non-interventional, long-term follow-up protocol that will assess safety and efficacy beyond Month 24 for a total of 15 years after drug product infusion. The end of Study HGB-206 will be defined as the last visit for the last subject.

3.2. Data Monitoring Committee

An independent Data Monitoring Committee (DMC) comprised of members with appropriate scientific and medical expertise to monitor the safety of subjects participating in the study will be convened before the study is opened. The DMC will make recommendations regarding study conduct (including stopping enrollment), but the ultimate decision is with the Sponsor. The composition, responsibilities, and meeting frequencies of the DMC will be described in the DMC charter.

Prior to myeloablative conditioning and subsequent infusion of drug product of the second subject in Group A, the DMC reviewed the safety data from the first subject treated after that subject had engrafted. The second subject was not enrolled into Group A until the DMC had determined that there were no safety concerns with the first subject. Thereafter, subject enrollment proceeded in parallel and the DMC reviewed safety data during their regular meetings.

During safety evaluation of plerixafor in Group B2, dosing of initial subjects was staggered. The DMC reviewed the AE profile during plerixafor treatment in mobilization cycle 1 for initial subjects, and evaluated on any issues of potential safety significance that could have affected determination of DLT and plerixafor dose before subsequent subjects were dosed with plerixafor (see also Section 5.2.3).

The first 2 subjects in Group C were infused with drug product in a staggered fashion: neutrophil engraftment was required to occur in the first subject treated in Group C before busulfan conditioning was initiated for the second subject.

3.3. Event Adjudication Committee

An independent Event Adjudication Committee comprised of members with appropriate scientific and medical expertise to review subject source documents will be convened during Group C. The Event Adjudication Committee will be responsible for adjudication of all investigator reported VOEs, irrespective of the severity, duration, or need for medical facility visit. The committee will review events that occurred prior to enrollment (retrospectively) as well as during the study for Groups A, B, and C. The committee may also be responsible for the adjudication of any subject deaths that occur after drug product infusion.

A charter describing the composition and conduct of this committee will be issued by the Sponsor and agreed to by all members prior to conducting any reviews.

3.4. Engraftment Failure

Neutrophil engraftment failure is defined as failure to achieve 3 consecutive ANC laboratory values $\geq 0.5 \times 10^9$ cells/L obtained on different days after the initial post-infusion nadir by Day 43, or receiving back-up cells at any time during the neutropenic phase, and is reported as an SAE (see Section 6.2.30.2).

3.5. Treatment Discontinuation and Enrollment Suspension Criteria

3.5.1. Stopping Rules for Plerixafor

Once mobilization with plerixafor has begun, there are no protocol-defined stopping rules for plerixafor. Mobilization with plerixafor followed by apheresis was introduced under Protocol HGB-206 Amendment 7.0. A plerixafor dose of 0.24 mg/kg was identified as having an acceptable safety profile by the DMC, and dose-limiting toxicities (DLTs) were no longer being assessed as of Protocol HGB-206, Amendment 8.0.

3.5.2. Stopping Rules for Busulfan

The drug product must be release tested and dispositioned for clinical use and stored at the study site, and a sufficient number of back-up cells must have been collected and stored at the study site before the subject can begin myeloablative conditioning with busulfan.

Once myeloablation with busulfan has begun, there are no protocol-defined stopping rules for busulfan. 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 treatment, the Medical Monitor should be contacted immediately. In such situations in which busulfan conditioning has not been completed per protocol, the drug product should not be given, and it is likely that rescue with back-up cells will be required.

3.5.3. Stopping Rules for bb1111

bb1111 is given as a single IV infusion; once the infusion has been given, there are no protocol-defined stopping rules for treatment of individual subjects.

Drug product infusion for an individual subject will not be initiated if:

- the drug product fails to meet criteria for dispositioning for clinical use. The Investigator, in consultation with the subject, may decide to proceed with an additional stem cell collection, with the approval of the Medical Monitor.

3.5.4. Enrollment Suspension Criteria

Enrollment in this study and other study procedures may be temporarily suspended at any time for safety reasons.

Following any of the events listed below occurring in a subject treated with bb1111 in a bluebird bio-sponsored clinical study, the Sponsor will convene an urgent safety review meeting to determine if enrollment (and other study procedures) in this study should be temporarily suspended, inform the DMC chair of the event, and convene an ad hoc DMC meeting as appropriate:

- Death
- Report of a hematologic malignancy (e.g., leukemia, lymphoma, myelodysplastic syndrome, or other hematologic malignancies)
- Detection of vector-derived RCL in any subject (confirmed on co-culture assay)
- Failure to achieve hematopoietic reconstitution with transduced cells requiring use of back-up cells

Subjects who have already been treated with drug product will continue in the study.

The Sponsor will inform the regulatory authorities, Investigators, and each site's Institutional Review Board (IRB)/ Independent Ethics Committee (IEC) and other appropriate institutional regulatory authorities if a decision to temporarily suspend enrollment is made.

4. STUDY POPULATION

4.1. Number of Subjects

A total of approximately 50 subjects will be treated with bb1111 in Study HGB-206.

Seven subjects have already been treated in Group A, and 2 subjects have been treated in Group B. A total of approximately 41 subjects in Group C (including subjects consented under Versions 7.0, 8.0, and 9.0 of this protocol) will be treated with bb1111, approximately 35 of which must meet the severe VOE criteria as set forth in Inclusion Criterion #3.1.

Subjects who withdraw prior to drug product infusion may be replaced.

4.2. Inclusion Criteria

Subjects must:

1. Be ≥ 12 and ≤ 50 years of age at time of consent.
2. Have a diagnosis of SCD, with either β^S/β^S or β^S/β^0 or β^S/β^+ genotype.
3. Previous Inclusion Criterion #3 is no longer applicable and has been replaced with Criterion #3.1.

3.1. In the setting of appropriate supportive care measures (e.g., pain management plan) have experienced **at least 4 severe VOEs in the 24 months prior to informed consent as defined below.**

For the purposes of this study, a severe VOE is defined as an event with no medically determined cause other than a vaso-occlusion, requiring a ≥ 24-hour hospital or emergency room (ER) observation unit visit or at least 2 visits to a day unit or ER over 72 hours with both visits requiring intravenous treatment. Exception: priapism does not require hospital admission but does require a medical facility visit; 4 priapism episodes that require a visit to a medical facility (without inpatient admission) are sufficient to meet criterion. Severe VOEs include:

a. an episode of acute pain with no medically determined cause other than a VOE
b. Acute chest syndrome (ACS), defined by an acute event with pneumonia-like symptoms (e.g., chest pain, fever [> 38.5°C], tachypnea, wheezing or cough, or findings upon lung auscultation) and the presence of a new pulmonary infiltrate consistent with ACS and requiring oxygen treatment and/or blood transfusion.
c. Acute hepatic sequestration, defined by a sudden increase in liver size associated with pain in the right upper quadrant, abnormal results of liver-function test not due to biliary tract disease, and reduction in Hb concentration by at least 2 g/dL below the baseline value
d. Acute splenic sequestration, defined as sudden enlargement of the spleen and reduction in Hb concentration by at least 2 g/dL below the baseline value.
e. Acute priapism: defined as a sustained, unwanted painful erection lasting more than 2 hours and requiring care at a medical facility (with or without hospitalization)

4. Previous Inclusion Criterion #4 is no longer applicable as of Protocol Amendment 8.0.

5. Have a Karnofsky performance status of ≥ 60 (≥ 16 years of age) or a Lansky performance status of ≥ 60 (< 16 years of age).
6. Previous Inclusion Criterion #6 is no longer applicable as of Protocol Amendment 8.0.
7. Have either experienced HU failure at any point in the past (defined as > 1 VOE or ≥ 1 ACS after HU has been prescribed for at least 6 months) or must have intolerance to HU (intolerance is defined as the patient being unable to continue to take HU per PI judgment).
8. Be treated and followed for at least the past 24 months prior to Informed Consent in medical center(s) that maintained detailed records on sickle cell disease history, including incidence of VOEs and severe VOEs, aplastic crises, infectious complications, SCD-related chronic complications, SCD-related surgery, neurovascular evaluation (including MRI/A and TCD), pRBC transfusions (including indications, volume and units of pRBCs, associated pre-transfusion HbS and total Hb values and post-transfusion AEs [including allo-immunization]), SCD-specific treatment history (e.g., HU, L-glutamine, iron overload, and chelation history), and use of pain medication.

4.3. Exclusion Criteria

Subjects are excluded if they meet any of the following criteria:

1. Positive for presence of human immunodeficiency virus type 1 or 2 (HIV1 or HIV2), hepatitis B, hepatitis C, human T-lymphotrophic virus1 (HTLV1) or -2 (HTLV-2), active syphilis. 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 antibody] are eligible. Subjects with past exposure to HBV [HBc Ab positive and/or HBe Ab positive] are also eligible for the study provided they are negative for HBV DNA. Subjects who are positive for anti-hepatitis C antibody are eligible as long as they have an undetectable hepatitis C viral load. Where clinically and/or regionally indicated, other tests may be performed, in which case relevant positive results suggesting active infection would exclude the subject from participating, depending on regional guidelines: for example, malaria, tuberculosis, active toxoplasmosis, Trypanosoma cruzi, or West Nile Virus.
2. Clinically significant, active bacterial, viral, fungal, or parasitic infection, as determined by the Investigator, e.g., active relapsing malaria.
3. Inadequate bone marrow function, as defined by an ANC of $< 1 \times 10^9$/L ($< 0.5 \times 10^9$/L for subjects on HU treatment) or a platelet count $< 100 \times 10^9$/L
4. Previous Exclusion Criterion #4 is no longer applicable as of Protocol Amendment 8.0.

4.1 Severe cerebral vasculopathy, defined by any history of: overt ischemic or hemorrhagic stroke, abnormal TCD (> 200 cm/sec) requiring chronic transfusion, occlusion or stenosis in the circle of Willis, or presence of Moyamoya disease. Subjects with radiologic evidence of silent infarction in the absence of any of the above criteria would still be eligible.

5. Previous Exclusion Criterion #5 is no longer applicable as of Protocol Amendment 8.0.

6. Previous Exclusion Criterion #6 is no longer applicable as of Protocol Amendment 8.0.
7. Previous Exclusion Criterion #7 is no longer applicable as of Protocol Amendment 8.0.
8. Baseline oxygen saturation < 90% without supplemental oxygen (excluding periods of SCD crisis, severe anemia or infection).
9. Baseline carbon monoxide diffusing capacity (DL_{CO}) < 50% (corrected for Hb) in the absence of infection. If DLco cannot be assessed due to age or cognition-related restrictions, there must be a normal respiratory exam, chest radiograph without pulmonary infiltrates, and oxygen saturation by pulse oximetry ≥ 90% on room air.
10. Baseline left ventricular ejection fraction (LVEF) < 45% measured by cardiac echography.
11. Clinically significant pulmonary hypertension at baseline, as defined by the requirement for ongoing pharmacologic treatment or the consistent or intermittent use of supplemental home oxygen.
12. Baseline estimated glomerular filtration rate (eGFR) < 70 mL/min/1.73 m^2, as determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation (see http://www.kidney.org/professionals/kdoqi/gfr_calculator.cfm).
13. Advanced liver disease, defined as:
    a. Persistent aspartate transaminase, alanine transaminase, or direct bilirubin value > 3 × the upper limit of normal (ULN), **or**
    b. Baseline prothrombin time or partial thromboplastin time > 1.5 × ULN, suspected of arising from liver disease, **or**
    c. Magnetic Resonance Imaging (MRI) of the liver demonstrating clear evidence of cirrhosis, **or**
    d. MRI findings suggestive of active hepatitis, significant fibrosis, inconclusive evidence of cirrhosis, or liver iron concentration ≥ 15 mg/g require follow-up liver biopsy in subjects ≥ 18 years of age. In subjects < 18 years of age, these MRI findings are exclusionary, unless in the opinion of the Investigator, a liver biopsy could provide additional data to confirm eligibility and would be safe to perform. If a liver biopsy is performed based on MRI findings, any evidence of cirrhosis, bridging fibrosis, or significant active hepatitis will be exclusionary.
14. For subjects who have history of iron overload or serum ferritin levels > 1000 ng/mL, a cardiac MRI is required. Cardiac T2* < 10 ms results in exclusion.
15. Contraindication to anesthesia.
16. Any contraindications to the use of plerixafor during the mobilization of HSCs and any contraindications to the use of busulfan and any other medicinal products required during the myeloablative conditioning, including hypersensitivity to the active substances or to any of the excipients.
17. Any prior or current malignancy or immunodeficiency disorder, except previously treated, non-life threatening, cured tumors such as squamous cell carcinoma of the skin.
18. Prior receipt of an allogeneic transplant.

19. 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).
20. Diagnosis of significant psychiatric disorder of the subject that, in the Investigator's judgment, could seriously impede the ability to participate in the study.
21. Pregnancy, or breastfeeding in a postpartum female, or absence of adequate contraception for fertile subjects. Females of childbearing potential must agree to use a medically acceptable method of birth control such as oral contraceptive, intrauterine device, barrier and spermicide, or contraceptive implant/injection from time of consent through at least 6 months after drug product infusion. Male subjects must agree to use effective contraception (including condoms) from Conditioning through at least 6 months after drug product infusion.
22. Participation in another clinical study with an investigational drug within 30 days of Screening.
23. Previous Exclusion Criterion #23 is no longer applicable as of Protocol Amendment 8.0.
24. Prior receipt of gene therapy.
25. Previous Exclusion Criterion #25 is no longer applicable as of Protocol Amendment 9.0.
26. An assessment by the Investigator that the subject or parents/caregivers (as required) will not be able to comply with the study procedures outlined in the study protocol.
27. Patients needing therapeutic anticoagulation treatment during the period of conditioning through platelet engraftment (patients on prophylactic doses of anticoagulants not excluded per this criteria).
28. Unable to receive RBC transfusion.
29. Any other condition that would render the subject ineligible for HSCT, as determined by the attending transplant physician.
30. Applicable to subjects < 18 years of age only: Availability of a willing, matched HLA-identical sibling hematopoietic cell donor.

4.4. Subject Screening and Registration

Subjects willing to participate in the study will provide written informed consent according to Good Clinical Practice (GCP). Written informed consent must be obtained before the conduct of any Screening tests.

Upon signing the informed consent, the subject will be registered and assigned a unique subject number. Once a subject number has been assigned, it cannot be reused. If the subject is re-enrolled after discontinuation, he/she will be assigned a new unique subject number.

4.5. Subject Withdrawal from the Study

Subjects have the right to withdraw from the study at any time for any reason. After giving informed consent and assent (as applicable), subjects may withdraw or be withdrawn from study

related procedures and treatments (e.g., apheresis, busulfan conditioning) under the following conditions:

- withdrawal of consent or assent
- the subject is unable to comply with protocol-defined visits or other requirements of the protocol
- pregnancy prior to conditioning
- adequate cells are not collected during harvests, or failure of transduced cells to be dispositioned for clinical use
- any medical condition which, in the opinion of the Investigators, would put the subject at risk for continuing treatment or follow-up studies

Although subjects have the right to withdraw from the study at any time, withdrawal after the start of conditioning and before administration of the drug product is strongly discouraged, as this would be considered deleterious to the subject. In such cases, it is likely that rescue will be required using the subject's stored unmanipulated HSCs (rather than transduced cells).

For subjects who withdraw consent or assent (if relevant), no further data will be collected on the subject; if they have received drug product, they are expected to enroll in the long-term follow-up study.

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. See also Section 6.2.30.1 for AEs monitoring of subjects who withdraw from the study.

- If withdrawal is before drug product infusion, subjects should remain on study for at least 30 days after any invasive study procedure (e.g., mobilization, liver biopsy) before withdrawal. In the rare case a subject undergoes myeloablation and receives back-up cells instead of bb1111, subject should remain on the study for at least 3 months post myeloablation.
- If subject withdraws after drug product has been manufactured but before infusion, the drug product may be shipped to bluebird bio for additional process development and characterization studies.
- If withdrawal is after drug product infusion, subjects will be asked to complete the same assessments as specified in the Schedule of Events (SOE) for Month 24 (Early Termination Visit assessments) and are expected to enroll in the long-term follow-up study.

Subjects withdrawn from the study prior to drug product infusion may be replaced.

4.6. Screen Failures

Subjects are Screen Failures if:

- They sign the main informed consent form, but cannot finish assessments for Screening or are ineligible based on those assessments

Data collected on Screen Failure subjects will only include:

- Demography
- Eligibility criteria for which subject was ineligible or did not finish assessment
- Note: Serious adverse events (SAEs) must still be immediately reported for Screen Failure subjects from consent until the time they are determined to be a Screen Failure

Upon consultation with the Medical Monitor, screen-failed subjects may be allowed to re-screen to be assessed for eligibility to enter the study.

5. STUDY TREATMENTS

5.1. Description of bb1111

BB305 Lentiviral Vector: BB305 LVV is a replication defective, self-inactivating, HIV-1 based LVV, pseudotyped with the vesicular stomatitis virus G envelope protein, carrying the human β-globin gene with a single modification in the coding region at codon 87 (β^{A87} Thr:Gln [$\beta^{A\text{-}T87Q}$]).

bb1111: bb1111 is defined as an autologous CD34+ cell-enriched population from patients with sickle cell disease that contains HSCs transduced with BB305 LVV encoding the $\beta^{A\text{-}T87Q}$-globin gene, suspended in cryopreservation solution.

5.2. Summary of Treatments to be Performed or Administered

5.2.1. Transfusion Regimen Prior to Drug Product Infusion

For at least 60 days prior to stem cell harvest and continuing through until the start of conditioning, subjects will undergo a transfusion regimen (exchange or simple, as available or needed) to reach a target Hb of 10 g/dL prior to mobilization (and 8 to 10 g/dL prior to conditioning; not to exceed 12 g/dL prior to either mobilization or conditioning) and a pre-transfusion target HbS proportion in the blood of < 30% to reduce the risk of SCD-related complications. The last exchange transfusion must occur within 4 days of start of mobilization, and the HbS proportion in the blood must be < 20% after the last exchange transfusion.

If a subject experiences an SCD-related crisis within 1 month of the start of mobilization, the transfusion regimen will be adjusted, and mobilization will be postponed until the subject is crisis-free for a period of 1 month. In case of iron overload, subjects should be adequately chelated; however, iron chelation must be discontinued at least 7 days prior to stem cell harvest. It may be restarted between completion of the stem cell harvest(s) and admission to the hospital for myeloablation but should be stopped again at least 7 days before initiating myeloablation. Subjects who are not documented to have met these parameters or who have experienced any significant new sickle-related complications within 1 month of the start of mobilization will require additional approval from the Medical Monitor before undergoing mobilization/apheresis.

While simple transfusions may be utilized for the majority of the transfusion period, it is strongly recommended that approximately 24 to 48 hours prior to receiving plerixafor for mobilization, an exchange transfusion (rather than simple transfusion) be performed in an attempt to ensure that the HbS proportion in the blood is < 20%.

5.2.2. Stem Cell Harvest: Bone Marrow

See flow diagram for study design in Section 3.1.

Subjects in Groups A and B1 underwent bone marrow harvest for both manufacture of drug product and back-up cells.

Subjects in Group B2 underwent mobilization with plerixafor and apheresis for collection of back-up cells and exploratory manufacturing development.

Subjects in Group C will undergo mobilization with plerixafor and apheresis for both manufacture of drug product and back-up cells. Group C subjects for whom adequate HSCs are collected to achieve the minimum cell dose but not for rescue, and who are not able to tolerate further plerixafor or did not mobilize well with plerixafor may be offered the option of bone marrow harvest for collection of back-up cells only, provided that drug product manufactured from mobilized cells has met criteria for dispositioning for clinical use. More than 1 bone marrow harvest is allowed if needed to obtain the minimum cell number required for potential rescue, provided that each bone marrow harvest is separated by at least 4 weeks.

Bone marrow harvest should be performed under general anesthesia and using sterile precautions, as per local procedures at the clinical site. Subjects should be monitored for safety as per local procedures for a bone marrow harvest, which must include vital signs and physical exam prior to the procedure and then again prior to discharge. Sickle red cells collected during the harvest procedure are prone to clumping when placed in the hypoxic environment of the bag; therefore, efficient collection, anti-coagulation, and frequent mixing of the collected bone marrow is strongly advised. The bone marrow aspirated should not exceed 20 mL/kg with the aim of obtaining a TNC count of $\geq 1 \times 10^8$/kg per subject.

Note: as of Protocol Amendment 8.0, bone marrow harvest can no longer be used to collect stem cells for drug product manufacture. Under Protocol Amendment 7.0, each subject in Group B, for whom bone marrow manufacture was planned underwent a minimum of 2 bone marrow harvests, separated by at least 4 weeks. More than 2 bone marrow harvests were allowed if needed to obtain additional cells for manufacture and potential rescue (to meet the cell dose minimum), provided that each bone marrow harvest was separated by at least 4 weeks. When more than 1 bone marrow harvest was used to produce bb1111, then separate drug products were manufactured, release tested and dispositioned from each harvest, but infused on the same day to meet criterion for a single dose.

5.2.3. Mobilization and Apheresis Procedure

See flow diagram for study design in Section 3.1.

Subjects in Group B2 underwent mobilization with plerixafor and apheresis for collection of back-up cells prior to undergoing bone marrow harvest for drug product manufacture (with bone marrow harvest for drug product manufacture occurring at least 14 days after the last apheresis session). Subjects in Group B2 who tolerated plerixafor mobilization and underwent successful apheresis with adequate collection of back-up cells were given the option to defer bone marrow harvest for drug product manufacture and instead wait for Group C to open and undergo drug product manufacture using plerixafor-mobilized and apheresed cells.

Subjects in Group C undergo mobilization and apheresis both for collection of back-up cells and manufacture of drug product. Administration of aspirin (80 to 325 mg) is recommended (between 12 hours and 30 minutes prior to initiation of apheresis). Subjects undergoing mobilization/apheresis should remain in the supine position to minimize symptoms (including hypotension) from plerixafor dosing through the end of apheresis each day. Placement of a central venous catheter prior to mobilization is highly recommended to ensure adequate flow rate during apheresis. Platelet count should be confirmed to be $\geq 100 \times 10^9$/L within 12 hours before the first day of apheresis session in the Cycle. Following plerixafor administration, subjects will be monitored for AEs, e.g., hypotension, syncope, hypoxia, per institutional

practice. Throughout apheresis, subjects will have frequent measures of heart rate, blood pressure, and pulse oximetry, e.g., every 20 minutes for adults, and every 10 minutes for pediatric subjects. If additional apheresis days are required, platelet counts must be confirmed to be $\geq 75 \times 10^9$/L within 24 hours of subsequent apheresis sessions, prior to administration of plerixafor on that day. If platelet counts do not meet these criteria, mobilization and apheresis should be deferred until platelet counts recover to $\geq 75 \times 10^9$/L. A separate manual on apheresis requirements and recommended parameters will be provided. During the entire time frame leading up to, during and after mobilization and apheresis, close attention should be paid to anticipation and management of pain and anxiety, repletion of electrolyte imbalances, and adequate hydration.

Group B2 (no further enrollment):

If fewer than 1.5×10^6 CD34+ cells/kg are obtained during the initial apheresis session, and the subject has tolerated this session, then the subject must wait for a period of at least 14 days to initiate the next apheresis procedure (Mobilization Cycle 2, Apheresis Day 1; see Figure 2).

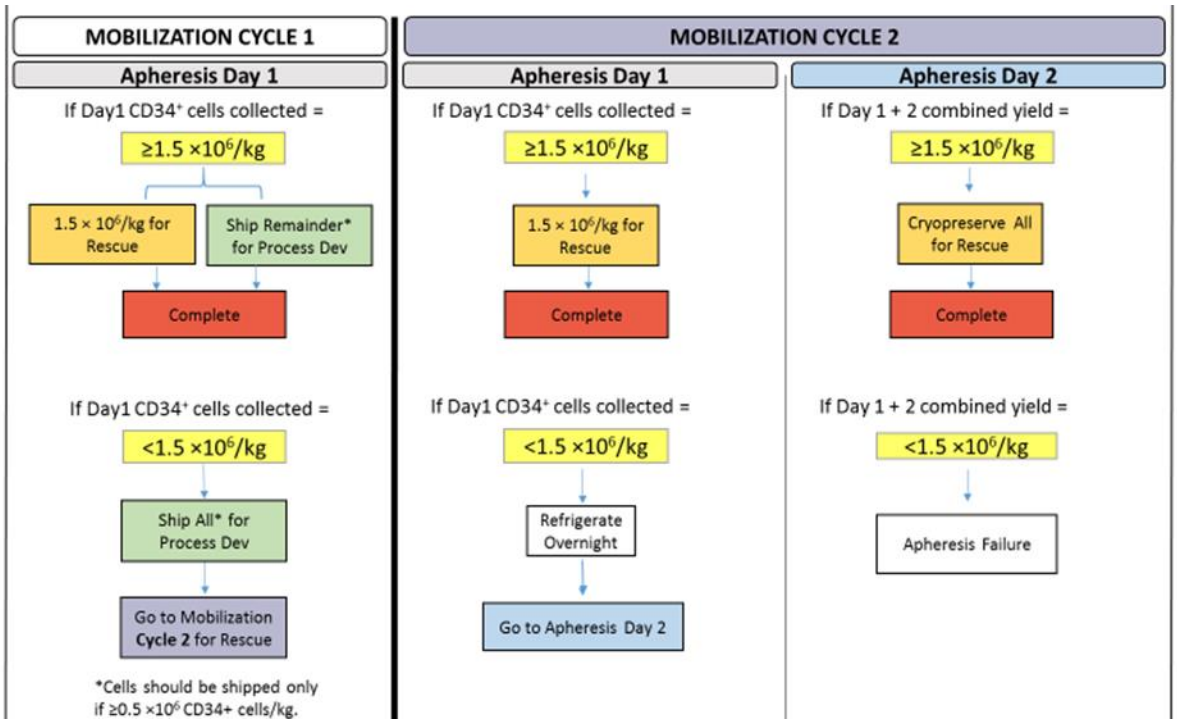
During mobilization/apheresis, subjects will be monitored for evidence of plerixafor-associated toxicity.

Plerixafor dosing of initial subjects in Group B2 (enrolled under Protocol Version 6.0) was staggered. Data Monitoring Committee members reviewed the AE profile during plerixafor treatment in mobilization Cycle 1 for the first subject treated in Group B2 and planned to adjudicate on any issues of potential safety significance that could affect determination of DLT before dosing of the second subject with plerixafor. A similar procedure was followed after dosing of the second subject in order to permit dosing of the third subject. The DMC reviewed aggregate safety data in Group B2 after 3 subjects had been dosed and confirmed that there are no safety concerns precluding dosing of subjects in Group C with plerixafor. See also Section 3.2.

Subjects unable to tolerate plerixafor mobilization may be offered bone marrow harvests for collection of back-up cells and for drug product manufacture. Subjects who do tolerate plerixafor mobilization will be given the option of waiting for Group C to open for drug product manufacture.

Figure 2 below depicts the mobilization and apheresis plan for collection of back-up cells from subjects in Group B2. If plerixafor is tolerated, and $\geq 1.5 \times 10^6$ CD34+ cells/kg are collected during Mobilization Cycle 1, then 1.5×10^6 CD34+cells /kg will be stored for rescue, and any remaining cells will be sent to the Sponsor for exploratory manufacturing development (minimum of 0.5×10^6 CD34+ cells/kg required in shipment).

Figure 2: Mobilization and Apheresis Plan for Back-up Cell Collection (Group B2)



If < 1.5 × 10^6 CD34+ cells/kg are collected during the first mobilization cycle, all of these cells will be sent to the Sponsor for exploratory process development work, and a second mobilization cycle may be performed (14 days after the completion of Mobilization Cycle 1), if the subject tolerated the first cycle of mobilization.

After successful mobilization and apheresis of back-up cells, subjects may undergo bone marrow harvests for drug product manufacture at least 14 days after the last apheresis session. See Section 5.2.2 for details surrounding bone marrow harvest. All cells collected from bone marrow will be sent to the transduction facility and utilized for drug product manufacture.

If < 1.5 × 10^6 CD34+ cells/kg are recovered in the second cycle of mobilization, as well as in the first, then the subject will be considered an apheresis failure, all recovered cells will be sent to the Sponsor (minimum of 0.5 × 10^6 CD34+ cells/kg required in shipment), and the subject will proceed to bone marrow harvest at least 14 days after completion of the last mobilization/apheresis cycle, for both back-up cell isolation and drug product manufacture.

If ≤ 1 of 3 subjects receiving plerixafor in Group B2 has a DLT during Cycle 1 of mobilization, then Group C may begin dosing with plerixafor.

If > 1 of 3 subjects receiving plerixafor in Group B2 has a DLT during Cycle 1, then a dose of plerixafor ≥ 0.16 and < 0.24 mg/kg may be investigated for mobilization in up to an additional 3 to 6 subjects. Treatment scheme will be as described above.

Group C:

Once the safety and tolerability of plerixafor had been confirmed in Group B2 and regulatory authorities approved the drug product manufacturing process with plerixafor-mobilized HSCs,

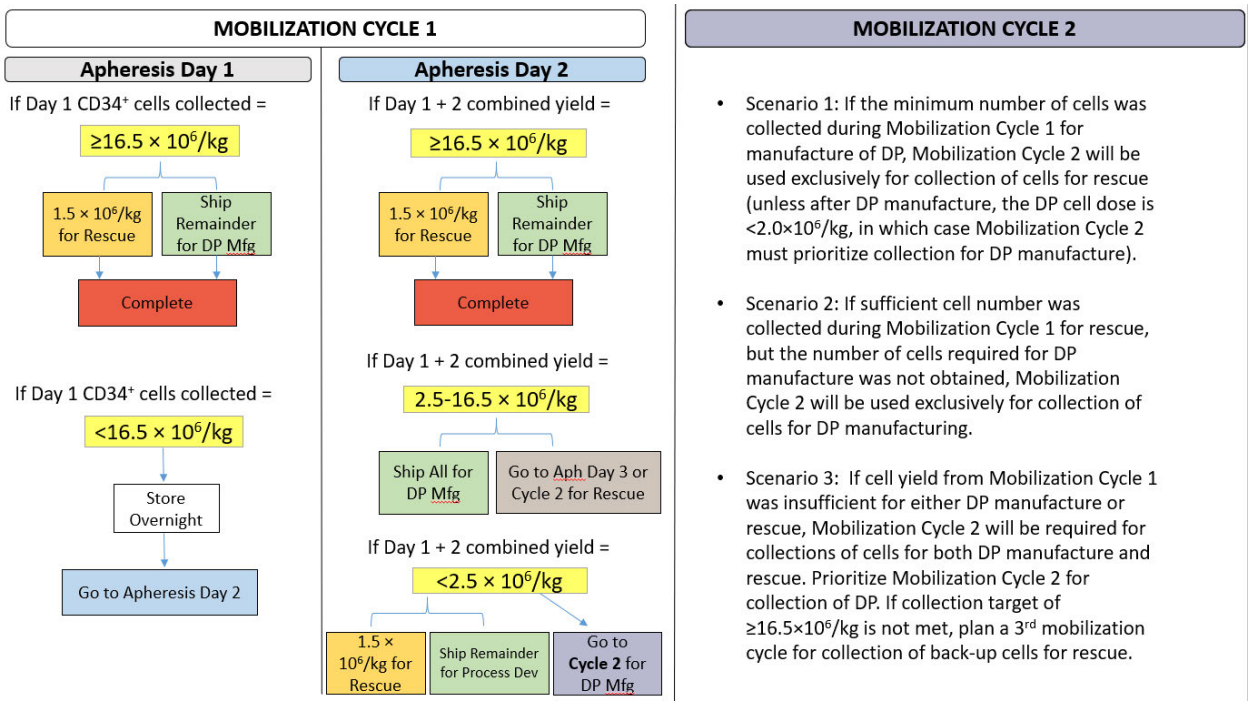
subjects began dosing with plerixafor in Group C. The first 2 subjects in Group C received drug product infusion in a staggered fashion: neutrophil engraftment was required to occur in the first subject treated in Group C before busulfan conditioning could be initiated for the second subject.

For subjects in Group C, plerixafor dose is 0.24 mg/kg. Apheresis should begin approximately 4 to 6 hours after plerixafor administration. If more than 1 apheresis day is required, platelet counts must be confirmed to be $\geq 75 \times 10^9$/L within 24 hours of subsequent apheresis sessions, prior to administration of plerixafor on that day. If platelet counts do not meet these criteria, mobilization and apheresis should be deferred until platelet counts recover to $\geq 75 \times 10^9$/L. For subjects undergoing more than 1 mobilization cycle, each cycle must be separated by at least 14 days. Subjects will receive daily plerixafor prior to each apheresis collection. A 3rd day of mobilization and apheresis (to be used for collection of back-up cells only) may be permitted only after prior discussion with the Medical Monitor and if the subject tolerated prior plerixafor and apheresis. If a sufficient number of cells are collected after the first mobilization cycle, no further mobilization/apheresis is required.

For Group C subjects, collection of cells for drug product manufacture will be prioritized over collection of cells for rescue. Subjects for whom back-up cells have already been collected (e.g., participation in Group B2) underwent a separate mobilization/apheresis cycle or bone marrow harvest during which all cells collected were submitted for drug product manufacture.

The schematic below (Figure 3) outlines the plans for plerixafor mobilization and apheresis in Mobilization Cycles 1 and 2 and beyond for subjects in Group C.

Figure 3: Mobilization and Apheresis Plan (Group C)



If plerixafor is tolerated, and ≥ 16.5 × 10^6 CD34+ cells/kg are collected after the first apheresis procedure of mobilization cycle 1, then 1.5 × 10^6 cells/kg will be saved for rescue and the remainder of cells will be sent to the transduction facility for drug product manufacture (as per the Study Operations Manual). If the site is unable to split the collection, then send all cells for drug product manufacture, and collect back-up cells during Apheresis Day 2 of mobilization cycle 1. CD34+ cells for rescue should be stored indefinitely at the clinical site or per local procedures.

If the Apheresis Procedure Day 1 collection of mobilization cycle 1 is < 16.5 × 10^6 CD34+ cells/kg, this collection should be held overnight, at a controlled storage facility at the clinical site (should be shipped immediately to the transduction facility if storage is not possible; minimum of 1.0 × 10^6 CD34+ cells/kg required in shipment), and the subject should return for Apheresis Procedure Day 2. If the combined yield from Days 1 + 2 of mobilization cycle 1 is ≥ 16.5 × 10^6 CD34+ cells/kg, then 1.5 × 10^6 cells/kg will be saved for rescue, and the remainder of cells will be sent to the transduction facility for drug product manufacture. If the combined yield from Days 1 + 2 is 2.5 to 16.5 × 10^6 CD34+ cells/kg, then all cells will be shipped for drug product manufacturing, and cells for rescue may be collected during a third day of apheresis in the mobilization cycle, or alternatively, during an additional mobilization cycle that occurs at least 14 days after the last apheresis day of the prior mobilization cycle, at the discretion of the Investigator and Medical Monitor. If the Day 1 + 2 combined yield is < 2.5 × 10^6 CD34+ cells/kg, then up to 1.5 × 10^6 CD34+ cells/kg will be saved for rescue, with remaining cells shipped for process development, and a second mobilization cycle will be required for collection of cells for drug product manufacturing. If fewer than 1.5 × 10^6 CD34+ cells/kg were collected during mobilization cycle 1, all cells will be sent to bluebird bio, and collection of cells for both drug product manufacturing and rescue will occur in mobilization cycle 2.

If after drug product manufacture is complete at the central manufacturing facility, a total of < 3.0 × 10^6 $CD34^+$cells/kg are obtained in the final drug product, or if drug product fails to meet specifications for any reason, subjects may undergo repeat apheresis and manufacture of new drug product, which should occur no sooner than 14 days after completion of the most recent mobilization/apheresis cycle.

Group C subjects for whom adequate HSCs are collected to achieve the minimum cell dose but not for rescue, and who are not able to tolerate further plerixafor or did not mobilize well with plerixafor may be offered the option of bone marrow harvest for collection of back-up cells only, provided that drug product manufactured from mobilized cells has met criteria for dispositioning for clinical use. More than 1 bone marrow harvest is allowed if needed to obtain the minimum cell number required for potential rescue, provided that each bone marrow harvest is separated by at least 4 weeks.

5.2.4. Transduction Process, Storage of Drug Product, Packaging and Labeling, and Traceability

All cell manipulation procedures will be performed in transduction facilities in accordance with Current Good Manufacturing Practices following process-specific procedures.

The subject's CD34+ cells transduced with BB305 LVV are stored and frozen in cryopreservative solution (containing 5% dimethyl sulfoxide) in the vapor phase of liquid nitrogen at the transduction facility until release testing and dispositioning.

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

The drug product will be labeled by the transduction facility according to Good Manufacturing Practices, and detailed records will be maintained to allow for accurate traceability of the drug product, to ensure that the drug product is administered to the original donor.

Refer to Section 5.7 (Product Accountability) and the Study Operations Manual (SOM) for details regarding traceability.

Note that samples routinely collected as part of the process of manufacturing the drug product may be used to study the manufacturing process. In particular, these samples may be used to understand how the process may be improved or made more robust. Additionally, these samples may be analyzed if clinically indicated.

If more than 1 stem cell harvest is necessary to produce the minimum cell dose of bb1111, they will be manufactured, release tested, and dispositioned independently as separate drug products, but infused on the same day to meet criterion for a single dose.

5.2.5. Conditioning

5.2.5.1. Busulfan Dosing

Myeloablative conditioning of the subject will be performed using busulfan. Conditioning will only begin after the following criteria are met:

- all drug product lots to be used for the subject have been release tested, dispositioned for clinical use, and stored at the clinical site
- a sufficient number of back-up cells has been collected and stored at the clinical site
- the subject has undergone interim assessments for updated medical history, AE, concomitant medications, physical examination, vital signs, and laboratory tests, all as per the SOE, and continues to meet the eligibility criteria based on these results
- subjects have undergone a transfusion regimen (Hb > 10 g/dL and HbS < 30%) and are off HU for at least 2 days and iron chelation for at least 7 days prior to the start of conditioning
- results of conventional cytogenetics (karyotyping) and cytogenetics by FISH from the bone marrow have been received and reviewed by the Investigator and the Medical Monitor; in the presence of a chromosomal abnormality or genetic mutation that does not render the subject ineligible for HSCT per the Investigator's judgment, approval of the Medical Monitor is required before the subject can proceed with conditioning

(see Section 6.2.11) and subject must sign appropriate informed consent before proceeding with conditioning and treatment with bb1111.

Busulfan will be administered at a starting dose of 3.2 mg/kg/day for 4 consecutive days via IV infusion on Days -6 through -3. The preferred schedule is busulfan via a central venous line (CVL) as a 3-hour infusion once daily for 4 consecutive days for a total of 4 doses. Divided dosing is permitted. In this case, busulfan should be administered as a 2-hour infusion every 6 hours for 16 consecutive doses. For subjects weighing < 35 kg, a q6h dose of 0.8 mg/kg is preferred; for subjects weighing ≥ 35 kg, either once daily (qd) or q6h dosing can be used, at the discretion of the Investigator. Please note that the timing of busulfan PK sample collection is based on whether a 2-hour or 3-hour infusion is used.

Clinical sites that use a test dose of busulfan before myeloablation to pre-determine the busulfan pharmacokinetics and predict an optimal dose may do so in this protocol. The recommended test dose of busulfan is 0.8 mg/kg, administered approximately 3 to 10 days before planned initiation of conditioning. Note that clinical sites must be able to measure first and third day busulfan PK, as described below.

5.2.5.2. Pharmacokinetics for Busulfan Dose Adjustments

The dose of busulfan will be adjusted based upon busulfan PK in order to maintain appropriate levels for myeloablation (AUC goal of 1250 [range 1100 to 1350] µM*min for a q6h dosing regimen, or 5000 [range 4400 to 5400] µM*min for a qd dosing regimen). The dosage should be calculated on the basis of the lower of the ideal versus actual body weight. The dose may be adjusted appropriately based upon actual plasma busulfan levels observed. Clinical sites must be able to measure first and third day busulfan PK. Based on busulfan PK results for day 1 and day 3 of busulfan dosing, busulfan dose adjustments should be made for subsequent dosing. If feasible, daily busulfan PK measurement is recommended. It is recommended that the busulfan clearance rate is also calculated.

Instructions for once daily dosing: Samples for busulfan concentration should be collected at the end of the 3-hour infusion, 195 minutes after **start** of infusion, and 4, 5, 6, and 8 hours after the start of the infusion. Samples should not be drawn from the lumen used to infuse busulfan.

Instructions for q6h dosing: Samples for busulfan concentration should be collected at the end of the 2-hour infusion, 135 minutes after **start** of infusion, 150 minutes after start of infusion, and 3, 4, 5, and 6 hours after the start of the infusion. Samples should not be drawn from the lumen used to infuse busulfan.

After completion of the 4-day course of busulfan, there must be a minimum of 48 hours before drug product infusion to achieve optimal engraftment. Busulfan levels will be measured 48 and 72 hours after final dose of busulfan for retrospective confirmation of adequate wash-out.

5.2.6. Drug Product Infusion Procedures, Dose, and Administration

Pre-hydration and pre-medication of the subject are not required and should follow local procedures for bone marrow infusion at the clinical site.

Prior to administration, bb1111 is thawed at the clinical site (typically in a 37°C water bath), and the infusion of the drug product should be completed immediately after thawing and within a

maximum of 4 hours of its thawing. All procedures involving bb1111 must be performed using aseptic techniques by trained personnel.

bb1111 will be administered on Day 1 via IV infusion at the clinical site. The dose to be administered is $\geq 3.0 \times 10^6$ CD34+ cells/kg (for mobilized apheresed cells). Subjects who undergo more than 1 stem cell collection procedure (and subsequent transduction of those cells) will have more than 1 drug product lot, which should be administered in sequence, with one administered immediately after the other. Disposition of back-up cells collected and/or partial drug product manufactured under a prior version of the protocol will be made after discussion between the Investigator and Medical Monitor.

Vital signs are to be monitored concurrently during drug product infusion no less frequently than at the start, once during, and upon completion of the infusion. Infusion reactions, including anaphylaxis, will be managed according to the medical judgment of the physician overseeing the infusion.

5.3. Method of Assigning Subjects to Treatment

Seven subjects who consented and received drug product under version 5.0 (or earlier) of the protocol were retrospectively assigned to Group A, in which both drug product and back-up cells were derived from bone marrow.

In addition to the above 7 subjects, 1 subject who had consented under version 5.0 of the protocol, but had not yet been treated with drug product, was reconsented under protocol version 6.0 and participated in Group B1.

Subjects initially consented under version 6.0 of this protocol were part of Group B or C. In Group B1, both drug product and back-up cells were derived from bone marrow. In Group B2, plerixafor mobilization and apheresis were used for collection of back-up cells and exploratory manufacturing development *prior* to undergoing bone marrow harvest for drug product manufacture. At least 3 subjects were required to enroll in Group B, and at least 3 subjects in Group B2 were required for evaluation of DLT at each dose of plerixafor.

Group C was opened upon (1) confirmation of the safety and tolerability of plerixafor mobilization in Group B2, and (2) regulatory authority approval of the drug product manufacturing process with plerixafor-mobilized HSCs. In Group C, both drug product and back-up cells are derived from HSCs collected by plerixafor-mobilized apheresis.

Subjects in Group B2 who tolerated and successfully mobilized with plerixafor for back-up cells, and who had not yet had drug product manufactured with bone marrow harvest, were given the option to delay collection of cells for drug product manufacture until Group C opened, and they could undergo drug product manufacture from cells obtained by a subsequent cycle of plerixafor mobilization.

Subjects in Group C will undergo mobilization with plerixafor and apheresis for both manufacture of drug product and back-up cells. Group C subjects for whom adequate HSCs are collected to achieve the minimum cell dose but not for rescue, and who are not able to tolerate further plerixafor or did not mobilize well with plerixafor may be offered the option of bone marrow harvest for collection of back-up cells only, provided that drug product manufactured from mobilized cells has met criteria for dispositioning for clinical use.

5.4. Blinding, Packaging and Labeling

5.4.1. Blinding and Breaking the Blind

This is an unblinded, open-label study.

5.4.2. Packaging and Labeling

bb1111 consists of an autologous CD34+ cell-enriched population from patients with SCD that contains HSCs transduced with BB305 LVV encoding the $\beta^{A\text{-}T87Q}$-globin gene, suspended in cryopreservation solution in the final infusion bag.

The drug product will be labeled by the transduction facility according to Good Manufacturing Practices. Refer to Section 5.7 for additional details regarding product accountability.

5.5. Duration of Subject Participation

Time between Screening and drug product infusion will be variable and is estimated generally to be between 3 to 5 months (e.g., up to 3 months between Screening and Mobilization, followed by approximately 2 months before drug product infusion). Thereafter the subject is planned to remain on study for approximately 24 months. Eligible subjects are then expected to enroll in a separate long-term follow-up study until approximately 15 years post-drug product infusion. For subjects enrolled in Group B2 who opted to defer manufacture of drug product until Group C opened, the time between Screening and drug product infusion was extended as long as the subject continued to meet eligibility criteria.

If the subject is unable to undergo mobilization for drug product manufacture within approximately 3 months of the initiation of Screening, some assessments may need to be repeated to confirm continuing eligibility or to provide a reliable baseline prior to study procedures. In particular, physical exam, vital signs, performance status, HRQoL, echocardiogram, complete blood count, chemistry, urinalysis, and liver function tests, and for women of child-bearing potential, tests for pregnancy, must be performed within 3 months of proceeding to evaluation for mobilization. For subjects with exposure risks for HIV, HBV and HCV, these tests should also be repeated. Some of these assessments may be performed immediately prior to mobilization, as specified in the SOE. Where clinically and/or regionally indicated, other tests for infectious pathogens may be performed, as required by regional regulations or at physician discretion. Other blood tests, including those for immune subsets, hormonal or dyserythropoiesis testing only need to be repeated if more than 6 months have transpired, unless there is clinical concern due to an initial screening test (e.g., borderline thyroid function). Cerebral MRA/MRI and echocardiogram performed within 3 months of ICF and PFT results performed within 6 months of ICF may be used, unless there is clinical indication for repeat testing, or initial testing was borderline. The need for liver biopsy should be guided by MRI findings, as described in the protocol, unless a biopsy performed within 1 year of mobilization exhibited no evidence of fibrosis or hepatitis. Sickle cell disease genotyping, baseline VCN, baseline RCL and baseline $\beta^{A\text{-}T87Q}$-globin, once performed, do not have to be repeated.

Subjects who enroll in the trial but discontinue prior to myeloablation should be followed for at least 1 month after completion of harvesting, or until resolution of any study procedure-related

AEs, whichever is later. In the rare case a subject undergoes myeloablation but does not undergo drug product infusion, follow up should continue on trial for at least 3 months, or until resolution of any study-procedure related AEs, whichever is later.

5.6. Assessment of Treatment Compliance

Eligible subjects will receive bb1111 on a single day as inpatients and thus will be monitored by hospital personnel.

5.7. Product Accountability

Drug product accountability and traceability is ultimately the responsibility of the Investigator and Sponsor. However, this responsibility may be delegated to suitably qualified personnel listed on Food and Drug Administration (FDA) Form 1572 who have had appropriate study-specific training and whose names have been appropriately listed on the Delegation of Responsibility Log for this task.

Detailed records will be maintained to allow for accurate accountability of the drug product as per applicable Sponsor and clinical site procedures. These records will include details of storage of the drug product, transfer of the drug product from the transduction facility, administration to subjects, and disposal of remaining materials.

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.

All material containing bb1111 will be treated and disposed of as hazardous waste in accordance with governing regulations and clinical site procedures

5.8. Prior and Concomitant Medication

5.8.1. Prior Medication

All medications taken within 30 days prior to the Screening Visit are to be recorded in the Prior and Concomitant Medication case report form (CRF). Note additional requirements for collection of HU and pRBC transfusion history as detailed in Section 6.2.1 and Section 6.2.2.

5.8.2. Concomitant Medications and Therapies

All concomitant medications and therapies (i.e., those that are taken by the subject after signing the informed consent form [ICF]) should be documented.

Instructions and/or restrictions regarding specific concomitant medications and therapies are listed below. Concomitant medications and therapies not explicitly listed below may be used at the discretion of the treating physician and as per local standard of care.

- Granulocyte colony stimulating factor (G-CSF): G-CSF should not be used prior to Day 43 without explicit approval from the bluebird bio Medical Monitor.
- Venous access: Subjects will have appropriate long-term central venous access placed, per institutional standard practice, prior to beginning the preparative regimen. For subjects planned to undergo apheresis, placement of central venous access prior

to the first dose of plerixafor is strongly advised. For access, a double lumen tunneled catheter is recommended.

- Hydroxyurea: Hydroxyurea must be discontinued at least 30 days prior to stem cell harvest. It may be restarted between completion of the stem cell harvest(s) and admission to the hospital for myeloablation but should be stopped again at least 2 days before initiating myeloablation. Following HSC transplant, HU is not permitted without prior discussion with and explicit approval of the Sponsor's Medical Monitor.
- Erythropoietin: Erythropoietin is excluded and must be discontinued 60 days prior to stem cell harvest, as it may prevent engraftment of the transduced cells. Following HSC transplant, erythropoietin is not permitted without prior discussion with and explicit approval of the Sponsor's Medical Monitor.
- L-glutamine, crizanlizumab, and voxelotor: Following HSC transplant, medications other than blood transfusions to treat sickle cell disease or anemia (e.g., HU, erythropoietin, L-glutamine, crizanlizumab, voxelotor) are not permitted without prior discussion with and explicit approval of the Sponsor's Medical Monitor.
- Iron chelation therapy: Iron chelation therapy must be discontinued at least 7 days prior to stem cell harvest and conditioning. Phlebotomy or iron chelation therapy using deferasirox or deferoxamine may be restarted at the discretion of the treating physicians no sooner than after discharge from the hospital post-transplant. Deferiprone use is not permitted without prior discussion with and explicit approval of the Sponsor's Medical Monitor. Deferiprone may be used no sooner than 3 months after transplant due to the potential risk of myelosuppression (as per prescribing information for Deferiprone).
- Seizure prophylaxis: Starting at least 12 hours prior to the first dose of busulfan for conditioning, subjects will be treated with anticonvulsants for seizure prophylaxis. The choice of anticonvulsant is at the discretion of the treating physician, but phenytoin is not allowed. Seizure prophylaxis can be discontinued 24 hours after the last dose of busulfan.
- Subjects should not use medications with anti-retroviral activity (such as those used for HIV prophylaxis) from within 1 month of initiating mobilization until after completion of stem cell collection for drug product manufacture.
- pRBC Transfusions: A transfusion regimen will be implemented for at least 60 days before stem cell harvest, and will continue through until busulfan conditioning (exchange or simple, as available or needed) to reach a target Hb of 10 g/dL prior to mobilization (and 8 to 10 g/dL prior to conditioning; not to exceed 12 g/dL prior to either mobilization or conditioning) and a pre-transfusion target HbS proportion in the blood of < 30% to reduce the risk of SCD-related complications. The last exchange transfusion must occur within 4 days of start of mobilization (or within 1 week of start of conditioning regimen), and the HbS proportion in the blood must be < 20% after the last exchange transfusion prior to mobilization, and < 30% after the last exchange transfusion prior to conditioning. Hemoglobin S should be quantified

prior to and (if possible) within 48 hours of transfusion. Subjects are recommended to continue transfusions during the pancytopenia phase to maintain Hb ≥ 8 g/dL. For subjects who were on a chronic transfusion regimen prior to study entry, once engrafted and discharged post-transplant, it is recommended that the frequency of routine pRBC transfusions be adjusted to maintain Hb ≥ 8 g/dL. Simple transfusions are recommended during this phase of follow-up and the period between transfusions will gradually be increased while maintaining safe HbS levels. Continued transfusion needs > 6 months post-treatment or any alternative plans should be discussed with the Medical Monitor. Following HSC transplant, medications other than blood transfusions to treat sickle cell disease or anemia (e.g., HU, erythropoietin, L-glutamine, crizanlizumab, voxelotor) are not permitted without prior discussion with and explicit approval of the Sponsor's Medical Monitor.

- Platelet transfusion parameters: during the hospitalization for transplantation, platelets should be transfused to maintain a platelet count of > 50,000/µL until platelet engraftment. After hospital discharge, subjects should get platelet count monitored approximately weekly and at least 8 days after the most recent platelet transfusion until platelet engraftment. Platelet engraftment is defined as 3 consecutive platelet count laboratory values ≥ 50×10^9/L obtained on different days while no platelet transfusions administered for 7 days immediately preceding and during the evaluation period. The day of platelet engraftment is the first day of the 3 consecutive platelet measurements.
- Blood pressure in patients with SCD is often lower than "normal" for age. Subjects should be closely monitored and antihypertensive medications started at the earliest sign of hypertension to maintain blood pressure (both systolic and diastolic) within 20% of the baseline.
- Other concomitant therapies during conditioning may include hyperdiuresis, prophylactic or therapeutic administration of anti-emetics, and veno-occlusive disease prophylaxis, e.g., ursodeoxycholic acid.
- See also next Section 5.8.3 for infection prophylaxis.

For the purposes of this study, vaccines (e.g., COVID-19 vaccines) are considered concomitant medications. Although interactions between a vaccine and bb1111 are not expected, the protocol includes use of immunomodulatory (plerixafor) and immunosuppressive medication (busulfan). Local guidelines should be followed regarding a minimum time period between any medication to be provided as part of treatment with bb1111 and any vaccine; it is recommended that vaccines are not administered to subjects within 1 month of initiating mobilization for stem cell collection, within 1 week after receiving mobilization agents, within 1 month of initiating myeloablative conditioning, or within 6 months after drug product infusion. Revaccination post-drug product infusion should be considered per Investigator's discretion due to potential loss of immunity after myeloablative conditioning and may be administered following local guidelines.

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

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

5.8.3. Infection Surveillance and Prophylaxis

Opportunistic infection surveillance and prophylaxis are recommended for subjects treated with bb1111 (see suggested approach below). Alternative approaches that follow institutional practices may be employed after discussion with the Medical Monitor.

- In subjects with past exposure to HBV, regular monitoring of HBV DNA by PCR and pre-emptive lamivudine is advised for any increase in HBV DNA titer (HBV reactivation). Subjects should not use medications with anti-retroviral activity from within 1 month of initiating mobilization until after completion of stem cell collection for drug product manufacture.
- Herpes simplex virus (HSV) prophylaxis: Acyclovir prophylaxis is recommended for at least 3 months after drug product infusion for patients who are sero-positive for HSV or varicella zoster virus (VZV)**.** Where systemic VZV is a concern, acyclovir may be continued until either T cell recovery or anti-VZV response can be documented.
- Pneumocystis pneumonia prophylaxis: Trimethoprim-sulphamethoxazole or an equivalent drug is recommended for at least 3 months after drug product infusion.
- Penicillin prophylaxis is allowed for asplenic or hyposplenic subjects per institutional guidelines.
- Fungal prophylaxis: Anti-fungal prophylaxis is recommended with agents such as Fluconazole for at least 3 months after drug product infusion.
- Cytomegalovirus (CMV) surveillance: CMV should be tested per institutional practices. Antiviral therapy for CMV reactivation should be commenced pre-emptively if CMV testing reveals a high or rising viral load. If CMV reactivation occurs at or before engraftment, Foscarnet may be considered to prevent marrow suppression.
- Adenovirus surveillance: Testing for adenovirus infection is recommended in the event of symptoms suspicious for infection such as diarrhea, hepatic dysfunction, respiratory symptoms, etc. If diagnosed with an active systemic infection, therapy should be instituted with cidofovir or other active agents.
- Epstein-Barr virus (EBV) surveillance: EBV monitoring by PCR is recommended per institutional practices. In the event of persistent EBV viremia or signs/symptoms consistent with EBV-related PTLD (adenopathy, fever, etc.) therapy such as Rituximab is recommended.
- BK virus, JC virus, and HHV-6 virus surveillance: Any patient diagnosed with progressive multi-focal leukoencephalopathy based on cerebral MRI will be monitored for BK, JC and HHV-6 viruses by blood PCR based methods. All patients with non-engraftment should be screened for HHV-6 infection.
- Maintain Intravenous Immune Globulin (IgG) levels at > 400 mg/dL or as per institutional standards.

- Follow institutional post-transplantation guidelines for re-immunization of subjects [see (Dykewicz et al. 2000)].

6. STUDY ASSESSMENTS

6.1. Schedule of Events

The study has 4 distinct stages, as follows.

- Stage 1: Screening and Eligibility Assessments
- Stage 2: Stem Cell Harvest and Drug Product Manufacture and Disposition
- Stage 3: Myeloablative Conditioning and Infusion of bb1111
- Stage 4: Follow-up through Month 24

The Schedule of Events (SOE) to be performed is outlined in Table 2 for Stages 1 and 2, Table 3 for Stage 3, and Table 4 for Stage 4.

Detailed descriptions of the efficacy, pharmacodynamics, and safety procedures to be conducted during this study are provided in the following subsections. Additional details, including administrative information, will be provided in the Study Operations Manual.

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.

If a subject is not able to visit the study site due to force of nature (e.g., COVID-19 pandemic), then a telehealth visit should be considered to ensure appropriate oversight and safety monitoring. In addition to this, if the subject is able to visit a local doctor and/or laboratory, then pre-defined assessments may be performed at a local laboratory or doctor's office, per Investigator discretion.

Table 2: Schedule of Events: Screening and Stem Cell Harvest

Procedure	**Screening (up to 90 days before harvest**	**Stem Cell Harvest**	
		Up to 7 days before harvest	**Day(s) of apheresis (or bone marrow harvest)**
Signing of informed consent form (ICF)[1]	X		
Demographics	X		
Medical History, including all VOEs[2]	X		
Physical examination[3]	X	X	X
Chronic pain assessment[4]	X		
Performance status[5]	X	X	
Tanner staging (for subjects < 18 years of age)	X		
Vital signs[6]	X	X	X[6]
Cardiovascular monitoring and oximetry			X[7]
Local lab: Blood for clinical laboratory tests:			

Table 2: Schedule of Events: Screening and Stem Cell Harvest

Procedure	Screening (up to 90 days before harvest	Stem Cell Harvest	
		Up to 7 days before harvest	Day(s) of apheresis (or bone marrow harvest)
Hematology[8]	X	X	X[9]
Serum Chemistry[10]	X	X	X
Coagulation Analysis[11]	X	X	
Iron Metabolism Studies[12]	X	X	
Haptoglobin	X	X	
Local lab: Blood for hormonal testing[13]	X		
Local lab: Urinalysis[14]	X		
Local lab: Blood for serology	X[15]	X	
Local lab: Blood for immunological studies	X[16]		
Screening for irregular antibodies	X		
Local lab: Blood for serum β-human chorionic gonadotropin for women of child-bearing potential (serum pregnancy test)	X	X	
Local lab: Blood for brain natriuretic peptide (BNP)	X		
Local lab: Blood for CD34+ cell count	X		X[17]
Central lab: Blood for SCD genotyping	X		
Central lab: Blood for RCL, VCN, and globin analysis	X[18]		
Central lab: Blood for potential biomarker analysis (optional)	X		
Specialty lab: Blood for methemoglobin assay	X		
bluebird bio lab: Blood for CCI PD	X		
bluebird bio lab: HPC-A sample for CCI PD			X[19]
bluebird bio lab: Bone marrow for CCI PD	X[20]		
Sperm / testicular tissue or oocyte banking, if requested	X[21]		
Liver MRI (and liver biopsy, depending on MRI results), and cardiac MRI, if required[22]	X		
Imaging studies: cerebral magnetic resonance angiography (MRA)/magnetic resonance imaging (MRI; TCD if ≤ 16 years of age), and echocardiogram (including LVEF and TRJV)	X[23]		
Imaging studies: Bone mineral density evaluation	X[24]		
Pulmonary function tests	X[25]		
6-minute walk test	X		

Table 2: Schedule of Events: Screening and Stem Cell Harvest

Procedure	Screening (up to 90 days before harvest	Stem Cell Harvest	
		Up to 7 days before harvest	Day(s) of apheresis (or bone marrow harvest)
Transfusion regimen	X[26]	X[26]	
Local lab: Blood for electrophoresis to assess globin fractions (i.e., %HbS, %HbA)	X[27]	X[27, 28]	X[28]
Plerixafor dosing and apheresis (Group C)			X[29]
Bone marrow harvest (for collection of back-up cells, as applicable)			X
Quality of life tests: EQ-5D-3L/Proxy Version 1, EQ-5D-Y /Proxy Version 1, PROMIS-57 Version 2.1, PROMIS Pediatric Profile/Parent Proxy Profile 49 Version 2.0	X[30]		
Work Productivity Assessment (WPAI)/Caregiver WPAI-GH	X[30]		
Cognitive Function Assessments: PROMIS Short Form 6a	X[30]		
Vaso-occlusive event collection	Continuous from ICF signing		
Adverse event collection	Continuous from ICF signing		
Prior and concomitant medications	Continuous from ICF signing		

[1] If a subject is < 18 years of age at signing of informed consent/assent and turns 18 while on study, the subject should re-consent to the adult ICF at the next scheduled study visit.
[2] See Section 6.2.1 and Section 6.2.2.
[3] Any leg (skin) ulceration noted upon physical exam or as part of medical history must include severity classification.
[4] Chronic pain will be assessed using the AAPT Diagnostic Criteria for Chronic Pain Associated with SCD (see Section 6.2.6).
[5] Karnofsky performance status (≥ 16 years of age) or a Lansky performance status (< 16 years of age).
[6] Vital signs include systolic/diastolic blood pressure, pulse, temperature, oxygen saturation, weight, and height (height only required at Screening).
[7] Following plerixafor administration, subjects will be monitored for AEs, e.g., hypotension, syncope, hypoxia, per institutional practice. Throughout apheresis, subjects will have frequent measures of heart rate, blood pressure, and pulse oximetry, e.g., every 20 minutes for adults, and every 10 minutes for pediatric subjects. Subjects should remain supine from plerixafor dosing through the end of apheresis each day.
[8] Hematology will include CBC with differential, nucleated erythrocytes, platelets, and reticulocytes.
[9] CBC with differential to be performed immediately before stem cell harvest, and immediately before and 2 hours after each dose of plerixafor. Complete blood count should be performed 24 hours post-plerixafor dose after the last day of apheresis. Complete blood count is also needed at the following 2 time points: 1) Within 1 hour prior to the start of apheresis (or if site is unable to do this, sample can be obtained upon initiation of apheresis). Complete blood count should be run STAT (the differential does not have to be STAT). 2) Immediately post-apheresis. If more than 1 apheresis day is required, platelet counts must be confirmed to be ≥ 75×10^9/L within 24 hours of subsequent apheresis sessions, prior to administration of plerixafor on that day. If platelet counts do not meet these criteria, mobilization and apheresis should be deferred until platelet counts recover to ≥ 75×10^9/L.
[10] Chemistry will include sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, calcium, magnesium, phosphate, albumin, total protein, uric acid, LDH, liver enzymes (GGT, ALP, ALT, AST, direct and

total bilirubin), glucose and CRP at Screening, Pre-mobilization, and Stem cell harvest. Erythropoietin to be performed at Screening only.

[11]Coagulation analysis will include PT/aPTT, D-Dimers.

[12]Iron studies include the following: serum iron, serum ferritin, serum transferrin, iron saturation (transferrin saturation), and serum transferrin receptor.

[13]Hormonal testing will include: Estradiol, (females only); total Testosterone (males only); TSH, free T3, free T4, AM cortisol, ACTH, 25 OH Vitamin D, PTH, FSH, LH (all subjects).

[14]Urinalysis includes color, appearance, specific gravity, pH, microalbumin, protein, creatinine, glucose, ketones, occult blood.

[15]Including HIV-1, HIV-2, HBcAb, HBsAb, HBsAg, HCV antibody, VZV, HTLV-1, HTLV-2, syphilis (RPR), toxoplasmosis, Trypanosoma cruzi, and West Nile Virus. Where clinically and/or regionally indicated, other tests for infectious pathogens may be performed, as required by regional regulations or at physician discretion.

[16]T cells (CD3), T cell subsets (CD4, CD8), B cells (CD19), natural killer cells (CD16/CD56), and antibody titers against tetanus, pneumococcus, and measles/mumps/rubella.

[17]CD34+ count required immediately prior to morning plerixafor dose, 2 hours post-plerixafor, as apheresis is being initiated, immediately after apheresis (and 24 hours post-plerixafor dose if no further apheresis procedures are planned in that mobilization cycle).

[18]Analysis of RCL, VCN, and globin chains does not need to be repeated if greater than 90 days between lab collection and mobilization/apheresis cycle

[19]Four mL HPC-A product must be collected from the first day of apheresis of all mobilization cycles where cells will be sent for drug product manufacturing.

[20]Bone marrow aspiration procedure will not be performed if not clinically appropriate per local standard of care (e.g., bone marrow aspirate would require general anesthesia which would be considered an unacceptable risk to the subject) or not considered by the Investigator to be in the best interest of the subject to perform.

[21]This can be done at any time before the start of conditioning once enough cells have been harvested for drug product manufacture. If surgery is required, this must be done a minimum of 2 weeks prior to initiation of conditioning. Subjects must be transfused within 4 days prior to surgery.

[22]Liver biopsy within 1 year and liver and cardiac MRIs within 3 months prior to consent may be used. If liver MRI findings suggest active hepatitis, significant fibrosis, inconclusive evidence of cirrhosis, or liver iron concentration ≥ 15 mg/g, subjects ≥ 18 years of age require a follow-up liver biopsy (in subjects < 18 years of age, these MRI findings are exclusionary, unless in the opinion of the Investigator, a liver biopsy could provide additional data to confirm eligibility and would be safe to perform). Subjects unable to undergo otherwise required liver MRI (e.g., have metal implants) can instead have a liver biopsy performed to rule out advanced liver disease. For subjects who have a medical history of iron overload or serum ferritin > 1000 ng/mL, a cardiac MRI is required at Screening and at Month 24 Visit.

[23]Cerebral MRA/MRI and echocardiogram results performed within 3 months of ICF may be used unless there is clinical indication for repeat testing. Cerebral MRA/MRI alternative procedure: For subjects unable to undergo cerebral MRA/MRI, a CT scan can be performed instead.

[24]If BMD evaluation was performed within 1 year of Informed Consent, these results may be used for Screening and do not need to be repeated.

[25]Including % predicted FVC, % predicted FEV1; % predicted RV; and % predicted DLCO (corrected for Hb). Adapt to pediatric use per institutional practices. If performed within 6 months prior to ICF, those results may be used and do not need to be repeated. In the event of pulmonary complications (e.g., ACS, pneumonia) between Screening and the start of conditioning, an unscheduled PFT must be performed.

[26]Transfusion regimen should be initiated at least 60 days prior to stem cell harvest. Last exchange transfusion must occur within 4 days of start of mobilization, and the HbS proportion in the blood must be < 20% after the last exchange transfusion.

[27]Hb electrophoresis will be performed within 48 hours prior and 48 hours post pRBC transfusions during the transfusion regimen.

[28]Hb electrophoresis will be performed within 48 hours of mobilization/bone marrow harvest (and after final transfusion before mobilization/bone marrow harvest).

[29]Apheresis should begin approximately 4 to 6 hours after plerixafor administration

[30]Baseline assessment should occur as close to Day -90 as possible, and where possible, must occur before start of pRBC transfusions. Subjects should complete PRO questionnaires at the beginning of the Visit (prior to undergoing any other procedures during the Visit.

Table 3: Schedule of Events: Conditioning and Drug Product Infusion

	Preconditioning Up to 7 days before busulfan	Conditioning Day -6 to -3 inclusive	Infusion Day 1	Day 2 until hospitalization discharge
Physical examination[a]	X	X	X[b]	X[b]
Performance status[c]	X			
Vital Signs[d]	X	X	X[e]	X[f]
Local lab: Blood for clinical laboratory tests				
Hematology[g]	X	X	X	X[h]
Serum Chemistry[i]	X	X	X	X
Coagulation Analysis[j]	X			
Iron Metabolism Studies[k]	X			
Haptoglobin	X			
Local lab: Blood for serology	X[l]			
Local lab: Blood for serum β-human chorionic gonadotropin for women of child-bearing potential (serum pregnancy test)	X			
Local lab: Blood for electrophoresis to assess globin fractions (i.e., %HbS, %HbA, %HbF)	X[m]			X[n]
Bone marrow aspiration[o]:				
Bone marrow smear[p]	X[q]			
Local lab: Bone marrow for conventional cytogenetics (karyotyping)	X[q]			
Central lab: Bone marrow for storage[r]	X[q]			
Central lab: Blood for next generation sequencing (NGS) mutational testing[s]	X[q]			
Central lab: Blood for storage[r]	X[q]			
Sperm / testicular tissue or oocyte banking, if requested	X			
Reconfirmation of suitability for transplant	X[t]			
Anti-seizure prophylaxis	X[u]	X[u]		
Busulfan chemotherapy (daily or q6h regimen, for 4 days)		X		
Local lab: Blood for busulfan pharmacokinetics[v]	X	X		
Infusion of bb1111			X	
Transfusion regimen	X[w]			

Table 3: Schedule of Events: Conditioning and Drug Product Infusion

	Preconditioning Up to 7 days before busulfan	Conditioning Day -6 to -3 inclusive	Infusion Day 1	Day 2 until hospitalization discharge
Vaso-occlusive event collection	Continuous from ICF signing			
Adverse event collection	Continuous from ICF signing			
Concomitant medication collection[x]	Continuous from ICF signing			

[a] Any leg (skin) ulceration noted upon physical exam or as part of medical history must include severity classification.
[b] To be performed on Day 1 before drug product infusion. A physical examination should be performed at least twice a week until discharge.
[c] Karnofsky performance status (≥ 16 years of age) or a Lansky performance status (< 16 years of age).
[d] Vital signs include systolic/diastolic blood pressure, pulse, temperature, oxygen saturation, weight (weight only required at preconditioning and conditioning), and height (height only required at preconditioning).
[e] To be measured on Day 1 no less frequently than at the start, once during, and upon completion of the infusion.
[f] Vital signs to be collected at least once a week during hospitalization post-infusion.
[g] Hematology will include CBC with differential, nucleated erythrocytes, platelets and reticulocytes. Reticulocytes are not needed from D2 to neutrophil engraftment.
[h] CBC daily during hospitalization. For pediatric subjects, daily measurements can be every other day due to blood volume concerns. After hospital discharge, subjects should get platelet count monitored approximately weekly and at least 8 days after the most recent platelet transfusion until platelet engraftment.
[i] During hospitalization, chemistry will include sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, calcium, magnesium, phosphate, albumin, total protein, liver enzymes (GGT, ALP, ALT, AST, direct and total bilirubin), and glucose daily, and CRP, uric acid, and LDH weekly. For pediatric subjects, daily measurements can be every other day due to blood volume concerns.
[j] Coagulation analysis includes PT/aPTT, D-Dimers.
[k] Iron studies include the following: serum iron, serum ferritin, serum transferrin, iron saturation (transferrin saturation), and serum transferrin receptor.
[l] For subjects with exposure risks for HIV, HBV and HCV, these assessments should also be repeated during the preconditioning visit.
[m] Hb electrophoresis will be performed within 48 hours prior and 48 hours post pRBC transfusions during the transfusion regimen.
[n] Weekly from Day 2 until hospital discharge.
[o] See Section 6.2.17.
[p] Bone marrow smear should be read locally; additional bone marrow slides are to be archived for potential central analyses.
[q] Collection of bone marrow aspirate and blood for conventional cytogenetics (karyotyping), NGS, and storage may be performed any time prior to conditioning and is not required to be within the 7 days prior to busulfan.
[r] See Section 6.2.21.
[s] In exceptional situations NGS mutational testing may be performed by a local lab upon agreement with the Medical Monitor.
[t] Review CBC, serum chemistry (chemistry will include sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, calcium, phosphate, albumin, total protein, uric acid, LDH, liver enzymes [GGT, ALP, ALT, AST, direct and total bilirubin], glucose and CRP), physical examination, cytogenetics, and performance status; verification that the drug product has been dispositioned for clinical use, is available on site and that back-up cells are available. If PFTs have not been performed within 3 months of initiation of conditioning, then these tests must be repeated prior to conditioning. While not mandatory, cardiac Doppler echocardiology (including left ventricular ejection fraction) and cerebral MRI should be repeated prior to conditioning if clinically indicated.

[u] Anti-seizure prophylaxis to start at least 12 hours before initiating busulfan and to continue at least 24 hours after completion of 4-day busulfan course.

[v] Blood for busulfan PK collected at Preconditioning time point only if a busulfan test dose was administered. Clinical sites must be able to measure first and third day busulfan PK. Based on busulfan PK results for day 1 and day 3 of busulfan dosing, busulfan dose adjustments should be made for subsequent dosing. If feasible, daily busulfan PK measurement is recommended. In addition, busulfan levels will be measured 48 and 72 hours after final dose of busulfan for retrospective confirmation of adequate wash-out.

[w] Transfusion regimen should be initiated at least 60 days prior to stem cell harvest and continue through to start of conditioning. Last exchange transfusion must occur within 1 week of start of conditioning.

[x] Hydroxyurea must be discontinued at least 30 days before stem cell harvest and at least 2 days before initiating busulfan. Iron chelation must be discontinued at least 7 days prior to stem cell harvest and 7 days before initiating busulfan.

Table 4: Schedule of Events: Follow-up

Procedures	Follow-Up: Day (D), Month (M) (Visit Window, days)										
	D30	D60	D90	D135	D180	D270	D360	D450	D540	D630	D720
	M1 (±7)	M2 (±7)	M3 (±7)	M4.5 (±14)	M6 (±14)	M9 (±14)	M12 (±30)	M15 (±30)	M18 (±30)	M21 (±30)	M24 (±30)
Physical Examination[1]	X	X	X	X	X	X	X	X	X	X	X
Chronic pain assessment[2]					X		X		X		X
Performance Status[3]					X		X		X		X
Tanner staging (for subjects < 18 years of age)					X		X		X		X
Vital signs[4]	X	X	X	X	X	X	X	X	X	X	X
Local lab: Blood for clinical laboratory tests[5]											
Hematology[6]	X	X	X	X	X	X	X	X	X	X	X
Serum Chemistry[7]	X	X	X	X	X	X	X	X	X	X	X
Coagulation Analysis[8]							X				X
Iron Metabolism Studies[9]			X			X	X		X		X
Haptoglobin	X	X	X	X	X	X	X	X	X	X	X
Local lab: Hormonal testing[10]							X				X
Local lab: Urinalysis[11]							X				X
Local lab: Blood for immunological studies[12]			X		X	X	X				X
Screening for new irregular antibodies[13]		X	X								
Local lab: Blood for BNP and Erythropoietin							X				X

Table 4: Schedule of Events: Follow-up

Procedures	Follow-Up: Day (D), Month (M) (Visit Window, days)										
	D30	D60	D90	D135	D180	D270	D360	D450	D540	D630	D720
	M1 (±7)	M2 (±7)	M3 (±7)	M4.5 (±14)	M6 (±14)	M9 (±14)	M12 (±30)	M15 (±30)	M18 (±30)	M21 (±30)	M24 (±30)
Central lab: Blood for globin and VCN[14]	X	X	X	X	X	X	X	X	X	X	X
Central lab: Blood for RCL detection			X		X		X				X[15]
Central lab: Blood for ISA[16]					X		X		X		X
Central lab: Blood for NGS mutational testing[17]							X				X
Central lab: Blood for storage[18]	X	X		X		X		X		X	
Central lab: Blood for potential biomarker analysis (optional)							X				X
Specialty lab: Blood for methemoglobin assay							X				X
bluebird bio lab: Blood for [redacted] PD			X		X		X		X		X

Table 4: Schedule of Events: Follow-up

Procedures	Follow-Up: Day (D), Month (M) (Visit Window, days)										
	D30	D60	D90	D135	D180	D270	D360	D450	D540	D630	D720
	M1 (±7)	M2 (±7)	M3 (±7)	M4.5 (±14)	M6 (±14)	M9 (±14)	M12 (±30)	M15 (±30)	M18 (±30)	M21 (±30)	M24 (±30)
Bone marrow aspiration[19]:											
Bone marrow smear[20]							X				X
Local lab: Bone marrow for conventional cytogenetics (karyotyping)[21]:							X				X
Central lab: Bone marrow for NGS[17], ISA[16], storage[22], and CCI PD							X				X
Imaging studies:											
Cerebral MRA/MRI[23], and, if ≤ 16 years of age, TCD							X				X
Echocardiogram (including LVEF and TRJV)											X
Cardiac MRI[24]											X
Liver MRI											X
Bone mineral density evaluation											X
Pulmonary function test[25]							X				X
6-minute walk test							X				X

Table 4: Schedule of Events: Follow-up

Procedures	Follow-Up: Day (D), Month (M) (Visit Window, days)										
	D30	D60	D90	D135	D180	D270	D360	D450	D540	D630	D720
	M1 (±7)	M2 (±7)	M3 (±7)	M4.5 (±14)	M6 (±14)	M9 (±14)	M12 (±30)	M15 (±30)	M18 (±30)	M21 (±30)	M24 (±30)
Quality of life tests:[26] EQ-5D-3L/Proxy Version 1, EQ-5D-Y /Proxy Version 1, PROMIS-57 Version 2.1, PROMIS Pediatric Profile//Parent Proxy Profile 49 Version 2.0			X		X		X		X		X
Work Productivity Assessment (WPAI)/Caregiver WPAI-GH[26]			X		X		X		X		X
Cognitive Function Assessments: PROMIS Short Form 6a[26]			X		X		X		X		X
Vaso-occlusive event collection	Continuous from ICF signing[27]										
Adverse event collection	Continuous from ICF signing										
Concomitant medication collection	Continuous from ICF signing										

[1] Any leg (skin) ulceration noted upon physical exam or as part of medical history must include severity classification.
[2] Chronic pain will be assessed using the AAPT Diagnostic Criteria for Chronic Pain Associated with SCD (see Section 6.2.6).
[3] Karnofsky performance status (≥ 16 years of age) or a Lansky performance status (< 16 years of age).
[4] Vital signs include systolic/diastolic blood pressure, pulse, temperature, oxygen saturation, weight, and height.
[5] CBC with differential should be carried out whenever ISA is performed.
[6] Hematology will include CBC with differential, nucleated erythrocytes, platelets and reticulocytes. Subjects should get platelet count monitored approximately weekly and at least 8 days after the most recent platelet transfusion until platelet engraftment.
[7] Chemistry will include sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, calcium, magnesium, phosphate, albumin, total protein, uric acid, LDH, liver enzymes (GGT, ALP, ALT, AST, direct and total bilirubin), glucose and CRP at every visit.
[8] Coagulation analysis includes PT/aPTT, D-Dimers.
[9] Iron studies include the following: serum iron, serum ferritin, serum transferrin, iron saturation (transferrin saturation), and serum transferrin receptor. Iron studies should be performed prior to restarting iron chelation, if needed.

[10]Hormonal testing will include: Estradiol, (females only); total Testosterone (males only); TSH, free T3, free T4, AM cortisol, ACTH, 25 OH Vitamin D, PTH, FSH, LH (all subjects).
[11]Urinalysis includes color, appearance, specific gravity, pH, microalbumin, protein, creatinine, glucose, ketones, occult blood.
[12]T cells (CD3), T cell subsets (CD4, CD8), B cells (CD19), natural killer cells (CD16/CD56), and antibody titers against tetanus, pneumococcus, and measles/mumps/rubella.
[13]For subjects who receive final pRBC transfusion after the Month 3 Visit, screening for new irregular antibodies must be performed at the scheduled visit occurring immediately after last pRBC transfusion.
[14]VCN is to be carried out whenever ISA is performed.
[15]If RCL screening tests are negative up to and including the Month 12 Visit, RCL screening will cease, and the Month 24 co-culture RCL sample only will be collected and archived.
[16]CBC with differential and VCN are to be carried out whenever ISA is performed.
[17]In exceptional situations NGS mutational testing may be performed by a local lab upon agreement with the Medical Monitor.
[18]See Section 6.2.21.
[19]Post drug product infusion, bone marrow aspirate is to be taken per SOE unless discussed with the Medical Monitor for exceptions, such as if not clinically appropriate per local standard of care (e.g., bone marrow aspirate would require general anesthesia which would be considered an unacceptable risk to the subject) or not considered by the Investigator to be in the best interest of the subject to perform.
[20]Bone marrow smear should be read locally; additional bone marrow slides are to be archived for potential central analyses.
[21]Conventional cytogenetics should be performed locally
[22]See Section 6.2.21.
[23]Cerebral MRA/MRI alternative procedure: For subjects unable to undergo cerebral MRA/MRI, a CT scan can be performed instead.
[24]For subjects who have a medical history of iron overload or serum ferritin > 1000 ng/mL, a cardiac MRI is required at Screening and at Month 24 Visit.
[25]Including % predicted FVC, % predicted FEV1; % predicted RV; and % predicted DLCO (corrected for Hb). Adapt to pediatric use per institutional practices.
[26]Subjects should complete PRO questionnaires at the beginning of the Visit (prior to undergoing any other procedures during the Visit).
[27]If the Month 18 Visit does not include 18 calendar months, a telephone contact assessment for VOEs is to take place after 18 calendar months (post-drug product infusion) have elapsed.
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6.2. Study Measures

6.2.1. Demographics and Medical History

Subject demographic data such as gender, age, race, and ethnicity, will be obtained at Screening, as per the SOE.

Medical history is to include all prior and current medical history, e.g., history of infectious events (including aplastic crises), iron overload, thromboembolism events, surgical procedures, medications (including pain medications).

6.2.2. SCD-specific History and Outcome Measures

Sickle cell disease-specific medical history will be obtained at Screening as per the SOE, including the following:

1. Any history of severe cerebral vasculopathy event, stroke, or TCD acceleration and any associated visits to medical facilities and/or hospitals prior to Informed Consent.

   History of any other acute or chronic SCD-related complications and any associated visits to medical facilities and/or hospitals in the 24 months prior to Informed Consent. Sickle cell disease-related complications may include, but are not limited to, any of the following:

   – VOEs/severe VOEs, pulmonary hypertension (TRJV), cardiomyopathy, osteonecrosis, gall bladder lithiasis, leg (skin) ulceration, sickle cell nephropathy, silent cerebral infarct, transient ischemic attack

2. Baseline Hb, as defined in Section 7.4.1
3. History of HU use, including any history of HU intolerance or failure.
4. History of L-glutamine use
5. History of pRBC transfusions (including date, indications, mL/kg and units of pRBCs, associated pre-transfusion HbS and total Hb values and post-transfusion AEs [including allo-immunization]) within the 24 months prior to Informed Consent will be obtained at Screening, as per the SOE. Iron chelator use within the 24 months prior to Informed Consent
6. In addition, for subjects who are receiving regular transfusions at the time of study entry:

   – other indication for chronic transfusions

   – if available, the history of VOEs/severe VOEs for the 2 years prior to the date at which regular transfusions started should be documented.

6.2.3. Physical Examination and Performance Status

A complete physical examination is to be conducted as per the SOE for the identification of medical history or AEs and reported on the appropriate CRF.

Screening PE and performance status should be performed within 90 days prior to mobilization. If > 90 days has elapsed, PE and performance status should be re-assessed during the screening period.

Any leg (skin) ulceration noted upon physical exam or as part of medical history must include severity classification.

Karnofsky and Lansky performance status (see Appendix 10.1) is to be assessed as per the SOE.

Additionally, Tanner staging (subjects < 18 years of age) should be performed at Screening and every 6 months after infusion during puberty.

6.2.4. Vital Signs

Vital signs are to be measured per the SOE and include systolic/diastolic blood pressure, pulse, temperature, oxygen saturation, weight, height, and pain evaluation. Screening vital signs should be performed within 90 days prior to mobilization. If > 90 days has elapsed, vital signs should be re-assessed during the screening period.

6.2.5. Vaso-occlusive Events (VOEs)

All VOEs 24 months prior to ICF and following ICF for the duration of the study will be recorded. VOE categories include acute pain with no medically determined cause other than VOE (e.g., VOC), acute chest syndrome (ACS), acute hepatic sequestration, acute splenic sequestration, and acute priapism. All VOEs that, in the opinion of the Investigator, represent at least one of these events (regardless of duration, severity, treatment, or need for medical facility visit) must be recorded. A VOE can be home-managed only, and does not require a visit to a medical facility or hospitalization to be reported. It is recognized that retrospective collection of VOE information may be challenging; however, complete collection and reporting of VOEs should be done to the best of the clinical site's ability.

Subject eligibility is determined by the Investigator based on that subject having at least 4 protocol-defined severe VOEs (i.e., protocol sVOEs) in the 24 months prior to ICF as set forth in Section 4.2.

For the purposes of this study, a protocol-defined VOE (i.e., protocol VOE) is any of the following events (with or without hospitalization):

a. an episode of acute pain with no medically determined cause other than a VOE (e.g., VOC) lasting more than 2 hours and requiring care at a medical facility
b. ACS, defined by an acute event with pneumonia-like symptoms (e.g., chest pain, fever [> 38.5°C], tachypnea, wheezing or cough, or findings upon lung auscultation), and the presence of a new pulmonary infiltrate consistent with ACS, and requiring oxygen treatment and/or blood transfusion
c. acute hepatic sequestration, defined by a sudden increase in liver size associated with pain in the right upper quadrant, abnormal results of liver-function test not due to biliary tract disease, and reduction in Hb concentration by at least 2 g/dL below the baseline value
d. acute splenic sequestration, defined as sudden enlargement of the spleen and reduction in Hb concentration by at least 2 g/dL below the baseline value

e. an event of acute priapism: defined as a sustained, unwanted painful erection lasting more than 2 hours and requiring care at a medical facility

A protocol sVOE is defined for the purposes of this study as a protocol VOE requiring ≥ 24-hour hospital or emergency room (ER) observation unit visit, or at least 2 visits to a day unit or ER over 72 hours with both visits requiring intravenous treatment, with the exception of priapism (all protocol VOEs of priapism are also protocol sVOEs).

All reported VOEs will also be adjudicated by an independent event adjudication committee for purposes of endpoint analysis (see Section 3.3).

6.2.6. Pain Assessment

Acute pain will be assessed as part of the patient-reported outcome assessment by PROMIS (see Section 6.2.26).

Chronic pain will be assessed per the SOE and includes evaluation of the following AAPT Diagnostic Criteria for Chronic Pain Associated with SCD [adapted from (Dampier et al. 2017)]:

1. Reports of ongoing pain present on most days over the past 6 months either in a single location or in multiple locations
2. Must display at least 1 sign:
   - Palpation of the region of reported pain elicits focal pain or tenderness
   - Movement of the region of reported pain elicits focal pain
   - Decreased range of motion or weakness in the region of reported pain
   - Evidence of skin ulcer in the region of reported pain
   - Evidence of hepatobiliary or splenic imaging abnormalities (e.g., splenic infarct, chronic pancreatitis) consistent with the region of reported pain
   - Evidence of imaging abnormalities consistent with bone infarction or avascular necrosis in the region of reported pain
3. There is no other diagnosis that better explains the signs and symptoms

6.2.7. Clinical Laboratory Tests

Blood samples for clinical laboratory tests, including hematology, serum chemistry, liver function tests, renal function tests, coagulation studies, and iron studies, are to be collected per the SOE. These tests are to be performed by the local laboratory and reviewed by the Investigator or qualified designee (e.g., physician's assistant, nurse practitioner).

Screening clinical labs should be performed within 90 days prior to mobilization. If > 90 days has elapsed, clinical labs should be re-assessed during the screening period.

The following clinical laboratory parameters are to be determined:

Hematology	Serum chemistry	Liver function tests	Coagulation
complete blood count [CBC] with differential, reticulocyte count, nucleated erythrocytes, platelets, haptoglobin	sodium potassium chloride bicarbonate creatinine albumin glucose CRP blood urea nitrogen calcium uric acid phosphate total protein magnesium lactate dehydrogenase erythropoietin estimated glomerular filtration rate (as determined using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation)	aspartate aminotransferase alanine aminotransferase alkaline phosphatase total and direct bilirubin gamma-glutamyl transferase	prothrombin time [PT]/activated partial thromboplastin time [aPTT] D-Dimers
Iron studies			
serum iron serum ferritin serum transferrin serum transferrin receptor iron saturation (transferrin saturation)			

Urine for urinalysis (sent to local lab) is to be collected per the SOE, and urinalysis includes color, appearance, specific gravity, pH, microalbumin, protein, creatinine, glucose, ketones, occult blood.

Additional clinical laboratory tests may be performed at the Investigator's discretion.

6.2.7.1. 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 and may include:

- Physical exam
- Blood tests
- Imaging tests
- Bone marrow biopsy and/or aspiration (may also be archived for additional analysis in the future; see Section 6.2.21)

6.2.8. Additional Laboratory Tests

Blood samples will be collected for additional eligibility-determining laboratory tests, according to the SOEs.

- **Serology**

  Screening serology will be evaluated using standard methods. The serology panel for eligibility includes HIV-1 and HIV-2; hepatitis B core antibody (HBcAb); hepatitis B surface antibody (HBsAb); hepatitis B surface antigen (HBsAg); hepatitis C virus (HCV) antibody; varicella zoster virus (VZV) IgG; HTLV-1 and HTLV-2, syphilis (RPR), toxoplasmosis, Trypanosoma cruzi, and West Nile Virus. Any other serology required by institutional guidelines before stem cell harvest and transplantation of drug product (including, for example, testing for CMV, EBV, and HSV) are permitted.

  For subjects with exposure risks for HIV, HBV and HCV, these assessments should also be repeated during the preconditioning visit. Where clinically and/or regionally indicated, other tests for infectious pathogens may be performed, as required by regional regulations or at physician discretion.

- **Serum β-human chorionic gonadotropin pregnancy test**

  Only required for females of child-bearing potential.

6.2.9. Hormonal Testing

Hormonal testing is to be performed as per the SOE and includes: estradiol, (females only); total testosterone (males only); TSH, free T3, free T4, AM cortisol, ACTH, 25 OH Vitamin D, PTH, FSH, LH (all subjects).

Hormonal testing should be performed within 90 days prior to mobilization. If > 90 days has elapsed, PE and performance status should be re-assessed during the screening period.

6.2.10. Sperm / Testicular Tissue or Oocyte Banking

Sperm or testicular tissue banking for males or oocyte aspiration following ovarian stimulation and cryopreservation for females will be done at the discretion of the subject. If a subject elects to undergo these procedures, the procedures must occur during the period between mobilization and conditioning and can be done at any time before the start of conditioning once enough cells have been harvested for drug product manufacture. If surgery is required, this must be done a minimum of 2 weeks prior to initiation of conditioning. Subjects must be transfused within 4 days prior to surgery.

6.2.11. Reconfirmation of Suitability for Transplant

At Pre-conditioning, review CBC, serum chemistry (chemistry will include sodium, potassium, chloride, bicarbonate, blood urea nitrogen, creatinine, calcium, magnesium, phosphate, albumin, total protein, uric acid, LDH, liver enzymes (GGT, ALP, ALT, AST, direct and total bilirubin), glucose and CRP), physical examination, cytogenetics, and performance status; verification that the drug product has been dispositioned for clinical use, is available on site and that back-up cells are available. If PFTs have not been performed within 3 months of initiation of conditioning, then these tests must be repeated prior to conditioning. While not mandatory, cardiac Doppler echocardiology (including LVEF) and cerebral MRI should be repeated prior to conditioning if clinically indicated.

Bone marrow aspirate will be collected prior to conditioning for assessment of chromosomal abnormalities or genetic mutations, particularly those associated with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML), by conventional cytogenetics (karyotyping). These results will be used to assess individual benefit-risk for a subject's suitability to proceed with HSCT. If a chromosomal abnormality that may put the subject at an increased risk of MDS/AML per the Investigator's judgment is identified in the bone marrow prior to conditioning, the subject will be withdrawn from the study and not proceed with conditioning or treatment with bb1111. If other chromosomal abnormalities are identified in the bone marrow prior to conditioning that do not render the subject ineligible for HSCT per the Investigator's judgment, approval of the Medical Monitor is required before the subject can proceed with conditioning, and the subject can proceed with conditioning and treatment with bb1111 after providing appropriate informed consent.

6.2.12. Immunological studies

Immunological testing includes measuring levels of T cells (CD3), T cell subsets (CD4, CD8), B cells (CD19), and natural killer cells (CD16/CD56), irregular antibodies, and antibody titers against tetanus, pneumococcus, and measles/mumps/rubella.

Screening for irregular antibodies is required for all subjects at Screening. Screening for new irregular antibodies is required at Month 2 and Month 3 and may also occur at physician's discretion. For subjects who receive their final pRBC transfusion after the Month 3 Visit, screening for new irregular antibodies must be performed at the scheduled visit occurring immediately after last pRBC transfusion.

6.2.13. Brain Natriuretic Peptide

Blood samples are to be collected per the SOE for BNP. This assay should be performed at the local laboratory.

6.2.14. Busulfan concentrations

Blood for measuring busulfan PK and making appropriate adjustments of the busulfan dose should be drawn for local testing as per Section 5.2.5.2.

6.2.15. Hemoglobin Analysis

Blood samples are to be collected per the SOE for assessment at a central laboratory of globin chain levels by HPLC, including $\beta^{A\text{-}T87Q}$-globin. Blood sample collection details are included in the Laboratory Manual for this study.

Blood samples will also be collected per the SOE to assay for methemoglobin concentration (specialty lab).

Additionally, blood samples will be analyzed by electrophoresis in local laboratories to monitor the percentages of HbS and HbA during the transfusion regimen through hospital discharge post-infusion (HbF results should be collected if available).

6.2.16. Testing for SCD Genotype, Vector Copy Number, Replication Competent Lentivirus, and Integration Site Analysis

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

- SCD genotype
- VCN
- RCL
- Integration site analysis (ISA)

Blood sample collection details are included in the Laboratory Manual for this study.

Note that ISA may be discontinued if a subject's VCN is less than 0.01 copies per cell.

Results from these analyses may be accessed by viewing the appropriate electronic case report forms within the electronic data capture system.

6.2.17. Bone Marrow Aspiration

Bone marrow aspirate is to be taken per SOE. After drug product infusion, a bone marrow aspirate is to be taken unless discussed with the Medical Monitor for exceptions, such as if not clinically appropriate per local standard of care (e.g., bone marrow aspirate would require general anesthesia which would be considered an unacceptable risk to the subject) or not considered by the Investigator to be in the best interest of the subject to perform. A bone marrow smear is to be collected at the time of each bone marrow aspiration; site monitoring to include: cell count and differential, cellularity, M:E ratio, peripheral smear, iron stores, and morphologic report at a minimum (refer to SOM for additional detail). The bone marrow aspiration will be performed in accordance with institutional practices. Bone marrow smears should be read locally; additional bone marrow slides are to be archived for potential central analyses. Bone marrow aspirate collection details are included in the Laboratory Manual.

Bone marrow samples will be used for cytogenetics and mutational testing (Section 6.2.18), and storage (including slides; see Section 6.2.21), and may also be used for CCI PD (Section 6.2.19).

6.2.18. Conventional Cytogenetics (Karyotyping) and NGS Mutational Testing

Bone marrow aspirate (see Section 6.2.17) and blood will be collected according to the SOE for assessment of chromosomal abnormalities or genetic mutations, particularly those associated with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML), by conventional cytogenetics (karyotyping) and NGS mutational testing.

If available, bone marrow and blood samples collected per SOE may be used for retrospective genetic testing.

Results from conventional cytogenetics (karyotyping) from the bone marrow prior to conditioning will be used to assess suitability for transplant (see Section 6.2.11).

6.2.19. Bone Marrow for Exploratory PD

Bone marrow samples (see Section 6.2.17) will be shipped to the Sponsor and used to evaluate biomarkers of the pathology of SCD in bone marrow (e.g., morphology, cellularity, cell count of bone marrow, CD34+ count). These samples may also be analyzed if clinically indicated.

6.2.20. Blood for Exploratory PD

Blood samples will be collected according to the SOE and sample collection details are included in the Laboratory Manual.

These samples will be shipped to the Sponsor and used to evaluate potential PD biomarkers after drug product infusion using exploratory techniques. Exploratory assays may include but not limited to single-colony VCN, single-cell analysis of percent lentiviral vector positive (%LVV+) cells, ex vivo sickling, and single-cell western for $\beta^{A\text{-}T87Q}$-globin.

These samples may also be analyzed if clinically indicated.

6.2.21. Blood and Bone Marrow for Storage

Bone marrow aspirates (see Section 6.2.17) and blood will be collected according to the SOE, and these samples will be shipped to a central laboratory for storage and may be analyzed if clinically indicated or may be used for exploratory PD. Leftover samples from protocol procedures (e.g., drug product manufacture, blood draw for ISA) may also be stored and analyzed if clinically indicated or used for exploratory PD.

Samples may be stored until the samples are exhausted or until the repository is discontinued. The Sponsor will be the custodian of the samples in the repository any unused samples will be destroyed at the Sponsor's discretion. The Sponsor may share these stored samples for future research with collaborators, such as research laboratories, academic or non-profit institutions, and scientific advisors.

6.2.22. Samples for Potential Biomarker Analyses

Optional blood samples will be collected per the SOE for future research. These samples may be used for biomarker analyses of proteins, DNA, RNA and other molecules to study SCD and/or gene therapy. Such samples may be stored until the samples are exhausted or until the 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.

Collection and storage of the 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.

As samples allow, leftover samples from protocol procedures (described in Section 6.2.21) may also be used for potential future analyses as described above. The Sponsor may share these stored samples for future research with collaborators, such as research laboratories, academic or non-profit institutions, and scientific advisors.

6.2.23. Imaging Studies

Liver MRI

Subjects with advanced liver disease (as described in the eligibility criteria) are excluded from participation in this study.

At Screening and at Month 24 Visit, a liver MRI is required for all subjects. For subjects who have a medical history of iron overload or serum ferritin > 1000 ng/mL, a cardiac MRI is required at Screening and at Month 24 Visit.

If a liver MRI was performed within 3 months prior to signing the informed consent, these results may be used as part of the eligibility assessment and the MRI does not need to be repeated.

Subjects unable to undergo otherwise required liver MRI (e.g., have metal implants) can instead have a liver biopsy performed to rule out advanced liver disease.

Cardiac MRI

For subjects who have a medical history of iron overload or screening serum ferritin > 1000 ng/mL, a cardiac MRI is required at Screening to evaluate for baseline cardiac disease due to iron overload. Patients with a Cardiac T2* < 10 ms are excluded from participation in this study.

If a cardiac MRI was performed within 3 months prior to signing the informed consent, these results may be used as part of the eligibility assessment and the screening MRI does not need to be repeated.

Subjects who have a cardiac MRI at screening will have a second cardiac MRI at Month 24 to evaluate cardiac iron stores.

Cerebral MRI/MRA

Cerebral vasculature and prior brain parenchymal injury evaluation (as measured by cerebral MRA/MRI will be performed per the SOE. Cerebral MRA/MRI results performed within 3 months of ICF may be used, unless there is clinical indication for repeat testing.

For subjects unable to undergo cerebral MRI (e.g., have metal implant), a CT scan can be performed instead.

Transcranial doppler ultrasound (TCD)

Subjects ≤ 16 years old (at time of ICF signing) will undergo a TCD per the SOE.

Cardiac echocardiogram

Cardiac Doppler echocardiography, including the assessment of LVEF and TRJV, will be performed per the SOE. Echocardiogram results performed within 3 months of ICF may be used, unless there is clinical indication for repeat testing.

Bone mineral density

Bone mineral density (BMD) evaluation will be performed per SOE unless not allowed by institutional standard of care. All attempts will be made to use the same DXA machine for all assessments of a given subject throughout the duration of the trial.

Bone mineral density will be performed per the SOE using dual x-ray absorptiometry (DXA) at 3 reference sites: the lumbar spine and the right femoral neck, or whole body less head for pediatric subjects. In subjects with a history of hip surgery, the contralateral hip is assessed. If BMD evaluation was performed within 1 year of Informed Consent, these results may be used for Screening and do not need to be repeated.

6.2.24. Liver Biopsy

Liver biopsy may be performed in subjects with liver MRI findings suggestive of active hepatitis, significant fibrosis, inconclusive evidence of cirrhosis, or liver iron concentration ≥ 15 mg/g to evaluate for presence of advanced liver disease and study eligibility.

Liver biopsy samples should be reviewed for liver pathology (e.g., portal fibrosis, cirrhosis, hepatitis) and measured for hepatic iron content. Any evidence of cirrhosis, bridging fibrosis, or significant active hepatitis will be exclusionary. If a liver biopsy was performed within the year prior to signing the informed consent, these results may be used as part of the eligibility assessment and the biopsy does not need to be repeated.

6.2.25. Pulmonary Function Tests

Pulmonary function testing will be performed per the SOE. Pulmonary function tests include: % predicted forced vital capacity (FVC); % predicted forced expiratory volume in 1 second (FEV_1); % predicted respiratory volume (RV); and % predicted DL_{CO} (corrected for Hb). If performed within 6 months prior to ICF, those results may be used and do not need to be repeated. In the event of pulmonary complications (e.g. ACS, pneumonia) between the Screening PFT and the start of conditioning, an unscheduled PFT must be performed. After resolution of pulmonary complications, and prior to conditioning, PFT results must meet the following requirements:

- Baseline oxygen saturation ≥ 90% without supplemental oxygen (excluding periods of SCD crisis, severe anemia or infection).
- Baseline carbon monoxide diffusing capacity (DL_{CO}) ≥ 50% (corrected for Hb) in the absence of infection.
- If DL_{CO} cannot be assessed due to age or cognition-related restrictions, then respiratory exam, chest radiograph and oxygen saturation should be performed.

6.2.26. Patient-reported Outcome Measures

The following assessments (see Table 5 and Appendix 10.2) are to be performed at Screening, Month 3, Month 6, and every 6 months thereafter up to Month 24:

- Quality of life, as measured by performance on PROMIS
  - For patients ≥ 18 years old, performance on PROMIS-57 Version 2.1
  - For patients < 18 years old, performance on PROMIS Pediatric Profile (age 12 to 17 years) or PROMIS Parent Proxy Profile 49 (age 12 to 17 years) Version 2.0, as applicable

- Work productivity, as measured by the Work Productivity and Activity Impairment Questionnaire-General Health (WPAI-GH; age ≥ 15 years)/Caregiver WPAI-GH
- Cognitive function as measured by PROMIS Short Form 6a (age ≥ 18 years)
- Overall health status as measured by EuroQol-5D (EQ-5D-3L, age ≥ 16 years or EQ-5D-Y, age 12 to 15 years). The EQ-5D-3L Proxy Version 1 or EQ-5D-Y Proxy Version 1 to be used instead only if subject unable to use the EQ-5D

Table 5: Patient-reported Outcome Measures

Age[1] ≥ 18 years	**Age[a] < 18 years**			
18+	**17**	**16**	**15**	**12 - 14**
PROMIS-57[2b]	PROMIS Pediatric Profile 49 *or* PROMIS Parent Proxy Profile 49	PROMIS Pediatric Profile 49 *or* PROMIS Parent Proxy Profile 49	PROMIS Pediatric Profile 49 *or* PROMIS Parent Proxy Profile 49	PROMIS Pediatric Profile 49 *or* PROMIS Parent Proxy Profile 49
PROMIS Short Form 6a[c]	N/A	N/A	N/A	N/A
WPAI-GH[d]	WPAI-GH *or* WPAI-Caregiver form	WPAI-GH *or* WPAI-Caregiver form	WPAI-GH *or* WPAI-Caregiver form	WPAI-Caregiver form
EQ-5D-3L[5e]	EQ-5D-3L	EQ-5D-3L	EQ-5D-Y	EQ-5D-Y

Abbrev.: EQ-5D, EuroQol-5D; N/A, not applicable; PROMIS, Patient Reported Outcomes Measurement Information System; WPAI, Work Productivity and Activity Impairment Questionnaire.

[a] For all assessments, age at time of assessment determines which form should be used. PRO form may change from baseline depending on subject age
[b] PROMIS-57 Version 2.1 or PROMIS Pediatric Profile/Parent Proxy Profile 49 Version 2.0. The numeric rating scale that assesses pain is part of the pain assessment tool
[c] PROMIS SF 6a is a measure of cognitive function
[d] Either the WPAI or WPAI-Caregiver form should be used depending which has the greatest impact on the family. Whoever completes the WPAI at baseline should be the person who completes it for the remainder of the trial
[e] EQ-5D-3L/EQ-5D-Y: Proxy form to be used instead only if subject unable to use the EQ-5D-3L or EQ-5D-Y

6.2.27. Six-Minute Walk Test

The six-minute walk test (6MWT) is to be performed per the SOE, and will be conducted by following the American Thoracic Society Guidelines for the Six-Minute Walk Test (2002). This test measures the distance that a patient can quickly walk on a flat, hard surface in a period of 6 minutes (6MWD). The 6MWT is a measure of moderate effort tolerance. Change in 6MWD equal to or greater than 50 m is used in pulmonary disease clinical trials.

6.2.28. Assessment of Oligoclonality by Integration Site Analysis

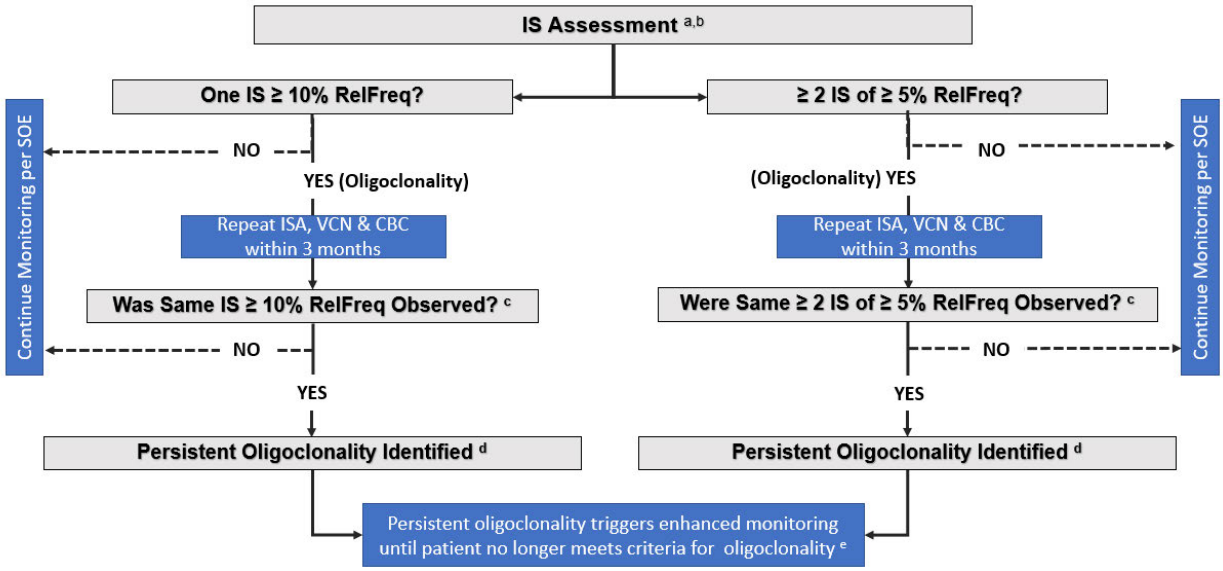
Integration site analysis (ISA) will be performed to determine the insertion site (IS) profile of subjects over time, as indicated in the SOE. Figure 4 shows the algorithm that determines the

frequency of monitoring by ISA to assess oligoclonality, in accordance with FDA Guidance (FDA 2020). While oligoclonality itself, or even monoclonality, will not a priori result in a malignancy, changes in IS relative frequency may be associated with an increase in the risk of a malignancy. Therefore ISA monitoring is performed every 6 months for the first 5 years after drug product administration, and annually thereafter. This is coupled with monitoring for hematological abnormalities via complete blood count (CBC) with differential every 6 months through 15 years after drug product administration (see Section 6.2.33 for information on long-term follow-up).

ISA monitoring may be repeated more frequently if there is an indication of oligoclonality. As shown in Figure 4, if an IS is detected at ≥ 10% relative frequency (RelFreq) for 1 IS, or ≥ 5% RelFreq for ≥ 2 IS each, ISA will be repeated within 3 months of receipt of this result. If the result is confirmed, a report of persistent oligoclonality will be submitted to the relevant Health Authorities within 30 days. This repeated observation will also trigger enhanced monitoring for hematological abnormalities, increasing the frequency of CBC with differential to every 3 months along with ISA and VCN every 6 months until the criteria for oligoclonality is no longer met.

Based on clinical and ISA findings, additional monitoring for malignancy may be instituted by the treating physician/Principal Investigator or Sponsor. See the following section for recommended clinical work-up for malignancy.

Figure 4: Algorithm for Frequency of ISA Monitoring



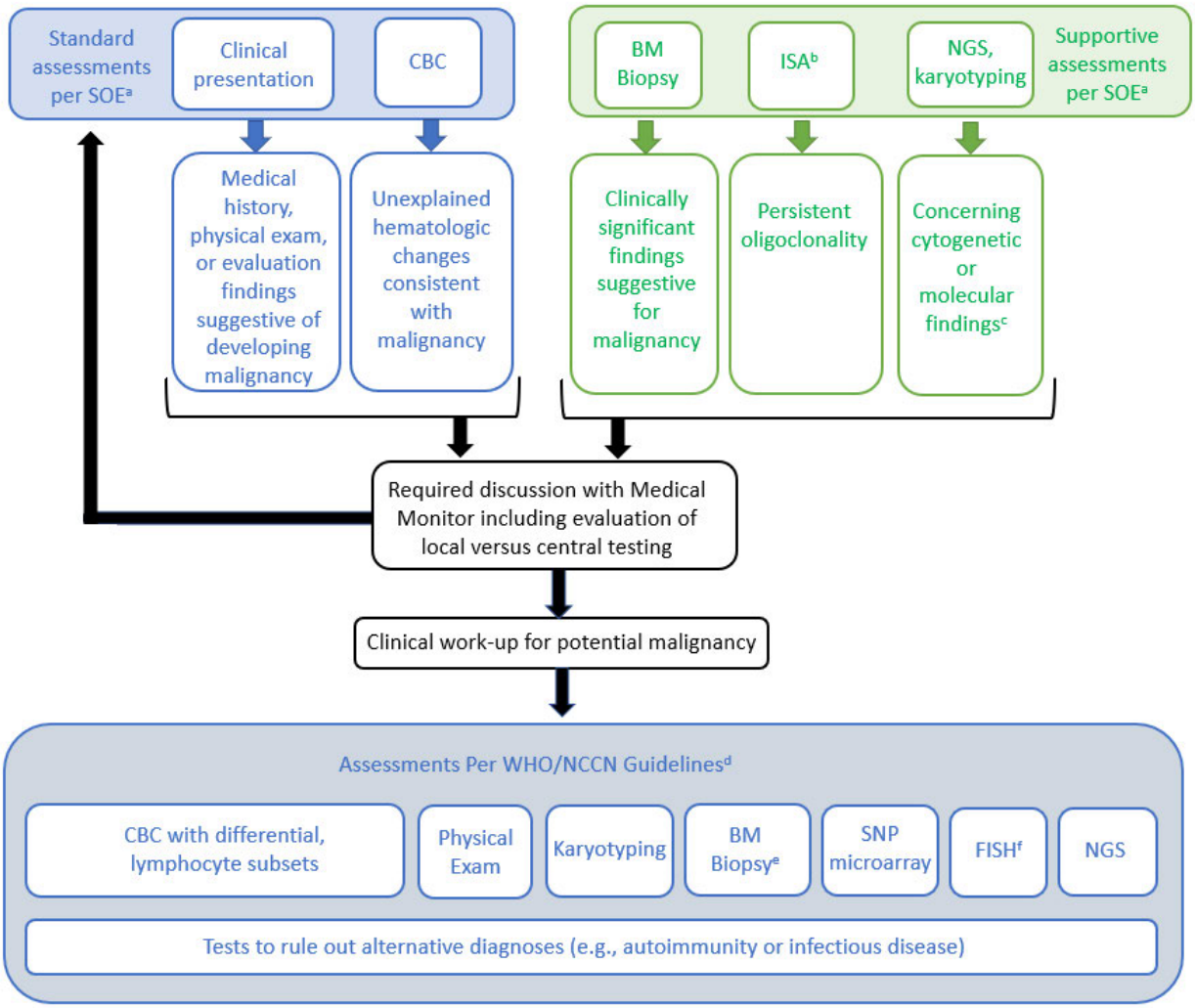
Abbrev.: IS, insertion site(s); ISA, integration site analysis; RelFreq, relative frequency
[a] ISA is assessed per the schedule of events; CBC with differential and VCN are to be carried out whenever ISA is performed (Table 4).
[b] If ISA is assessed concurrently from PB and BM, the assessment with higher RelFreq values should be used.
[c] Samples obtained one month or more apart can be considered consecutive measurements.
[d] Persistent oligoclonality will be reported to the relevant Health Authorities within 30 days.

[e] At a minimum, enhanced monitoring includes CBC with differential every 3 months and ISA and VCN every 6 months. Additional monitoring for malignancy may be instituted by the treating physician/Principal Investigator (see Section 6.2.29).

6.2.29. Assessments for Risk of Hematologic Malignancy and Clinical Work-up

Routine assessments carried out per the SOE (Table 4) for risk of potential hematologic malignancy are presented in Figure 5. Clinical presentation and CBC with differential are considered standard assessments, whereas BM biopsy, ISA, NGS, and karyotyping are considered supportive assessments in the context of gene therapy. In the event of suspicion of hematologic malignancy (i.e., myelodysplasia, leukemia, or lymphoma), discussion with the Medical Monitor is required and should include an evaluation of local versus central testing. Clinical work-up is to be performed by the Investigator per appropriate standard of care and in alignment with WHO and NCCN guidelines (Arber et al. 2016; Greenberg et al. 2022).

Figure 5: Assessments for Risk of Hematologic Malignancy per Schedule of Events and Clinical Work-up



Abbrev.: AML, acute myeloid leukemia; BM, bone marrow; CBC, complete blood count; FISH, fluorescence in situ hybridization; IS, insertion site; ISA, integration site analysis; MDS, myelodysplastic syndrome; NCCN, National

Comprehensive Cancer Network; NGS, next generation sequencing; RNA-seq, RNA sequencing; SNP, single nucleotide polymorphism; VCN, vector copy number; WHO, World Health Organization

[a] Routine monitoring include assessments per SOE (Table 4) and ongoing patient education on risk of malignancy.

[b] Turnaround times for ISA results can vary in duration, typically taking approximately 10 weeks from sample collection to data delivery. ISA may be done on PB or BM per the SOE (Table 4).

[c] In addition to NGS and karyotype, other genetic testing results that are suggestive of potential risk for malignancy may be taken into account.

[d] If a subject has persistent oligoclonality (see Section 6.2.29) and other cause for clinical work-up, additional assessments such as BM biopsy, RNA-seq, lineage-specific ISA (to be performed on BM only if as available), or IS-specific VCN can be considered; (Arber et al. 2016; Greenberg et al. 2022).

[e] ISA should be done on BM if taken as part of clinical work-up.

[f] In the context of clinical work-up for hematologic malignancy, FISH assessments should be local, according to guidelines, as local laboratories are certified in this context. Central FISH may be performed retrospectively on stored samples if results from local work-up are unclear.

Under specific circumstances, after discussion with the Medical Monitor, BM biopsy, RNA-seq/RT-PCR, lineage-specific ISA, and IS-specific VCN may be considered appropriate assessments during clinical work-up, specifically if there is persistent oligoclonality as identified with ISA AND at least one of the following:

- Medical History, physical exam, or evaluation findings suggestive of developing malignancy OR
- Unexplained hematologic changes consistent with malignancy OR
- Clinically significant findings suggestive for malignancy on BM pathology OR
- Concerning cytogenetic or molecular findings on karyotype, NGS, or other genetic testing results that are suggestive of potential risk for malignancy

Note at any time, regardless of clonality measurements, any additional clinical work-up may be performed at the Investigator's discretion if there is suspicion of malignancy. In the event of unclear diagnosis, additional supportive assessments (e.g., whole genome sequencing or whole exome sequencing) can be considered in individual, specific cases.

If the clinical work-up indicates no evidence of myelodysplasia, leukemia, or lymphoma, the subject will continue to be monitored as per the protocol-defined SOE, or more frequently at discretion of the treating physician/Principal Investigator. If the clinical work-up indicates a diagnosis of myelodysplasia, leukemia, or lymphoma, the Sponsor will convene an urgent safety review meeting. Further analyses will be determined by the Sponsor, in consultation with the DMC. All efforts should be made to confirm the source of malignancy. It should be noted that it may not be possible to distinguish the source of malignancy (e.g., arising from underlying pathophysiology of the disease, transplant-related medications or procedures, or from expansion of gene-modified cells due to insertional oncogenesis).

6.2.30. Adverse Events

6.2.30.1. Assessments During Study

Monitoring of AEs will be conducted from the signing of informed consent. Adverse events, as defined in Section 6.2.30.2 will be documented from the time informed consent is signed through the following time points for Groups A and B:

- ≥ Grade 1 AEs: through 30 days after drug product infusion.
- ≥ Grade 2 AEs: through 12 months after drug product infusion.
- All serious adverse events (SAEs), ≥ Grade 3 AEs, and drug product-related AEs: through 24 months after drug product infusion.

For Group C, AEs for all subjects (excluding screen failures) will be recorded in the CRFs starting from the time informed consent is signed through the Month 24 Visit. All SAEs (including screen failures) will be reported from the signing of informed consent/assent on the SAE report form. Group C subjects enrolled under Version 7.0 of Protocol HGB-206 will have AEs retrospectively documented to be in accordance with Version 8.0 and Version 9.0 of Protocol HGB-206.

All AEs should be monitored until they are resolved or are clearly determined to be due to a subject's stable or chronic condition or inter-current illness(es). See also Section 4.5 for AE monitoring of subjects who withdraw from the study.

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. If withdrawal is before drug product infusion, subjects should remain on study for at least 30 days after any invasive study procedure (e.g., mobilization, liver biopsy) before withdrawal and ongoing AEs should be monitored for the 30-day duration. In the rare case a subject undergoes myeloablation and receives back-up cells instead of bb1111, subject should remain on the study for at least 3 months post-myeloablation and AEs should be followed for the 3-month duration.

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

Note that at the completion of Study HGB-206, subjects are expected to enroll into a long-term follow-up study, that will monitor the safety of subjects (including drug product-related AEs) through a total of 15 years after drug product infusion. If there is a gap of time between the final visit in Study HGB-206 (e.g., Month 24 study visit) and participation in the long-term follow-up study (i.e., signature on long-term follow-up ICF), all SAEs that start during that gap should be reported on the Study HGB-206 SAE report form and submitted according to Section 6.2.30.5.

6.2.30.2. Adverse Events Definitions

Adverse Events

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

For the purposes of this study, neutrophil engraftment failure is defined as an SAE and is to be reported as such. For this protocol, neutrophil engraftment failure is defined as failure to achieve an ANC laboratory value of $\geq 0.5 \times 10^9$ cells/L for 3 consecutive measurements on different days by Day 43 or receiving back-up cells at any time during the neutropenic phase.

Unexpected Adverse Events

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

Conditioning-related Adverse Events

Busulfan IV is a cytotoxic drug that causes profound myelosuppression. Accordingly, subjects will experience intended hematologic events (e.g., neutropenia, thrombocytopenia, anemia) and expected non-hematologic events (e.g., mucositis, nausea, vomiting, alopecia, pyrexia) as a result of receiving busulfan IV. For the purposes of this protocol, these events, which are familiar to transplant physicians and are described in the busulfan prescribing information, are considered conditioning-related events (CREs).

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

Serious Adverse Events

An SAE is any AE that:

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

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.

6.2.30.3. Adverse Event Assessment of Severity and Relationship

For all AEs, the Investigator must determine both the severity of the event and the relationship of the event to treatment with drug product.

Severity will be assessed by the Investigator, including for AEs that are a result of a laboratory abnormality:

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

If the Grade changes within a day, the maximum Grade should be recorded.

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

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

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

6.2.30.4. Procedures for AE and SAE Collection and Reporting

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

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

6.2.30.5. Immediate Reporting of SAEs

All SAEs for all subjects, including screen failures, must be immediately reported on the SAE report form to the Sponsor (or designee) within 24 hours of the Investigator (or designee) becoming aware of the SAE. All SAEs must be reported whether or not they are considered causally related to drug product. For immediate reporting of SAEs, the Investigator must provide assessments of relationship and serious criteria at the time of SAE report submission to the Sponsor. The SAE report form and completion guidelines can be found in the Study Operations Manual (SOM). All follow-up information on SAEs must also be immediately reported to the Sponsor (i.e., within 24 hours).

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

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

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

If there are suspected, unexpected serious adverse reactions (SUSARs) associated with the use of bb1111 or plerixafor, the Sponsor will notify the appropriate regulatory agency(ies) and all participating investigators on an expedited basis and in accordance with applicable regulations.

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

Reporting of SUSARs to regulatory agencies will be performed within 7 or 15 days as indicated by the event and in accordance with local regulations. Annual reporting of safety information will also be performed by the Sponsor, as required by local regulation.

6.2.31. Pregnancy and Contraception

Pregnancy 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 occurring during this study (in subjects or female partners of male subjects) are to be reported in the same time frame as SAEs using the Pregnancy Form. Serious adverse events during the course of a pregnancy experienced by a subject or female partner of male subject are required to be immediately reported (i.e., within 24 hours) on the SAE report form.

The course of all* pregnancies, including perinatal and neonatal outcome, regardless of whether the subject has discontinued participation in the study, will be followed until outcome, including

follow-up of the health status of the newborn at 6 weeks of age and annually thereafter for 2 years.

*Exceptions include:

In cases where the MALE was the study subject, pregnancies resulting from sperm banking prior to receipt of drug product will not be followed.

In cases where the FEMALE was the study subject, pregnancies resulting from oocyte banking prior to receipt of drug product in which the pregnancy is carried to term by surrogacy will not be followed.

Busulfan has been shown in animal studies to be teratogenic (see package insert for additional details). The effects of administration of bb1111 on the pregnant female or the developing fetus are unknown.

Female subjects of child-bearing potential are required to use 1 method of highly effective contraception from Screening to at least 6 months after drug product infusion, and male subjects of child-bearing potential are required to use 1 method of highly effective contraception from Conditioning to at least 6 months after drug product infusion. Beyond 6 months after drug product infusion, subjects should discuss with their physician prior to resuming unprotected intercourse.

Acceptable forms of highly effective contraception for female participants of childbearing potential or for partners of male participants who are of childbearing potential include:

- Combined (estrogen and progestogen containing) oral, intravaginal, or transdermal hormonal contraception associated with inhibition of ovulation
- Oral, injectable, or implantable progestogen-only hormonal contraception associated with inhibition of ovulation
- Intrauterine device
- Intrauterine hormone-releasing system
- Bilateral tubal ligation or bilateral tubal occlusion (performed at least 3 months prior to screening)
- Vasectomized partner (performed at least 3 months prior to screening)
- Sexual abstinence (no sexual intercourse)

Acceptable forms of highly effective contraception for male participants include:

- Sexual abstinence (no sexual intercourse)
- History of vasectomy (performed at least 3 months prior to screening)
- Condom with spermicide used together with highly effective female contraceptive methods if the female partner(s) is of childbearing potential (see above for list of acceptable female contraceptive methods)

6.2.32. 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. If the Month 18 Visit does not include 18 calendar months, a telephone contact assessment for VOEs is to take place after 18 calendar months (post-drug product infusion) have elapsed. Other evaluations and procedures to be performed at unscheduled visits will be at the Investigator's discretion in consultation with the Sponsor as appropriate and may be based on those listed in the SOE. Unscheduled visits, including any unscheduled assessments or procedures performed at the visit, should be promptly entered into the CRF.

6.2.33. Long-term Follow-up Protocol

Subjects will be followed for 24 months after drug product infusion under this protocol. Then, if appropriate consent is obtained, subjects will be followed for an additional 13 years under a separate long-term follow-up protocol, which will focus on long-term safety, with an emphasis on the assessment of factors associated with risk of hematologic malignancies, and long-term efficacy. This follow-up includes recording of SAEs, patterns of vector integration (ISA) in DNA from whole blood, and potentially testing peripheral blood leukocytes for RCL as clinically indicated.

7. STATISTICAL PROCEDURES

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

7.1. Sample Size Estimation

The sample sizes for Groups A and B were not determined by formal statistical methods. Approximately 41 subjects will be enrolled in Group C, approximately 35 of whom must meet the severe VOE criteria as set forth in Inclusion Criterion #3.1.

Assuming 80% of subjects in Group C who have at least 4 VOEs in the 24 months prior to Informed Consent will meet the primary efficacy endpoint of VOE-CR, 35 subjects will provide more than 99% power to reject the null hypothesis of 40% at 1-sided alpha of 0.025, using the Exact Test per EAST® (Version 6). The success criterion is 60% (21 out of 35) of subjects meeting the primary efficacy endpoint VOE-CR, if exactly 35 subjects with at least 4 VOEs in the 24 months prior to Informed Consent are enrolled and receive drug product in Group C.

Power calculations were also performed for the key secondary efficacy endpoints of sVOE-CR and Globin Response.

Assuming 85% of subjects in Group C with at least 4 VOEs in the 24 months prior to Informed Consent will meet sVOE-CR, 35 subjects will provide more than 99% power to reject the null hypothesis of 50% at 1-sided alpha of 0.025, using the Exact Test per EAST® (Version 6). The success criterion is 69% (24 out of 35) of subjects meeting sVOE-CR, if exactly 35 subjects with at least 4 VOEs in the 24 months prior to Informed Consent are enrolled and receive drug product in Group C.

Assuming 70% of subjects will meet Globin Response, 41 subjects will provide approximately 96% power to reject the null hypothesis of 40% at 1-sided alpha of 0.025, using the Exact Test per EAST® (Version 6). The success criterion is 59% (24 out of 41) of subjects meeting Globin Response, if exactly 41 subjects are enrolled and receive drug product in Group C.

7.2. Populations for Analysis

The following subject populations will be evaluated and used for presentation and analysis of the data:

- Intent-to-Treat (ITT) Population: All subjects who initiate any study procedures, beginning with stem cell collection procedures (mobilization/apheresis or bone marrow harvest).
- Transplant Population (TP): All subjects who receive drug product.
- Transplant Population for VOE (TPVOE): Subset of TP subjects with at least 4 VOEs in the 24 months prior to Informed Consent.
- Successful Engraftment Population (SEP): A subset of TP subjects who, following busulfan myeloablation and drug product infusion, successfully engraft with drug product, defined as 3 consecutive absolute neutrophil count (ANC) $\geq 0.5 \times 10^9$/L

laboratory values obtained on different days after the initial post-infusion nadir by Day 43.

The ITT is the primary population for the analysis of safety, the TPVOE is the primary population for VOE-related endpoints including the primary efficacy endpoint and the key secondary endpoint, the TP is the primary population for the key secondary efficacy endpoint of Globin Response and the other secondary efficacy endpoints and pharmacodynamic endpoints. The SEP will be used to provide supportive data for subjects who engraft.

7.3. Planned Analyses

7.3.1. Interim Analyses

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 input 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 complete the study.

7.3.3. Additional Data Review

Safety data are reviewed on an ongoing basis for signal detection, DMC meetings and to support preparation of regulatory submission documents. Analyses of study data may also be performed for the purposes of internal data review, regulatory agency interactions, 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 if applicable, analyses will be performed to measure the effect of disruptions due to the pandemic on these assessments.

7.4. Statistical Methods

7.4.1. General Methods

Tabulations will be produced for appropriate demographic, baseline, safety, efficacy, and PD parameters. For categorical variables, summary tabulations of the number and percentage within each category of the parameter will be presented. For continuous variables, the number of observations, mean, standard deviation, median, minimum and maximum values will be presented.

Descriptive summary statistics as well as 2-sided, 95% confidence intervals will be presented on selected parameters. By-subject listings of data for all completed and discontinued subjects will be provided.

For Hb, baseline will be defined as the average of the 2 most recent Hb assessments made at or prior to the Screening evaluation, which meet the following criteria:

i. Assessments must be separated by at least 1 month from each other.

ii. Assessments must have been drawn no earlier than 24 months prior to Informed Consent and may include the Hb result from Screening.

iii. There should be no pRBC transfusion within 3 months prior to each Hb assessment.

For subjects who are on chronic, recurrent transfusions, and do not have 2 Hb assessments which meet the above criteria, the following criteria can be used: 2 Hb values which meet criteria (i) and (iii) that are found within 24 months prior to the start of a regular transfusion program.

All reported VOEs will be adjudicated (see Section 3.3 for details on the event adjudication committee). All sVOE- or VOE-related efficacy endpoint analyses will be based on adjudicated sVOEs or VOEs. Supplementary analyses will be performed using protocol sVOEs/VOEs and investigator VOEs, where applicable.

For VOE analysis, baseline will be defined as the annualized number of VOEs over the 24 months before Informed Consent.

Unless otherwise specified, for other efficacy, pharmacodynamic, and safety parameters, including shifts in key laboratory parameters, baseline will be defined as the first assessment during screening (which can be identified by the first record on or after date of ICF, but before initiation of stem cell collection). Longitudinal data (collected serially over time on study and follow-up) will be presented by appropriate time intervals, such as monthly, quarterly and so forth, depending on the nature of the data.

7.4.2. Disposition of Subjects

A tabulation of the disposition of subjects will be presented, including the number harvested, infused, and the number with any post-drug product infusion data available for analysis, and the extent of data available. Tables and listings will be provided for subjects in each analysis data set, including the distribution of subjects according to genotype. Subject data will also be displayed by site. The number of subjects completing the study through 2 years after drug product infusion and reasons for study discontinuation will be reported. Deviations from protocol treatment and assessment specifications will be tabulated and listed.

7.4.3. Demographic and Baseline Characteristics

The following demographic and baseline characteristic factors will be summarized: age (at diagnosis and at Informed Consent), country of birth, race and ethnicity, SCD-specific medical history, and the time from diagnosis to initiation of conditioning, number of VOEs, baseline Hb, and chronic transfusion status.

7.4.4. Efficacy Analysis

In the calculation of non-transfused total Hb for some efficacy endpoints, any subject with a β+ allele will produce some endogenous HbA which will not be distinguishable from residual transfused HbA, and therefore their contribution to the amount of non-transfused total Hb will be underestimated. For these subjects only, HbA will be included as "non-transfused Hb" only for samples taken ≥ 60 days after last transfusion. For subjects without a β+ allele, non-transfused total Hb is the sum of HbS + HbF + HbA_2 + HbA^{T87Q}.

7.4.4.1. Analysis of Primary Efficacy Endpoint

The primary endpoint of VOE-CR will be tested against the null hypothesis of 40% using a 1-sided exact binomial test. The number and percentage of subjects reaching the primary endpoint will be presented. The associated 2-sided exact 95% confidence interval will be provided. The primary endpoint will be analyzed in the TPVOE for Group C subjects. The subject must have been or would have been followed for a minimum of 18 calendar months after drug product infusion before the subject can be evaluated for the primary endpoint. If the Month 18 Visit does not include 18 calendar months, a telephone contact assessment for VOEs can take place after 18 calendar months (post-drug product infusion) have elapsed.

The primary estimand examines the proportion of subjects achieving complete resolution of VOEs between 6 months and 18 months after drug product infusion among all subjects who receive bb1111 and have at least 4 VOEs in the 24 months prior to Informed Consent.

Supplementary analyses for the primary endpoint will be detailed in the statistical analysis plan (SAP).

7.4.4.2. Analysis of Key Secondary Efficacy Endpoints

7.4.4.2.1. Analysis of sVOE-CR

The key secondary endpoint of sVOE-CR will be tested against the null hypothesis of 50% using a 1-sided exact binomial test. The number and percentage of subjects reaching this key secondary endpoint will be presented. The associated 2-sided exact 95% confidence interval will be provided. The endpoint will be analyzed in the TPVOE for Group C subjects. The subject must have been or would have been followed for a minimum of 18 calendar months after drug product infusion before the subject can be evaluated for the endpoint. If the Month 18 Visit does not include 18 calendar months, a telephone contact assessment for VOEs can take place after 18 calendar months (post-drug product infusion) have elapsed.

The estimand examines the proportion of subjects achieving complete resolution of severe VOEs between 6 months and 18 months after drug product infusion among all subjects who receive bb1111 and have at least 4 VOEs in the 24 months prior to Informed Consent.

7.4.4.2.2. Analysis of Globin Response

This key secondary endpoint of Globin Response will be tested against the null hypothesis of 40% using a 1-sided exact binomial test. The number and percentage of subjects reaching this key secondary endpoint will be presented. The associated 2-sided exact 95% confidence interval will be provided. The endpoint will be analyzed in the TP for Group C subjects.

Globin Response is defined as meeting the following criteria for a continuous period of at least 6 months after drug product infusion:

- Weighted average HbA^{T87Q} percentage of non-transfused total Hb $\geq$ 30%; AND
- Weighted average non-transfused total Hb increase of $\geq$ 3 g/dL compared to baseline total Hb OR weighted average non-transfused total Hb $\geq$ 10 g/dL.

The estimand examines the proportion of subjects achieving Globin Response for a continuous period of at least 6 months after drug product infusion starting ≥ 60 days after the last pRBC transfusion among all subjects who receive bb1111.

Subjects must meet these criteria for a continuous period of ≥ 6 months after drug product infusion (starting ≥ 60 days after last pRBC transfusion).

The weighted average HbA^{T87Q} percentage is defined as:

$[(t_1-t_0) \times ((p_0+p_1)/2) + (t_2-t_1) \times ((p_1+p_2)/2) +\ldots+ (t_k-t_{k-1}) \times ((p_{k-1}+p_k)/2)]/(t_k-t_0)$,

where

- t_0 represents the time of the first HbA^{T87Q} assessment where there is no pRBC transfusion in the previous 60 days, and t_1, t_2, … t_k represent continuous time points for HbA^{T87Q} assessments after t_0 where no pRBC transfusion occurs between these timepoints.
- p_0, p_1, p_2, … p_k represent the HbA^{T87Q} percentage at each of these time points.
- t_k-t_0 must be at least 6 months.

HbA^{T87Q} is assessed every 3 months starting from Month 3 after drug product infusion. In the event that there are 3 consecutive scheduled HbA^{T87Q} assessments that are within visit window but are less than 6 months apart, the ≥ 6-month requirement will not be needed. The weighted average may be considered as an average AUC calculation for HbA^{T87Q} percentage.

Note that the weighted total Hb can be calculated in a similar way.

Sensitivity and/or supplementary analyses for the key secondary endpoints will be detailed in the SAP.

7.4.4.3. Analyses of Other Secondary Efficacy Endpoints

Analyses of the following secondary efficacy endpoints will be descriptively summarized. More details can be found in the SAP.

Clinical and Disease Evaluation Endpoints:

(Only for subjects with at least 4 VOEs in the 24 months prior to Informed Consent):

- Change in the annualized number of VOEs in the 24 months after drug product infusion compared to the 24 months prior to Informed Consent.

(Only for Group C subjects with at least 4 VOEs in the 24 months prior to Informed Consent):

- Change in the annualized number of severe VOEs in the 24 months after drug product infusion compared to the 24 months prior to Informed Consent.
- VOE-CR24, defined as complete resolution of VOEs between 6 months and 24 months after drug product infusion
- sVOE-CR24, defined as complete resolution of severe VOEs between 6 months and 24 months after drug product infusion

- sVOE-75, defined as at least a 75% reduction in annualized severe VOEs in the 24 months after drug product infusion compared to the 24 months prior to Informed Consent

Note: The analyses regarding severe VOEs will be based on Group C subjects who have at least 4 VOEs in the 24 months prior to Informed Consent.

Characterization of Globin Response (only for Group C subjects who have achieved Globin Response):

- Proportion of subjects who meet the definition of Globin Response at Month 24
- Duration of Globin Response

Hematologic Endpoints:

- Weighted average for the following at Month 6, 12, 18, and 24:
  - non-transfused total Hb
  - HbS percentage of non-transfused total Hb
  - HbS percentage of non-transfused total Hb $\leq 70\%$, $\leq 60\%$, $\leq 50\%$
  - HbA^{T87Q} percentage of non-transfused total Hb
  - non-HbS percentage of non-transfused total Hb
- Assessment of the following over time:
  - non-transfused total Hb
  - HbS percentage of non-transfused total Hb
  - HbA^{T87Q} percentage of non-transfused total Hb
  - non-HbS percentage of non-transfused total Hb
- Change from baseline in hemolysis markers, including absolute reticulocyte count, % reticulocytes/erythrocytes, total bilirubin, haptoglobin, and lactate dehydrogenase
- Change from baseline in markers of iron stores including ferritin, liver iron content, and if assessed at baseline, cardiac iron content
- Change from baseline in annualized frequency and volume of packed red blood cell (pRBC) transfusions between 6 months and 24 months after drug product infusion
- Change from baseline in markers of stress erythropoiesis, including erythropoietin and serum transferrin receptor

SCD Burden and Chronic Complications Assessment:

- Change from baseline in renal function as measured by eGFR
- Change from baseline in cardiac-pulmonary function via echocardiogram (tricuspid regurgitant jet velocity [TRJV], LVEF) and pulmonary function tests
- Change from baseline in meters walked during 6-minute walk test

Hospitalizations and Quality of Life

- Change from baseline in annualized VOE-related hospital admissions and days
- Change from baseline in patient-reported quality of life, as measured by PROMIS

7.4.4.4. Analyses of Exploratory Efficacy Endpoints

Analyses of exploratory efficacy endpoints will be descriptively summarized. Details can be found in the SAP.

7.4.4.5. Correlation Analyses between Efficacy Endpoints

The following correlation analyses will be performed:

- Globin Response vs VOE-CR
- Globin Response vs sVOE-CR
- HbA^{T87Q} percentage at Month 6 vs percent change in annualized sVOEs

Details of the correlation analyses will be provided in the SAP.

7.4.5. Safety Analyses

The safety analyses will include evaluation of the incidence of AEs by Preferred Term and System Organ Class coded using the Medical Dictionary for Regulatory Activities (MedDRA). Adverse events will be summarized for the ITT population for those events that occur from signing of informed consent through the Month 24 visit. All AEs will be listed and summarized for the following time periods: 1) from Informed Consent up to the initiation of the stem cell harvest procedure (first administration of mobilization agent, or bone marrow harvest); 2) from initiation of the stem cell harvest procedure up to start of conditioning; 3) from start of conditioning (approximately D-6) through neutrophil engraftment; 4) from neutrophil engraftment through Month 24 Visit; 5) from the day of drug product infusion (Day 1) through Month 24 Visit; 6) from Informed Consent through Month 24 Visit. All AEs will be listed in by-patient, by-time data listings, including any events that may have occurred after signing Informed Consent but prior to stem cell harvest.

Changes in laboratory parameters over time will be summarized for the ITT population with descriptive statistics, including laboratory shifts tables.

Additional summaries of safety data may be produced, if warranted for further characterizing the safety profile of study subjects.

7.4.6. Pharmacodynamic Analyses

Analyses of PD endpoints and other exploratory endpoints will be described in the SAP.

8. ADMINISTRATIVE REQUIREMENTS

8.1. Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonisation (ICH) Guideline for GCP and the appropriate regulatory requirement(s). The Investigator will be thoroughly familiar with the appropriate use of bb1111 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. 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 and other appropriate institutional regulatory bodies will review all appropriate study documentation in order to safeguard the rights, safety, and wellbeing of the subjects. The study will only be conducted at sites where IRB/IEC and other appropriate institutional regulatory body approval has been obtained. The protocol, Investigator's Brochure, informed consent, 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 and other appropriate institutional regulatory bodies by the Investigator.

8.3. Subject Information and Informed Consent

After the study has been fully explained, written informed consent will be obtained from the subject or their legal representative prior to study participation. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICHGCP and all applicable regulatory requirement(s). If a subject is < 18 years of age at signing of informed consent/assent and turns 18 while on study, the subject should re-consent to the adult ICF at the next scheduled study visit.

Consent to this study indicates acknowledgement that follow-up is expected to last 15 years, with the first 2 years in this Study HGB-206 and 13 additional years in a long-term follow-up study. A brief summary of expected study procedures in the long-term follow-up study will be described in the Study HGB-206 consent so that the subject and/or their guardian or legal representative are aware of expectations in the long-term follow-up study. Consent for participation in the long-term follow-up study will be obtained at the time of enrollment in the long-term follow-up study.

If any concerning chromosomal abnormality or genetic mutation is identified prior to conditioning, and the subject intends to continue to stay on study (see Section 6.2.11), appropriate informed consent must be obtained after the results have been fully discussed.

8.4. Subject Confidentiality

In order to maintain subject privacy, all CRFs, 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 the Sponsor 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 the Sponsor, that has been given approval/favorable opinion by the IRB/IEC and other appropriate institutional regulatory bodies. Modifications to the protocol should not be made without agreement of both the Investigator and the Sponsor. Changes to the protocol will require written IRB/IEC and other appropriate institutional regulatory body 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 that have the approval /favorable opinion of the IRB/IEC and other appropriate institutional regulatory bodies. The Sponsor 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 the Sponsor, if circumstances permit, to discuss the planned course of action. Any departures from the protocol must be fully documented in the CRF and source documentation.

8.6. Direct Access to Source Data

Monitoring and auditing procedures developed by the Sponsor will be followed, in order to comply with GCP guidelines.

The study will be monitored by the Sponsor or its designee. Monitoring will be done by personal visits from a representative of the Sponsor (site monitor) and will include onsite review of the CRFs for completeness and clarity, crosschecking with source documents, and clarification of administrative matters will be performed. 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, email, telephone, and fax).

Regulatory authorities, the IRB/IEC and other appropriate institutional regulatory bodies, and/or the Sponsor may request access to all source documents, CRFs, and other study documentation for onsite 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. Case Report Form Completion

CRFs will be completed for each study subject. It is the Principal Investigator's responsibility to ensure the accuracy, completeness, and timeliness of the data reported in the subject's CRF. Source documentation supporting the CRF 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 Principal Investigator or designated representative, should complete the CRF screens 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 Principal Investigator must sign and date the Investigator's Statement at the end of the CRF to endorse the recorded data.

8.8. Record Retention

The Investigator will maintain all study records according to ICHGCP and applicable regulatory requirement(s). Records will be retained for at least 2 years following 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. The Sponsor 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

The Sponsor 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 BB305 LVV and bb1111 supplied by the Sponsor 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 the Sponsor. It is understood that there is an obligation to provide the Sponsor with complete data obtained during the study. The information obtained from the clinical study will be used towards the development of BB305 LVV and bb1111 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 the Sponsor, as appropriate, will be formed to oversee the publication and presentation of the study results, which will reflect the experience of all participating clinical sites. No publication or disclosure of study results will be permitted except under the terms and conditions of a separate written agreement between Sponsor and the Investigator and/or the Investigator's institution.

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10. APPENDICES

10.1. Performance Status Assessments

10.1.1. Karnofsky Performance Status Scale

The following table presents the Karnofsky performance status scale and should be used for subjects ≥ 16 years of age.

Points	Description
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self; unable to carry on normal activity or to do active work
60	Required occasional assistance but is able to care for most of his/her needs
50	Required considerable assistance and frequent medical care
40	Disabled; required special care and assistance
30	Severely disabled; hospitalization indicated. Death not imminent
20	Very sick; hospitalization necessary; active support treatment necessary
10	Moribund; fatal process progressing rapidly
0	Dead

Source: (Mor et al. 1984)

10.1.2. Lansky Performance Status Scale

The following table presents the Lansky performance status scale and should be used for subjects < 16 years of age.

Points	Description
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both greater restriction of, and less time spent in, active play
60	Up and around, but minimal active play; keeps busy with quieter activities
50	Gets dressed, but lies around much of the day; no active play; able to participate in all quiet play and activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities
10	No play; does not get out of bed
0	Unresponsive

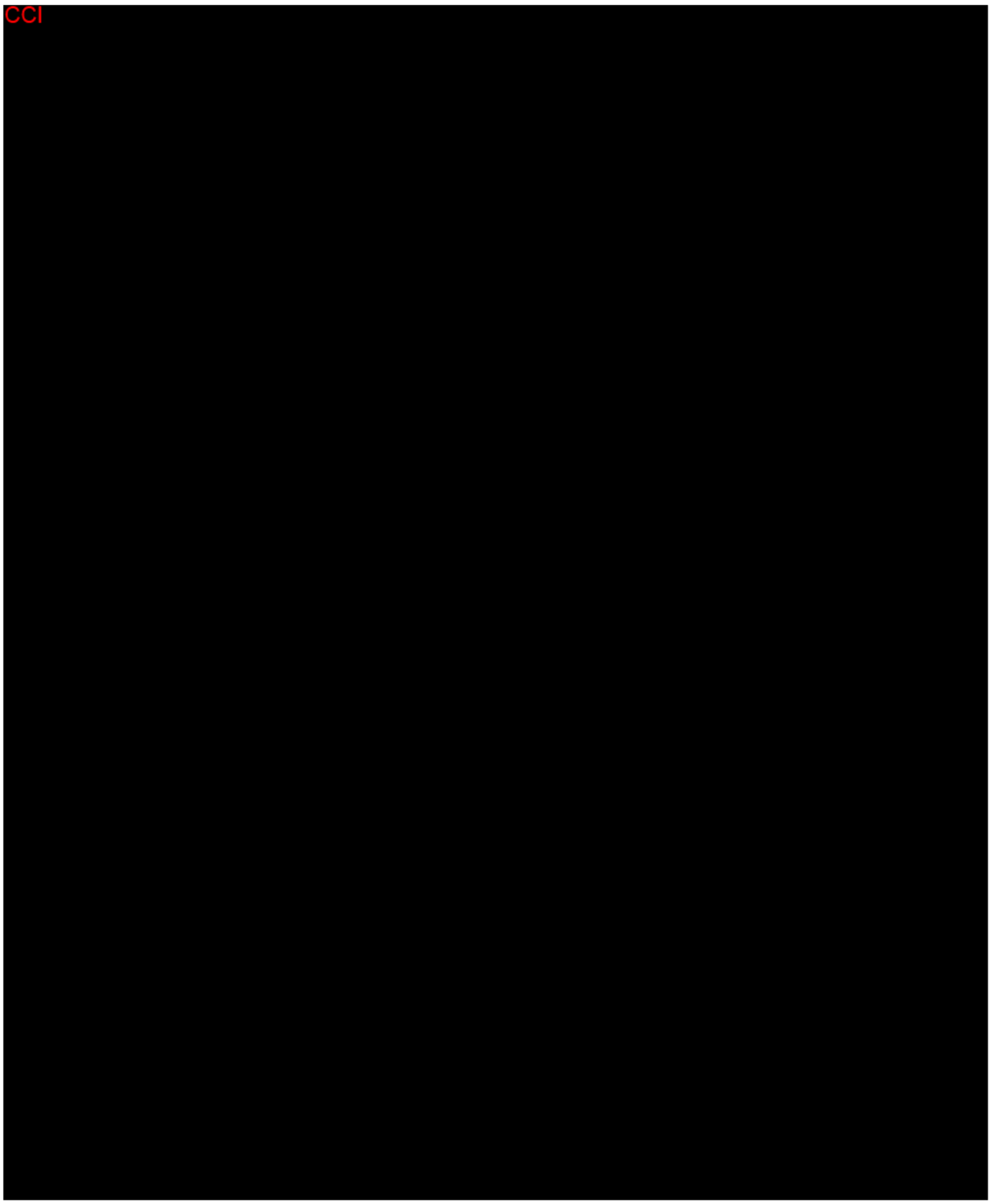
Source: (Lansky et al. 1987)

10.2. Health-Related Quality of Life, Cognitive Function, and Work Productivity Assessments

- Patient Reported Outcomes Measurement Information System (PROMIS)-57 measures Pain Interference, Pain-Intensity, Physical Function, Sleep Disturbance, Satisfaction with Participation in Social Roles, Anxiety, Depression and Fatigue.
- PROMIS Pediatric Profile/PROMIS Parent Proxy Profile assess the beliefs of a pediatric subject/proxy about Physical Function/Mobility, Anxiety, Depressive Symptoms, Fatigue, Peer Relationships, Pain Interference, and Pain Intensity.
- PROMIS Short Form 6a measures perceived cognitive functioning.
- EuroQol-5D (EQ-5D-3L) or EuroQol-5D Youth version (EQ-5D-Y) provide a general measure of overall health status. The EQ-5D-3L Proxy Version 1 or EQ-5D-Y Proxy Version 1 may also be used as appropriate.
- Work Productivity and Activity Impairment-General Health (WPAI-GH/Caregiver WPAI-GH) measures impairments in work and usual activities. The results can provide a quantitative summary of the amount of work time missed, reduced on-the-job effectiveness and amount of other usual activities missed due to general health problems.

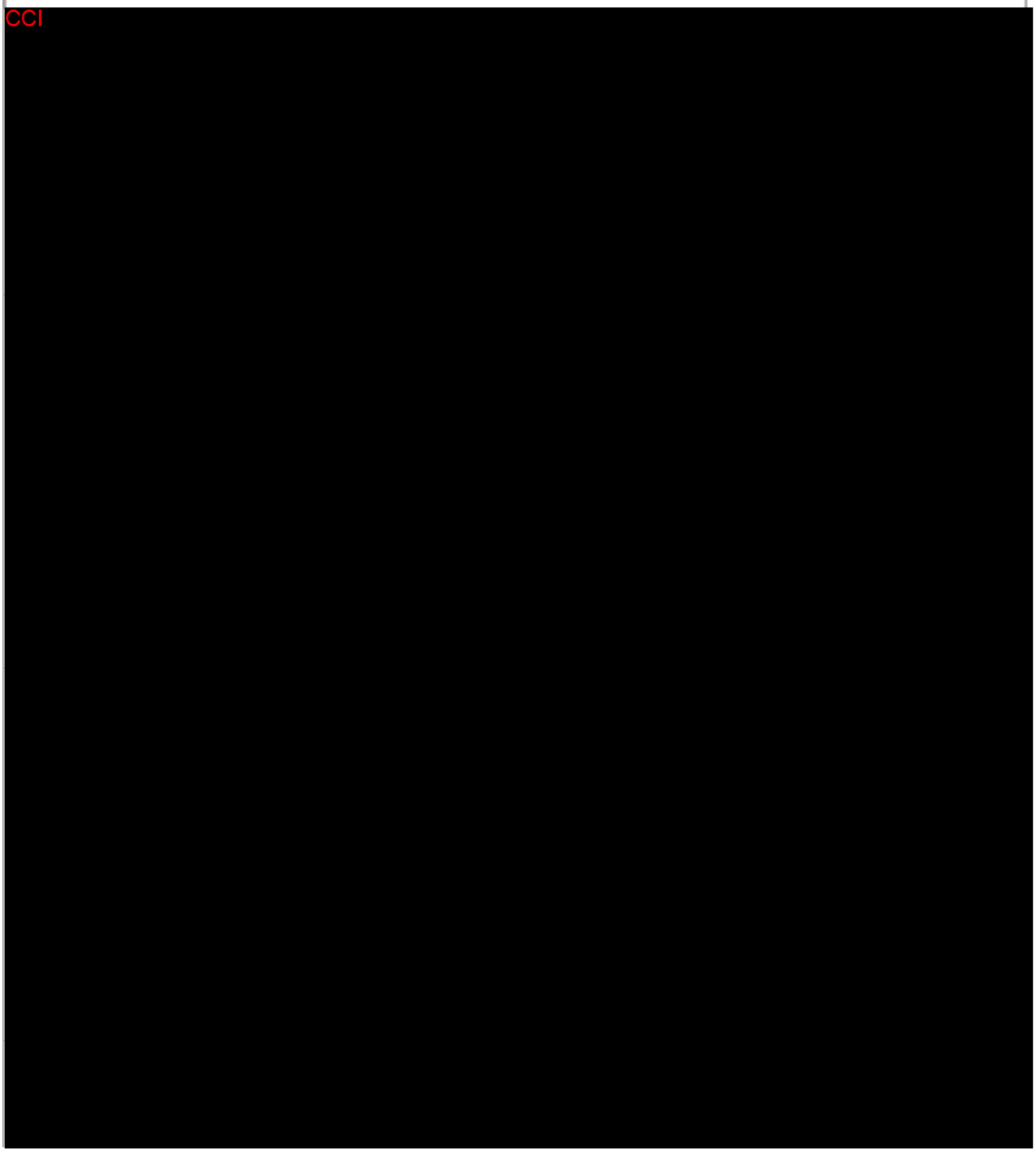
See sample forms below.

PROMIS–57 Profile v2.1

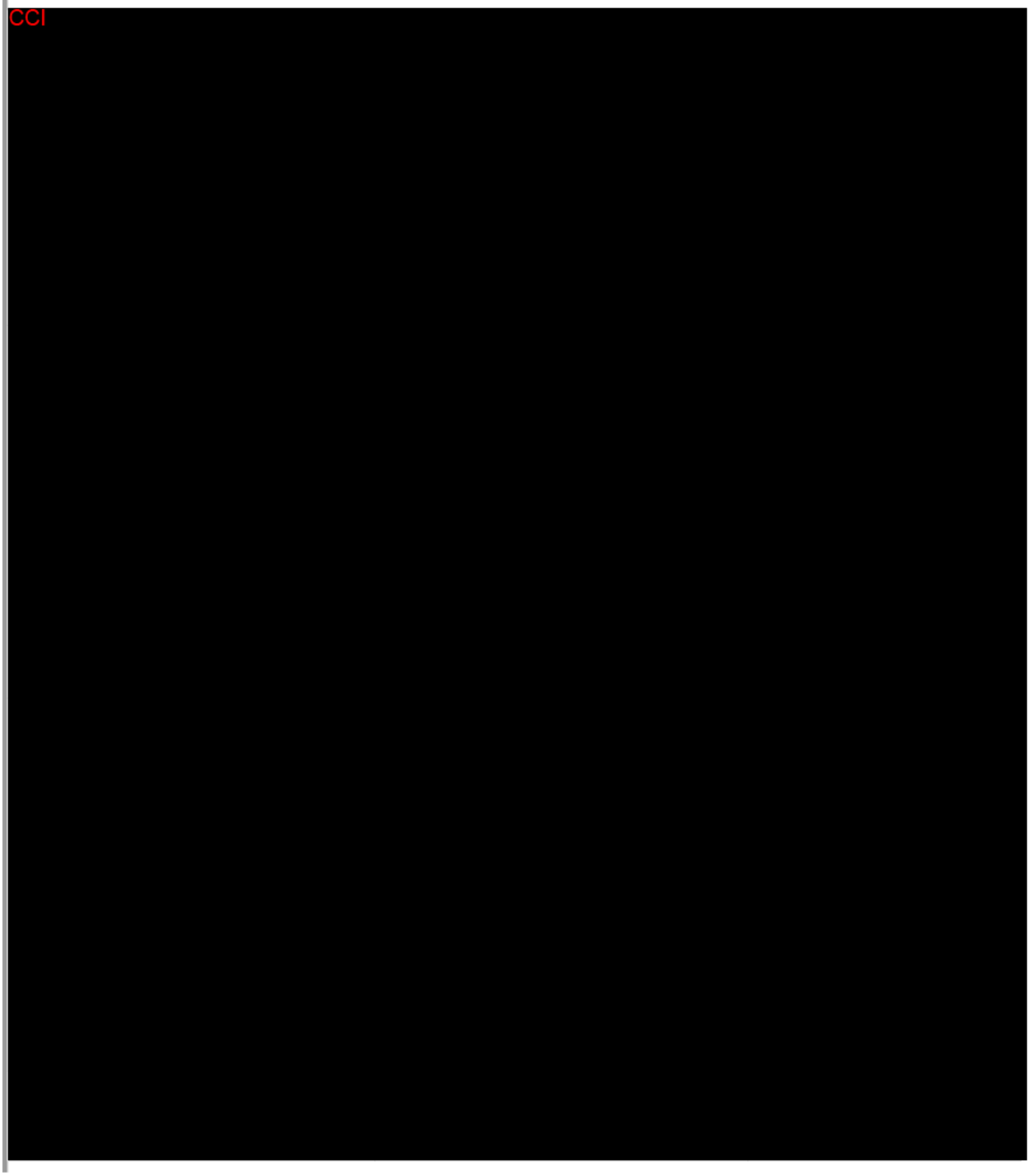


PROMIS–57 Profile v2.1

CCI



PROMIS–57 Profile v2.1



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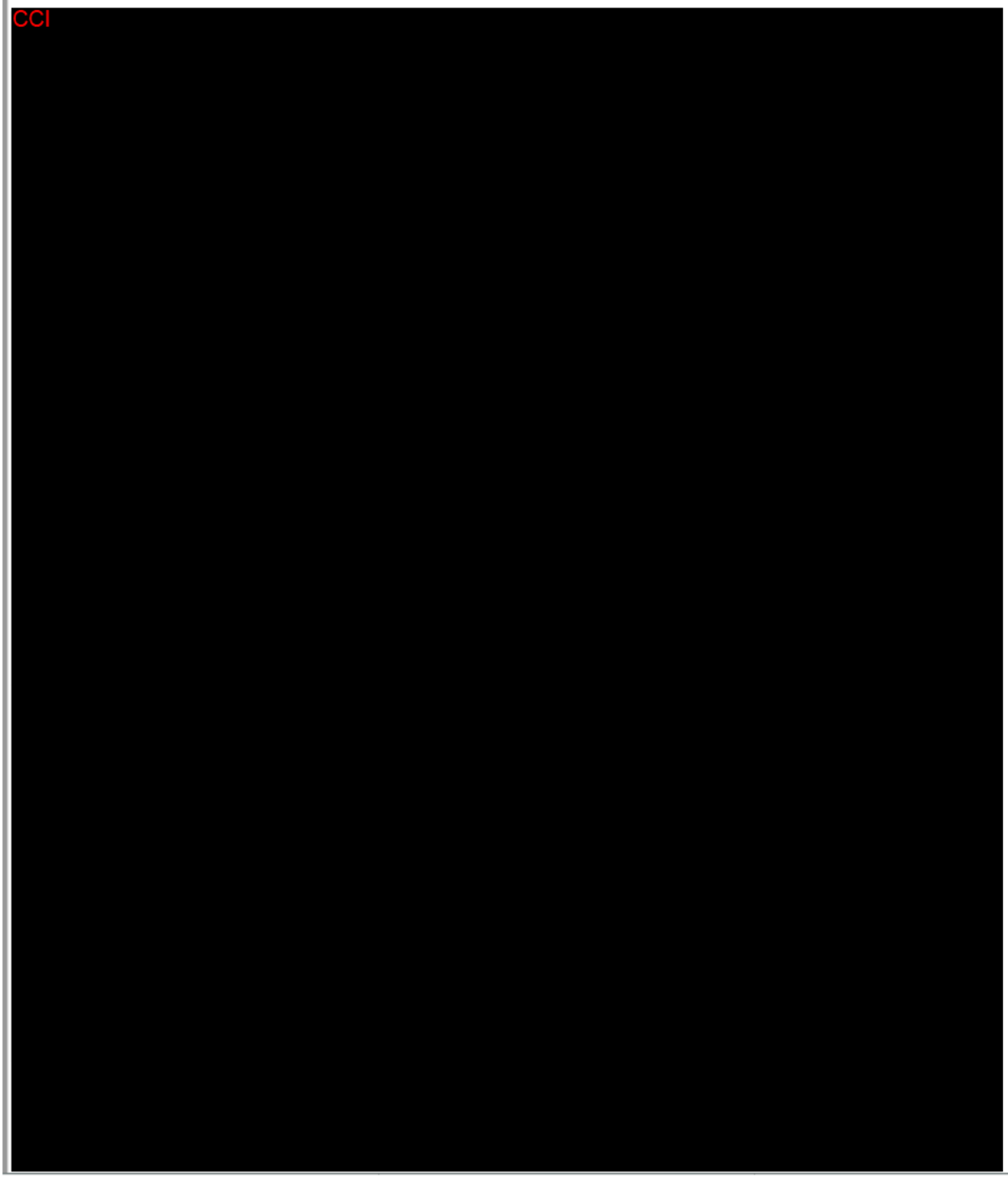
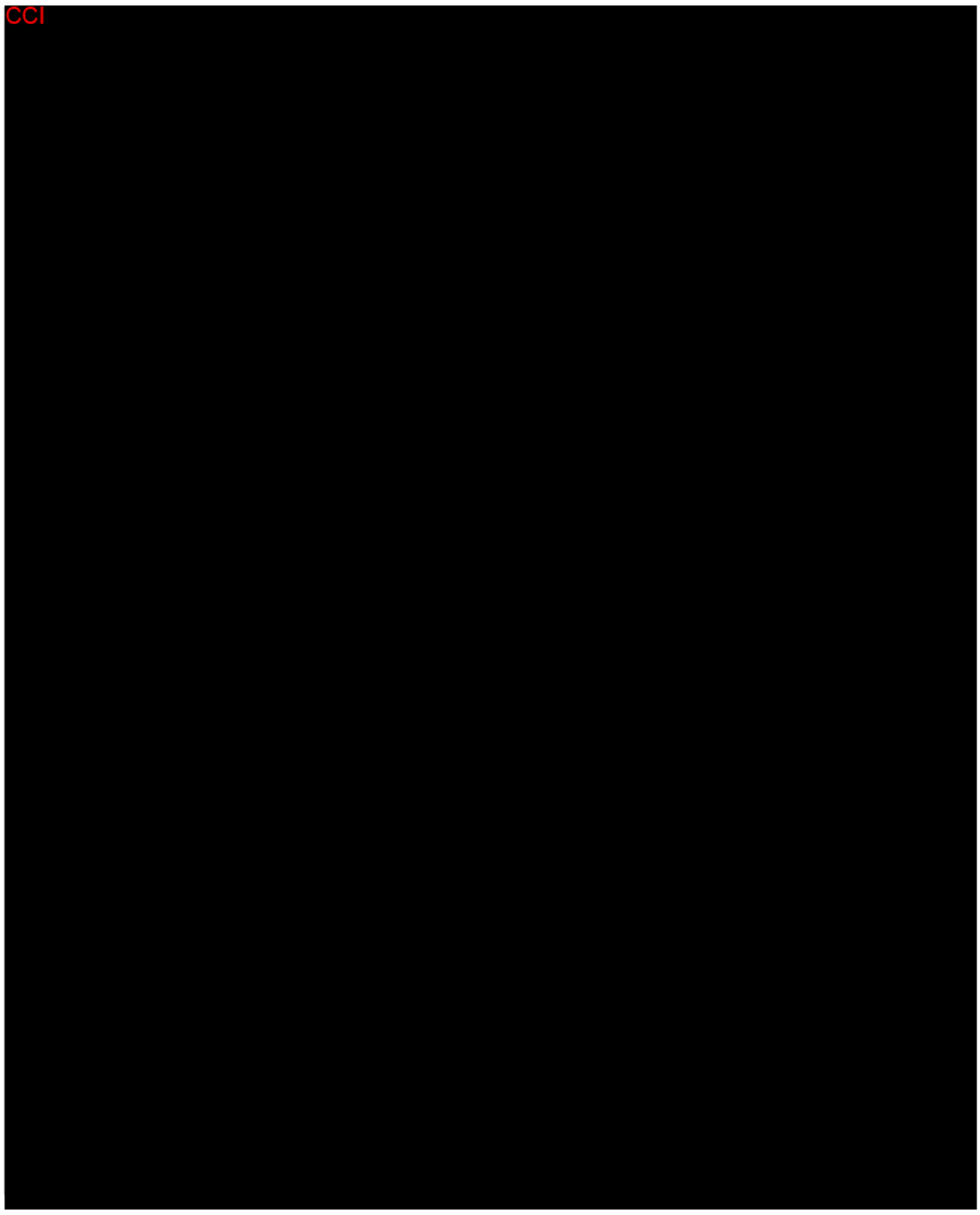
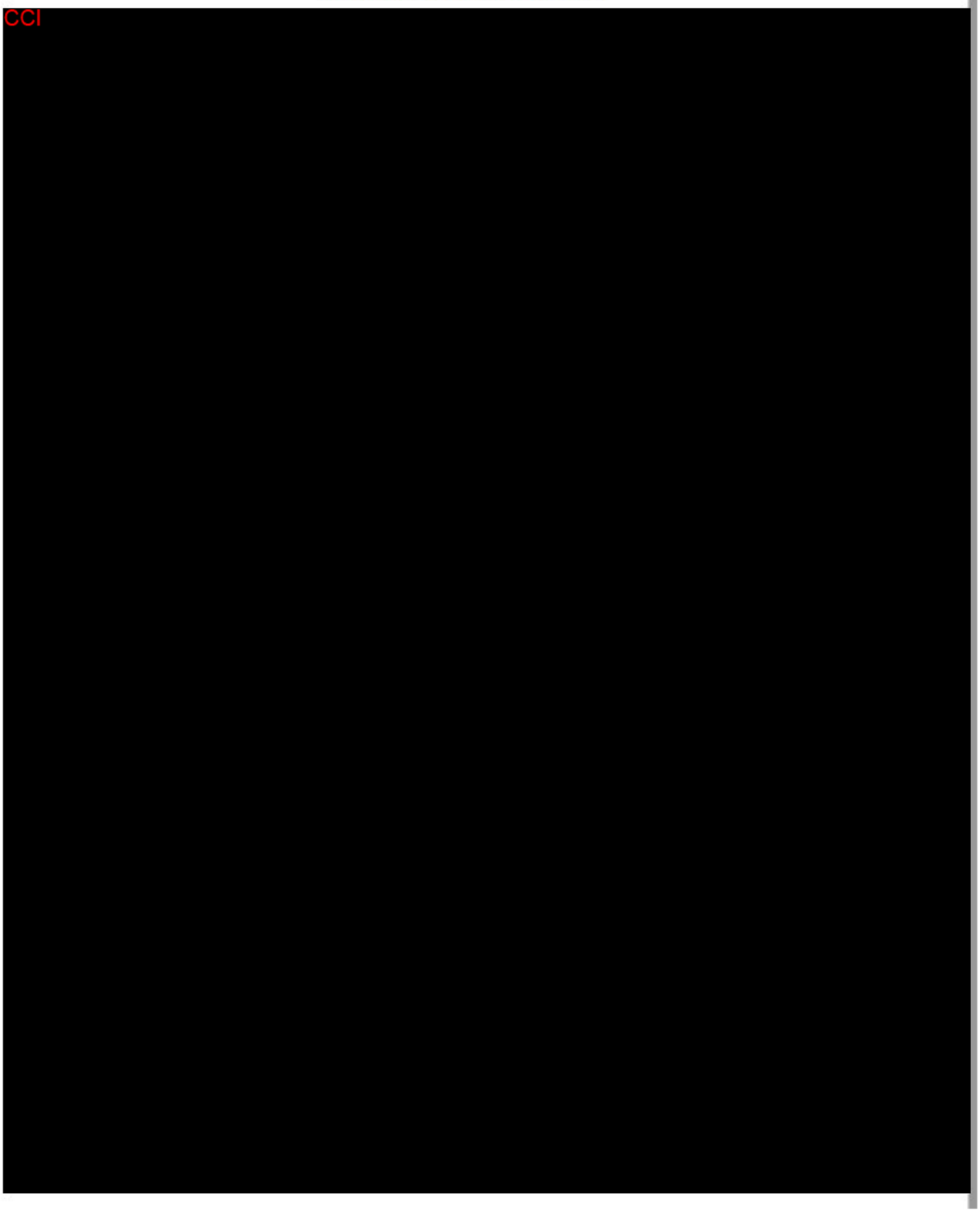


PROMIS Pediatric Profile v2.0 – Profile-49

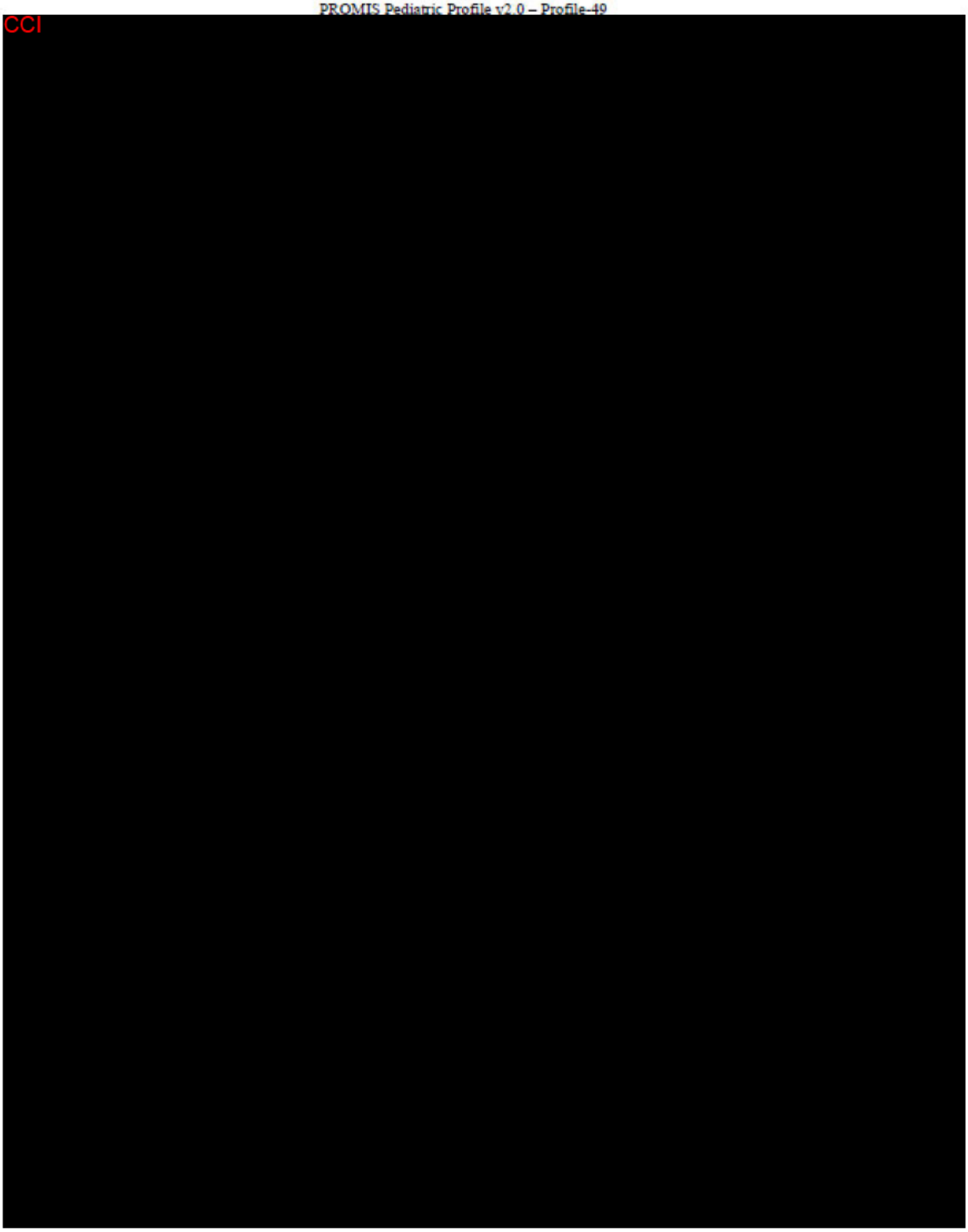
Pediatric Profile – 49



PROMIS Pediatric Profile v2.0 – Profile-49

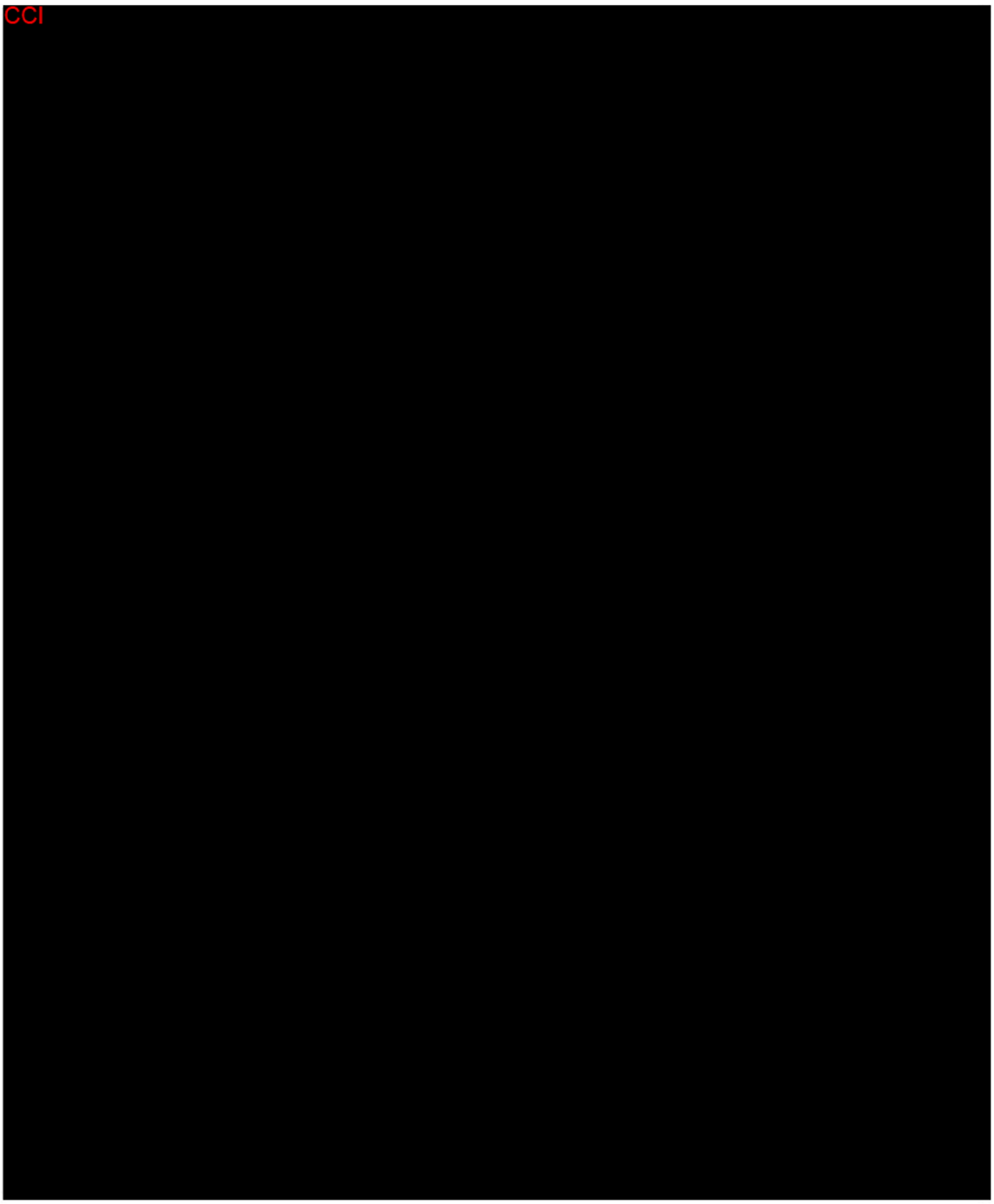


PROMIS Pediatric Profile v2.0 – Profile-49



PROMIS Parent Proxy Profile v2.0 – Profile-49

Parent Proxy Profile – 49



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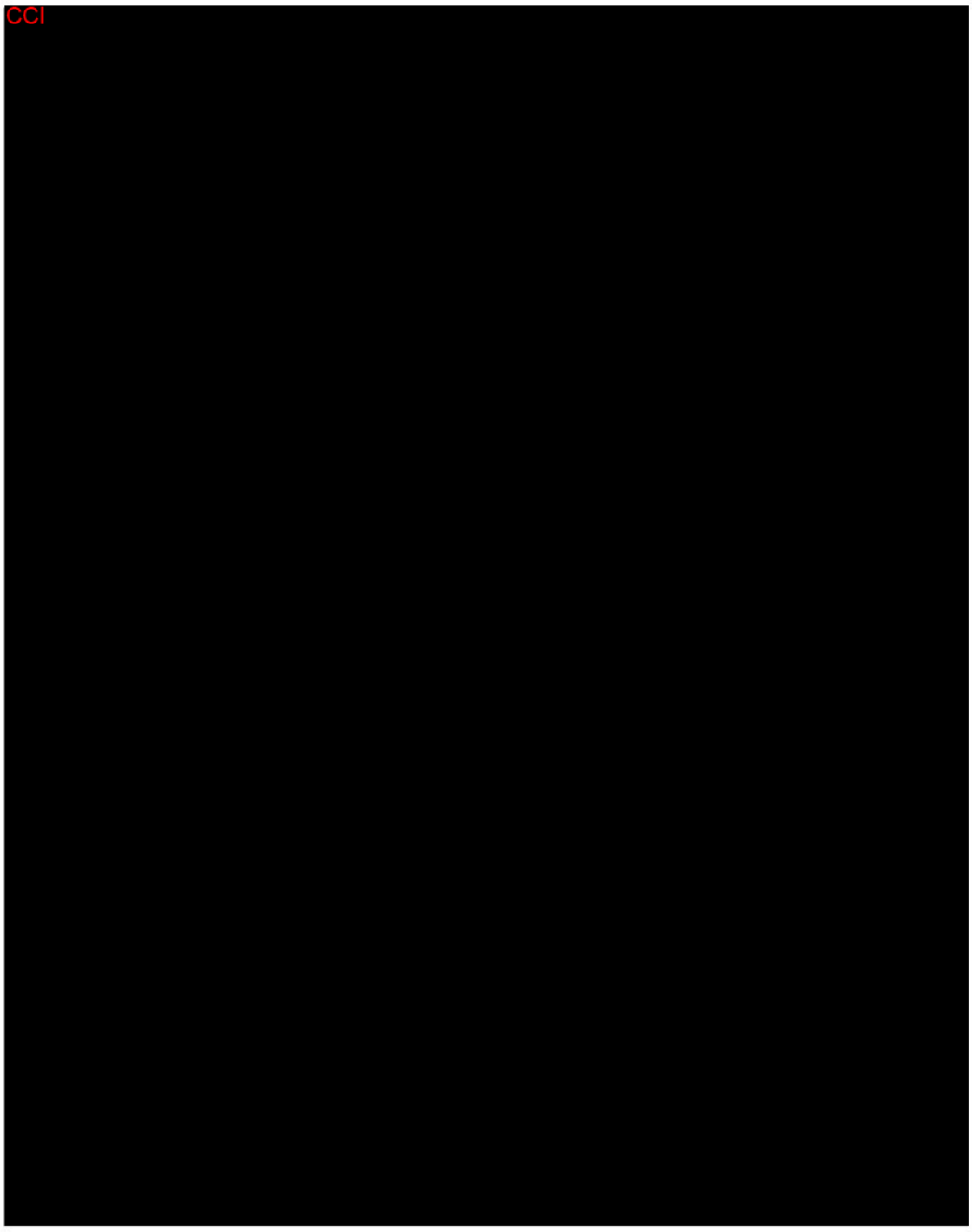
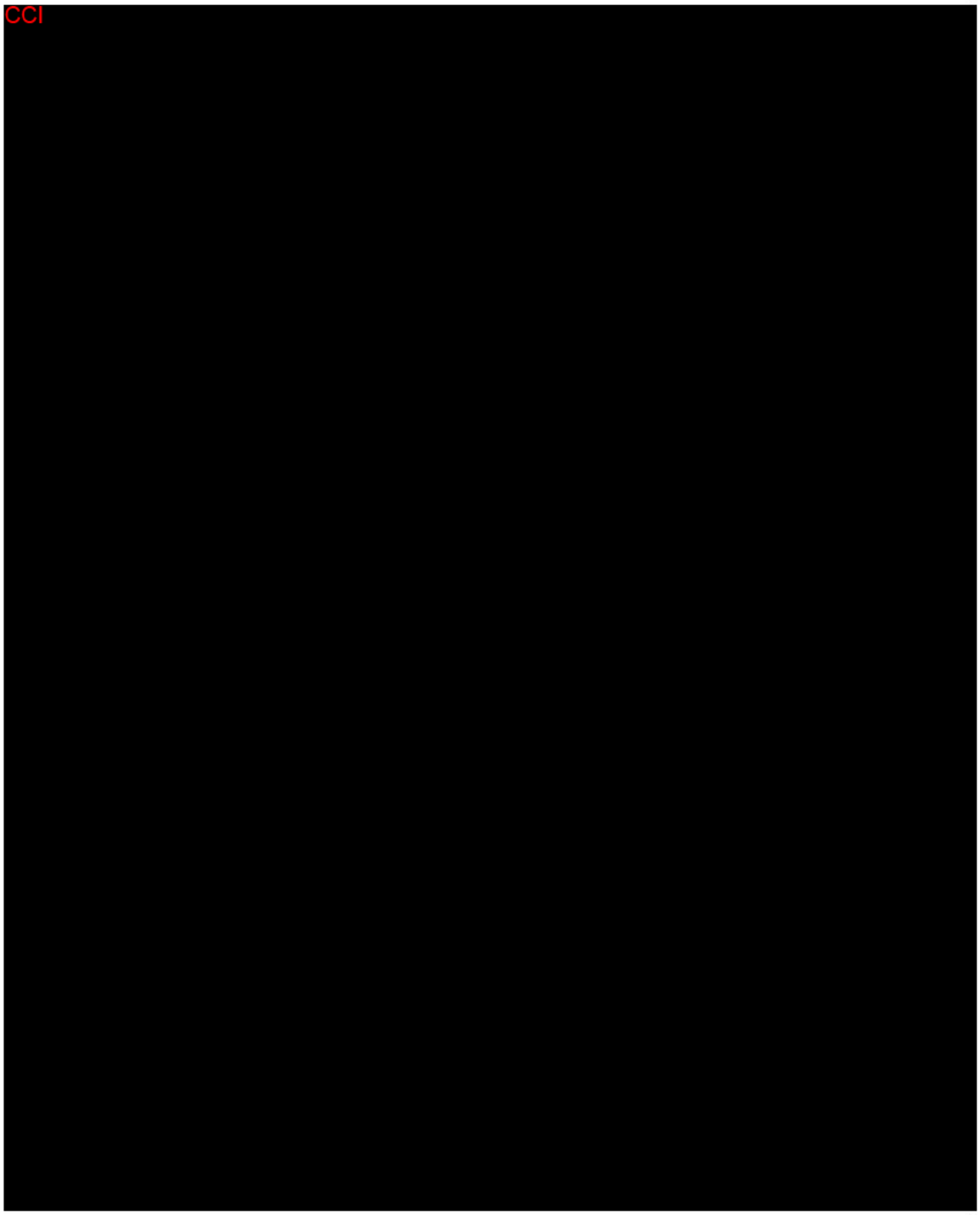
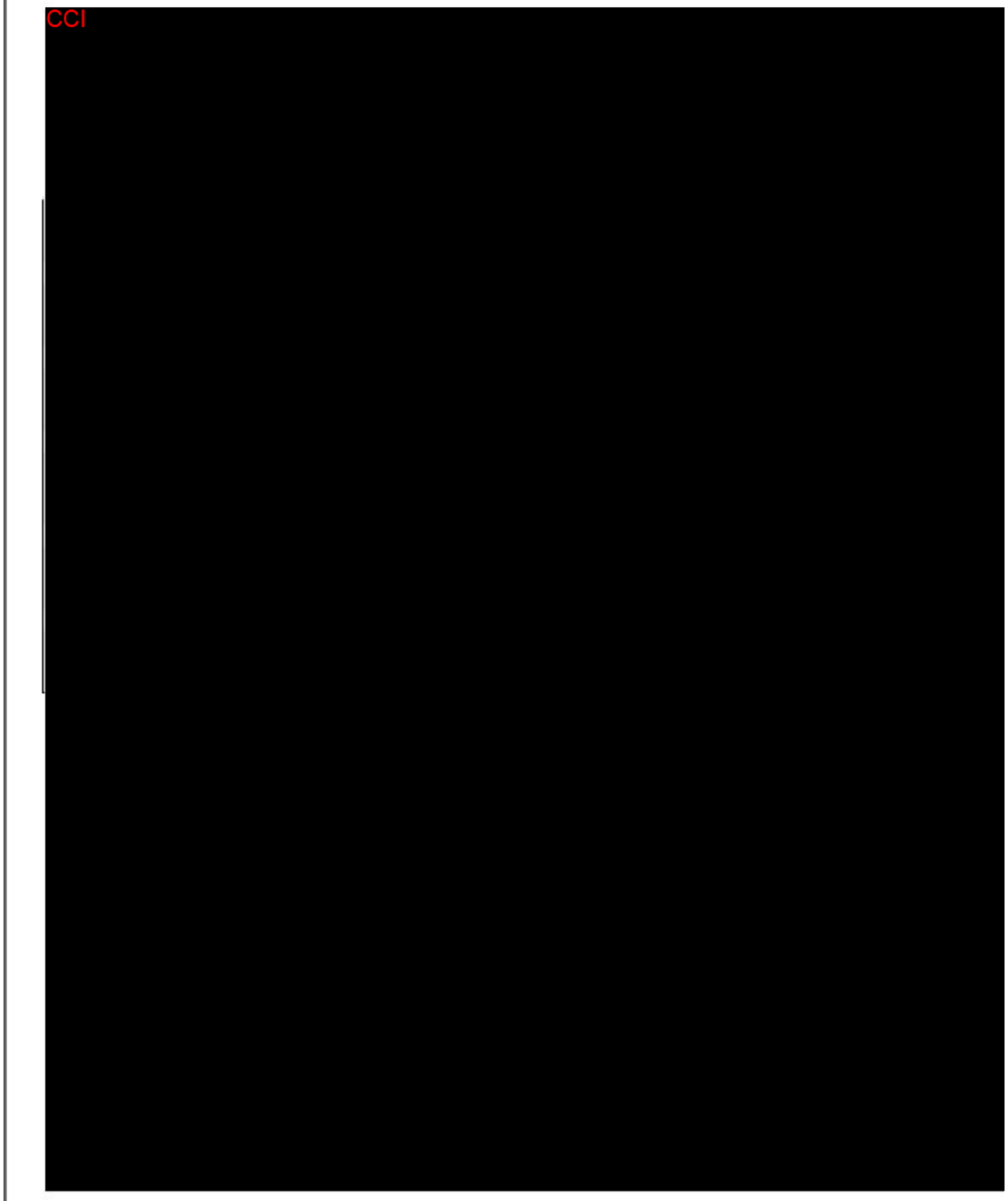


PROMIS Parent Proxy Profile v2.0 – Profile-49



PROMIS Parent Proxy Profile v2.0 – Profile-49

PROMIS Item Bank v2.0 – Cognitive Function- Short Form 6a

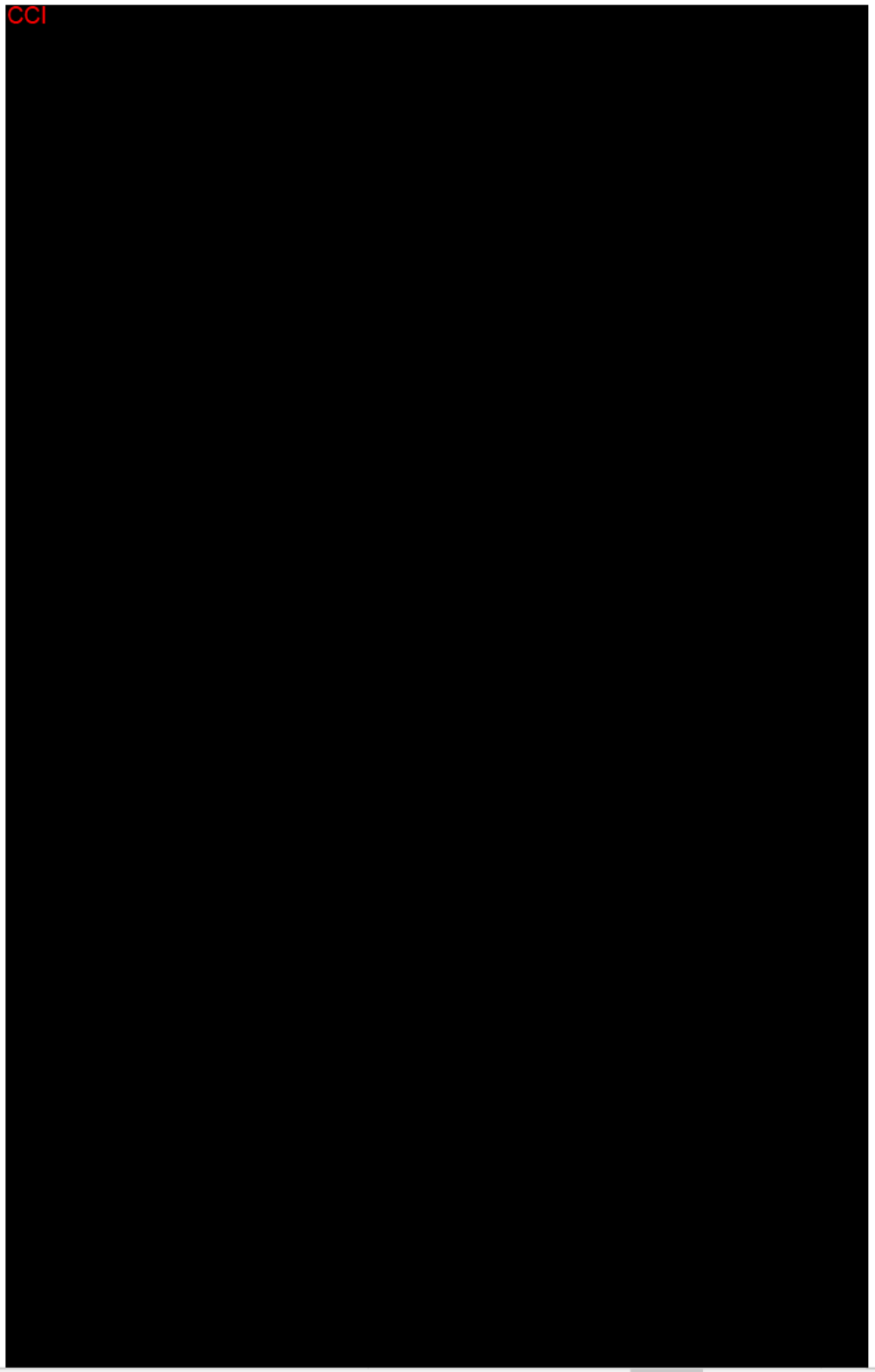


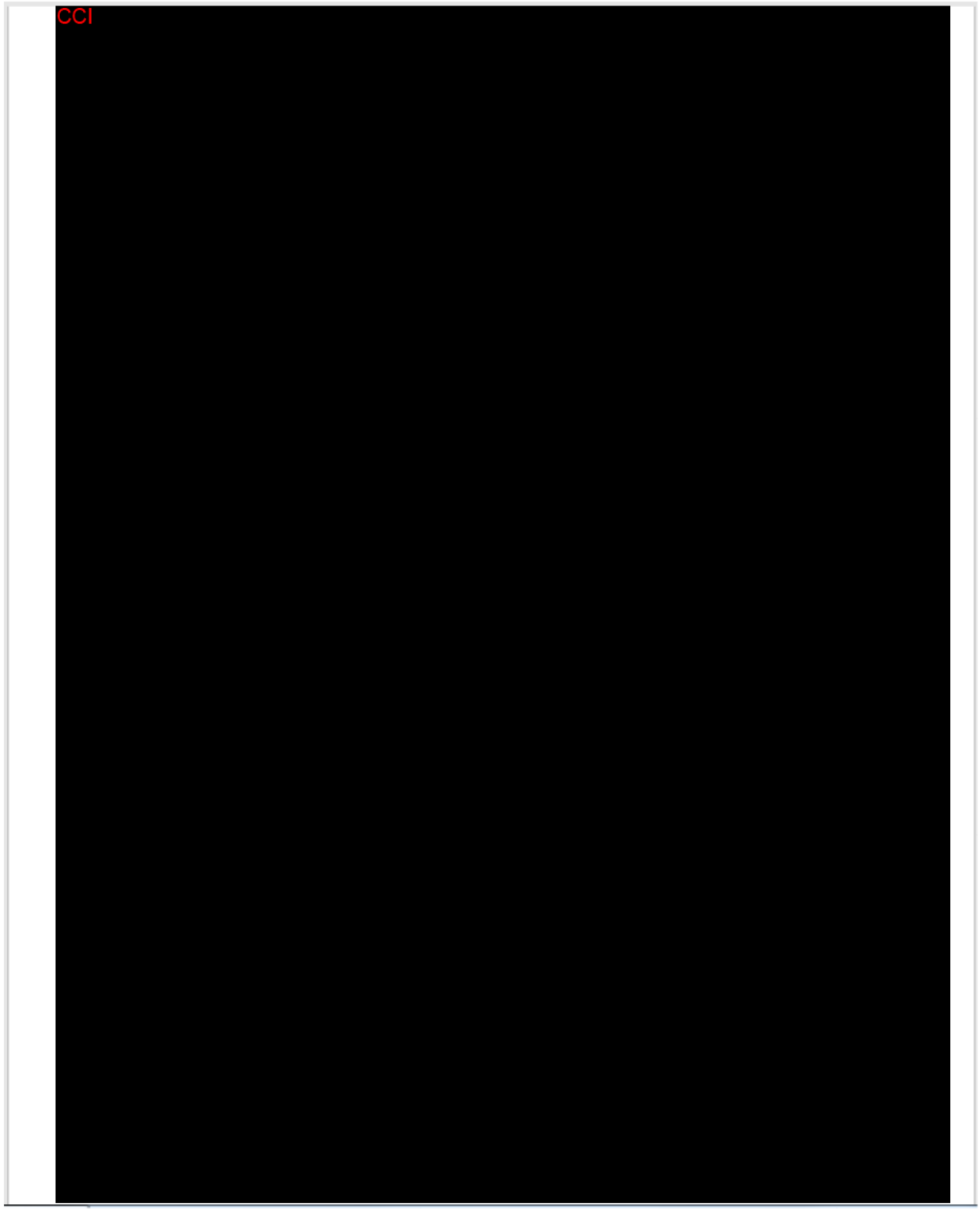
Health Questionnaire

English version for the USA

CCI

CCI




CCI

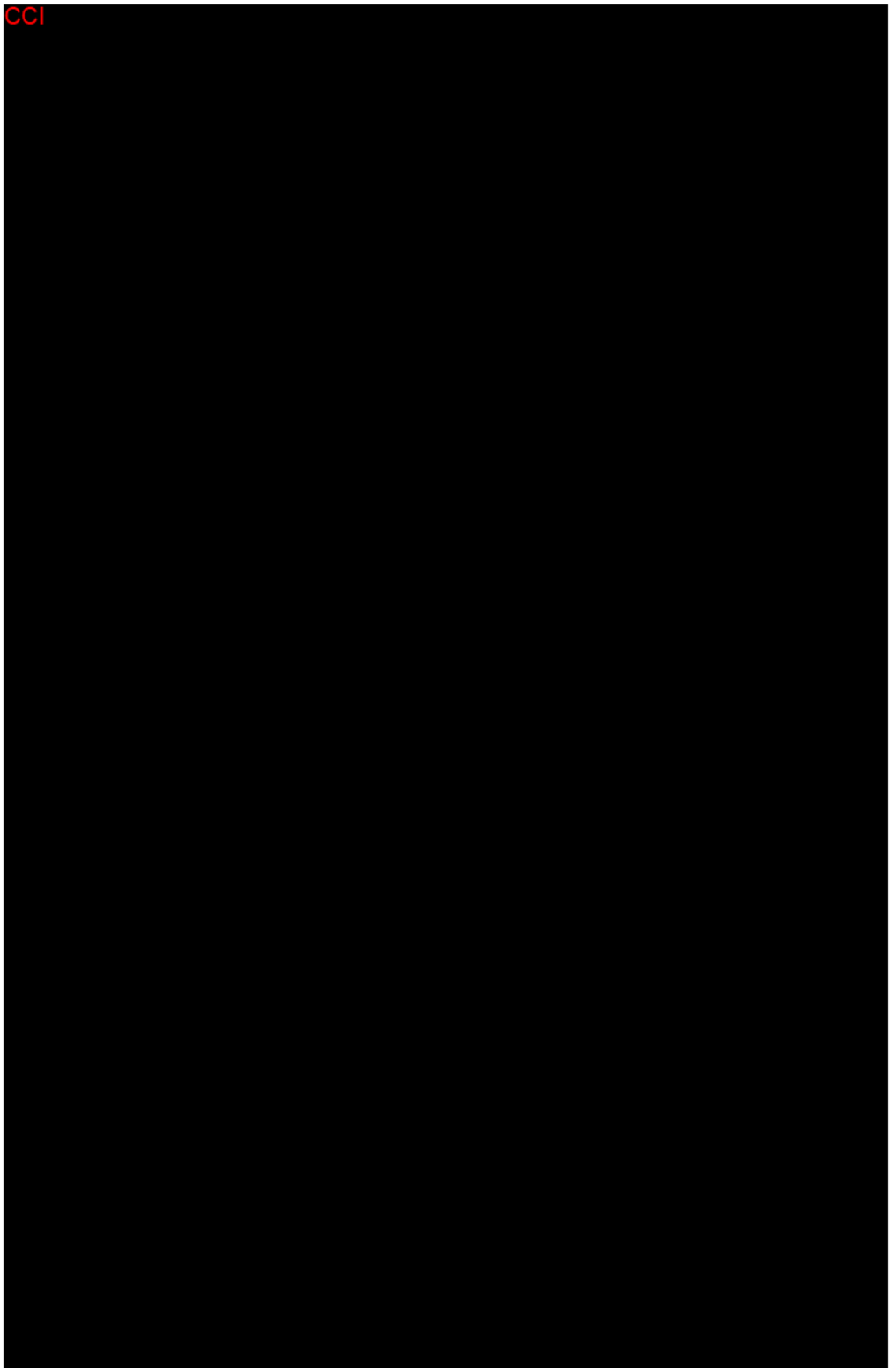
Health Questionnaire

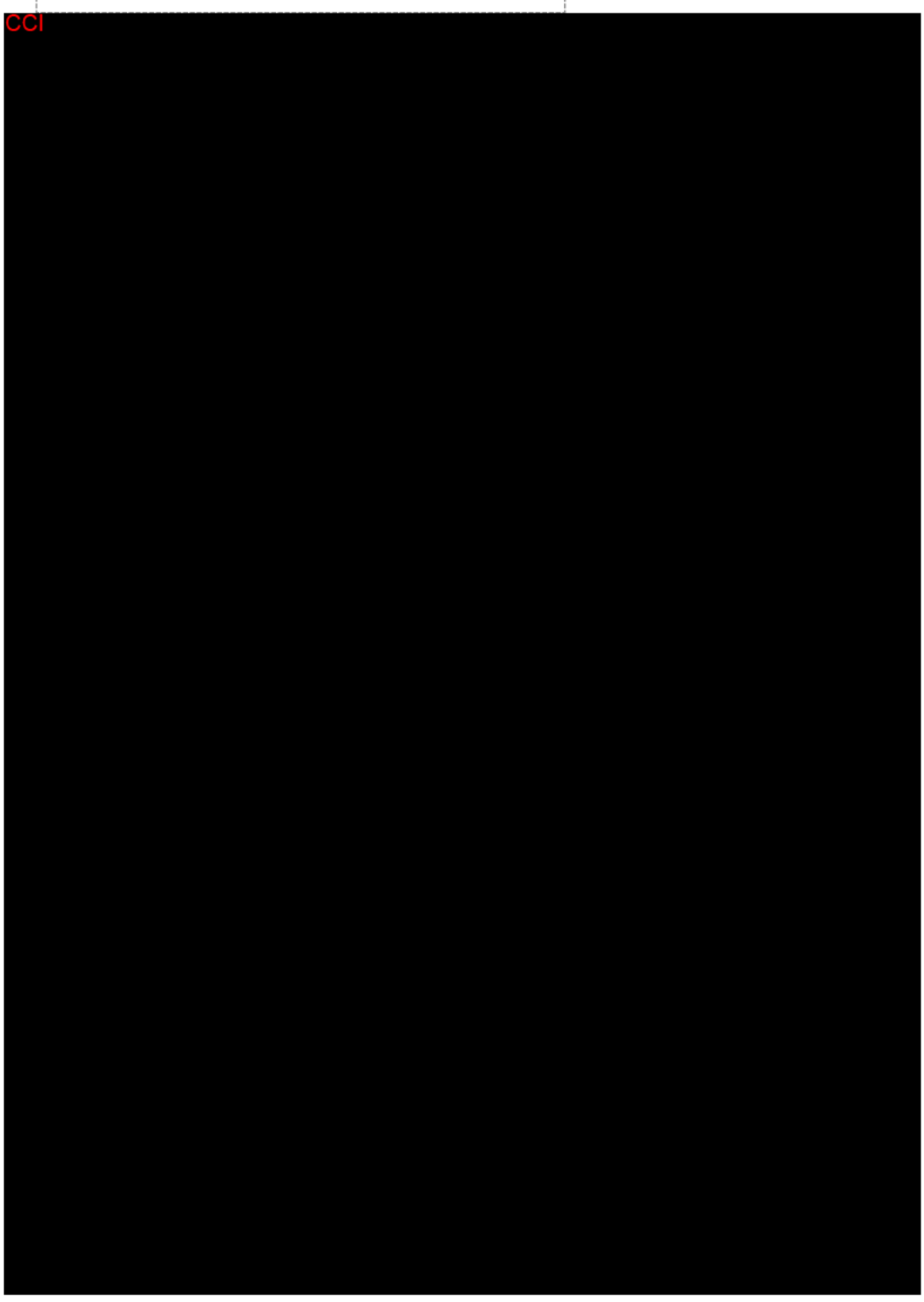
English version for the USA

Script for caregiver version of the EQ-5D: 1
(asks the caregiver to rate how he or she, (i.e. the caregiver), would rate the patient's health)

CCI

CCI




CCI

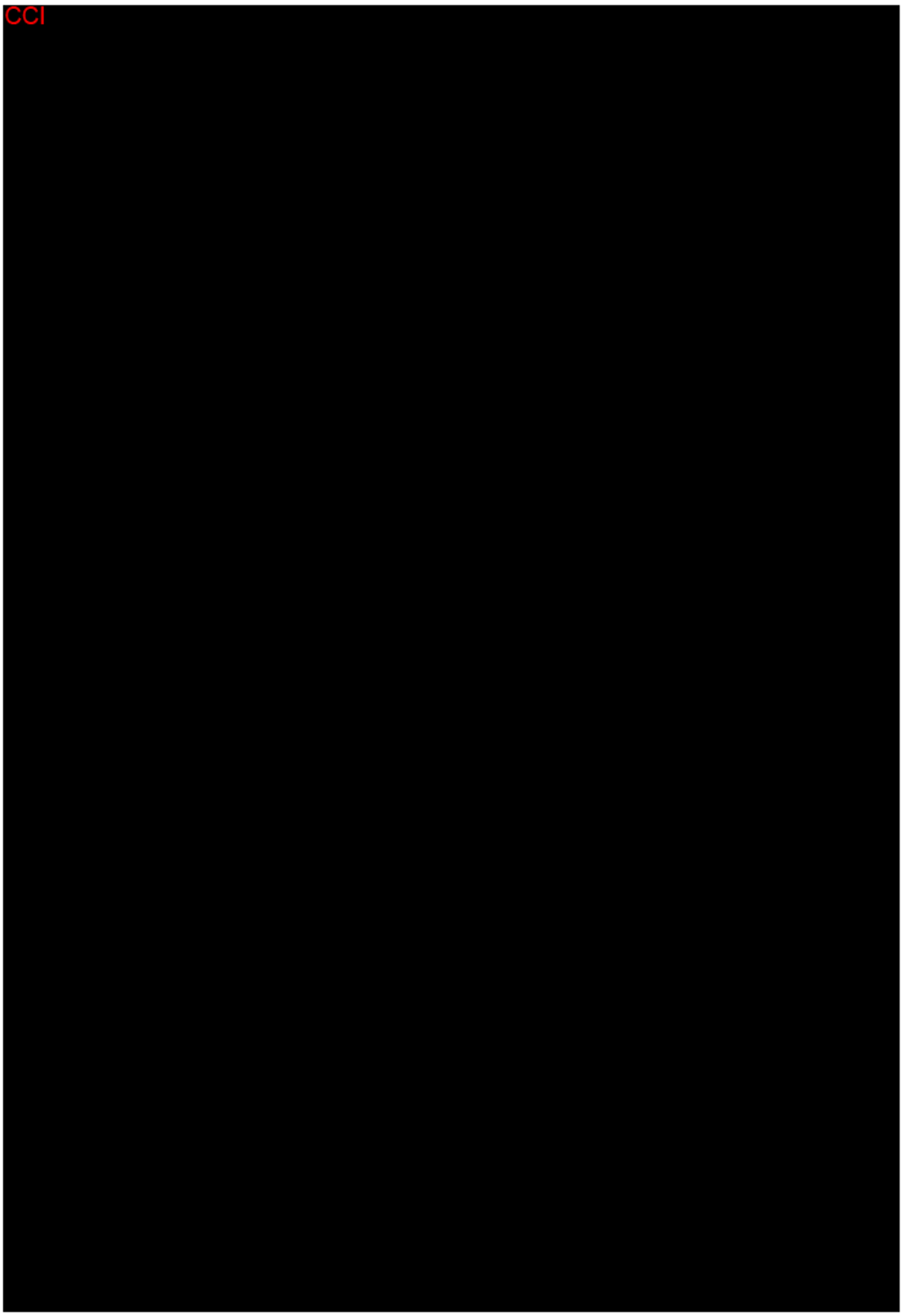
Health Questionnaire

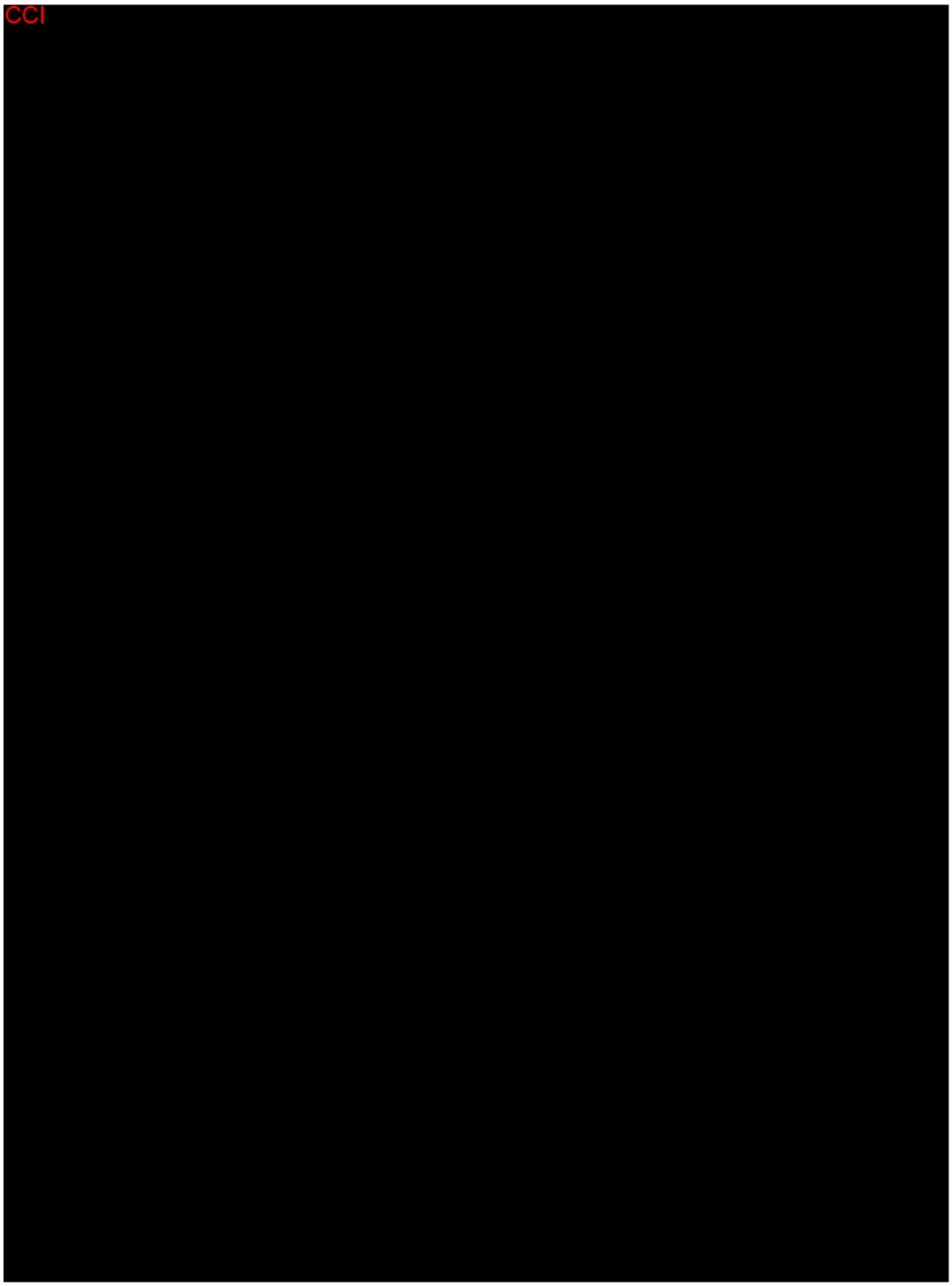
English version for the USA

CCI

EQ-5D-Y

CCI




CCI

Health Questionnaire

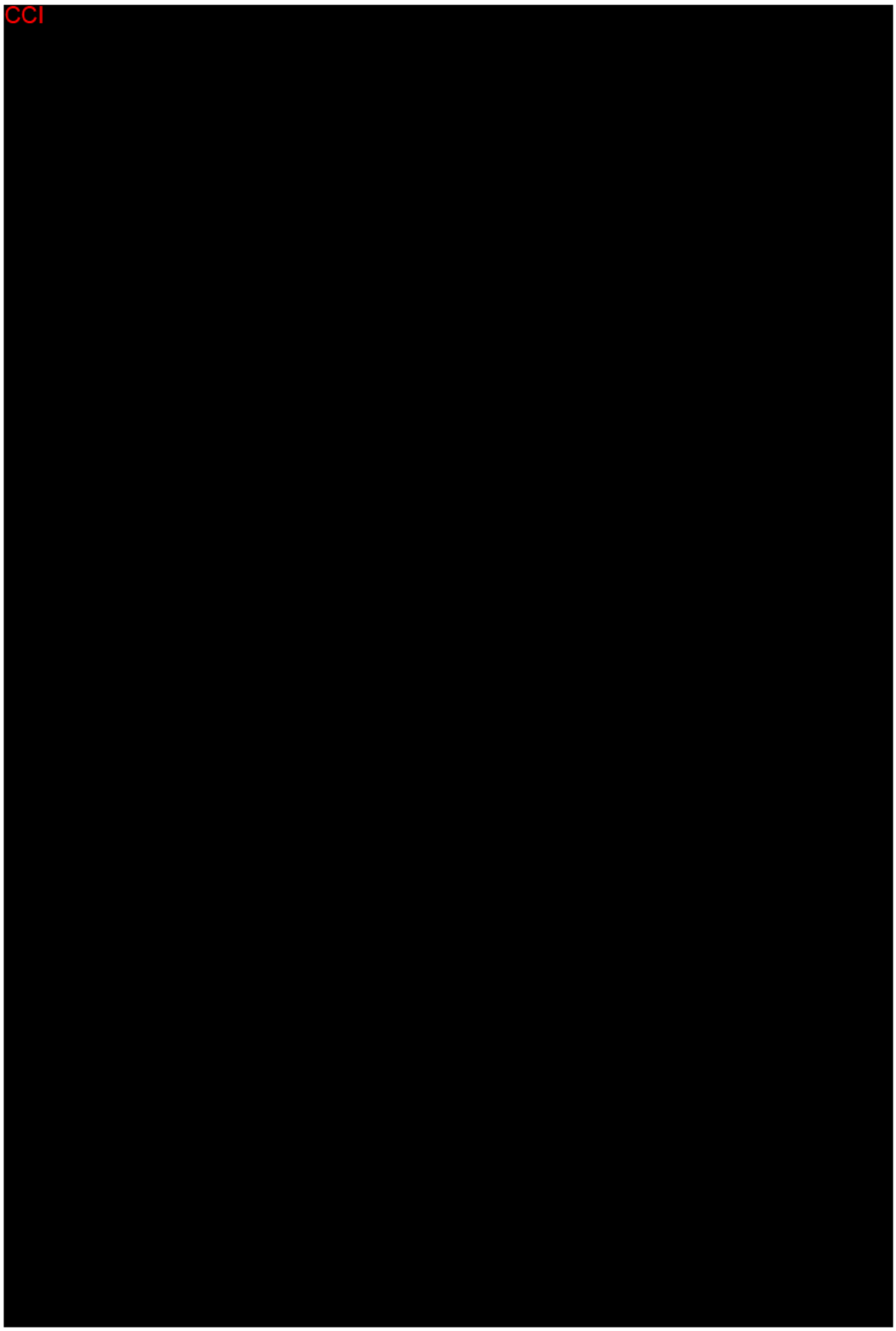
English version for the USA

Proxy version of the EQ-5D-Y: 1

(The purpose of this questionnaire is to explore how a care-giver or someone who knows the child well (proxy), would rate the health of the child. The proxy should not answer on behalf of the child, but rather rate the child's health status as the proxy sees it)

EQ-5D-Y

CCI



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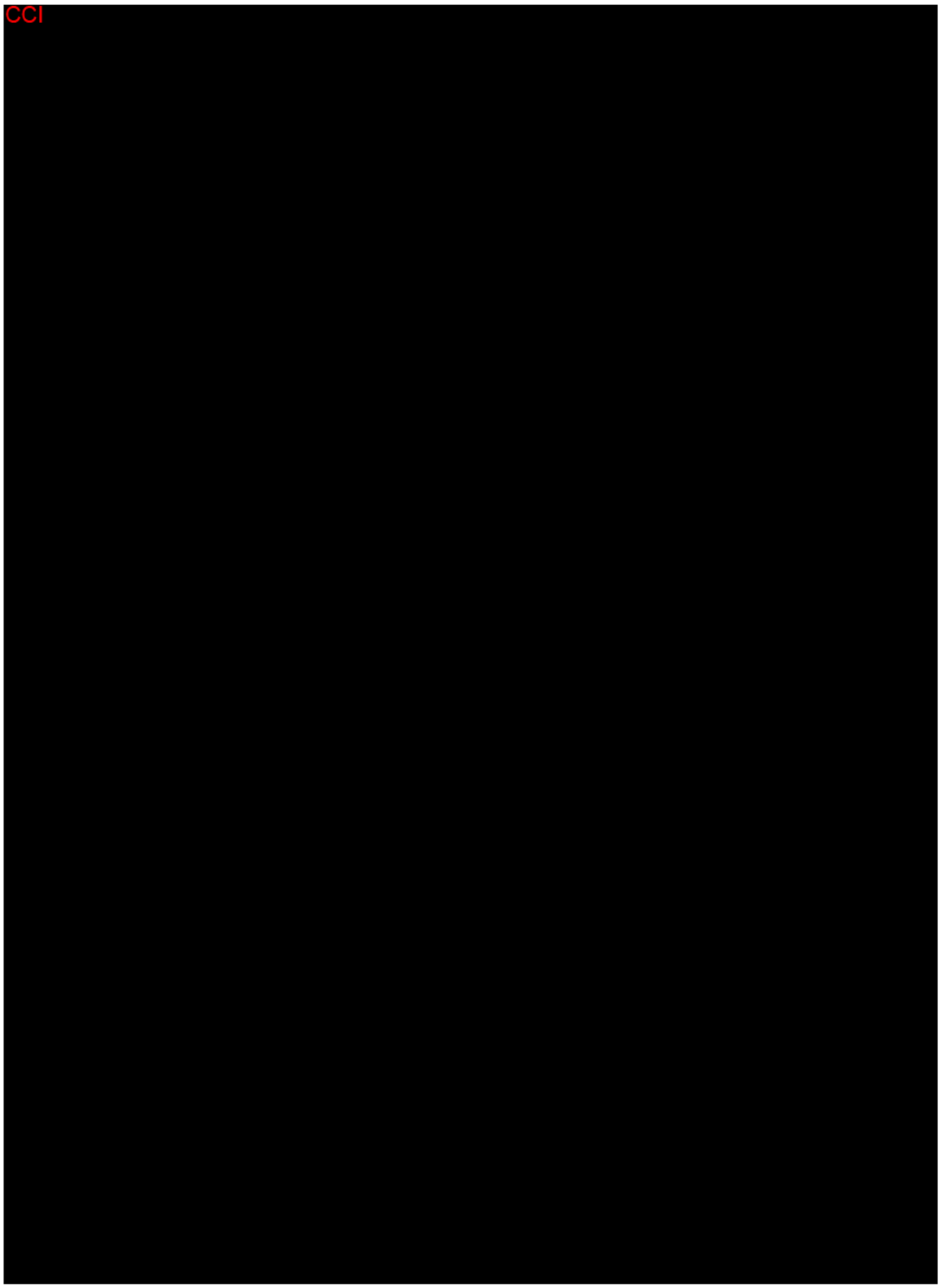
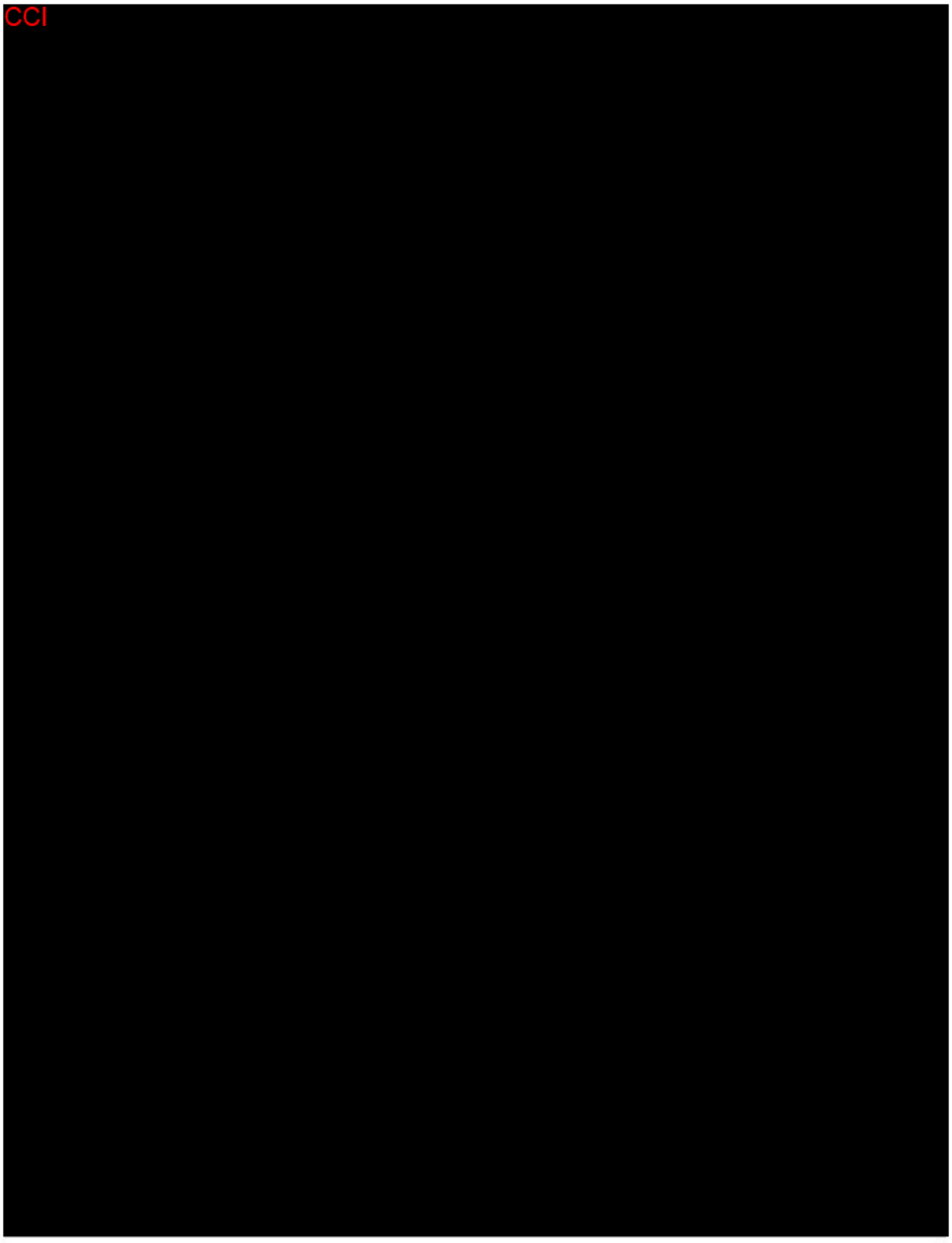
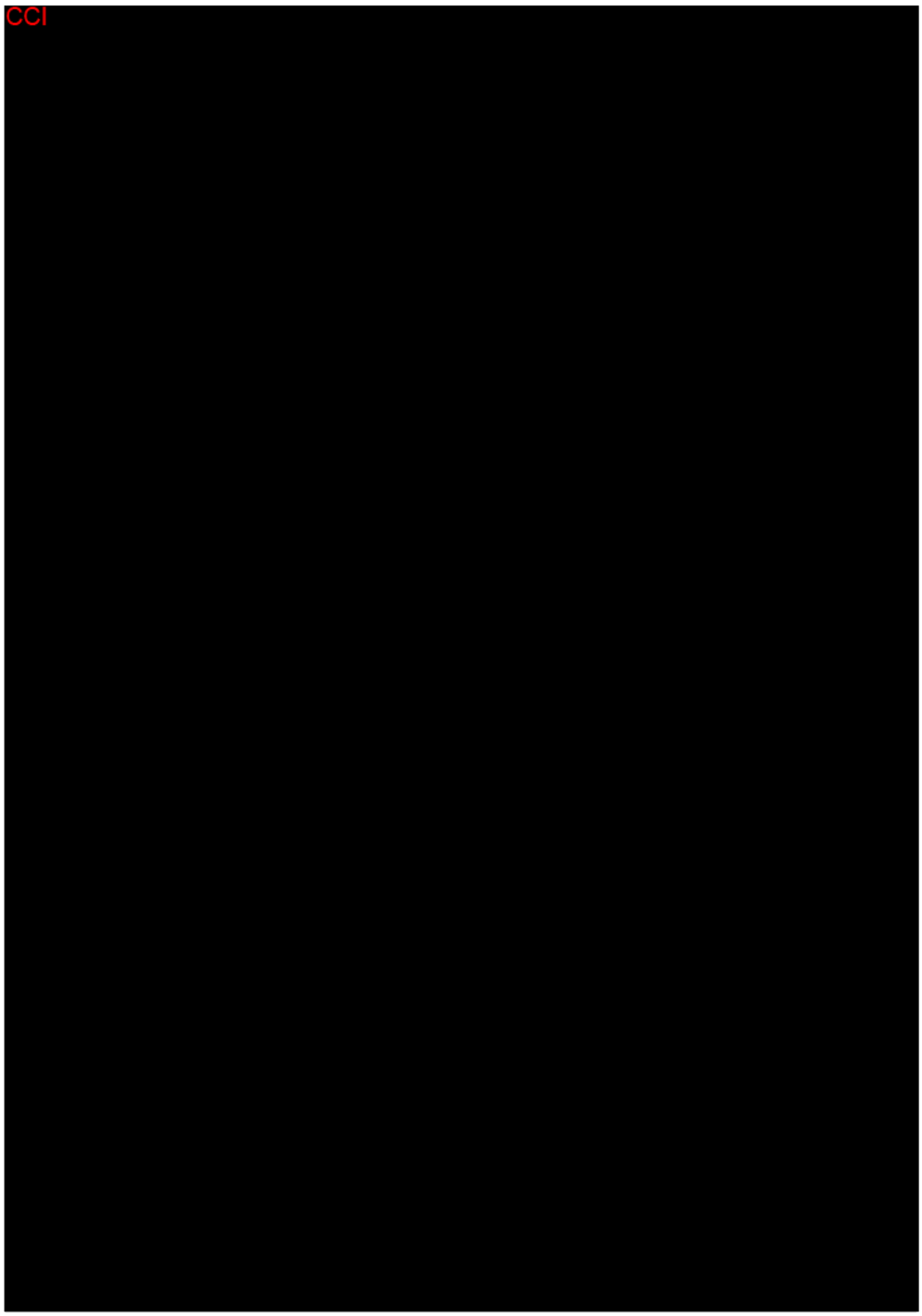


Work Productivity and Activity Impairment Questionnaire: General Health V2.0 (WPAI:GH)

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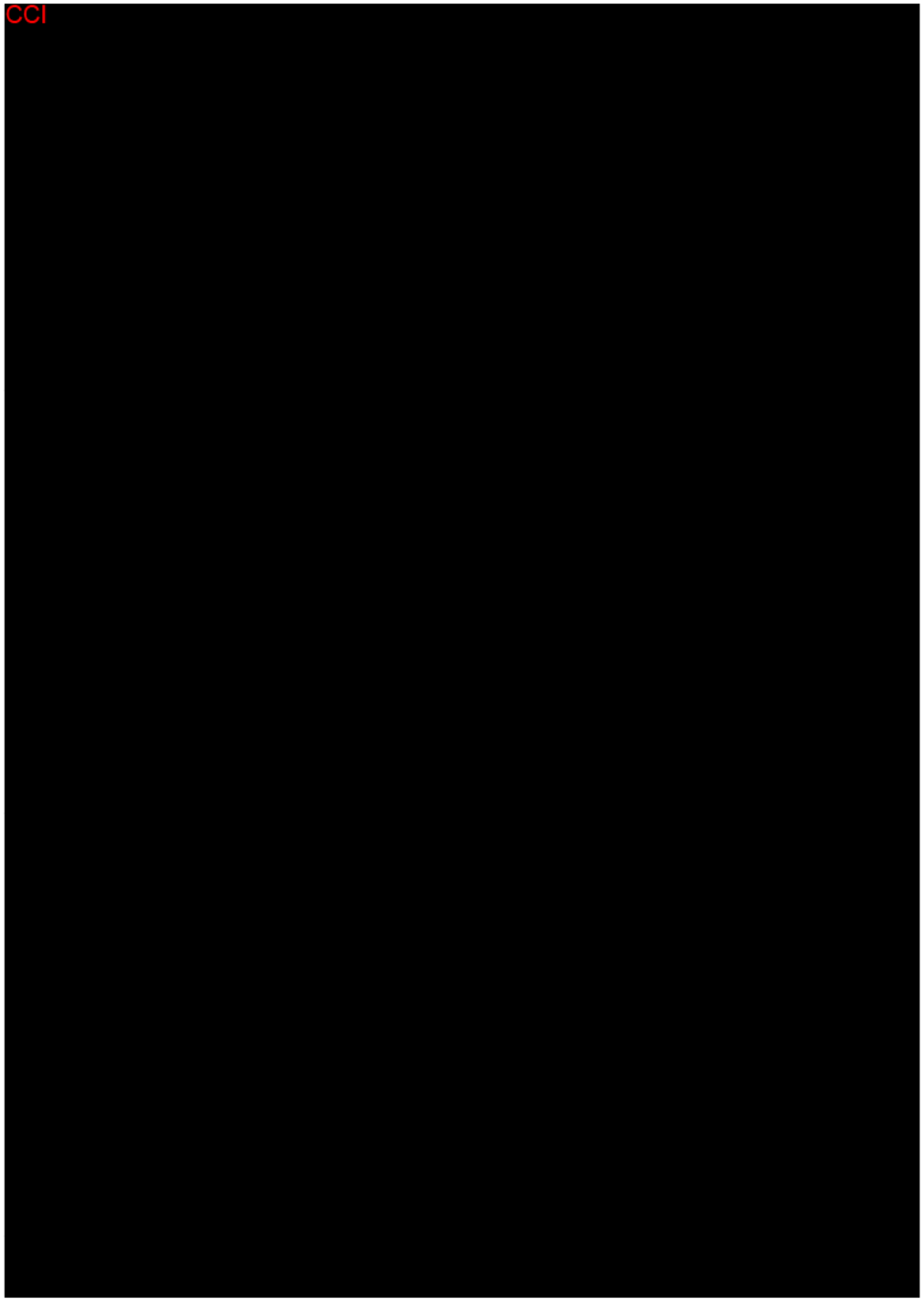
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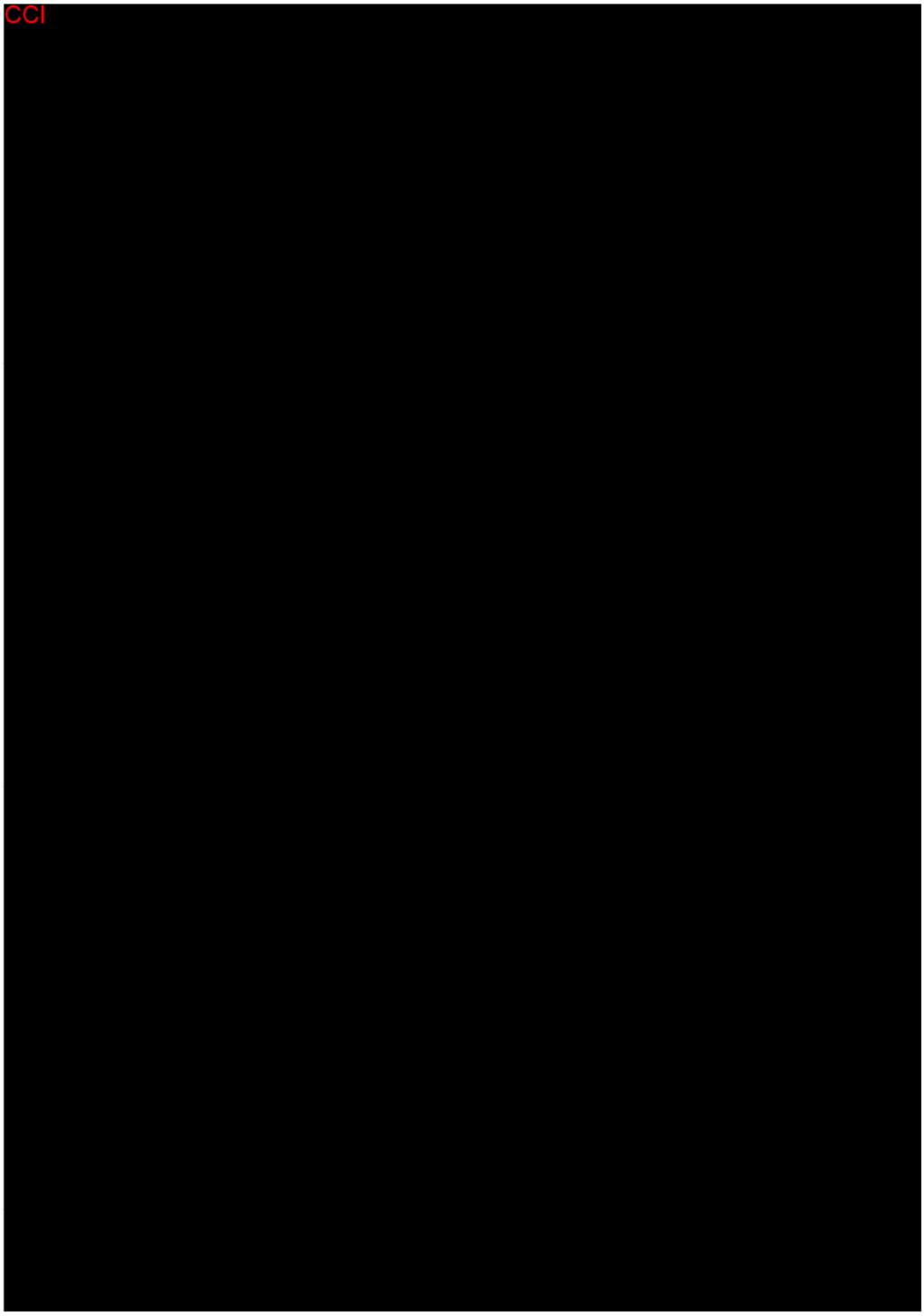
CCI

Protocol Title:	A Phase 1/2 Study Evaluating Gene Therapy by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with the LentiGlobin BB305 Lentiviral Vector in Subjects with Severe Sickle Cell Disease
Protocol Number:	HGB-206 Version 13.0 (17 January 2023)

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)

Signature Page for HGB-206 Protocol V13 v1.0

Approval Task	PPD CTSE 27-Jan-2023 20:59:12 GMT+0000

Approval Task	PPD Clinical Research Development 27-Jan-2023 21:16:44 GMT+0000

Approval Task	PPD Biostatistics 27-Jan-2023 22:22:36 GMT+0000

Approval Task	PPD Pharmacovigilance/Safety 27-Jan-2023 23:15:15 GMT+0000

Approval Task	PPD Regulatory Strategy 30-Jan-2023 17:46:54 GMT+0000

Signature Page for HGB-206 Protocol V13 v1.0