bluebirdbio™

Transforming the Treatment
OF SEVERE GENETIC DISORDERS

PROTOCOL ALD-102

AN OPEN LABEL, NON-RANDOMIZED, SINGLE DOSE, MULTI-CENTER PHASE 2/3 STUDY OF THE SAFETY AND EFFICACY OF LENTI-D MODIFIED AUTOLOGOUS STEM CELLS (LENTI-D DRUG PRODUCT) FOR THE TREATMENT OF SUBJECTS WITH CHILDHOOD CEREBRAL ADRENOLEUKODYSTROPHY (CCALD)

PI David A. Williams, M.D.,

Children's Hospital Boston
Harvard Medical School

DEC 7, 2010

RAC Meeting

PRESENTATION OVERVIEW

Outline

1. *Background on Childhood Cerebral Adrenoleukodystrophy (CCALD)*
2. *Overview of supporting preclinical and clinical data*
3. *Outline of clinical protocol*
4. *Responses to specific reviewer questions*

CCALD: A MONOGENIC, BRAIN DEMYELINATING DISEASE

Clinical Presentation: natural course of the disease

Progressive Demyelination

Loes Score = 7

← at diagnosis

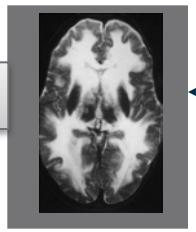
Loes Score = 10

← at 12 months

Loes Score = 16

← at 18 months

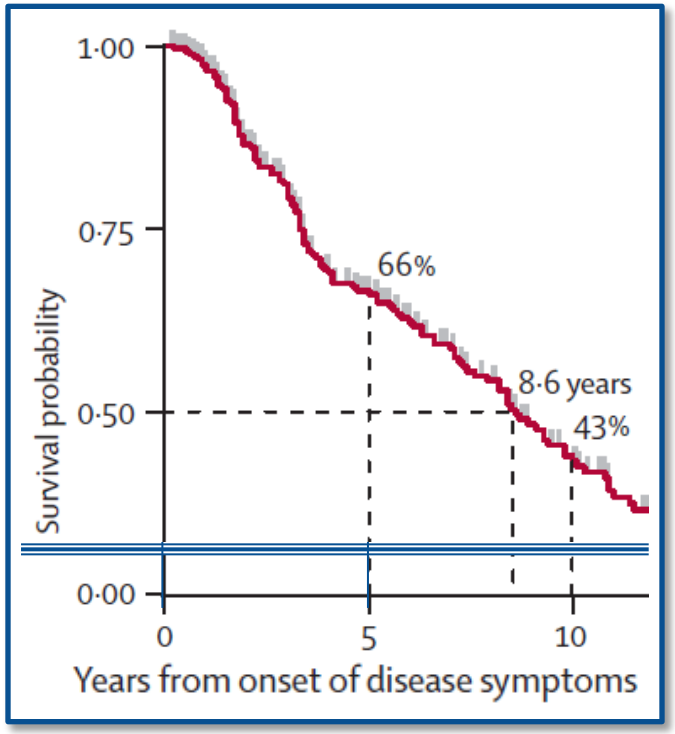
Loes Score = 28



← at 30 months

Modified: Science 2009 Nov 6;326:818-23

Survival



Modified: Lancet Neurol 2007; 6:687-92

- **Disease progression is marked by an initial decline in neurocognitive function, which progresses to a vegetative state, and ultimately, death.**

bluebirdbio

Clinical Presentation: natural course of the disease

Progressive Demyelination

Loes Score = 7

at diagnosis

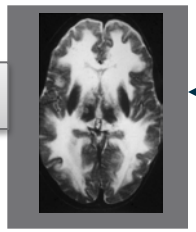
Loes Score = 10

at 12 months

Loes Score = 16

at 18 months

Loes Score = 28



at 30 months

Modified: Science 2009 Nov 6;326:818-23

Loes Score is a continuous scale 0 to 34 points based on the volume of myelin loss manifest on brain MRI. The higher the number the more myelin is destroyed.

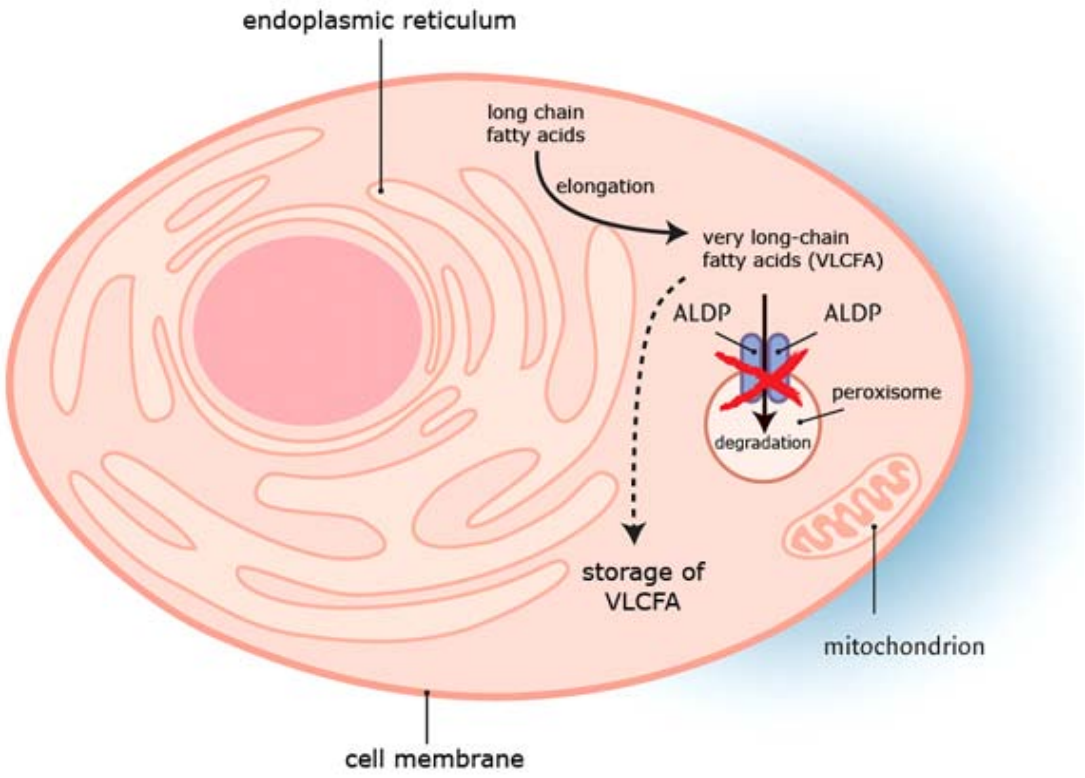
There is a good relationship between Loes score > 4-5 and functionality:

- LS between 1 and 5 : usually cognitive functions are normal ; no neurologic deficits.
- 5 < LS < 10 : cognitive deficits; with LS at 9 or 10, patients may begin to develop obvious visual deficits.
- LS > 12 : patients always have severe cognitive deficits and also various neurologic deficits.
- LS > 15-16 : relationship depends on the age. Often have lost ability to walk and talk.

bluebirdbio

CCALD: CAUSE, PRESENTATION, AND TREATMENT

Defects in the ALD protein lead to a toxic buildup of very long chain fatty acids (VLCFA)



Kennedy Krieger Institute
Research. Treatment. Education.

http://www.x-ald.nl/biochemistry-genetics/vlcfa/

Incidence and Presentation

- Affects boys age 3-15 and manifests with neurocognitive deficits and adrenal insufficiency, diagnosed by elevated plasma VLCFA
- 1/17,000 live births carry mutation; 1/40,000 symptomatic in childhood

Treatment

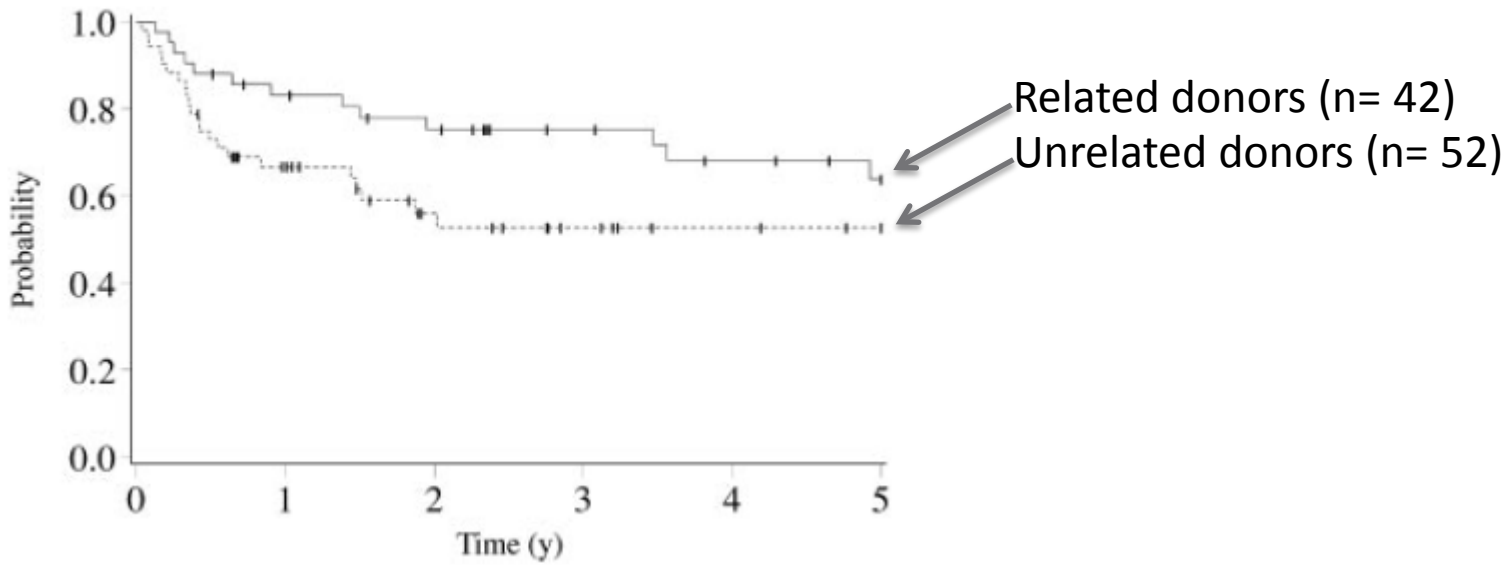
- Hematopoietic Stem Cell Transplant (HSCT) can arrest the course of the disease, but carries a significant risk of procedure related death and SAEs – especially GVHD
- A safer treatment is needed

bluebirdbio

RATIONALE FOR GENE TRANSFER IN CCALD

Hematopoietic cell transplant (HCT) experience: 1982 – 1999

Transplant related adverse events are a significant cause of morbidity and mortality



Blood. 2004 Aug 1;104(3):881-8.

- The most recent study reports survival 6 months post cord blood HCT at only 67% (n=12; Beam et al. 2007, Biol Blood Marrow Transplant)
- Efficacy from HCT is thought to result from the replacement of brain microglia with ALDP-producing cells derived from donor bone marrow.
- **Gene transfer restores ALDP function in a patient's own cells, and can potentially arrest disease progression without some of the safety risks of allogeneic HCT**

bluebirdbio

SUPPORTING CLINICAL DATA FOR GENE TRANSFER IN CCALD

Promising data for lentivirus mediated gene transfer in CCALD

Science 2009

Hematopoietic Stem Cell Gene Therapy with a Lentiviral Vector in X-Linked Adrenoleukodystrophy

Nathalie Cartier,[1,2]* Salima Hacein-Bey-Abina,[3,4,5]* Cynthia C. Bartholomae,[6] Gabor Veres,[7] Manfred Schmidt,[6] Ina Kutschera,[6] Michel Vidaud,[1] Ulrich Abel,[6] Liliane Dal-Cortivo,[3,5] Laure Caccavelli,[3,5] Nizar Mahlaoui,[8] Véronique Kiermer,[9] Denice Mittelstaedt,[10] Céline Bellesme,[2] Najiba Lahlou,[11] François Lefrère,[3] Stéphane Blanche,[8] Muriel Audit,[12] Emmanuel Payen,[13,14] Philippe Leboulch,[13,14,15] Bruno l'Homme,[1] Pierre Bougnères,[2] Christof Von Kalle,[6] Alain Fischer,[4,8] Marina Cavazzana-Calvo,[3,4,5]* Patrick Aubourg[1,2]*†

Subjects

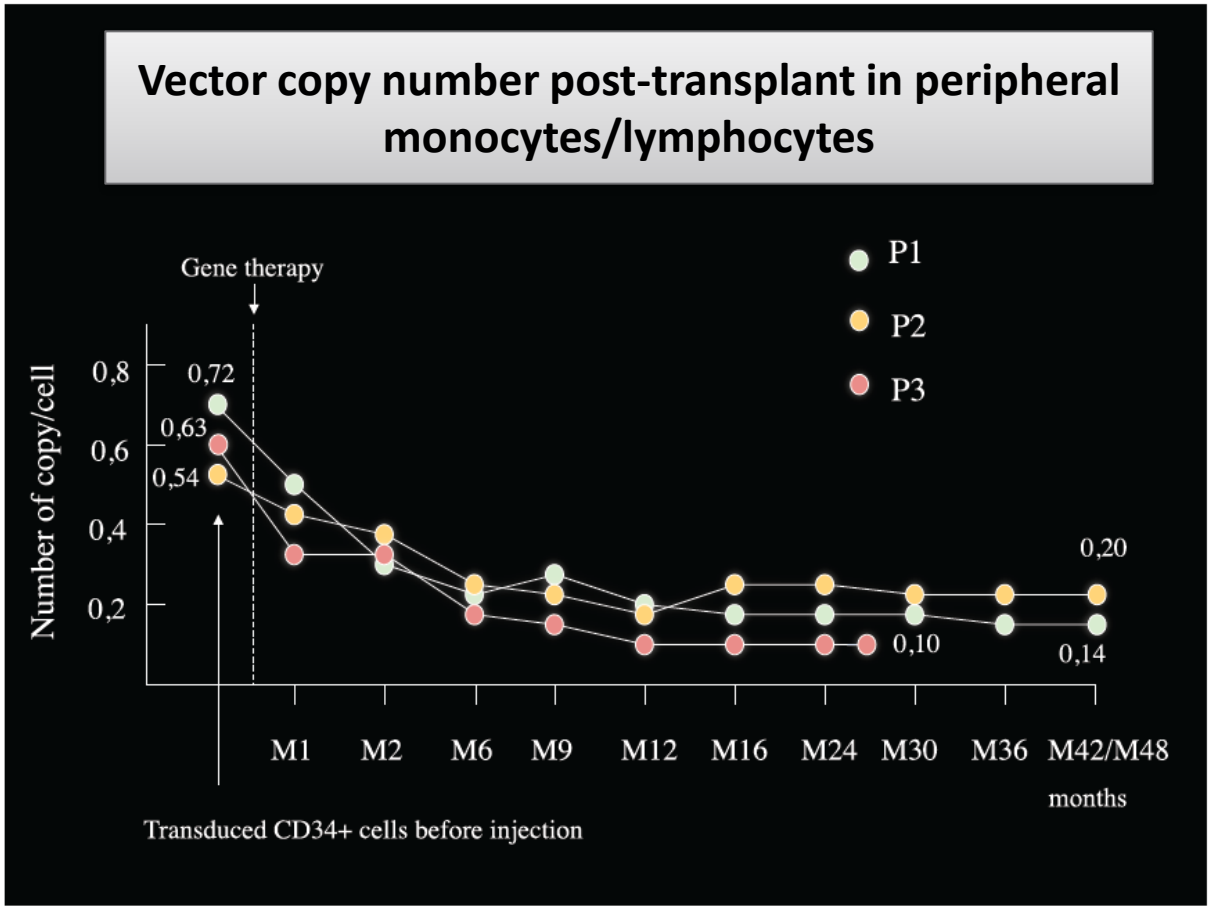
- Four CCALD subjects now treated with lentiviral gene transfer to restore function of ALDP.
- Three subjects with long term follow-up of 2.5 to 4.5 years, and the fourth recently treated.

Treatment

- Lentiviral gene transfer into GCSF mobilized $CD34^+$ cells.
- Transfer vector expresses a functional copy of the ALDP encoding ABCD1 gene – defective in CCALD.

SUPPORTING CLINICAL DATA FOR GENE TRANSFER IN CCALD

Long term reconstitution with gene modified HSCs



- **Identical insertion sites in lymphoid and myeloid cells, and long term marking suggest transduction of primitive pluripotent cells**

bluebirdbio

SUPPORTING CLINICAL DATA FOR GENE TRANSFER IN CCALD

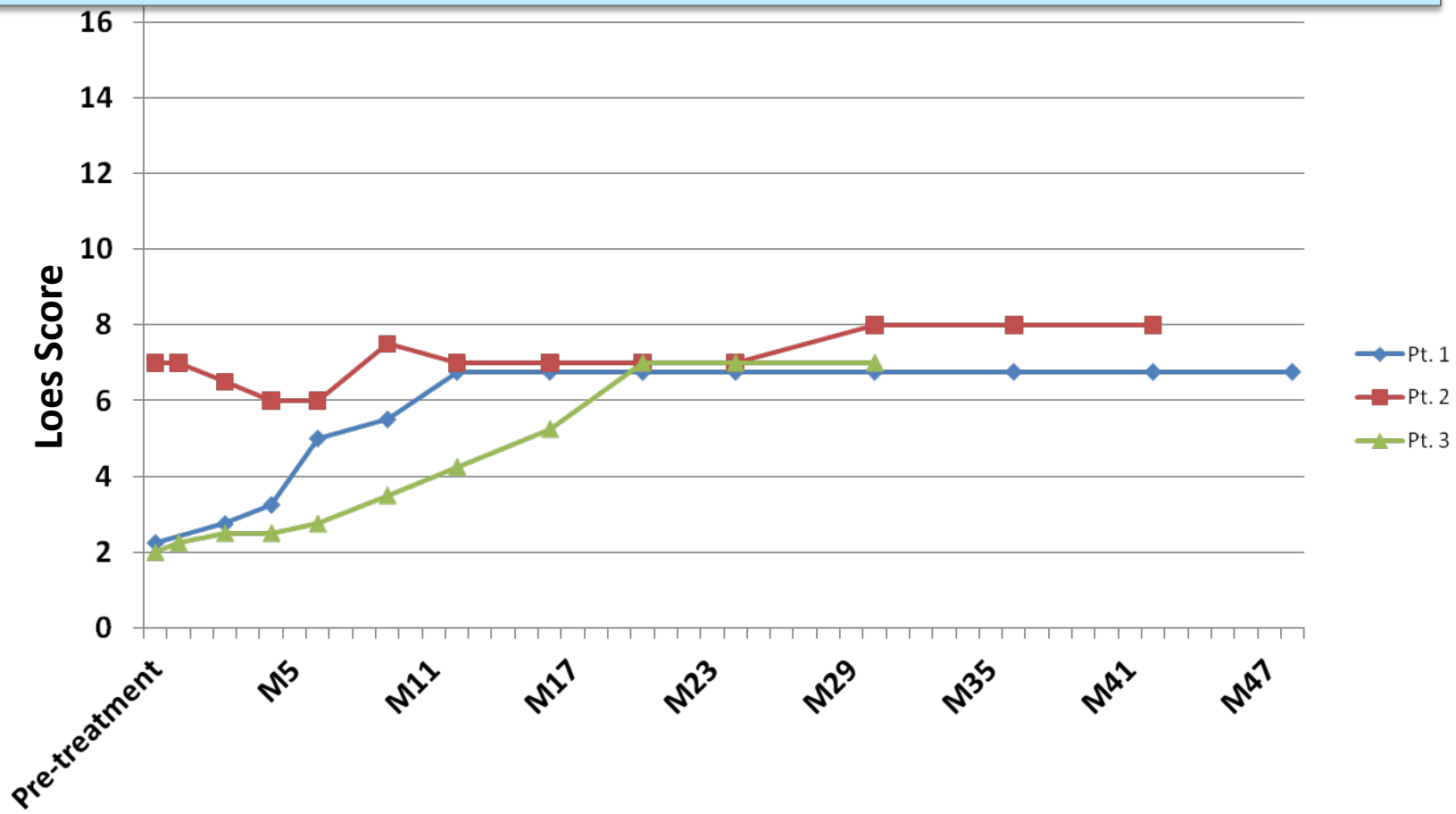
Excellent long term safety data

- **No procedural or long term serious adverse events encountered.**
- **Ongoing integration site analyses in HSC derived lineages demonstrate maintained polyclonality, with no evidence of clonal skewing or dominance.**

bluebirdbio™

SUPPORTING CLINICAL DATA FOR GENE TRANSFER IN CCALD

Evidence of disease stabilization in all subjects with long term follow-up



***Loes Score is a continuous scale 0 to 34 points based on the volume of myelin loss manifest on brain MRI. The higher the number the more myelin is destroyed.**

bluebirdbio

SUPPORTING CLINICAL DATA FOR GENE TRANSFER IN CCALD

Evidence of disease stabilization in all subjects with long term follow-up

	Subject 1	Subject 2	Subject 3
Months since treatment	48	42	28
Loes Score*	6.75 (stable since M14)	8 (stable since M16)	7 (stable since M20)
Performance IQ	74 (last tested at M24)	86	76
Verbal IQ	101 (last tested at M24)	114	72

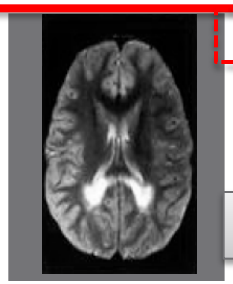
***Loes Score is a continuous scale 0 to 34 points based on the volume of myelin loss manifest on brain MRI. The higher the number the more myelin is destroyed.**

bluebirdbio™

SUPPORTING CLINICAL DATA FOR GENE TRANSFER IN CCALD

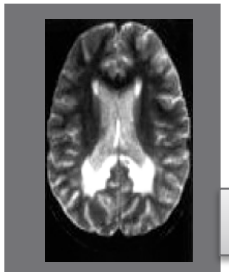
ARREST OF DEMYELINATION

Untreated ALD patient



at diagnosis

Loes Score = 7

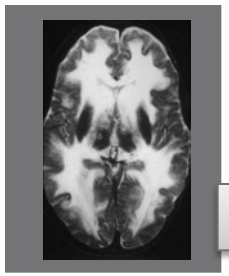


12 months after, untreated

Loes Score = 10

18 months after, untreated

Loes Score = 16



30 months after, untreated

Loes Score = 28

P1 **P2**

Before gene therapy

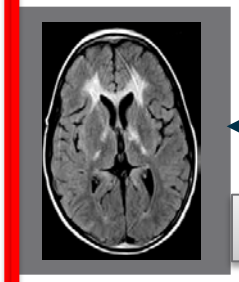
Loes Score =2.25

Loes Score = 7

12 months after gene therapy

Loes Score =6.75

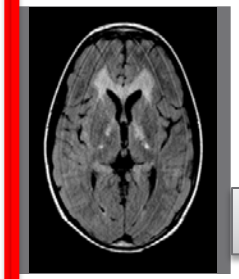
Loes Score = 7



16 months after gene therapy

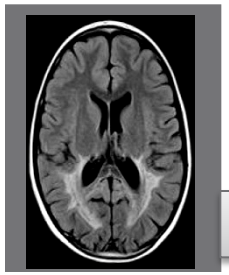
Loes Score = 6.75

Loes Score = 7.5



24 months after gene therapy

Loes Score = 6.75



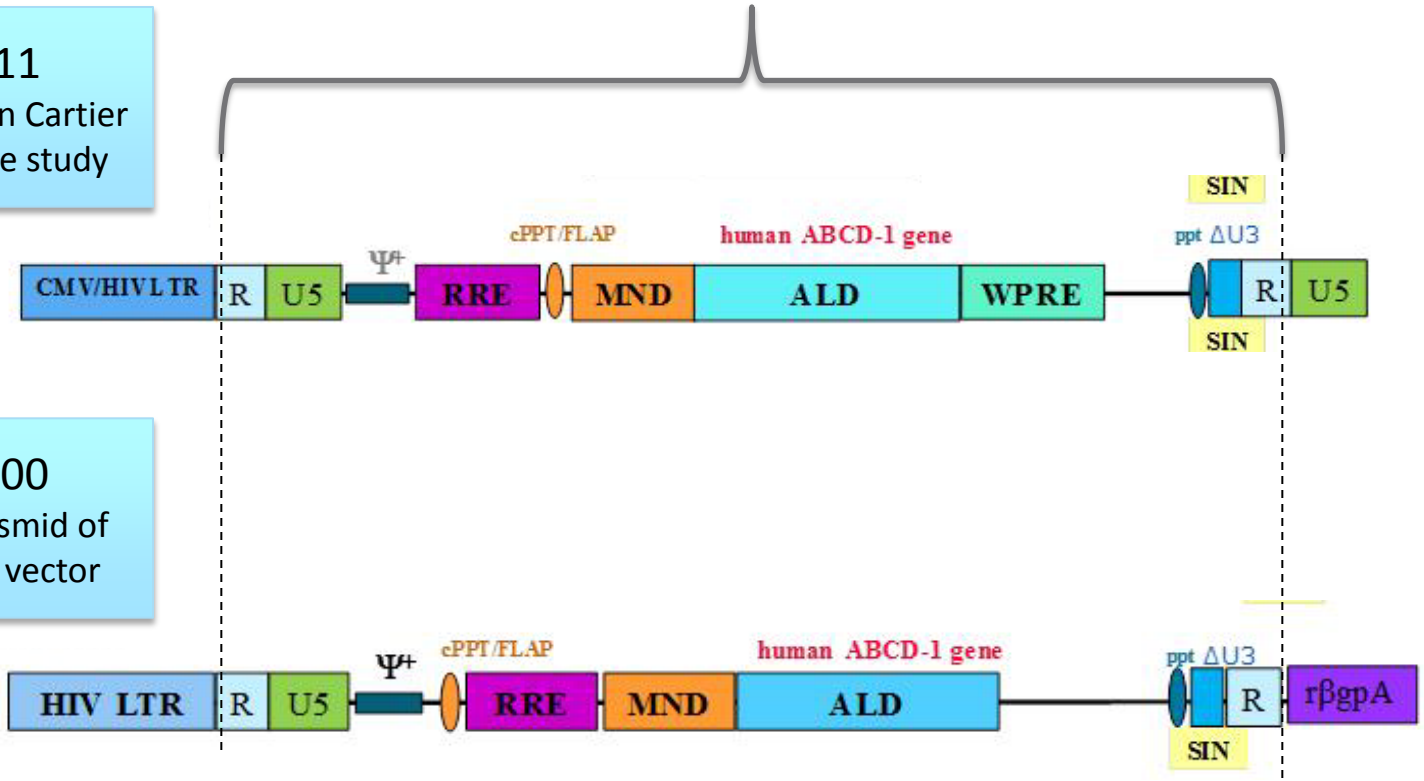
Loes Score = 7.5

COMPARISON OF LENTI-D TO PREVIOUSLY USED VECTOR

Integrated Provirus

CG1711
Vector used in Cartier et al. Science study

pLBP100
Transfer plasmid of the Lenti-D vector

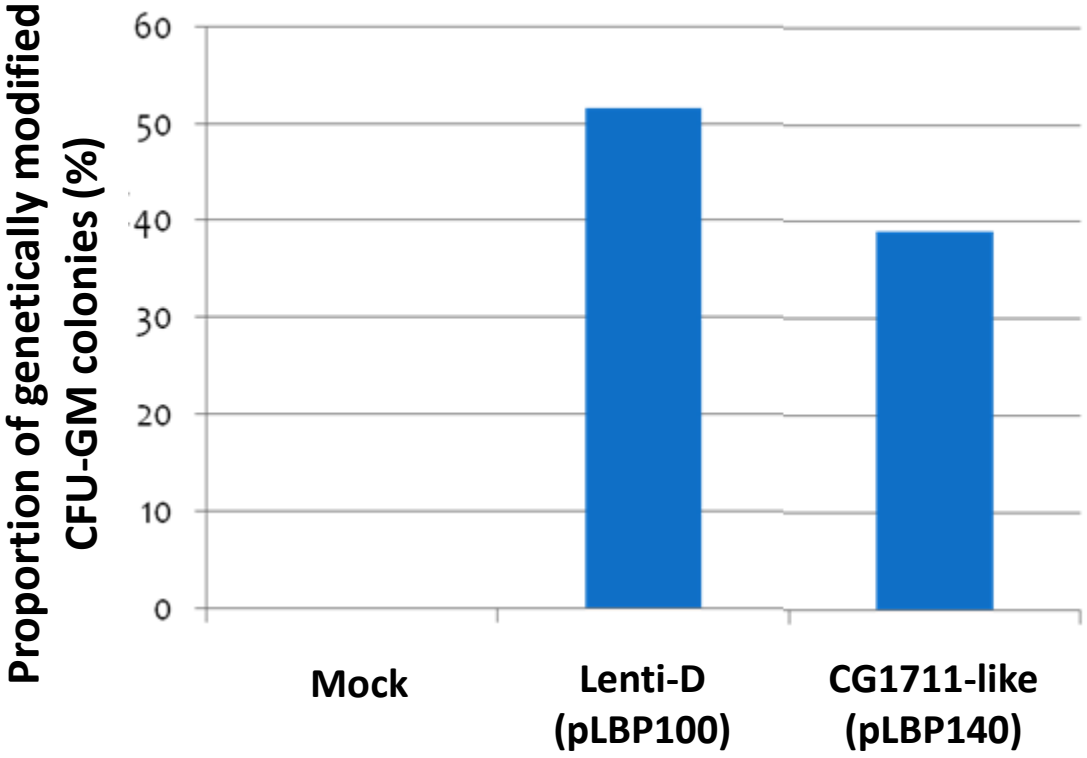


- **Vectors constructed with the same promoter and cDNA**
- **WPRE element removed from pLBP100**

bluebirdbio

COMPARABLE TRANSDUCTION EFFICIENCY IN LENTI-D AND CG1711-LIKE TRANSDUCED HUMAN CD34 CELLS

Percent transgene-positive CD34-derived CFU-GM

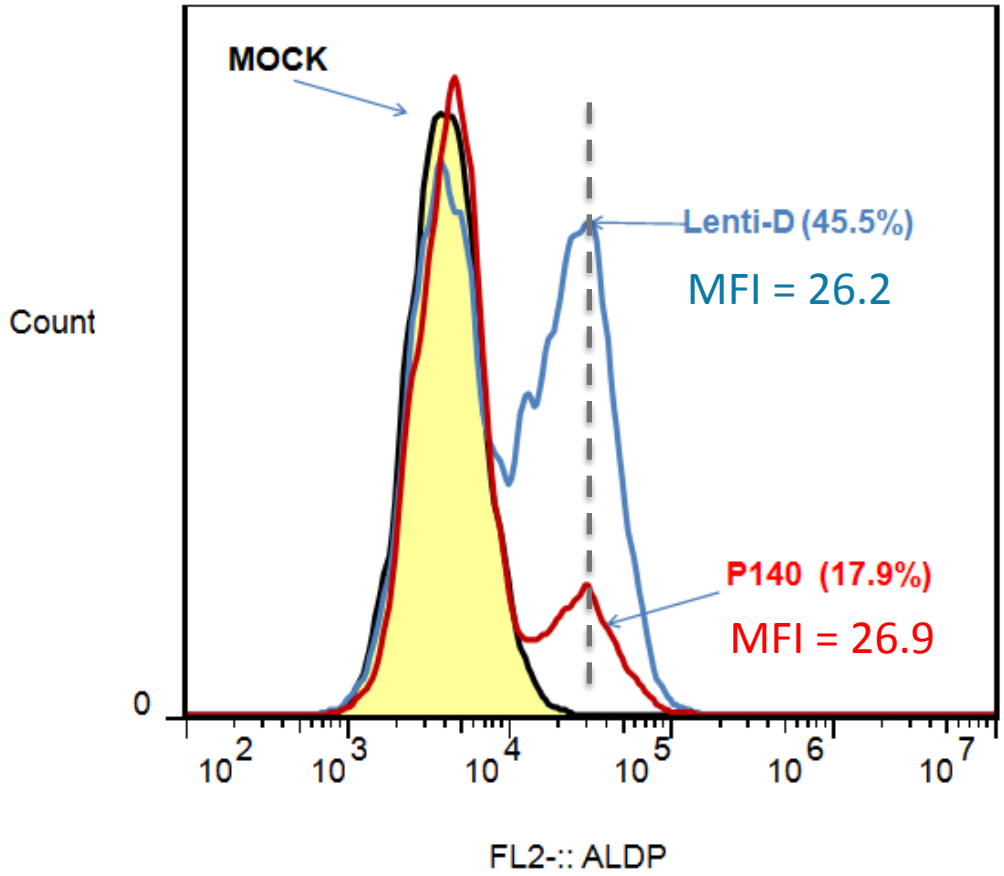


- **MOI = 25 for both vectors**

bluebirdbio™

EQUIVALENT EXPRESSION IN HUMAN CD34 CELLS TRANSDUCED WITH LENTI-D AND P140 (CG1711-LIKE)** VECTOR

ALDP expression as measured by flow cytometry at day 31 of culture



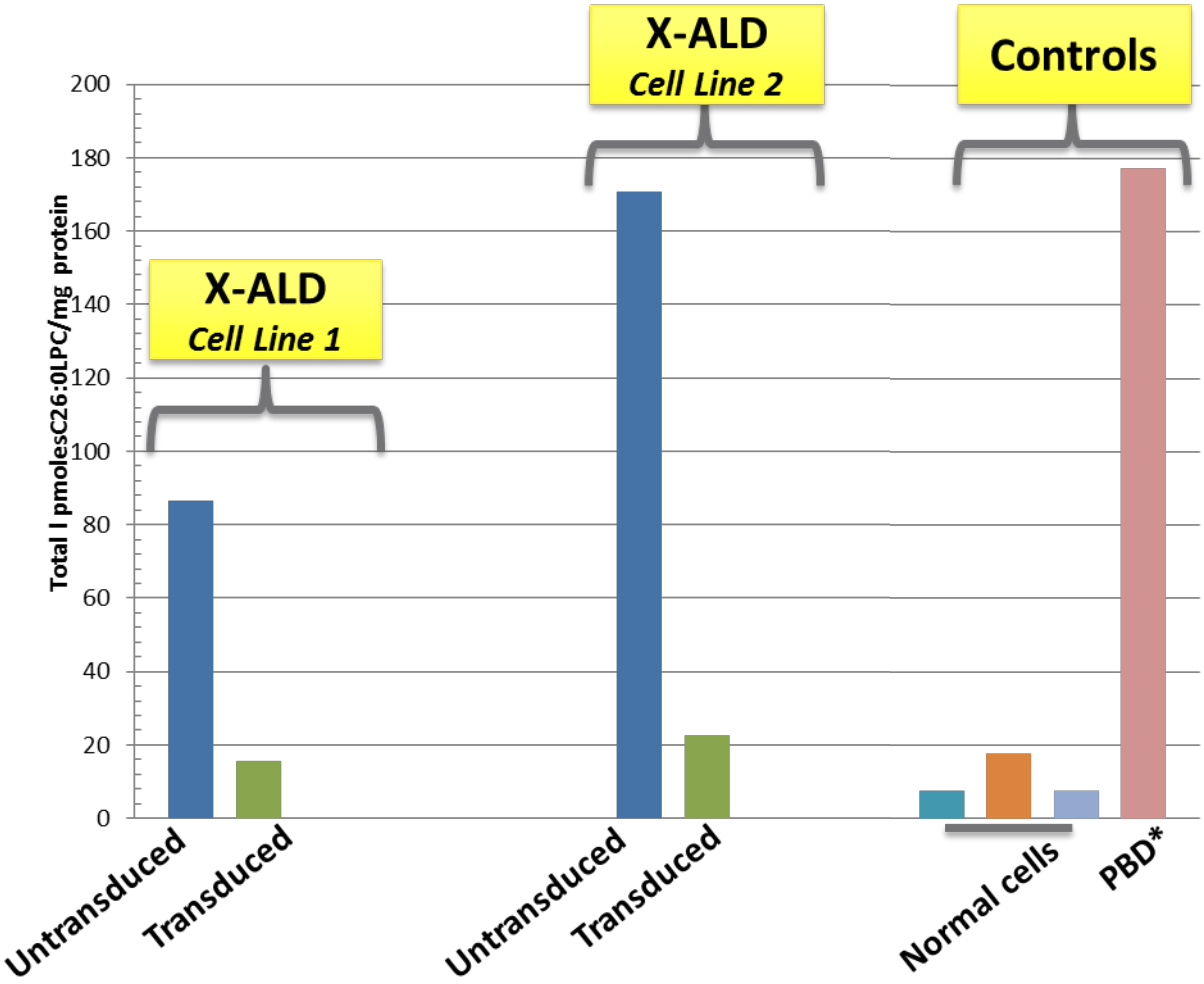
****P140 vector contains the WPRE element, and was used as a surrogate for the CG1711 vector**

- **MOI = 25 for both vectors**

bluebirdbio

LENTI-D TRANSDUCTION OF X-ALD FIBROBLASTS RESTORES ALDP ACTIVITY TO NORMAL LEVELS

Reduction in VLCFA levels as measured by LC-MS



*** PBD: Peroxisomal Biogenesis Disorder cells – unable to break down VLCFAs (negative control)**

bluebirdbio

OVERVIEW OF THE ALD-102 CLINICAL PROTOCOL

An open label, non-randomized, single dose, multi-center phase 2/3 study of the safety and efficacy of Lenti-D modified autologous stem cells (Lenti-D Drug Product) for the treatment of subjects with childhood cerebral adrenoleukodystrophy (CCALD).

Stage	Control Population	Objectives	Endpoint	Study Size
Phase II/III (registration)	Single Arm (but definition of success informed by retrospective data)	(a) To demonstrate evidence of efficacy as compared to untreated patients (b) To demonstrate efficacy comparable to that observed with allogeneic HSCT (c) To show that treatment with Lenti-D is safer than allogeneic HSCT	(a) Efficacy – Stabilization of pIQ and MRI over a minimal 6 out of 30 month period (b) Safety – Significantly fewer transplant related SAEs than typically observed with HSCT	12 patients

Safety and Efficacy are co-primary endpoints

bluebirdbio™

OVERVIEW OF THE ALD-102 CLINICAL PROTOCOL

Eligibility Criteria

- Boys between 3 and 15 years old
- Positive diagnosis of CCALD
  - Elevated levels of VLCFAs
  - Identification of a mutation in the ABCD1 gene
  - Evidence of demyelination: Loes > 1
- Early in the progression of the disease
  - Loes score < 10
  - Data from retrospective studies suggests that patients with a Loes score of 10 or greater respond poorly to HCT.

bluebirdbio™

ANSWERS TO REVIEWER QUESTIONS

OVERVIEW

Outline

1. *Clinical*
2. *Preclinical*
3. *Consent*

bluebirdbio™

RETROSPECTIVE DATA

The primary endpoints are a less-rapid rate of decline, measured by a change in Loes score over any consecutive 6 month period, and a decline of less than 15 points in performance IQ over any consecutive 6 month period. **My only request is for control data for the rate of change in those measures in untreated children, and/or children who received stem cell transplants.**

Study Sponsor is carrying out a retrospective collection of de-identified data to refine the selected IQ and MRI endpoints.

- **Subjects**
  - Both HCT treated and untreated subjects
  - Estimated number of records: 30-60 untreated; 30-60 treated
- **Subject record eligibility**
  - Loes score between 1 and 15 at the time of transplant or diagnosis (if untreated)
  - Follow-up of 2 years or more from the time of treatment or diagnosis
- **Functional outcomes selected**
  - Loes score
  - Verbal and performance IQ
  - Ambulation, feeding, schooling
  - Neurological disability score
- **Study sites:**
  - 4-5 major transplant sites in US and Europe

INTEGRATION SITE ANALYSIS

The protocol was not clear on whether LAM-PCR to look at integration site analysis would be done routinely or only on those patients that show CBC abnormalities. Could the investigators please clarify?

The timing and methodology of integration site analysis will depend on the marking and clonality of circulating white blood cells (WBCs) in each subject.

Percent of WBCs with detectable provirus

- None detected
  - No clonal analysis
- Detected, but under 1%
  - LAM-PCR to assess clonality at a minimum of every 6 months for the first year
- Greater than 1%
  - LAM-PCR to assess clonality at a minimum of every 6 months for the first two years

If LAM-PCR profile suggests the existence of a dominant clone and Q-PCR indicates that a clone contributes to > 1/5th of hematopoiesis, the following studies will be performed: clinical evaluation of RBC, WBC, and platelet counts, bone marrow analysis, cytogenetic studies, and immunophenotypic analysis to rule out leukemia.

- If no leukemia, clonality analyzed every 3 months for 3 years.

bluebirdbio™

STUDY ENDPOINTS

The pace of engraftment of modified progeny cells will be slow. Please review with the committee and justify the definition of pIQ stabilization as a decline in performance of less than 15 points in a 6-month period.

Pediatric neurologists expert in this disease consider a less than 15 point decline of PIQ over a 6 month period as indicative of stabilization of the disease.

- Note: PIQ declines between 1 and 2 years following allogeneic hematopoietic stem cell transplantation.

PIQ is an excellent predictor of neurocognitive function and quality of life after allogeneic hematopoietic stem cell transplantation for cerebral X-ALD. (Peters et al., Blood ,2004; Shapiro et al., Lancet, 2000).

bluebirdbio™

STUDY ENDPOINTS

Among the secondary endpoints, are there potential surrogate markers of performance improvement that could be validated during this study for future use as primary endpoints?

- While patients possess elevated levels of VLCFAs, the levels have not been found to correlate with the presentation of the disease (Loes score).
- Decrease of plasma VLCFA after allogeneic hematopoietic stem cell transplantation is not correlated with clinical outcome.
- The following surrogate markers are currently being investigated by a number of investigators:
  - measurement of cytokines and growth factors in serum using "Luminex" assay
  - customized myelin and neuronal as well as lipid array
  - plasma oxysterol levels

bluebirdbio™

PHARMACOLOGY STUDIES

The investigators state they plan to conduct additional pharmacology studies to examine Lenti-D GTP transduction and potential toxicity studies with human CD34+ cells. Will these be carried out prior to initiating the gene therapy trials? Can the investigators more explicitly describe what additional studies will be carried out?

GLP Toxicology and Biodistribution Study

Rationale

- Submit study as part of safety package for Lenti-D IND filing.
- Assesses whether treatment of CD34 cells ex-vivo with Lenti-D adversely affects bone marrow engraftment.

Study Design

- Transduced (and nontransduced control) cells are administered to mice myloablated with busulfan.
- Mice are sacrificed at 30 or 90 days post treatment to allow for assessment of engraftment .
- The study employs NSG mice (lacking B, T, NK cells, and cytokine signaling), which achieve higher of HSC engraftment than other immunocompromised mouse strains.

Readouts

- Flow cytometry to estimate bone marrow engraftment via quantitation of human HSCs (CD45 marker), and lymphoid and myeloid lineages.
- qPCR to measure the fraction of HSCs and progeny harboring integrated provirus (VCN).
- Histopathology of transplanted human HSCs and derived lineages across tissues.

WITHDRAWAL OF SUBJECTS FROM STUDY

Could the investigators elaborate on what basis the sponsor would request withdrawal of a patient?

Up until the point of myeloablation:

-General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator

-If in consultation with the investigator, the subject is deemed to be non-compliant with the study requirements

-If despite support efforts and multiple attempts the subject did not complete the pretransplant required screening per institutional standard, then we could not admit the child for the transplant aspect of the study. The specific screening requirements are: infectious disease markers, echocardiogram, pulmonary function tests (when age appropriate), glomerular filtration rate, audiogram, ophthalmologic exam, and dental examination (when age appropriate).

Given that support systems will be put in place to assure and facilitate compliance with the study, we anticipate that such events should be rare.

bluebirdbio™

CONTINGENCY IF CELL TRANSDUCTION FAILS

The protocol states that transduced autologous cells with a vector copy number of 0.5 to 3 copies per CD34+ cells will be engrafted. If this is not achieved with a patient's cells, will they be engrafted with the untransduced back-up HSC?

- Failure to achieve the release specification of transduced cells would require the head of the cell manipulation laboratory to notify the site PI, who subsequently notifies the study sponsor.
- Since vector copy number is determined before myeloablation, backup transplantation with untransduced HSCs would not be needed.
- Given the progressive nature of the disease, the risks of waiting 4-8 weeks for a second round of GCSF mediated HSC mobilization would be discussed with the subject.
- The subject would be given the choice to leave the trial and seek treatment with allogeneic HCT if desired.

RISK OF GVHD WITH AUTOLOGOUS CELL TRANSPLANT

It is stated in the text that GVHD may occur in the second phase of study. Is this meant to refer to the potential for engraftment syndrome post-autologous HCT?

- Although extremely rare, there are isolated case reports of what appears to be GVHD in autologous stem cell transplants (Drobyski, BBMT, (43) 2009 page 169).

bluebirdbio™

SUBJECTS WITH MATCHED SIBLING DONORS

Since HSCT seems to have a high success rate in such patients, will patients who can undergo matched sibling HSCT be eligible for this trial?

- Yes, we propose for patients with matched sibling donors will be eligible for the trial.
- Even with matched siblings, there is a risk of morbidity and mortality with HCT.
- We believe that in consultation with their physician, parents should have the right of enrolling their son with CCALD in the trial.

bluebirdbio™

HEMATOPOIETIC STEM CELL HARVEST

Please provide a discussion of the mobilization and collection procedures, e.g. days of mobilization and collection that will be used to collect sufficient PBSC in this pediatric population. The purpose of this question is to estimate the likelihood that 1-2 days of collection will be sufficient.

- Stem cell mobilization and collection will be performed according to the Foundation for the Accreditation of Cellular Therapy (FACT) guidelines.
- Previous mobilization and collection data suggest this will not be an issue:
  - 57 patients had pre-HSCT apheresis in 2009-2010.
  - Three did not acheive their goal, which gives us a 94.7% success rate
  - The average number of days to acheive goal was 1.7 with a range of 1-7 days.
  - 56% met their goal in one day, 31% in two days.
  - 94% of patients successfully apheresed in 3 or fewer days.
- While there is no upper limit on the number of days for apheresis, if the attending transplant physicians determines that the desired harvest is unlikely to be reached, then a bone marrow collection may be performed instead.

LONG TERM FOLLOW-UP

Is there a contingency plan in case the investigators or the sponsor are not available for this length of time?

- It is standard of care for patients with a complex medical condition such as CCALD to be followed throughout their life.
- In order to comply with the FDA requirement of long-term follow-up in gene transfer studies, Children's Hospital Boston is developing an NIH grant application to enable monitoring of safety and efficacy across multiple gene transfer studies (SCID-X1, WAS, ALD).
  - It will be recognized by the committee that there is no conforming NIH mechanism to provide funding for more than 5 years.
- Should bluebird bio be merged into or acquired by another entity, its obligations to the study will be assumed by the new entity.
- In the case of bankruptcy, bluebird bio would move to protect sufficient assets to guarantee that sufficient follow-up is performed.

DIFFERENCES BETWEEN LENTI-D AND CARTIER STUDIES

How this study is different from the study described by Cartier et al. For example, exactly how do the vectors differ, selection of CD34 cells, conditioning regimen, etc?

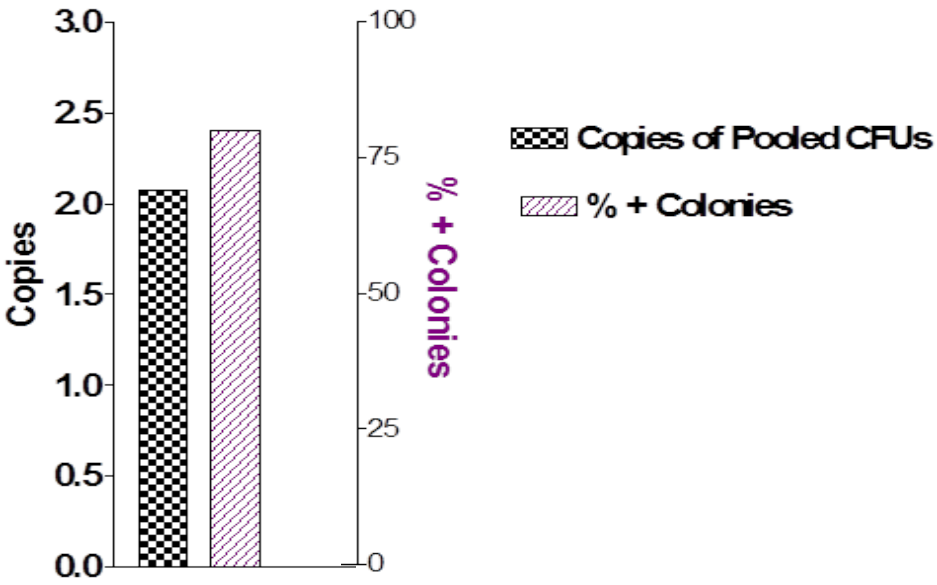
Study Feature	Comparison
Vector	• Promoter and cDNA preserved • Lenti-D vector lacks WPRE element, and differs in positioning of cPPT and RRE sequences
GTP formulation*	• Cartier: X-VIVO20 (Lonza) • ALD-102: SCGM (CellGenix)
RCL analysis for release of DP	• Cartier: performed before dosing of DP • ALD-102: in parallel with dosing, because of the low risk of RCL
Transduction conditions and cytokines	No change
CD34 cell collection	No change
Conditioning regiment	No change
Subject eligibility	• Cartier: Loes score between 2 and 8; no HLA-identical donor • ALD-102: Loes score between 1 and 9; irrespective of donor availability

bluebirdbio

DISTRIBUTION OF COPY NUMBER ACROSS TRANSDUCED CELLS

When vector copies per cell are shown, this is an average. Did you perform a limiting dilution assay to determine the range of copies/cell among the transduced population? This question is asked to help determine if the MOI=25 could have extremes of high copies/cell that could be of concern.

- While we have not performed a limiting dilution assay to determine the range of copies/cell, we have compared copy number in 14 day methylcellulose bulk culture to the fraction of transduced colonies to answer this question.
- The data demonstrate that approximately 75% of colonies are transduced, and that colonies in bulk have a copy number of about 2.
- We are developing new qPCR assays to assay for copy number in individual colonies.



bluebirdbio™

QUESTIONS REGARDING CONSENT FORM

Regarding equipoise in presenting results from the Cartier gene transfer study: *"The consent notes that progress of the disease has been interrupted".*

The consent will emphasize uncertainty as to both short and long term risks and benefits; the consent process will neutrally describe the post-treatment disease trajectory of the treated boys.

Regarding alternatives to participation: *"Please indicate whether allogeneic HCT remains an alternative after choosing the research autologous HCT approach."*

It is standard practice to wait up to a 6 month interval between autologous and allogeneic transplants because of the risk of procedure related organ toxicity. However, transplant could be carried out sooner if the subject's physician deemed the risks of disease progression to outweigh those of transplant.