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Clinical Use of ZSCAN4 For Telomere Elongation in Hematopoietic Stem Cells

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ABSTRACT

BACKGROUND: Extremely short telomeres in patients with dyskeratosis congenita and related telomere biology disorders (TBD) lead to premature cellular senescence and bone marrow failure. ZSCAN4 elongates telomeres by recombination.
METHODS: We report a clinical study (NCT04211714) in which EXG34217, autologous CD34+ hematopoietic stem cells from patients with TBD exposed to a temperature-sensitive Sendai virus vector encoding human ZSCAN4 at 33°C for 24 hours, was infused into patients without preconditioning.
RESULTS: Four patients were enrolled; two experienced successful CD34+ mobilization during the second mobilization attempt and underwent apheresis and EXG34217 infusion, with follow-up of 24 and 5 months (both ongoing). We observed telomere elongation (1.06- to 1.34-fold) in CD34+ cells ex vivo. In one patient, the treatment was associated with a change in the mean absolute neutrophil count (ANC) from $1.78 \times 10^3/\mu l$ to $3.18 \times 10^3/\mu l$; the lymphocyte subpopulations telomere length changed from 3.6 kb to 6.7 kb (50th percentile for age). In another patient, the treatment was associated with a change in the lowest ANC from $0.6 \times 10^3/\mu l$ to $1.2 \times 10^3/\mu l$; this has occurred in 5 months without the patient receiving her prior intermittent low-dose granulocyte-colony-stimulating factor injections. During mobilization, all patients experienced mild to moderate bone pain or pain after line replacement, and one patient had a blood infection associated with fever and hypoxemia. After EXG34217 infusion, no acute safety issues were noted; in one patient mild-moderate long-term cardiac and pulmonary adverse events were noted; these were similar to the patient's underlying conditions.
CONCLUSIONS: Although definitive conclusions cannot be drawn from the two EXG34217-treated patients, these results warrant further investigation of CD34+ cells exposed to ZSCAN4 for treating TBD.

INTRODUCTION

Telomeres are repeat sequences at the end of each chromosome that play a critical role in genome stability, cell proliferation, and various biological functions.[1] Telomeres are considered markers of aging and shorten throughout the lifespan,[1] although actions of the enzyme telomerase[2] and recombination[3] can lengthen them. Telomeres must be maintained within a certain length; those that are shorter or longer than normal cause disorders.[4,5] Extremely short telomeres in patients with telomere biology disorders (TBD), including prototypical dyskeratosis congenita, lead to premature cellular senescence and a high risk of bone marrow failure.[4,6-16] Hematopoietic stem cell transplant (HSCT) is the only curative intervention for bone marrow failure,[17] but as TBD patients are susceptible to DNA damage, conditioning regimens required for HSCT may lead to accelerated deterioration of disease co-morbidities.[18] The recent introduction of reduced-intensity conditioning regimens has reduced toxicity, but long-term mortality after HSCT remains high.[19-22]

Zinc Finger and SCAN Domain Containing 4 (ZSCAN4) is a protein expressed transiently in preimplantation embryos,[23,24] germ cells during meiosis,[25] embryonic stem cells,[23] and adult tissue stem cells.[26] Transient expression of ZSCAN4 elongates telomeres in mouse embryonic stem cells[27] and human fibroblast cells.[28] Telomere elongation by ZSCAN4 is independent of telomerase and is mediated by telomere recombination.[27] Here, we report a gene and cell therapy experimental study in a 37-year-old male patient and a 33-year-old female patient with TBD to extend telomere length and its impact on various facets of their condition.

METHODS

EXG-001 is a non-transmissible, temperature-sensitive variant of the Sendai virus (SeV) vector (SeV18/TS15ΔF)[29] that encodes the cDNA sequence for the human *ZSCAN4* gene.[28] SeV has never demonstrated pathogenicity in humans.[30] Two previous clinical trials using wild-type SeV and F-gene-deleted SeV reported no adverse events in humans.[31,32]

The entire ex vivo manufacturing procedure of the patients CD34+ cells was performed using the CliniMACS Prodigy (Miltenyi Biotec),[33] a semi-automated, functionally closed tubing/bag system without open manipulation. CD34+ cells were isolated[33] and incubated ex vivo with EXG-001 at 33°C for 24 hours, incubated at 37°C for 1 hour, washed to remove free EXG-001, and resuspended in Plasma-Lyte A. The final cellular product for infusion is called EXG34217.

Telomere measurement was performed by Telomere qPCR,[34] Telomere flow-FISH (fluorescence in-situ hybridization)[35], and single-molecule single telomere length analysis (SM-STELA) which provides the distribution of the actual telomere lengths of chromosome 17p.[36]

For detailed methods, see the Supplementary Appendix (Methods, p. 6).

Statistical considerations

The data provided are descriptive and no statistical inferences are drawn. The findings are not meant to be clinically directive but rather describe the experience following EXG34217 infusion in these two patients.

RESULTS

THERAPEUTIC PROCEDURE DESIGN AND CLINICAL STUDY

The temperature-sensitive SeV vector used in this study allows viral replication and gene expression when held at 33°C, but raising the temperature to 37°C inactivates the virus[28,29] (Text S1; Fig. S1). Delivery efficiency of ZSCAN4 is high (> 70%) when CD34+ cells are in contact with EXG-001 at a multiplicity of infection (MOI) of 25 at 33°C for 24 hours (Fig. S1). EXG-001 elongates telomeres in mobilized peripheral CD34+ cells from healthy individuals (Text S2; Fig. S2). Importantly, EXG-001 elongates telomeres of primary fibroblast cells derived from TBD patients (7- and 11-year-old males[37], unrelated to the current patients) (Fig. S3). EXG-001 treatment did not alter the differentiation ability of mobilized peripheral CD34+ cells derived from healthy individuals (Fig. S4).

We report our experience to date in an industry-sponsored Phase I/II study of autologous CD34+ cells modified by contact with EXG-001 (EXG34217) for adults with TBD and mild to moderate bone marrow failure (Supplementary Protocol). Initial enrollment criteria included that patients be 18 years of age or older , which changed to 12 years of age or older on April 3, 2024. This clinical study (ClinicalTrials.gov: NCT04211714) is being conducted under US IND 19748. IRB approval and informed consent were obtained. The primary endpoint is to assess the safety and incidence of adverse events (AEs) related to EXG34217 treatment. Secondary endpoints include assessing the feasibility of stem cell collection, ex vivo transduction, and reinfusion of EXG34217, as well as telomere extension and clinical benefit assessed as peripheral blood count improvement.

Because patients will not receive any preconditioning regimen, an autologous transplant of EXG34217, if successfully engrafted, is expected to cause mosaicism of treated and untreated CD34+ cell populations in the bone marrow, resulting in mosaicism of peripheral blood cells, such as white blood cells (Text S3). We tested whether it is possible to detect such mosaicism by measuring telomere lengths in control experiments, where blood cells from two individuals with different average telomere lengths were mixed at different ratios (Text S3). The standard analyses (i.e., median telomere length) by both telomere flow-FISH (Text S3; Fig. S5, S6) and SM-STELA (Text S3; Fig. S7, S8, S9) can detect two cell populations at 50%:50% mixture, but cannot detect them at 25%:75% mixture (Fig. S10). However, when more cells with longer telomeres were mixed with cells with shorter telomeres, telomere distribution curves morphed from a single sharp curve into a broader curve (Fig. S5, Fig. S9). We found that the deconvolution of a telomere distribution curve into multiple Gaussian curves can detect two cell populations at >10%:90% mixture (Text S3, Fig. S5, S9).

Study procedures are shown in Fig. 1A. Autologous peripheral blood CD34+ cells are isolated from mononuclear cells (MNCs) collected via apheresis after mobilization with granulocyte-colony-stimulating factor (G-CSF) and Plerixafor prior to each apheresis collection. Due to challenges with collecting CD34+ cells from TBD patients,[6] if circulating CD34+ cells are below 5 cells/µl, patients are not subjected to apheresis and are withdrawn from the study.

REPORT ON THE FIRST FOUR STUDY PATIENTS AS OF SEPTEMBER 30, 2024

This clinical study was designed to enroll 12 patients. There was no formal interim analysis planned for this study, rather this is a report the results of the first four study patients enrolled as of September 30, 2024 (Table 1) because of the safety profile to date and treatment novelty,. Although 4 patients were entered into the protocol, we report data from the two patients who received EXG34217, i.e., patients R02 and R05 with outcome data of 24 months and 5 months post-EXG34217 treatment, respectively.

Initial attempts to mobilize CD34+ cells by the standard G-CSF and plerixafor injection failed in all four patients, resulting in no apheresis and no EXG34217 treatment (R01, R03, R04, R06 in Table 1). Modifications of the mobilization strategy to optimize stem cell collection were applied to patient R01 (now called R02) and R04 (now called R05), resulting in an increased peripheral CD34+ cell count to > 5 cells/µl, thus allowing adequate CD34+ cell collection by apheresis to treat two of the patients with EXG34217 (Table 1). Mobilization attempts occurred between June 27, 2021 (first attempt for R01) and July 31, 2024 (attempt for R06). Follow-up data for patients R02 and R05 treated with EXG34217 are included through September 30, 2024 (Table 1).

REPORT ON THE FIRST EXG34217-TREATED PATIENT

The first EXG34217-treated patient (R02) is a 37-year-old male diagnosed with TBD (telomere length < 1st percentile) 10 years before enrollment in 2021. Eight months after the first mobilization attempt failed (peripheral CD34+ cells; 3 cells/µl), a second mobilization attempt using an improved protocol yielded more peripheral CD34+ cells (10 cells/µl), resulting in two days of apheresis, ex vivo processing of pooled MNCs, and a single 30-minute infusion of $0.85x10^6$ EXG34217 cells/kg (93.3% cell

viability). Each apheresis yielded MNCs containing CD34+ cells estimated to be $1.67x10^6$/kg and $1.10x10^6$/kg.

Immunohistochemistry of the final product using an anti-ZSCAN4 antibody[28] showed 89.7% of cells were ZSCAN4-positive (Fig. 1B). Colony-forming cell (CFC) assays of EXG34217 demonstrated maintenance of normal differentiation potential after treatment (Fig. 1C). qPCR results show that EXG-001 treatment was associated with a change in the average telomere length of the patient's CD34+ cells by 1.24-fold (Fig. 1D). SM-STELA results similarly showed that mean and median telomere length increased from 3.35 kb to 3.59 kb (1.07-fold) and 3.26 kb to 3.46 kb (1.06-fold), respectively, through EXG-001 treatment (Fig. 1E; Fig. S11). SM-STELA analysis further deconvoluted the telomere distributions into distinct telomere populations, most likely attributed to each parent allele, both of which were elongated from 2.72 kb and 3.50 kb to 3.20 kb and 4.37 kb (Fig. 1E).

Telomere flow-FISH was performed on patient (R02)'s blood at a CLIA-certified laboratory 3 days before EXG34217 dosing (M0) and 1 month (M1) to 24 months (M24) after EXG34217 dosing (Fig. 2A). The median values of each profile were plotted on a nomogram of age-adjusted telomere length (Fig. 2B; Fig. S12). Median telomere length decreased slightly over two years (lymphocytes: 3.6 kb (M0), 3.1 kb (M12), 3.2 kb (M24)), similarly to that in healthy individuals (Fig. 2B). However, the telomere flow-FISH analysis identified gradual broadening of telomere distribution curves from M0 to M24 (Fig. 2A).

Deconvolution of the patient's peripheral blood cells shows that M0 and M1 profiles constitute a single major peak, i.e., cell population, whereas starting from M3, a cell population with longer telomeres emerges (Fig. 2A; Text S4; Fig. S13, S14). At M6, a cell population with 6.6 kb telomeres was identified, indicating the emergence of a cell population with normal telomere length (10th – 50th percentile for age). In the M24 profile, cell populations with 6.7 kb, 6.3 kb, 5.0 kb, 3.4 kb, and 2.4 kb telomere length were identified. The nomogram of age-adjusted telomere length of M0 and M24 indicate the sustained presence of cell populations with longer telomeres after EXG34217 treatment (Fig. 2C).

The fractions (%) of lymphocytes with telomeres longer than a certain length were also examined. For example, at M0 and M1, there were no cells with telomeres > 6 kb in length, whereas all time points from M3 to M24 reported cells with telomeres > 6 kb in length (Fig. 2D). The same trends were observed from the telomere flow-FISH assay of granulocytes (Text S4, Fig. S15).

Results from the SM-STELA assay also showed the emergence of cell populations with longer telomeres over time following EXG34217 treatment (Fig. S16, S17, and S18).

A description of the patient's clinical phenotypes before and after EXG34217 treatment is included in the Supplementary Appendix (Text S5). The patient is genetically undefined but has a family history of aplastic anemia and liver cirrhosis in a brother (deceased from liver transplant complications following HSCT) and pulmonary fibrosis in his mother, consistent with TBD.

The patient had mild neutropenia and thrombocytopenia for 15 years before participating in this study. The patient's ANC changed from a mean of $1.78x10^3$/μl to $3.18x10^3$/μl following EXG-34217 treatment (Fig. 2F). The period between M0 and M6 was excluded from the analyses to account for possible effects of G-CSF and Plerixafor that were administered before EXG34217 infusion to facilitate CD34+ cell mobilization. White blood cell counts also increased (Fig. S19). Red blood cells, hematocrit, and hemoglobin did not change, remaining just below the normal range for age. The platelet count remained slightly below the normal range, with a marginal reduction after treatment. The patient remained transfusion-independent without serious infections. The patient's bone marrow cellularity has remained stable or improved slightly since treatment, although bone marrow cellularity is challenging to assess in patients with inherited bone marrow failure. Cellularity is often patchy, varying extensively throughout different locations within the bone marrow (Table S1, Fig. S20). The percentage of CD34+ cells was low but stable (from <0.1% to 0.2%, Table S1). The karyotype and MDS FISH/cytogenetics were

normal, and the somatic mutation panel was negative (Table S1).

The patient was followed annually with spirometry because of mild subclinical pulmonary fibrosis and pulmonary arteriovenous malformations. A clinically asymptomatic decline was noted at -M40 in % predicted forced expiratory volume in one second (FEV_1) from 106% to 88% with the normal diffusing capacity of the lungs for carbon monoxide (DLCO) from 87% to 90% (Text S5). CT imaging showed mild pulmonary fibrosis at -M13 before EXG34217 treatment. At M12 and M22 after EXG34217 treatment, clinical pulmonary monitoring demonstrated stable FEV1 (97% and 104%, respectively) and slightly decreased DLCO (68% and 78%, respectively). CT identified increased moderate fibrosis at M12, and the patient was started on Pirfenidone one year after the EXG34217 treatment (Text S5).

At -M4, a screening echo revealed mild left ventricle dilation with a 45% ejection fraction (Table S1). This was ~1 month after infection with COVID-19. Further cardiac evaluation, including MRI, revealed no ongoing inflammation and was consistent with the echocardiogram. The patient was diagnosed with presumed post-viral cardiac dysfunction leading to nonischemic cardiomyopathy. After EXG34217 treatment, the left ventricular ejection fraction was 37%, 36%, and 32% at M9, M21, and M23, respectively. He is currently on Spironolactone, Sacubitril-valsartan, and Metoprolol with diminished but stable function. Genetic evaluation revealed a separate likely pathogenic mutation in the *TTN* gene, indicating genetic susceptibility to cardiomyopathy.

The patient demonstrates no functional liver impairment (Table S1). Before EXG34217 treatment, there was intermittent transaminitis, with aspartate aminotransferase (AST) elevation of 40-100 units/l over many years (Fig. S21). Other liver chemistries were within the normal ranges (Fig. S21). Abdominal ultrasound showed some progression of liver disease. Liver imaging has not been performed after EXG34217 treatment.

REPORT ON THE SECOND EXG-34217-TREATED PATIENT

The second EXG34217-treated patient (R05) is a 33-year-old woman diagnosed with TBD (telomere length < 1st percentile) 5 years before initial enrollment when she was noted to have mild intermittent neutropenia with hypocellular bone marrow. She was subsequently found to have a mutation in the *TERT* gene. Eight months after the first mobilization attempt failed (peripheral CD34+ cells; 3 cells/µl), a second mobilization attempt using an improved protocol yielded more peripheral CD34+ cells (11-17 cells/µl), resulting in two days of apheresis, ex vivo processing of pooled MNCs, and a single 30-minute infusion of a total of 2.50×10^6 EXG34217 cells/kg. Analyses of ex vivo processed CD34+ cells showed 94.2% ZSCAN4+ cells (Fig. 3A), normal CFC assay results (Fig. 3B), elongation of average telomere length (1.34-fold) by qPCR (Fig. 3C), and elongation of mean telomere length from 3.76 kb to 3.97 kb (1.06-fold) or median telomere length from 3.50 kb to 3.78 kb (1.08-fold) by SM-STELA (Fig. 3D; Fig. S22).

For patient R05, telomere flow-FISH data were available from M0 to M3 as of September 30, 2024. Age-adjusted telomere nomograms showed a rapid decrease in telomere length (Fig. S23). For example, telomere length in granulocytes decreased from M0 (5.2 kb) to M1 (4.2 kb), but partially recovered by M3 (4.7 kb). However, telomere distribution and deconvoluted profiles of lymphocytes (Fig. S24) and granulocytes (Fig. 3E; Fig. S25) showed the emergence of subpopulations of cells with longer telomeres. Granulocytes showed a transition from a single 4.9 kb peak (M0) to two peaks of 4.9 kb and 4.1 kb (M1) and two peaks of 5.8 kb and 4.5 kb (M3) (Fig. 3E, F). This was also noted by a slight increase in the fraction (%) of granulocytes with telomeres > 7 kb in length from M0 to M1 and M3 (Fig. 3G).

A description of the patient's clinical phenotypes before and after EXG34217 treatment is included in the Supplementary Appendix (Text S7). Fig. S26 shows changes in blood parameters over time before and after EXG34217 treatment. Intermittent low-dose (150-300 µg) G-CSF injection was first initiated 6 years before EXG34217 treatment for fatigue,

aphthous ulcers, and occasional grade 1-2 bone pain. Nine months before the first mobilization attempt G-CSF was stopped and the ANC declined as expected, with recurrence of mouth ulcers and fatigue. G-CSF injections of 300 µg 2-3 times per week was restarted. Between the first and second mobilization attempts, the patient was clinically treated with intermittent G-CSF (480 µg) every 2-8 weeks in order to maintain an ANC above $1.0x10^3$ cells/µl. Assessment of peripheral blood counts after EXG34217 treatment is limited as follow-up is short and ongoing; however, the patient has not received G-CSF treatment since the EXG34217 treatment and remains free from previous symptoms (fatigue, aphthous ulcers, bone pain) at the most recent (M5) follow-up visit. Before EXG34217 treatment, the lowest ANC without low-dose G-CSF was $0.6x10^3$ cells/µl, whereas after the EXG34217 treatment, the lowest ANC was $1.2x10^3$ cells/µl (M1) with the ranges of 1.2 $x10^3$-$5.6x10^3$ cells/µl up to M5 (Fig. 3H). Total white blood cell counts showed trends similar to ANC (Fig. S26). Red blood cells, hematocrit, and hemoglobin were below the normal ranges before and after the EXG34217 treatment. Comparing before (-M8 to M0) to after the EXG34217 treatment (M0 to M5) red blood cell counts were 3.19-3.63 $x10^6$/µl before and 3.39-3.84 $x10^6$/µl after; the hematocrits were 32.2-37.0% before and 35.4-40.1% after; the hemoglobin was 11.2-12.8 g/dl before and 11.7-13.4 g/dl after (Fig. S26). Platelets remained in the normal range before and after EXG34217 treatment except for M0 ($87x10^3$/µl), most likely due to the apheresis (Fig. S26). Before and after EXG34217 treatment, the patient was and has remained transfusion-independent without serious infections or evidence of myeloproliferation on examination of her peripheral blood.

Pulmonary fibrosis and function have not yet been assessed following EXG34217 treatment. The patient's high-resolution chest CT scan 6 years before EXG34217 treatment was normal without evidence of pulmonary fibrosis. Serial pulmonary function testing by spirometry 17 months before EXG34217 treatment was normal. As of 5 months after EXG34217 treatment, the patient was without shortness of breath or difficulty breathing (Text S7).

The patient demonstrates no functional liver impairment with normal functional markers before and after EXG34217 treatment (Fig. S27). Magnetic resonance imaging (MRI) elastogram 6 years before EXG34217 treatment was negative for liver fibrosis. Liver imaging has not yet been performed after EXG34217 treatment (Text S7).

The patient has had endometriosis and chronic pelvic pain requiring ablation for several years before EXG34217 treatment. A hysterectomy was recommended before EXG34217 treatment but was performed 5 months after treatment, as intended (Text S7).

The patient's hyperextensible joints, present since early childhood, have caused recurrent joint subluxations requiring multiple surgeries. After EXG34217 treatment, the patient has not reported additional joint injuries or concerns (Text S7).

SAFETY AND ADVERSE EVENTS

Table 2 shows all AEs for the four study patients. No deaths have been reported. 70% of AEs (Common Terminology Criteria for Adverse Events (CTCAE) were grade 1-2, mild to moderate) occurred during mobilization and resolved. They included bone pain, pain after line replacement, nausea, skin sensitivity, and edema for patients R01/R02, R4/R5, and R06 (Table 2). During mobilization, patient R03 experienced mild pain after line placement (grade 1) and serious AEs (SAEs) of fever (grade 2), hypoxemia (grade 3, severe), and blood infection (*s. capitis*: grade 3). These SAEs were subsequently resolved. Patients R02 and R05 were dosed with EXG34217 and followed up to M24 and M5, respectively. Patient R02 experienced cardiac AEs: mild prolonged QT interval by electrocardiogram (grade 1, at M3, M11), ectopic atrial rhythm (grade 1, at M5), and worsening of pre-existing non-ischemic cardiomyopathy (grade 2, , M24). Patient R02 also showed pulmonary AEs: lung infection (grade 2, at M7) and worsening of pulmonary fibrosis (grade 1, at M12). Patient R05 showed an increase in thrombin time (grade 1, at 19 days after infusion) (Table 2).

DISCUSSION

We report a novel therapeutic approach for treating TBD and a summary of the experience for four patients who have participated in the study thus far. Two patients were treated with EXG34217 after successful mobilization and were followed for 24 and 5 months. Most AEs, mild to moderate, occurred during mobilization and resolved. After EXG34217 infusion, no acute safety issues were noted, and mild-moderate long-term cardiac and pulmonary AEs in one patient may be attributable to the patient's underlying conditions. Due to the limited number of patients, the conclusions we can draw are limited. However, in both EXG34217-treated patients, we observed ex vivo telomere elongation in CD34+ cells and the emergence of subpopulations of cells with longer telomeres in peripheral blood cells following EXG34217 infusion. Notably, patient R05, who required intermittent injection of low-dose G-CSF, has not required G-CSF injection since the EXG34217 treatment and has remained without prior symptoms of fatigue, aphthous ulcers, or bone pain at the last follow-up visit (M5). We speculate that these results suggest hematopoiesis derived from the possible persistence of autologous CD34+ cells treated with ZSCAN4. Persistence may have been facilitated by G-CSF and Plerixafor injection opening the bone marrow before EXG34217 infusion, as demonstrated previously in a mouse study.[38] Importantly, an effective therapy that does not require a preparative regimen is desirable as TBD patients are radiation- and chemotherapy-sensitive.[27] These data support further investigation and translation of telomere elongation by ZSCAN4 exposure in patients with TBD as a potential therapeutic intervention for preventing or treating bone marrow failure.

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FIGURE LEGENDS

Figure 1. Ex vivo telomere elongation of patient R02 CD34+ cells after contact with EXG-001

Panel A shows the EXG34217 therapeutic procedure.

Panel B shows the immunohistochemistry of patient R02's CD34+ cells before contact with EXG-001 (Tx-) and after contact with EXG-001 (Tx+: EXG34217) using an anti-ZSCAN4 antibody. Efficiency of ZSCAN4 delivery was 89.7% (4532/5052) in EXG34217. DAPI denotes 4',6-diamidino-2-phenylindole for DNA staining.

Panel C shows the colony-forming cell (CFC) assay of EXG34217 (patient R02). BFU-E denotes Burst-forming unit-erythroid; CFU-E, Colony-forming unit-erythroid; CFU-G, Colony-forming unit-granulocyte; CFU-M, Colony-forming unit-macrophage; CFU-GM, Colony-forming unit-granulocyte, macrophage; CFU-GEMM, Colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte.

Panel D shows the telomere length of the patient R02's CD34+ cells before contact with EXG-001 (Tx-) and after contact with EXG-001 (Tx+: EXG34217) using qPCR assay. Relative telomere length: Tx-, Mean 0.779, 95% CI [0.634, 0.918]; Tx+: Mean 0.964, 95% CI [0.780, 1.148]. PBMC from a healthy donor (Female, Age 38), mobilized peripheral blood CD34+ cells (#1) from a healthy donor (Male, Age 29), and mobilized peripheral blood CD34+ (#2) from a healthy donor (Male, Age 28) were used as controls. qPCR denotes quantitative polymerase chain reaction; PBMC denotes peripheral blood mononuclear cells.

Panel E shows the telomere length distribution of chromosome 17p analyzed by SM-STELA for the same Tx- and Tx+ samples used in Panel D. Left panel: Scatter plots of Tx- (Mean 3.35 kb, 95% CI [3.16, 3.54]) and Tx+ (Mean 3.59 kb, 95% CI [3.46, 3.72]). Middle and right panel: Telomere length distribution profiles (black curve) obtained by plotting the number of DNA fragments with specific telomere length (0.2 kb bin) (y-axis) against the telomere length of these DNA fragments (x-axis) for Tx- (Middle panel) and Tx+ (Right panel). The Gaussian curves, Fit Peak 1 (green) and Fit Peak 2 (pink), show distinct telomere populations identified by the deconvolution analysis of the density curve (black). Cumulative Fit Peak (blue).

Figure 2. In vivo persistence of hematopoiesis with telomere elongation in patient R02 after EXG34217 infusion.

Panel A shows telomere flow-FISH profiles (black curves) of lymphocytes from the patient R02 at M0, M1, M3, M6, M9, M12, M20, and M24. Curves with colors indicate Gaussian curves (Fit Peaks) obtained by deconvolution analyses of the black curves, indicating cell populations with different telomere lengths. Cumulative Fit Peak (blue). The numbers to the right of the curves are the average values of each Gaussian curve, i.e., the average telomere length (kb) of each cell population.

Panel B shows a nomogram of telomere flow-FISH results (M0, M1, M3, M6, M9, M12, M20, and M24) based on the standard method (35-45 years range). The median values of the profiles are considered the telomere length (kb).

Panel C shows a nomogram of telomere flow-FISH results (M0 and M24) based on the mean telomere length of each cell subpopulation obtained by the deconvolution.

Panel D shows the fractions (%) of lymphocytes with telomeres longer than 6 kb.

Panel E shows SM-STELA telomere profiles (black curves) of lymphocytes from the patient R02 at M0, M6, and M24. Curves with colors indicate Gaussian curves (Fit Peaks) obtained by deconvolution analyses of the black curves, indicating cell populations with different telomere lengths. Cumulative Fit Peak (blue). The numbers to the right of the curves are the average values of each Gaussian curve, i.e., the average telomere length (kb) of each cell population.

Panel F shows the ANC of patient R02 over 4 years before and after EXG34217 treatment. A dotted vertical line at -M8 indicates the first mobilization attempt, resulting in no apheresis and no EXG34217 treatment. A vertical line and a blue arrow at M0 indicate the second mobilization attempt, followed by apheresis and EXG34217 treatment. Normal ranges of ANC are shown in pink (1.80-7.70x10^3/µl). Right panel: Difference of ANC before (Pre) and after

(Post) EXG34217 treatment. Immediately before EXG34217 treatment, the patient received G-CSF and Plerixafor for mobilization, which may affect blood counts, and thus, the period after M0 and before M6 was excluded. Pre (-M27-M0): mean 1.78x10^3/µl, 95% CI [1.43, 2.12]. Post (M6-M24): mean 3.18x10^3/µl, 95% CI [2.13, 4.22].

Figure 3. Ex vivo and in vivo telomere elongation in patient R05.
Panel A shows the immunohistochemistry of patient R05's CD34+ cells before contact with EXG-001 (Tx-) and after contact with EXG-001 (Tx+: EXG34217) using an anti-ZSCAN4 antibody. The efficiency of ZSCAN4 delivery was 94.2% (1807/1917) in EXG34217.
Panel B shows the colony-forming cell (CFC) assay of EXG34217.
Panel C shows the telomere length of the patient R05's CD34+ cells before contact with EXG-001 (Tx-) and after contact with EXG-001 (Tx+: EXG34217) using qPCR assay. Relative telomere length: Tx-, mean 2.134, 95% CI [1.975, 2.293]; Tx+: mean 2.622, 95% CI [2.228, 3.016]. PBMC from a healthy donor (Female, Age 38), mobilized peripheral blood CD34+ cells (#1) from a healthy donor (Male, Age 29), and mobilized peripheral blood CD34+ (#2) from a healthy donor (Male, Age 28) were used as controls.
Panel D shows the telomere length distribution of chromosome 17p analyzed by SM-STELA for the same Tx- and Tx+ samples used in Panel C. Left panel: Scatter plots of Tx- (Mean 3.76 kb, 95% CI [3.63, 3.90]) and Tx+ (Mean 3.97 kb, 95% CI [3.83, 4.10]). Middle and right panel: Telomere length distribution profiles (black curve) obtained by plotting the number of DNA fragments with specific telomere length (0.1 kb bin) (y-axis) against the telomere length of these DNA fragments (x-axis) for Tx- (Middle panel) and Tx+ (Right panel). The Gaussian curves, Fit Peak 1 (green) and Fit Peak 2 (pink), show distinct telomere populations identified by the deconvolution analysis of the density curve (black). Cumulative Fit Curve (blue).
Panel E shows telomere flow-FISH profiles (black curves) of granulocytes from patient R05 at M0, M1, and M3. Curves with colors indicate Gaussian curves (Fit Peaks) obtained by deconvolution analyses of the black curves, indicating cell populations with different telomere lengths. Cumulative Fit Peak (blue). The numbers to the right of the curves are the average values of each Gaussian curve, i.e., the average telomere length (kb) of each cell population.
Panel F shows the telomere lengths of the deconvoluted peaks.
Panel G shows the fractions (%) of granulocytes with telomeres longer than 7 kb.
Panel H shows the ANC of the patient R05 over 5 years before and after EXG34217 treatment. A dotted vertical line at -M8 indicates the first mobilization attempt, resulting in no apheresis and no EXG34217 treatment. A vertical line and a blue arrow at M0 indicate the second mobilization attempt, followed by apheresis and EXG34217 treatment. Normal ranges of ANC are shown in pink (1.80-7.70x10^3/µl). Red squares denote data points when blood counts were performed while the patient received a low-dose G-CSF treatment.

Table 1. Changes of mobilization strategy and the number of mobilized CD34+ cells in peripheral blood

Patient ID	Sex	Age (Years)	Race	Mobilization protocol[3]	Draw Time (hours post plerixafor)	CD34+ cells/µl[4]	Apheresis	EXG34217 Infusion
R01[1]	Male	36	White	P1	12 hours (day 5)	3	No	No
R02[1]		37		P2	9 hours (day 5)	10	2 days	Yes
R03	Male	27	White	P2	9 hours (day 5)	1	No	No
R04[2]	Female	32	White	P2	9 hours (day 5)	3	No	No
R05[2]		33		P3	2.5 hours (day 5)	11	2 days	Yes
					3.5 hours (day 5)	15		
					6 hours (day 5)	17		
					2 hours (day 6)	14		
					4 hours (day 6)	17		
R06	Male	18	White	P3	3 hours (day 5)	2	No	No
					3.5 hours (day 6)	2		
					7 hours (day 6)	2		

1) R01 (first mobilization) and R02 (second mobilization) are the same patient, mobilized with different protocols at separate time points.

2) R04 (first mobilization) and R05 (second mobilization) are the same patient, mobilized with different protocols at separate time points.

3) Mobilization and peripheral blood stem cell collection are challenging in patients with telomere biology disorders. To optimize stem cell collection, the mobilization protocol has been changed throughout the duration of the study as follows:

P1: G-CSF 10 µg/kg daily for 5 days, plerixafor 0.24 mg/kg on day 4 in the evening.

P2: G-CSF 10 µg/kg daily for 2 days, G-CSF 20 µg/kg daily for 3 days, plerixafor 0.24 mg/kg on day 4 and day 5 in the evening.

P3: G-CSF 20 µg/kg daily for 6 days, plerixafor 0.24 mg/kg on day 5 and day 6 in the morning.

4) Study criteria for initiation of apheresis and CD34+ cell collection is peripheral blood CD34+ cell count of ≥5 cells/µl.

Table 2. Adverse events

Patient ID	AE	Start date	SAE	CTCAE Grade	Preexisting condition	Outcome
R01[1]	Skin sensitivity	Day 1 of mobilization		1		Resolved
	Bone pain	Day 1 of mobilization		1		[3]
	General edema	Day 1 of mobilization		1		Resolved
R02[1]	Bone pain	Day 2 of mobilization		1		Resolved
	ECG QT interval prolonged	94 days after infusion		1		Ongoing
	Ectopic atrial rhythm	158 days after infusion		1	Yes	Resolved
	Lung infection (possible pneumonia)	214 days after infusion		2		Resolved
	ECG QT interval prolonged	335 days after infusion		1		Ongoing
	Worsening pulmonary fibrosis[4]	1 year after infusion		1	Yes	Ongoing
	Worsening non-ischemic cardiomyopathy[5]	2 years after infusion		2	Yes	Ongoing
R03	Pain after line placement	Day 4 of mobilization		1		Resolved
	Fever	Day 4 of mobilization	Yes	2		Resolved
	Hypoxemia	Day 4 of mobilization	Yes	3		Resolved
	Blood infection (*S. capitis*)	Day 4 of mobilization	Yes	3		Resolved
R04[2]	Bone pain	Day 3 of mobilization		2		[3]
	Pain after line placement	Day 4 of mobilization		1		Resolved
R05[2]	Bone pain	Day 2 of mobilization		2		Resolved
	Pain after line placement	Day 2 of mobilization		1		Resolved
	Lower extremity edema	Day 5 of mobilization		1		Resolved
	Increased TT	19 days after infusion		1		Resolved
R06	Bone pain	Day 4 of mobilization		1		Resolved
	Pain after line placement	Day 6 of mobilization		1		Resolved
	Nausea	Day 6 of mobilization		1		Resolved

1) R01 and R02 are the same patient.

2) R04 and R05 are the same patient.

3) These patients were withdrawn from the study immediately after mobilization. There is no follow-up assessment after withdrawal.

4) See Table S5, pulmonary section.

5) See Table S5, cardiac section.

Abbreviation: ID, identification; AE, adverse event; CTACE, common terminology criteria for adverse events; ECG, electrocardiogram; SAE, serious adverse event; TT, thrombin time.

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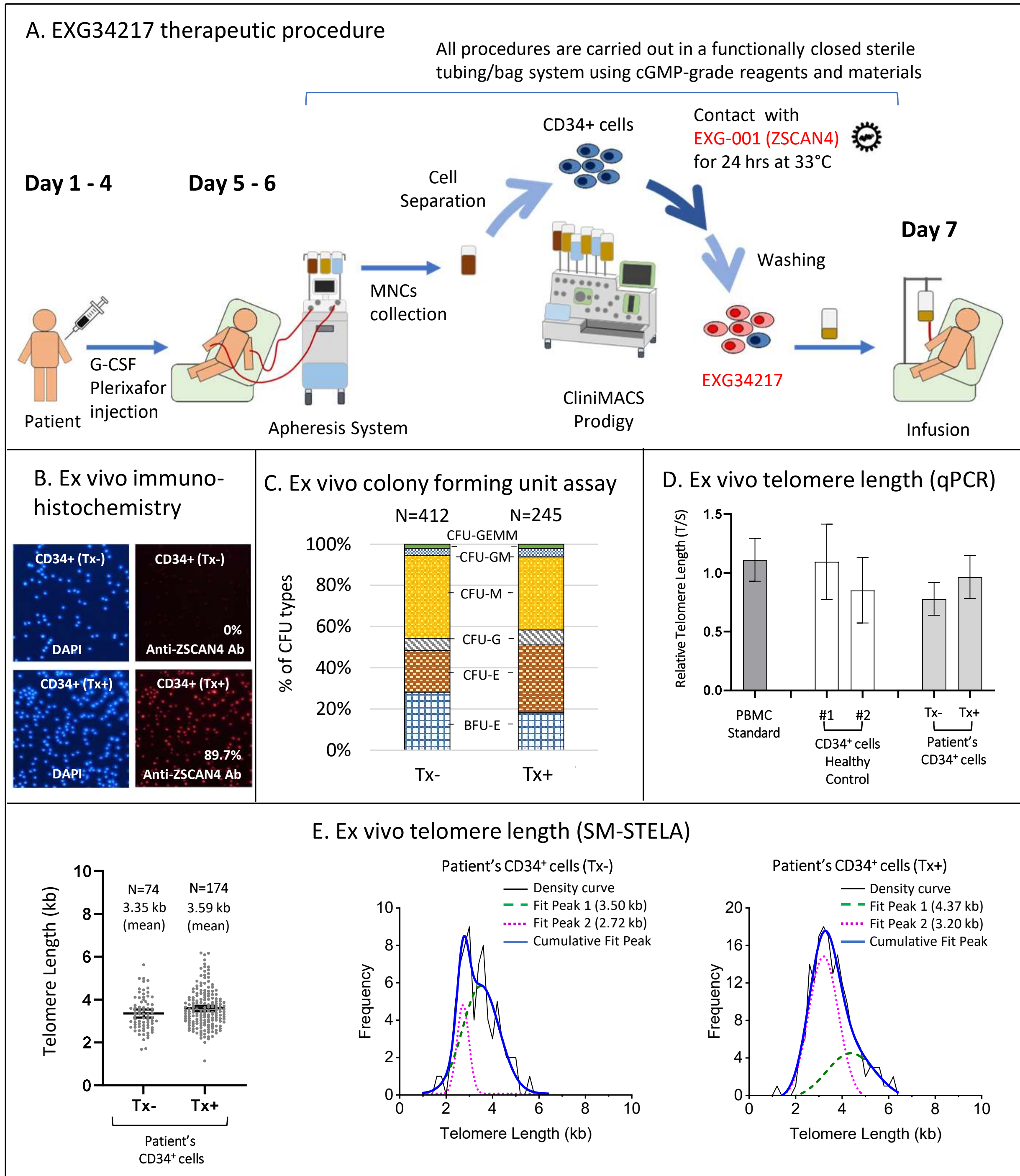

FIG. 1

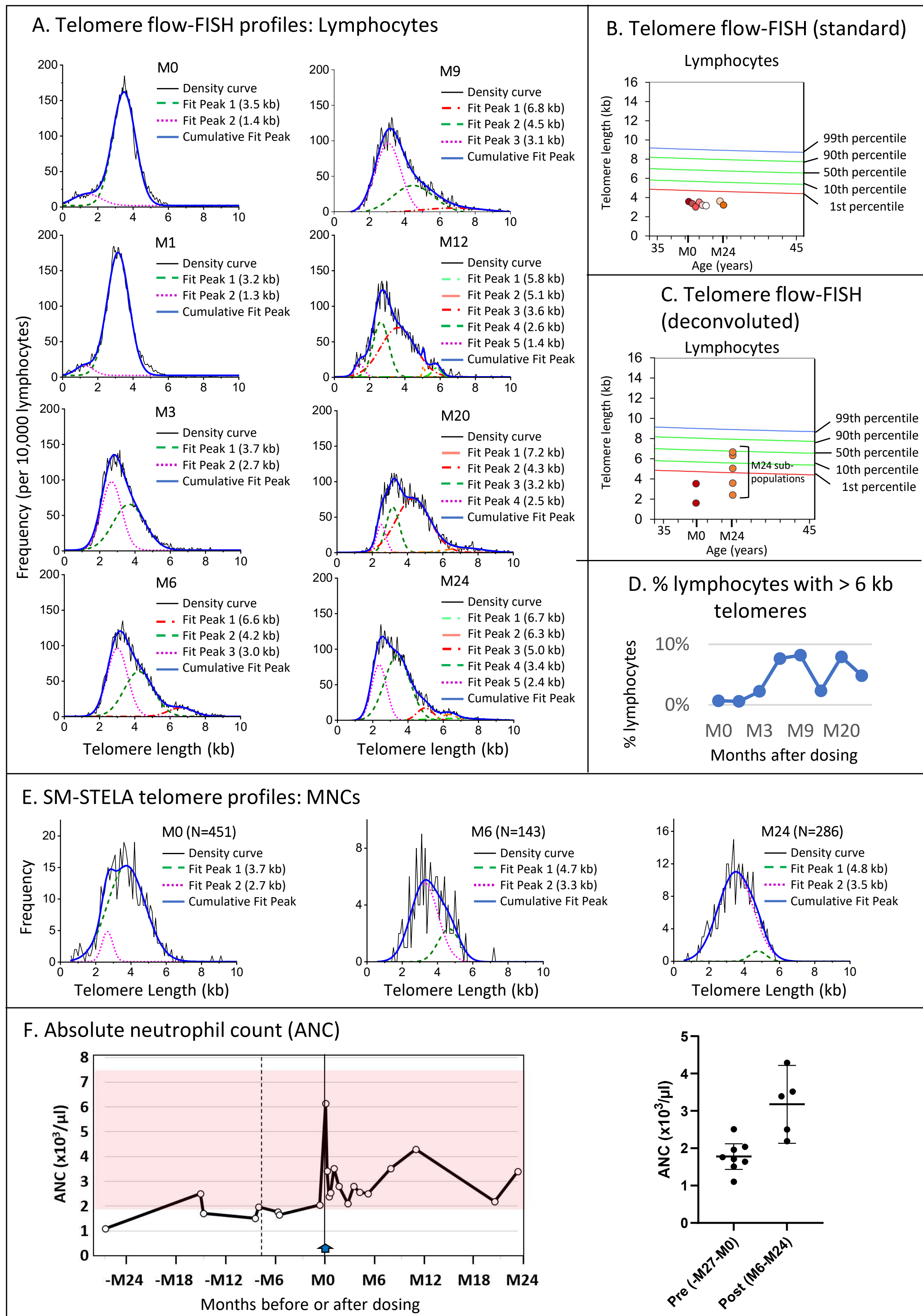

FIG. 2

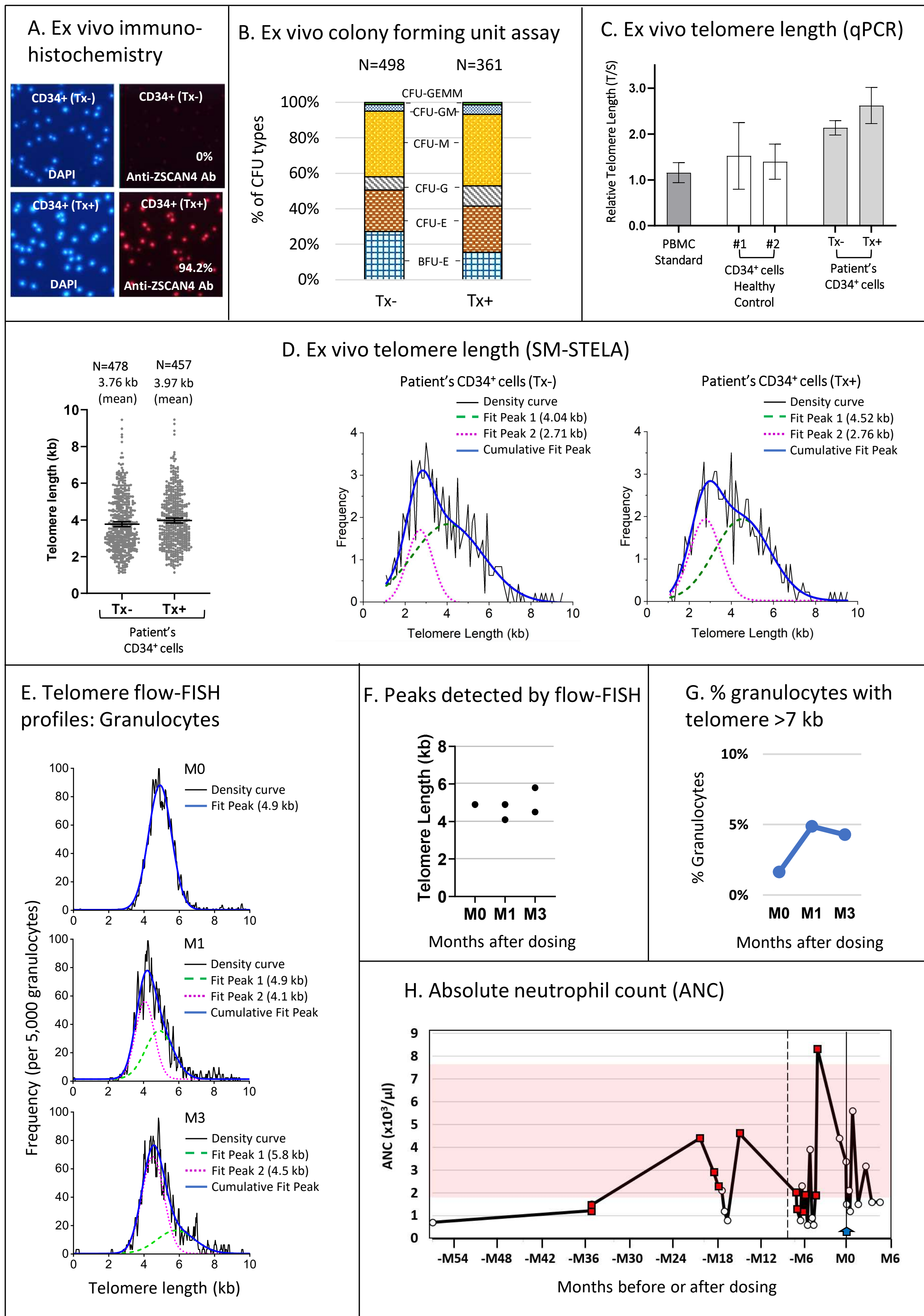

FIG. 3

Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Myers et al. Clinical Use of ZSCAN4 For Telomere Elongation in Hematopoietic Stem Cells

Table of Contents:

List of Investigators Page 5

Methods Page 6

Author Contributions and Acknowledgements Page 9

Supplementary Data

Section S1: Preclinical Page 10

Text S1: Treatment strategy. Page 11

Figure S1: Control experiments showing the rapid reduction of ZSCAN4 protein in CD34+ cells via immunofluorescence after increasing the temperature to 37°C. Page 12

Text S2: Preclinical data. Page 13

Figure S2: Control experiments showing telomere elongation by EXG-001 in CD34+ cells derived from three healthy donors. Page 14

Figure S3: Control experiments showing telomere elongation by EXG-001 in human primary fibroblast cells derived from patients with dyskeratosis congenita, i.e., telomere biology disorders (TBD). Page 15

Figure S4: Control colony-forming cell (CFC) assays showing normal broad differentiation potential in CD34+ cells derived from three healthy donors after EXG-001 treatment. Page 16

Text S3: Control telomere assays to detect mosaicism. Page 17

Figure S5: Deconvoluted telomere profiles of the lymphocytes from blood cell mixtures analyzed by telomere flow-FISH. Page 19

Figure S6: Alternative analyses of telomere flow-FISH profiles obtained from blood cell mixtures. Page 20

Figure S7: Representative SM-STELA images of a mixture of MNCs obtained from a TBD patient (unrelated to the clinical study patient) and from a healthy individual. Page 21

Figure S8: Scatter plots of telomere lengths from the MNC mixtures analyzed by SM-STELA. Page 22

Figure S9: Deconvoluted telomere profiles of the MNC mixtures analyzed by SM-STELA. Page 23

Figure S10: Comparison of median telomere lengths in blood mixtures detected by telomere flow-FISH and SM-STELA. Page 24

Section S2: Patient R02 Page 25

Text S4. Patient R02's telomere data Page 26

Figure S11: Representative SM-STELA images of the patient R02 CD34+ cells with (CD34 Tx+) or without (CD34 Tx-) EXG-001 treatment. Page 27

Figure S12. Nomograms of telomere flow-FISH results of patient R02's peripheral blood cells. Page 28

Figure S13: Telomere profiles of the patient R02's lymphocytes analyzed by telomere flow-FISH. Page 29

Figure S14: Deconvoluted telomere profiles of the patient R02's lymphocytes analyzed by telomere flow-FISH. Page 30

Figure S15: Telomere profiles of the patient R02's granulocytes analyzed by telomere flow-FISH. Page 31

Figure S16: Representative images of the patient R02's MNCs analyzed by SM-STELA. Page 32

Figure S17: Scatter plots of the patient R02's MNCs telomere length as analyzed by SM-STELA. Page 33

Figure S18: Deconvoluted telomere profiles of the patient R02's MNCs analyzed by SM-STELA. Page 34

Text S5: Patient R02's clinical data. Page 35

Figure S19: Changes in blood counts of the patient R02 over time before and after EXG34217 treatment. Page 37

Table S1: Analyses of patient R02's bone marrow cells before and after EXG34217 treatment. Page 38

Figure S20: Representative images of bone marrow biopsies. Page 39

Figure S21: Changes in liver function markers of the patient R02 over time before and after EXG34217 treatment. Page 40

Section S3: Patient R05 Page 41

Text S6. Patient R05's telomere data. Page 42

Figure S22. Representative SM-STELA images of the patient R05 CD34+ cells with (CD34 Tx+) or without (CD34 Tx-) EXG-001 treatment. Page 43

Figure S23. Nomograms of telomere flow-FISH results of patient R05's peripheral blood cells. Page 44

Figure S24: Telomere profiles and deconvoluted telomere profiles of the patient R05's lymphocytes analyzed by telomere flow-FISH. Page 45

Figure S25: Telomere profiles and deconvoluted telomere profiles of the patient R05's granulocytes analyzed by telomere flow-FISH. Page 46

Text S7. Patient R05's clinical data Page 47

Figure S26: Changes in blood counts of the patient R05 over time before and after EXG34217 treatment. Page 49

Table S2. Analyses of patient R05's bone marrow cells before EXG34217 treatment. Page 50

Figure S27: Changes in liver function markers of the patient R05 over time before and after EXG34217 treatment. Page 51

References Page 52

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Methods

Study Medication

EXG-001 is a non-transmissible, temperature-sensitive variant of the Sendai virus (SeV) vector (SeV18/TS15ΔF)[1] that encodes the cDNA sequence for the human ZSCAN4 gene.[2] EXG-001 is manufactured at a cGMP facility (ID Pharma Co., Ltd., Tokyo, Japan) and stored in a sterile glass vial below 80°C until used. SeV vectors are based on the Sendai virus (now also called murine respirovirus) - a single-stranded RNA virus of the Paramyxovirus subfamily. The Sendai virus has never been shown to be pathogenic to humans.[3] The vector backbone lacks the F protein (ΔF), eliminating its ability to form infectious virus particles, thereby abolishing transmission from cell to cell in humans.[3] Two previous clinical trials using wild-type SeV[4] and another clinical trial using F-gene-deleted SeV[5] have reported no adverse events in humans. The virus completes its entire infection cycle as RNA (i.e., without DNA involvement) so that the recombinant gene of interest introduced by this vector type cannot be integrated into the host genome.

Mobilization

Autologous peripheral blood CD34+ cells are isolated from mononuclear cells (MNCs) collected via apheresis after mobilization with G-CSF and Plerixafor. Due to the expected challenges of collecting CD34+ cells from TBD patients, a go-no-go decision point is set before the apheresis: if circulating CD34+ cells are below 5 cells/μl, patients are not subjected to the apheresis and withdrawn from the study. The details of the mobilization condition are described in Table S1. The mobilization strategy was changed twice during the study to improve mobilization outcomes (Table S1).

Ex vivo cell manufacturing procedure

The ex vivo manufacturing procedure used a semi-automated, functionally closed tubing/bag system without open manipulation. CD34+ cells were isolated using the CliniMACS Prodigy (Miltenyi Biotec) [6] from mononuclear cells (MNCs) collected via apheresis. Subsequently, the CD34+ cells were incubated with EXG-001 at 33°C for 24 hours, incubated at 37°C for 1 hour, washed three times to remove free EXG 001, and resuspended in 100 ml of Plasma-Lyte A. The final cellular product is called EXG34217. After taking an aliquot for post-infusion analyses, the EXG34217 cells were transported to the bedside at 2 - 8°C and infused into the patient within 3.5 hours of manufacturing.

Immunostaining for ZSCAN4 expression

This assay measured the efficiency of ZSCAN4 protein production in EXG34217 by identifying the percent of ZSCAN4+ cells. Immunostaining was performed as previously described [2]. In brief, $2x10^5$ cells were plated on sterilized Poly-L lysine-coated glass coverslips in a 12-well plate. The cells were fixed with 8% paraformaldehyde (PFA) and stained with an antibody against human ZSCAN4. The cells were subsequently counterstained with DAPI. Two individuals manually counted the number of DAPI+ and ZSCAN4+ cells independently.

Colony-forming cell (CFC) assay of CD34+ cells

This assay measured whether EXG34217 could produce various types of blood cells in vitro in methylcellulose-based media. The patient's CD34+ cells (before EXG-001 treatment) and EXG34217 (after EXG-001 treatment) were analyzed using methylcellulose-based media (R&D Systems, Catalog #HSC003) according to the manufacturer's protocol. In brief, $2x10^3$ cells were added to 35 mm cell

culture dishes and cultured at 37°C for 14 days. The cultured cells were then used for CFU assays using methylcellulose-based media (R&D Systems, Catalog #HSC003). Each colony was assessed under the microscope and grouped into BFU-E (Burst-forming unit-erythroid), CFU-E (Colony-forming unit-erythroid), CFU-G (Colony-forming unit-granulocyte), CFU-M (Colony-forming unit-macrophage), CFU-GM (Colony-forming unit-granulocyte, macrophage), CFU-GEMM (Colony-forming unit-granulocyte, erythroid, macrophage, megakaryocyte) categories according to the standard morphological criteria.

Telomere qPCR assay

Telomere qPCR was performed using SYBR Green master mix (ThermoFisher Scientific) as previously described[7]. In brief, qPCR was performed using primer pairs for telomeres (T) (5′-GGTTTTTGAGGGTG AGGGTGAGGGTGAGGGTGAGGGT-3′; 5′-TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCC TA-3′) and the 36B4 single copy gene (S) (5′-CAGCAAGTGGGAAGGTGTAATCC-3′; 5′-CCCATTCTATCATCAACGGGTACAA-3′). Relative telomere length was presented as a T/S ratio.

For the Telomere qPCR results presented in Fig. 1C, the assay measured whether telomeres were extended in EXG34217 compared to a patient's pretreatment CD34+ cells. For comparison, the following cells were purchased from vendors: peripheral blood MNCs (mononuclear cells) from a healthy donor (Female, Age 38, Lot 3007181, Donor 8866: AllCells), CD34+ cells (#1, Male, Age 29, mobilized peripheral blood CD34+ cells, Lot 3008831, Donor 6764, 98.9% purity: AllCells); and CD34+ cells (#2, Male, Age 28, mobilized peripheral blood CD34+ cells, Lot 3007720, Donor 9157, 99.5% purity: AllCells). For MNCs, genomic DNA was directly extracted and used for telomere qPCR. The patient's CD34+ cells, EXG34217, control CD34+ cells (#1), and control CD34+ cells (#2) were plated separately in a 12-well plate and cultured for 9 days at 37°C. These cells were cultured in StemMACS HSC Expansion Media XF (Miltenyi Biotec) supplemented with StemMACS HSC Expansion Cocktail (recombinant human Flt3-Ligand, recombinant human stem cell factor, recombinant thrombopoietin) (Miltenyi Biotec). Cells were harvested for genomic DNA isolation using DNeasy Blood and Tissue Kit (Qiagen) according to the manufacturer's protocol.

Telomere flow-FISH assay

The Telomere Flow-FISH assay identified whether telomeres were extended in the patient's peripheral blood after treatment. Telomere Flow-FISH was performed at the CLIA-certified laboratory of Repeat Diagnostics (North Vancouver, Canada) using the established method[8]. The obtained FACS data were replotted after normalizing to total cell counts and converting the values of each bin to telomere length (kb). The telomere size distribution curves were deconvoluted into multiple Gaussian curves using OriginPro (Version 2024, OriginLab Corporation, Northampton, MA, USA). Assuming that each Gaussian curve represents cell populations with specific telomere lengths, each cell population's average telomere length (kb) was mapped to a telomere nomogram.

Single-molecule single telomere length analysis (SM-STELA)

SM-STELA allows high-resolution telomere analysis[9]. DNA was extracted using the Maxwell RSC Blood DNA kit (Promega) and quantified using the QuantiFluor ONE dsDNA system (Promega). SM-STELA was carried out at the 17p telomere as described previously[10]. For each sample, 1 µM of the Telorette-2 linker was added to 10 ng of purified genomic DNA in a final volume of 40 µl. Multiple PCRs were performed for each test DNA in volumes of 10 µl, which incorporated 1 µl of the DNA/Telorette-2 mix and 0.5 µM of the telomere-adjacent and Teltail primers in 75 mM Tris-HCl pH 8.8, 20 mM (NH4)2SO4, 0.01% Tween-20, and 1.5 mM MgCl2, with 0.5 U of a 10:1 mixture of Taq (Life Technologies) and Pwo polymerase (Roche Molecular Biochemicals). The reactions were subjected to 22 cycles at 94°C for 20 s, 59°C for 30 s, and 68°C for 8 min in a Tetrad2 Thermal Cycler (Bio-Rad). DNA fragments were resolved

using 0.5% Tris-acetate-EDTA agarose gel electrophoresis and identified via Southern hybridization using a random-primed dCTP α-33P-labeled (Hartmann Analytic) TTAGGG repeat probe, together with probes specific for the 1 kb (Stratagene) and 2.5 kb molecular weight markers (Bio-Rad). Hybridized fragments were detected using a Typhoon FLA 9500 Phosphorimager (GE Healthcare). The molecular weights of the DNA fragments were calculated using the Phoretix 1D Quantifier software (Nonlinear Dynamics). The telomere size distribution curves were deconvoluted into multiple Gaussian curves using OriginPro (Version 2024, OriginLab Corporation, Northampton, MA, USA).

Graphing and Statistical Analyses

Graphing and statistical analyses were performed using GraphPad Prism version 10.0.0 for Windows (GraphPad Software, Boston, MA). Data analyses and graphing were also performed using Microsoft Excel (Microsoft Corporation, Redmond, WA).

Deconvolution Analyses of Telomere Distribution Curves

Deconvolution analyses were performed using OriginPro (Version 2024, OriginLab Corporation, Northampton, MA, USA).

Author Contributions and Acknowledgements

Kasiani Myers, MD: Principal Investigator of this Clinical Study (NCT04211714), helped design the clinical study, analyzed the data, and wrote the manuscript.

Stella M. Davies, MBBS, PhD: Conducted the clinical study and reviewed the manuscript.

Carolyn Lutzko, PhD: Conducted the cellular therapy production for the clinical study and reviewed the manuscript.

Robin Wahle: Conducted the cellular therapy production for the clinical study and reviewed the manuscript.

David D. Grier, M.D.: Analyzed the bone marrow biopsy data.

Geraldine Aubert, PhD: Performed telomere flow-FISH, analyzed the data, and reviewed the manuscript.

Kevin Norris, PhD: Performed single-molecule single telomere length assay (SM-STELA) and analyzed the data.

Duncan M. Baird, PhD: Analyzed the SM-STELA data and reviewed the manuscript.

Minako Koga: Designed and coordinated the clinical study and reviewed the manuscript.

Akihiro C. Ko: Designed the therapeutic strategy, designed and coordinated the clinical study, analyzed the data, and reviewed the manuscript.

Tomokazu Amano, PhD: Performed and analyzed control experiments, performed the ex vivo analyses of EXG34217, and reviewed the manuscript.

Misa Amano: Performed and analyzed control experiments, performed the ex vivo analyses of EXG34217, and reviewed the manuscript.

Hong Yu: Performed and analyzed control experiments, performed the ex vivo analyses of EXG34217, and reviewed the manuscript.

Minoru S.H. Ko, MD, PhD: Conceived therapeutic strategy, designed a clinical study, analyzed the data, and wrote the manuscript.

Section S1: Preclinical

Text S1. Treatment Strategy

We designed the procedure for treating CD34+ cells with ZSCAN4 with the following points in mind.

1. Transient expression of ZSCAN4. The natural mode of action of ZSCAN4 is transient expression, originally discovered in two cell embryos[11] and mouse ES cells[12]. In fact, prolonged expression of ZSCAN4 in cells slows cell proliferation, which is released by the downregulation of ZSCAN4[12]. We expect that ZSCAN4 is required to extend telomeres but not required to maintain the extended telomere length. That is, once the telomeres are extended by ZSCAN4, the telomere length can be maintained for some time without the presence of ZSCAN4.

2. Rapid and permanent inactivation of ZSCAN4 and SeV viral proteins. Immunogenicity against viral vectors is a common concern when using viral vector systems for therapy. To mitigate this issue, we developed a strategy that uses a temperature-sensitive Sendai virus vector to express ZSCAN4. After ZSCAN4 is expressed in hematopoietic stem cells for a fixed period of time using the temperature-sensitive Sendai virus vector within the permissive temperature range (30°C - 35°C), the vector can be inactivated by increasing the temperature to 37°C, the non-permissive temperature. To test this temperature-switching method, we carried out an experiment where CD34+ cells from a healthy individual were treated with EXG-001 at 33°C for 24 hours, followed by incubation at 37°C (Figure S1.) These conditions simulated the cell manufacturing procedure in this clinical study: 24-hour incubation of CD34+ cells with EXG-001 at 33°C to express ZSCAN4 (alongside viral vector proteins), followed by CD34+ infusion into a patient (37°C). Temperature switching provides transient exposure of ZSCAN4 to CD34+ cells and causes viral proteins to rapidly disappear from CD34+ cells. The rapid disappearance of viral proteins helps reduce potential immunogenicity.

3. No bone marrow ablation. In the standard HSCT, preconditioning, such as bone marrow ablation, is required to open a bone marrow niche for CD34+ cell engraftment. However, preconditioning procedures have severe negative impacts on this patient group. We reasoned that mobilization of CD34+ cells with G-CSF and Plerixafor could open the bone marrow niche, providing a narrow window of opportunity for engraftment without preconditioning. Therefore, CD34+ cells treated with EXG-001 for 24 hours are immediately infused back into a patient to minimize the time from the Plerixafor injection to the EXG34217 infusion, taking advantage of the open bone marrow niche. As there is no delay in reintroducing the CD34+ cells to the patient, there is no cryopreservation step for the current EXG34217 therapy.

4. Functionally enclosed system. The current EXG34217 therapeutic procedure is completed in a functionally enclosed tubing system using an apheresis machine and a CliniMACS Prodigy Instrument[6]. A closed system is possible because, unlike most viral vector systems, SeV can infect non-dividing cells in suspension cell culture, and only transient exposure to ZSCAN4 is required.

Figure S1

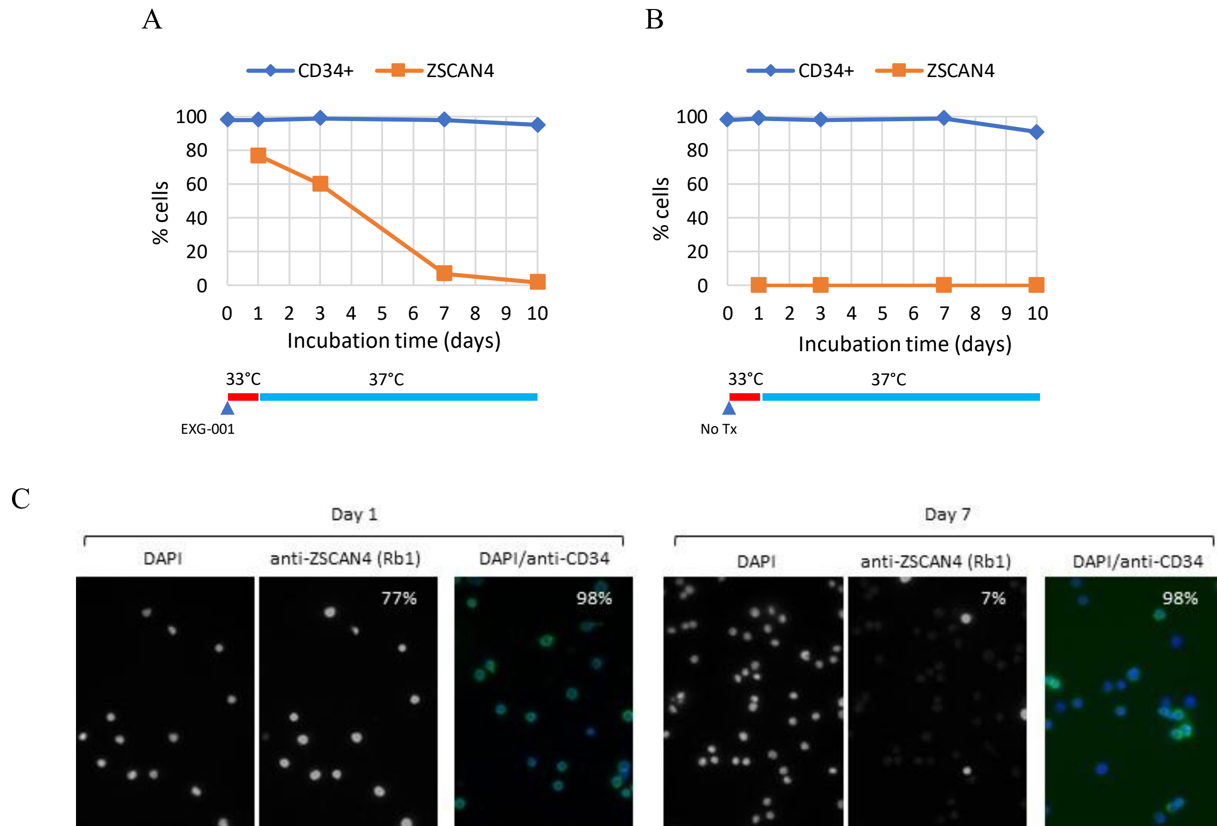


Figure S1. Control experiments showing the rapid reduction of ZSCAN4 protein in CD34+ cells via immunofluorescence after increasing the temperature to 37°C.

CD34+ cells were incubated with (Panel A) or without (Panel B) EXG-001 at 33°C for 24 hours and further incubated at 37°C for 9 days (see Methods section: Telomere qPCR assay). Tx (EXG-001 contact). Panel C shows examples of immunohistochemistry. Cells were sampled on days 1, 3, 7, and 10 and immunostained with antibodies against CD34 and ZSCAN4. To closely mimic the clinical study conditions, CD34+ cells isolated by mobilization of peripheral blood HSCs were purchased from Hemacare (Male, Age 50, Lot 15037441, Donor D111). DAPI (4',6-diamidino-2-phenylindole).

During the 9-day incubation period, nearly 100% of cells retained their CD34 marker, which indicates that contact with EXG-001 did not change the nature of CD34+ cells in terms of the fraction of cells with CD34 marker expression (Panels A, C). In this experiment, after 24-hour contact with EXG-001 (MOI=25), 77% of CD34+ cells expressed ZSCAN4 protein (Panels A, C). As expected, once the temperature shifted to the non-permissive 37°C, the proportion of ZCSAN4+ cells decreased to 7% of the total cell population by day 7 and 2% by day 10 (Panels A, C). By contrast, CD34+ cells without EXG-001 contact had no ZSCAN4+ cells, but nearly 100% remained CD34+ (Panels B, C). The rapid decline of ZSCAN4 protein after switching to 37 °C (core body temperature) was not caused by cell division as the number of cells only increased by 3.5-fold (fewer than 2 cell divisions on average) over 10 days, whereas the number of control cells (no EXG-001 contact) increased by 6.1-fold.

Text S2. Preclinical data

Various preclinical data support this clinical study.

First, we demonstrate that transient expression of ZSCAN4 can increase telomere length in human CD34+ hematopoietic stem cells (Figure S2).

Second, we demonstrate telomere elongation by EXG-001 in human primary fibroblast cells derived from patients with dyskeratosis congenita, i.e., telomere biology disorders (TBD) (Figure S3).

Third, we demonstrate that EXG-001 treatment does not change the differentiation potential of CD34+ cells derived from healthy individuals (Figure S4).

Figure S2

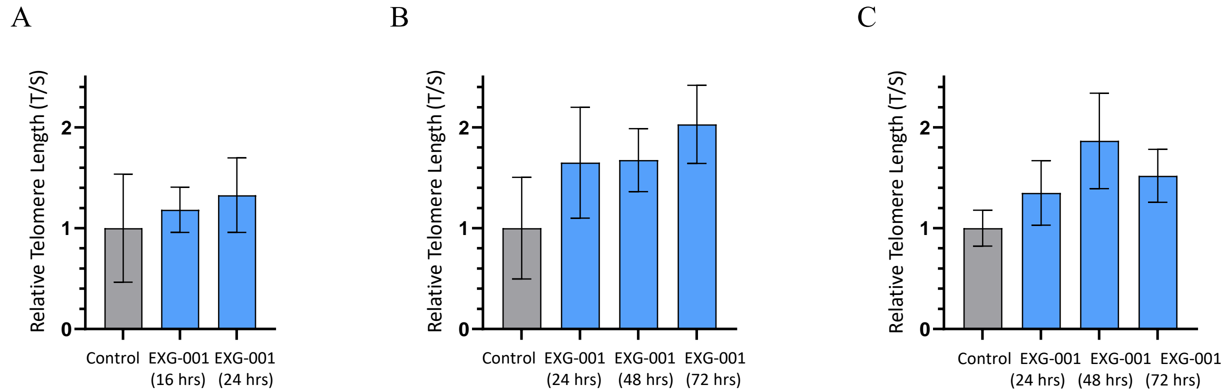
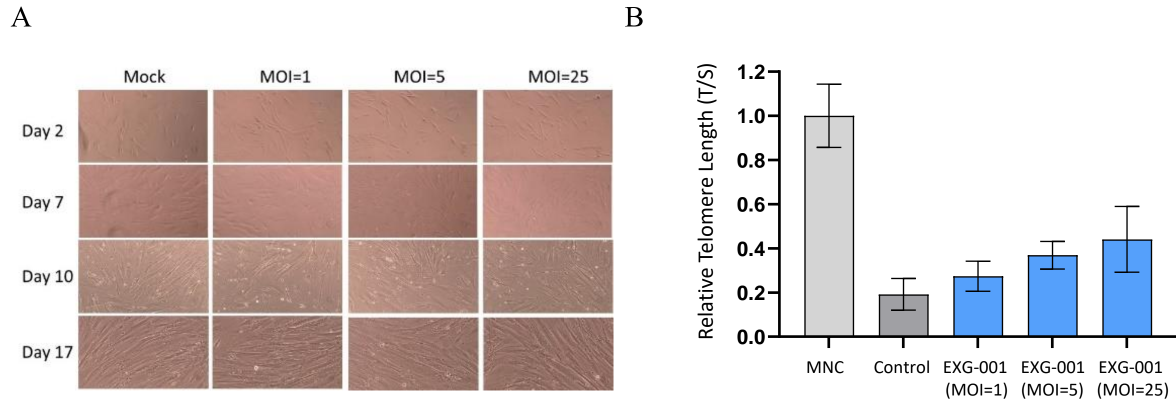


Figure S2. Control experiments showing telomere elongation by EXG-001 in CD34+ cells derived from three healthy donors.

The biological activity of ZSCAN4 in the therapeutic target cells (human CD34+ cells) was examined in CD34+ cells isolated from healthy, unaffected donors. Frozen mobilized peripheral blood CD34+ cells from healthy donors were purchased from AllCells (CA, USA) (Lot# A4934A, Donor 6386 [Panel A], Lot# A4754, Donor 8201 [Panel B]), and Hemacare (CA, USA) (Lot# 16039717, Donor D316069 [Panel C]). CD34+ cells were thawed and remained either untreated or were treated with EXG-001 (MOI=25) for 16, 24, 48, or 72 hours at 33°C. The cells were then cultured at 37°C for 9 days and harvested for genomic DNA extraction. The genomic DNA was analyzed using a quantitative real-time PCR method that used a telomere-specific primer (T) and a single copy gene-specific primer set (S), as previously described, to determine telomere lengths[2,7]. Relative telomere length was calculated as a T/S ratio and further normalized by the T/S ratio of a control sample (untreated control). Mean with 95% CI is shown.

Compared to the untreated CD34+ cells (control), 16-hour contact with EXG-001 was enough to induce telomere elongation (1.18-fold), whereas 24-hour contact induced even more telomere elongation (~1.33-fold) (Panel A). Further tests using CD34+ cells from another donor showed telomere elongation with EXG-001 contact (at 33°C) for 24 hours, 48 hours, and 72 hours (Panel B). For this donor, 24-hour contact with EXG-001 induced ~1.65-fold telomere elongation, which was slightly enhanced by extending the EXG-001 exposure time to 48 or 72 hours. This test was repeated using CD34+ cells from another donor (Panel C). For this donor, 24-hour contact with EXG-001 was enough to induce telomere elongation (~1.35-fold); however, extending the EXG-001 exposure time to 48 hours induced more robust telomere elongation (~1.87-fold), but extending it further to 72 hours showed less telomere elongation. This may be within the range of sample-to-sample fluctuation. Taken together, experiments using CD34+ cells obtained from four healthy individuals showed that treatment and 24-hour incubation at 33°C consistently extended telomeres.

Figure S3



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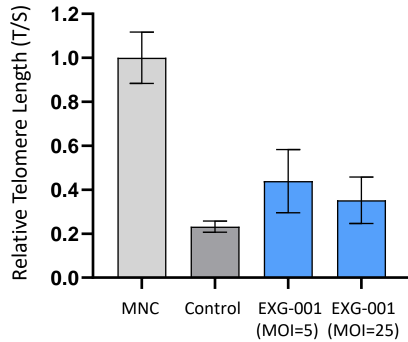


Figure S3. Control experiments showing telomere elongation by EXG-001 in human primary fibroblast cells derived from patients with dyskeratosis congenita, i.e., telomere biology disorders (TBD).

(Panel A) Phase-contrast microscopic images of primary fibroblast cells derived from a 7-year-old male TBD patient (Coriell ID: GM01774, X-linked, DKC1 mutated, passage 8) after contact with EXG-001 at 33°C for 24 hours and then cultured at 37 °C for 17 days. No apparent morphological differences among these cells were observed. Mock (untreated); MOI (multiplicity of infection) =1, 5, or 25.

(Panel B) EXG-001 extended the short telomeres of human primary fibroblast cells derived from a 7-year-old male TBD patient (Coriell ID: GM01774, X-linked, DKC1 mutated). Cells had contact with EXG-001 at 33°C for 24 hours, were cultured at 37°C for 14 days, and were then harvested for genomic DNA extraction and telomere qPCR assays. MNCs (peripheral blood mononuclear cells) from a healthy donor were used for normalization. TBD fibroblast cells made contact with EXG-001 for three MOIs (MOI=1, MOI=5, and MOI=25) and showed telomere elongation by 1.4-fold, 1.9-fold, and 2.3-fold compared to untreated TBD fibroblast cells (Control), respectively. Mean with 95% CI is shown.

(Panel C) EXG-001 extended the short telomeres of human primary fibroblast cells derived from an 11-year-old male TBD patient (Coriell ID: AG04646, X-linked, DKC1 mutated). Cells had contact with EXG-001 for two MOIs (MOI=5, MOI=25) at 33°C for 24 hours, were then cultured at 37°C for 4 days, and harvested for genomic DNA extraction and telomere qPCR assays. TBD fibroblast cells had contact with EXG-001 at two MOIs (MOI=5 and MOI=25), which showed telomere elongation by 1.9-fold and 1.5-fold, respectively, compared to the untreated TBD fibroblast cells (Control). Mean with 95% CI is shown.

Figure S4

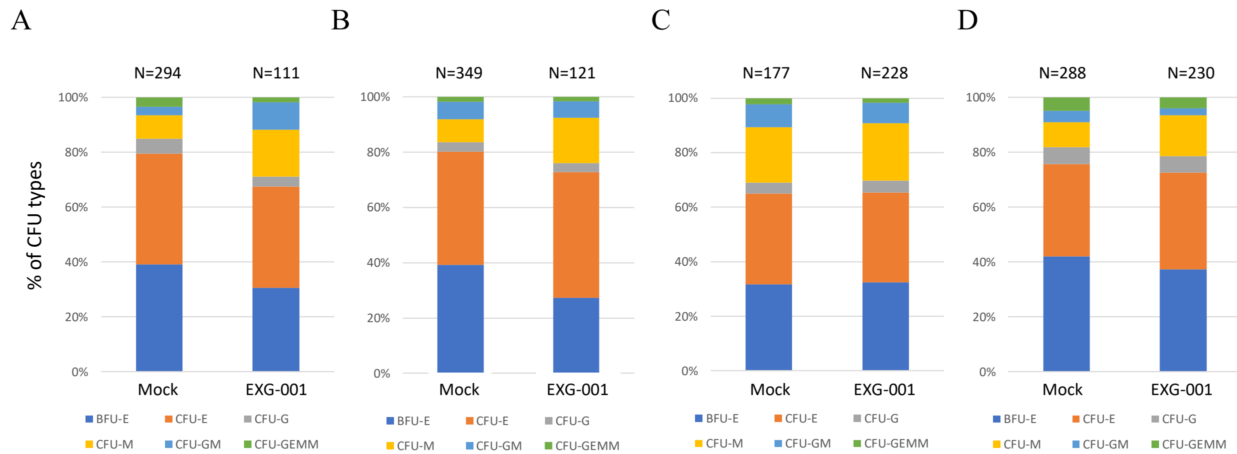


Figure S4. Control colony-forming cell (CFC) assays showing normal broad differentiation potential in CD34+ cells derived from three healthy donors after EXG-001 treatment.

(Panels A and B) Replicates of CFC-types (also called CFU) differentiated from mobilized peripheral blood CD34+ cells in a single healthy individual (Male, age 28, Lot A4727, Donor 6581, purchased from AllCells). (Panel C) CFC-types differentiated from mobilized peripheral blood CD34+ cells in a single healthy individual (Female, age 40, Lot 17044088, Donor D270150, purchased from HemaCare). (Panel D) CFC-types differentiated from mobilized peripheral blood CD34+ cells in a single healthy individual (Male, age 24, Lot 18049664, Donor D316203, purchased from HemaCare).

BFU-E denotes Burst-forming unit-erythroid; CFU-E, Colony-forming unit-erythroid; CFU-G, Colony-forming unit-granulocyte; CFU-M, Colony-forming unit-macrophage; CFU-GM, Colony-forming unit-granulocyte, macrophage; CFU-GEMM, Colony-forming unit - granulocyte, erythroid, macrophage, megakaryocyte.

Text S3. Control telomere assays to detect mosaicism.

In the current therapeutic procedure, a patient receives G-CSF and Plerixafor for CD34+ mobilization but no radiation, chemotherapy, or immune suppression. Therefore, if successfully engrafted, autologous transplant of CD34+ cells treated with EXG-001 (EXG34217) is expected to cause mosaicism with treated and untreated CD34+ cell populations in the bone marrow. After the treatment, blood cells with longer telomeres (as expected by the EXG-001 treatment) are expected to emerge in the peripheral blood against the backdrop of blood cells with shorter telomeres (the patient's untreated endogenous blood cells). The presence of blood cells with longer telomeres in the patient's circulation would indicate successful engraftment and hematopoiesis of EXG34217 (CD34+ cells treated with EXG-001).

Control assays were conducted to confirm that mosaicism could be detected, as peripheral blood cells differentiated from treated CD34+ cells may be only a small fraction of a patient's blood cells. Unlike commonly used retroviruses or other viruses for gene therapy, the SeV used in the current procedure is a non-integrating RNA virus and, thus, does not leave genetic markers. Therefore, there is no easy way to identify blood cells derived from the treated CD34+ cells or to isolate such cells for subsequent analyses. The only method of detection is to analyze the telomere length of the patient's blood cells and to demonstrate the presence of cells with longer telomeres against the backdrop of cells with shorter telomeres. However, this posed a challenge because telomere length can only be measured as a distribution of different telomere lengths, thus, the median telomere length is usually used as the average telomere length. Therefore, it becomes a question of whether telomere distribution curves of two or more cell populations with different telomere length distributions can be identified and to what sensitivity.

To test this possibility, we performed control experiments measuring telomere lengths of blood cells with two different methods, where blood cells from two different individuals were mixed at different ratios (100%:0%, 99%:1%, 95%:5%, 90%:10%, 75%:25%, 50%:50%, 25%:75%, 10%:90%, 5%:95%, 1%:99%, 0%:100%). Blood cells isolated from a TBD patient (unrelated to the patient treated in this study) were mixed with blood cells isolated from a healthy individual. The former represents cells with shorter telomeres, whereas the latter represents cells with longer telomeres.

Telomere Flow-FISH detects two cell populations with different telomere lengths.
The telomere flow-FISH method calculates the sum of telomere DNA amounts in a single cell by fluorescence-activated cell sorting (FACS)[8,13]. It is the standard telomere assay for clinical samples and is used to diagnose TBD. All telomere flow-FISH assays presented in this paper were performed by Repeat Diagnostics (North Vancouver, Canada).

Figure S5 shows the telomere distributions of lymphocytes at different mixing ratios (black curves). No clear-cut bimodal peaks that represent two distinct cell populations were observed. However, when cells with longer telomeres were mixed with cells with shorter telomeres, the single sharp curve of shorter telomeres (100%:0%) gradually morphed into curves with a broader peak and finally into a single sharp curve of longer telomeres (0%:100%).

The broader peak, e.g., 50%:50%, seems to be a composite of two peaks representing 50% of cells with shorter telomeres and 50% of cells with longer telomeres, as these cells were mixed at this particular ratio. One mathematical method for identifying such peaks is the deconvolution of the curve into multiple Gaussian curves. To this end, we used the statistical software OriginPro (Version 2024, OriginLab Corporation, Northampton, MA, USA). As shown in Figure S5 (color curves), the deconvolution method detects the cell population with longer telomeres starting from 5% (95%:5%), whereas the standard median telomere length cannot detect the difference until the cells with longer telomeres make up ≥25% (75%:25%).

Visual inspection of the telomere distribution (black curves) also indicated that the fraction of cells with longer telomeres gradually increased when a greater number of cells with longer telomeres were present in the blood cell mixtures (Figure S5). We selected 6 kb as a threshold because few cells exceeded 6 kb in the 100%:0% condition (Figure S5). Figure S6 shows the fraction of lymphocytes with telomeres >6 kb (y-axis) plotted against the blood mixing ratio. As a general trend, the higher the percentage of cells with telomeres >6 kb, the more cells with longer telomeres were present in the mixture of blood samples. This suggests that the percent of cells with telomeres >6 kb (or another suitable telomere size) indicates the presence of a cell population with longer telomeres.

SM-STELA detects two cell populations with different telomere lengths.
SM-STELA determines the telomere length distributions of specific chromosome ends by PCR-amplification of a single telomere[9]. An adaptation of this method is used for clinical samples and diagnosis of TBD[14]. All SM-STELA presented in this paper were performed by TeloNostiX Ltd. (Cardiff, UK) using a chromosome 17p-specific primer. The same MNC samples used in telomere flow-FISH analyses were used; however, the mixing of two MNC samples (without separating into lineages such as lymphocytes) was carried out at TeloNostiX at ratios (patient:healthy individual) ranging from 100:0% to 0:100%.

Figure S7 shows images of the SM-STELA results, and Figure S8 shows the scatter plot of the results. Although visual inspection of the scatter plots shows some differences between the different MNC mixtures, mean telomere length only shows a clear difference between 25%:75% and 75%:25%.

To further examine the SM-STELA data, we first plotted the number of DNA fragments with specific telomere length (0.2 kb bin) (y-axis) against the telomere length of these DNA fragments (x-axis) (black curve) (Figure S9). The telomere size distribution curves were deconvoluted into multiple Gaussian curves using OriginPro (Version 2024, OriginLab Corporation, Northampton, MA, USA). The deconvolution analyses of SM-STELA profiles (black curve) identified distinct telomere populations (pink, green, and red Gaussian curves with their mean telomere sizes) in the mixtures of two MNC populations (from a TBD patient and a healthy individual). The deconvolution analyses showed three distinct telomere lengths in the mixture of these two MNCs, which were detected only in 75%:25%, 50%:50%, and 25%:75%. As described[9], SM-STELA detects two distinct telomere lengths of parental alleles even in 100%:0% and 0%:100% MNC mixtures. The detection of three peaks (three alleles), rather than 4 peaks (4 alleles), can be interpreted as similarity in the telomere size range of one of the parental alleles (4-5 kb) depicted as green curves. An insert (bottom right) shows the telomere sizes of the deconvoluted peaks for the different MNC mixtures.

Detection of two cell populations by median telomere lengths
Figure S10 compares median telomere lengths detected by telomere flow-FISH and SM-STELA. Both flow-FISH and SM-STELA results show similar trends and can detect the presence of a cell population with longer telomeres within a cell population of shorter telomeres. However, in both methods, the detectable range is limited: 50%:50% can be detected, but 25%:75% or 75%:25% cannot be detected.

Figure S5

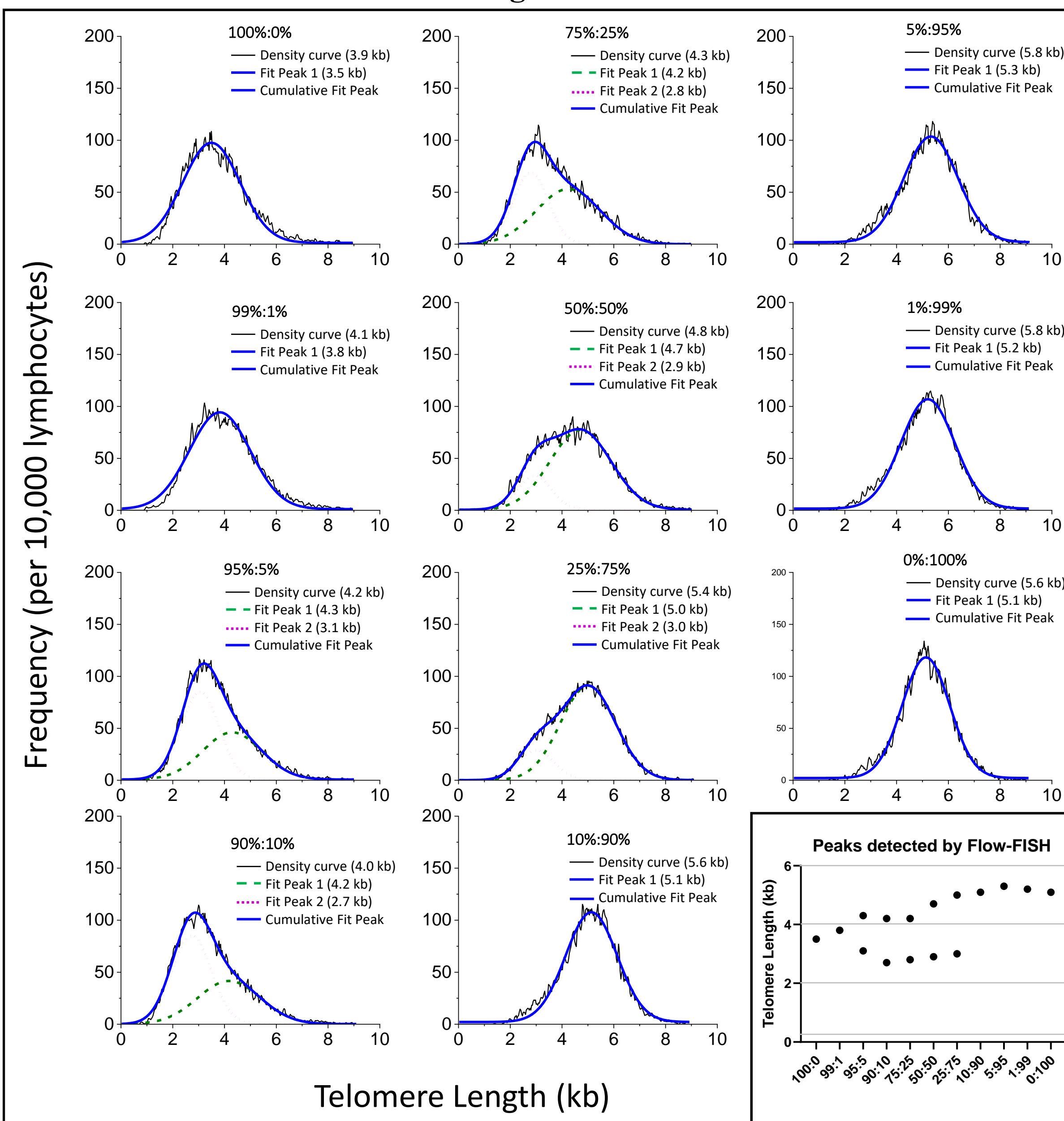


Figure S5. Deconvoluted telomere profiles of the lymphocytes from blood cell mixtures analyzed by telomere flow-FISH.

Deconvolution analyses of telomere flow-FISH profiles (black curve) identified two distinct cell populations (green and red curves) of lymphocytes from mixed blood samples. Blue curves indicate the composite of green and red curves. Mixed blood samples ranging from 100:0% to 0:100% were subjected to telomere flow-FISH. The telomere flow-FISH data were replotted after being normalized to total cell counts and the values of each bin were to telomere length (kb). The standard telomere readouts based on median kb length are shown in the brackets. The telomere size distribution curves were deconvoluted into multiple Gaussian curves using OriginPro 2024. Assuming that each Gaussian curve represents cell populations with specific telomere lengths, the average telomere length (kb) of each cell population is shown (without brackets).

Figure S6

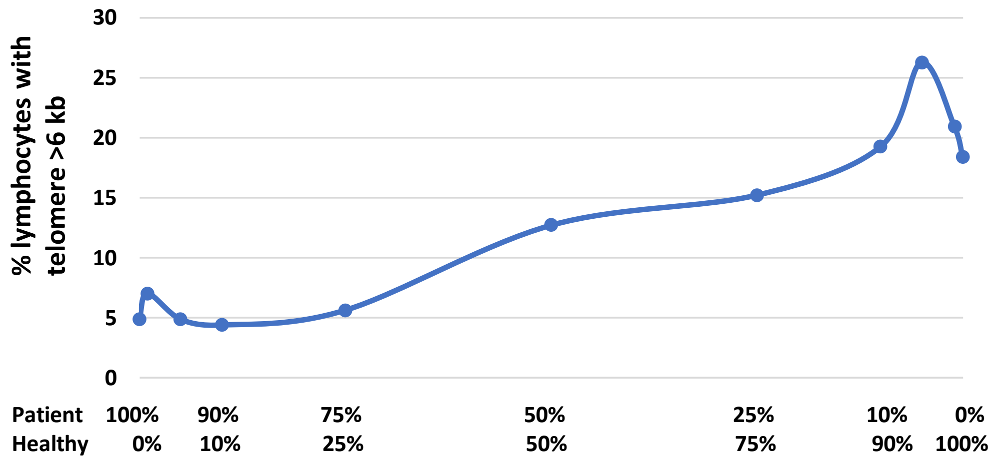


Figure S6. Alternative analyses of telomere flow-FISH profiles obtained from blood cell mixtures.

The analyses detected the proportion of lymphocytes from a healthy individual (with longer telomeres) in a blood sample containing cells from a healthy individual and a patient (with shorter telomeres). Each telomere flow-FISH profile of lymphocytes (the black curves in Figure S5) was reanalyzed to calculate the fraction of lymphocytes with telomeres >6 kb (y-axis) and plotted against the proportion of blood samples from a healthy individual mixed with blood samples from a TBD patient (unrelated to the clinical study patient) at ratios (patient:healthy individual) ranging from 100:0% to 0:100% (x-axis). There was a general trend that the higher the percent of cells with telomeres >6 kb, the more cells with longer telomeres were present in the mixture of blood samples. This suggests that the percentage of cells with telomeres >6 kb (or another suitable telomere size) can indicate the presence of a cell population with longer telomeres. Notably, this method may be effective only when a sufficient fraction of cells have different telomere lengths; for example, ratios (patient: healthy individual) from 100%:0% to 75%:25% and from 10%:90% to 0%:100% could not be distinguished.

Figure S7

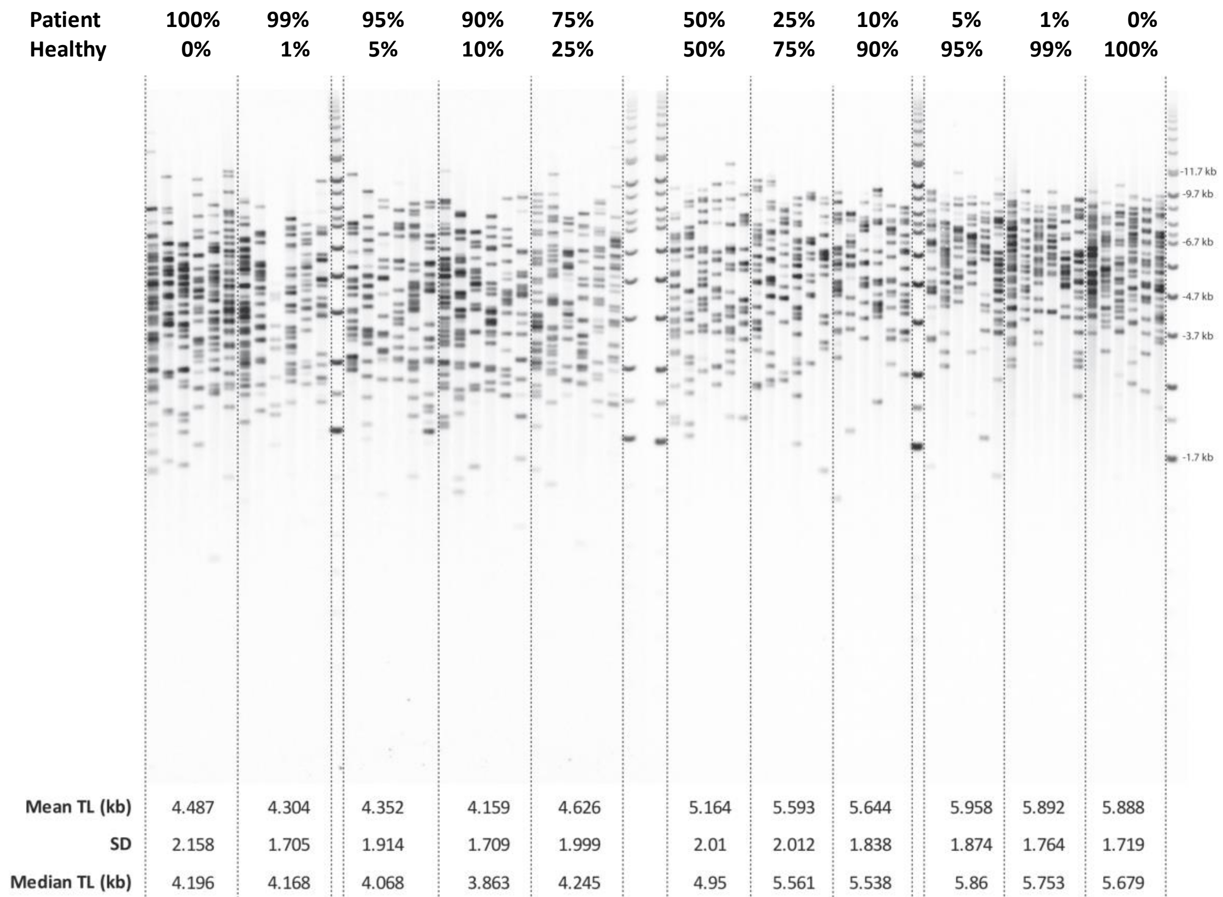


Figure S7. Representative SM-STELA images of MNC mixtures obtained from a TBD patient (unrelated to the clinical study patient) and a healthy individual.

The MNC samples used in the telomere Flow-FISH analyses shown in Figure S5 were used; however, the mixing of two MNC samples was carried out by TeloNostiX at ratios (patient: healthy individual) ranging from 100:0% to 0:100%. Telomeres of chromosome 17p were analyzed according to the established method[9] by TeloNostiX.

Figure S8

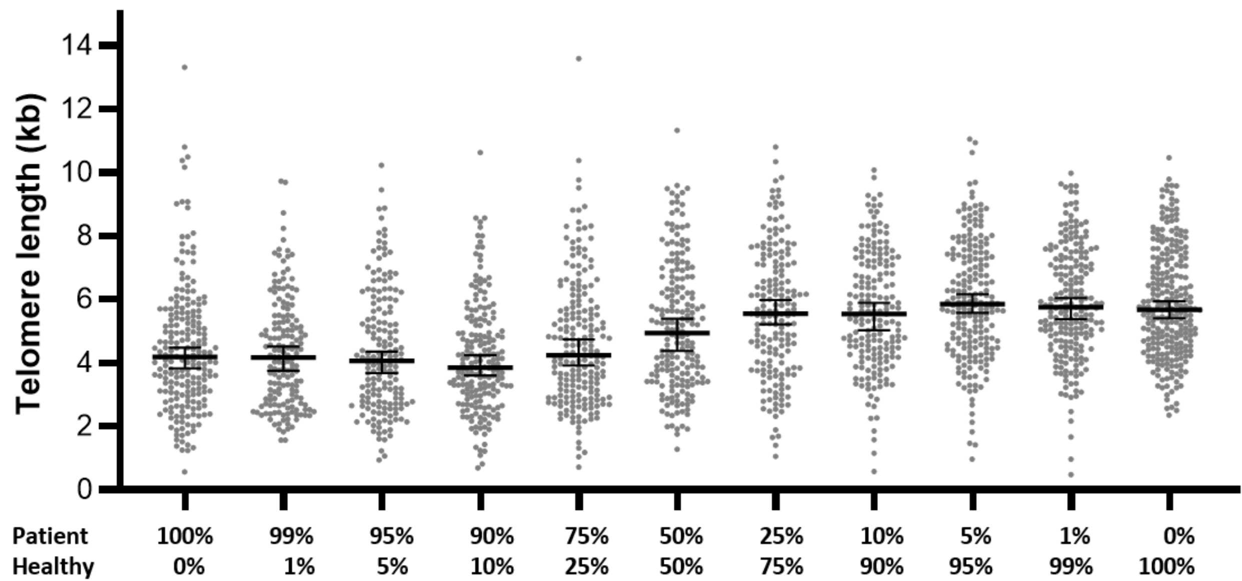


Figure S8. Scatter plots of telomere lengths from the MNC mixtures analyzed by SM-STELA.

MNC mixtures (also shown in Figure S7.) were obtained from a TBD patient (unrelated to the clinical study patient) and a healthy individual. Each dot represents the telomere length of a single chromosome 17p telomere. Bars indicate the mean with 95% CI.

Figure S9

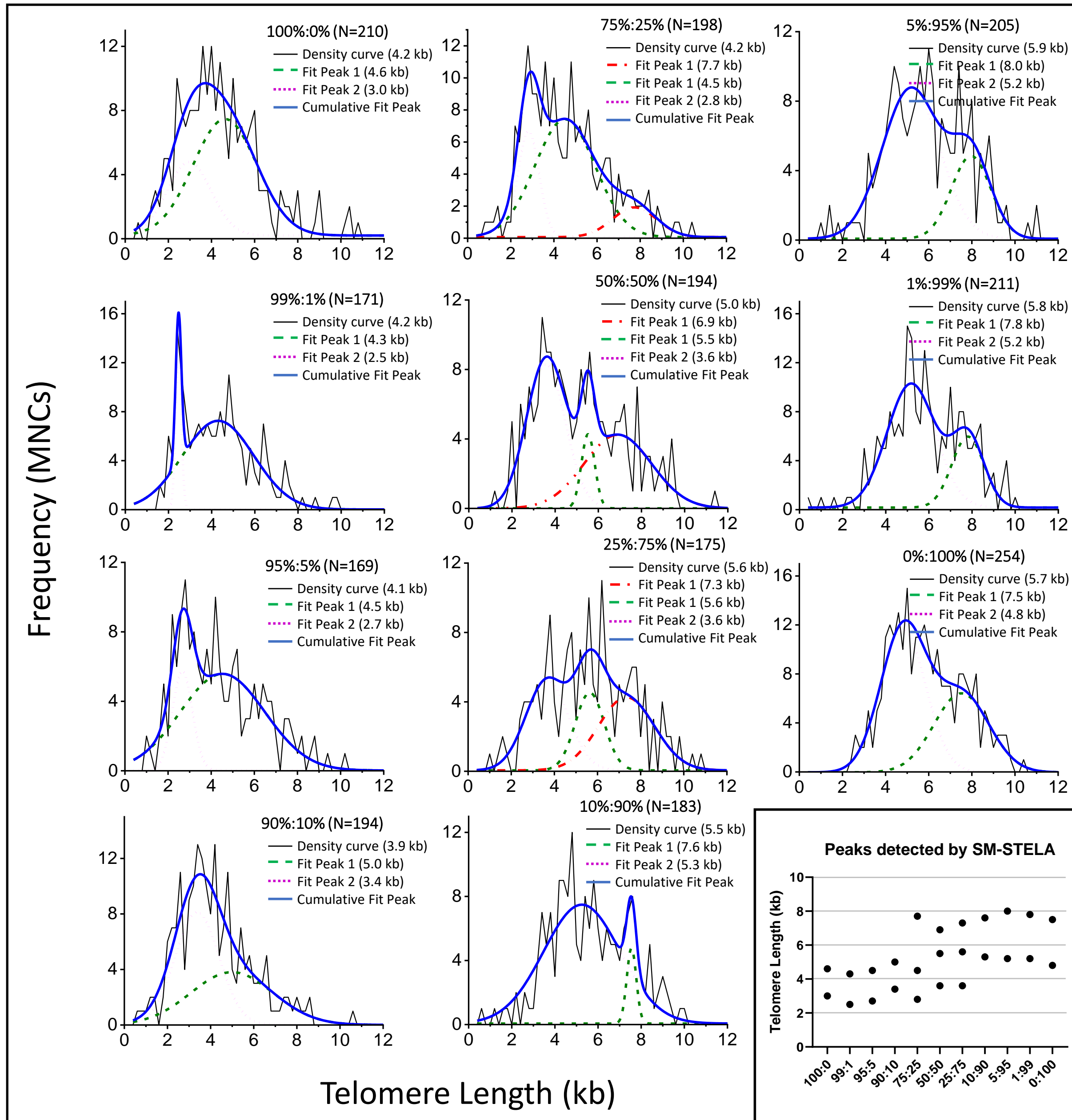


Figure S9. Deconvoluted telomere profiles of the MNC mixtures analyzed by SM-STELA.

SM-STELA data from MNC mixtures (described in Figures S7 and S8) were analyzed, and the number of DNA fragments with a specific telomere length (0.2 kb bin) (y-axis) were plotted against the telomere length of these DNA fragments (x-axis) (black curve). The telomere size distribution curves were deconvoluted into multiple Gaussian curves using OriginPro (Version 2024, OriginLab Corporation, Northampton, MA, USA). The deconvolution analyses of SM-STELA profiles (black curve) identified distinct telomere populations (pink, green, and red curves with their mean telomere sizes) in the mixtures of two MNC populations (from a TBD patient unrelated to the clinical study and a healthy individual). The standard telomere readouts based on median kb length are shown in the brackets. An insert (bottom right) shows the telomere sizes of the deconvoluted peaks for different MNC mixtures.

Figure S10

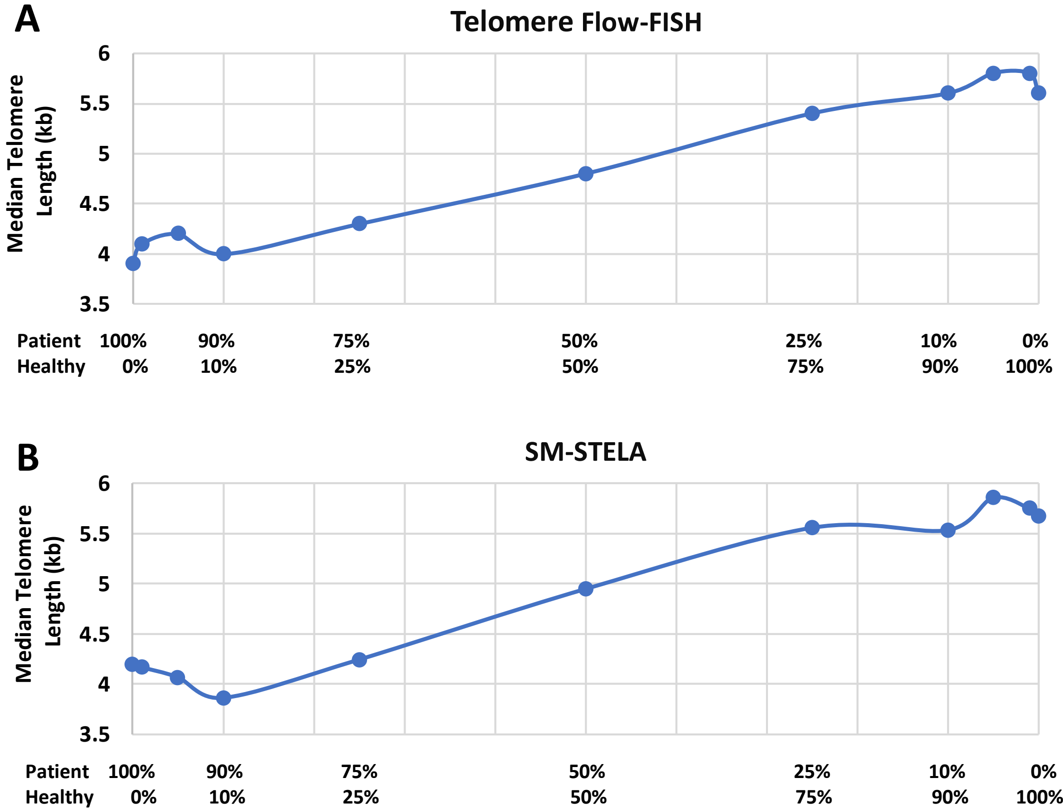


Figure S10. Comparison of median telomere lengths in blood mixtures detected by telomere flow-FISH and SM-STELA.

The data sets presented in Figures S5-S9 were used for the analyses. For comparison purposes, the median telomere lengths obtained by telomere flow-FISH and SM-STELA were plotted against the ratio of cell mixtures: blood obtained from a TBD patient (unrelated to the clinical study patient) and blood obtained from a healthy individual. Both methods show similar trends and can detect the presence of a cell population with longer telomeres in a cell population with shorter telomeres. However, in both methods, the detectable range is limited (25% to 75%).

Section S2: Patient R02

Text S4. Patient R02's telomere data

SM-STELA of the patient CD34+ cells ex vivo.
Figure S11 shows images of SM-STELA of the patient CD34+ cells, which are presented in Fig. 1E.

Nomograms of telomere flow-FISH results.
Nomograms of lymphocytes, granulocytes, naïve T cells, memory T cells, B cells, and NK cells from M0, M1, M3, M6, M9, M12, M20, and M24 are based on the standard telomere flow-FISH analysis (x-axis: 35-45 years range) (Figure S12). The median values of the profiles are considered the telomere length (kb). Telomere shortening is known to be accelerated in TBD patients, but no accelerated shortening was observed from M0 (3 days before the EXG34217 infusion) to M24 (24 months after the EXG34217 infusion) in the six cell types examined here.

Changes in telomere profiles of the patient's lymphocytes measured by telomere flow-FISH.
Figure S13 is the same figure presented in Fig. 2A but without the deconvoluted curves (shown in color), allowing the telomere profiles to be seen more clearly. The plot showing the fraction (%) of lymphocytes with telomeres longer than 6 kb clearly indicates the increase of lymphocytes with longer telomeres from M3 (post-treatment month 3), which reaches a plateau at M6 and then sustains to M24 (except for a dip at M12).

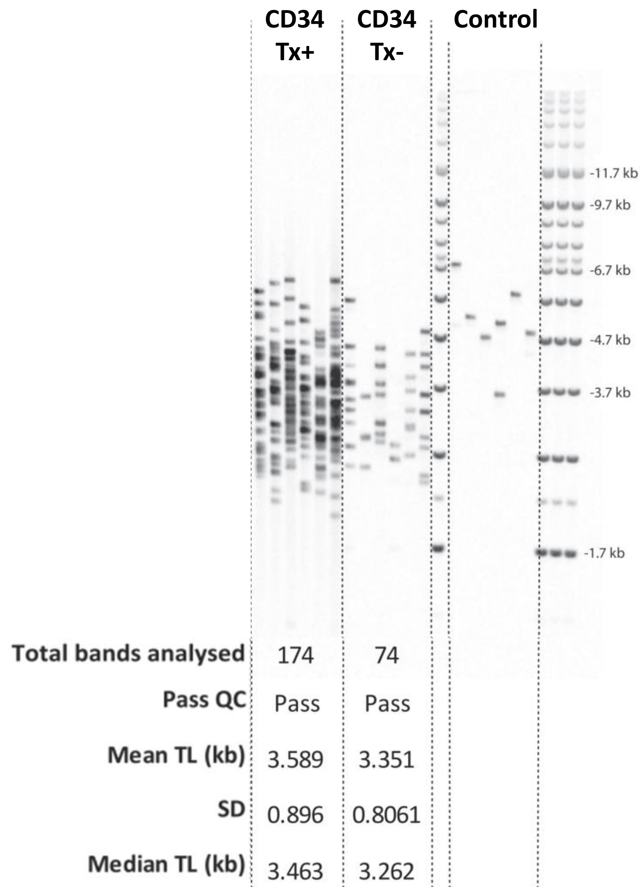
Deconvolution analyses of telomere profiles of the patient's lymphocytes (telomere flow-FISH).
Figure S14 is the same figure presented in Fig. 2A, with an additional plot showing the deconvoluted peaks of telomeres. There is a general trend of peaks with longer telomeres after EXG34217 infusion (M1-M24) compared to those before the EXG34217 infusion (M0). Notably, the shortest telomere peak (1.4 kb) detected at M0 and M1 disappeared after M3 (except M12), indicating that the EXG34217 treatment indeed extended critically short telomeres, a desirable effect of the treatment of TBD patients.

Changes in telomere profiles of the patient's granulocytes measured by telomere flow-FISH.
Figure S15 shows the telomere profiles of the patient's granulocytes. The plot showing the fraction (%) of granulocytes with telomeres longer than 6 kb clearly indicates that the proportion of granulocytes with longer telomeres was increased at M3 and further increased from M6 to M24 (except for dips at M9 and M12).

Changes of telomere profiles of the patient's MNCs measured by SM-STELA.
Figure S16 shows the gel images of SM-STELA, and Figure S17 shows scatter plots of the results.

Deconvolution analyses of telomere profiles of the patient's MNCs (SM-STELA).
Figure S18 shows the telomere distribution profiles and their deconvolution analyses. Similar to the deconvolution analyses of telomere flow-FISH profiles, the deconvolution analyses of SM-STELA profiles (black curve) could identify distinct cell populations with different telomere lengths. Also, similar to the telomere flow-FISH profiles, there is a general trend of peaks with longer telomeres after the treatment (M1-M24) compared to peaks from cells obtained 3 days before the EXG34217 infusion (M0). Notably, the shortest telomere peak (2.7 kb) detected at M0 disappeared after M6, indicating that the EXG34217 treatment extended critically short telomeres, a desirable effect in the treatment of TBD patients.

Figure S11



	CD34 Tx+	CD34 Tx-	Control
Total bands analysed	174	74	
Pass QC	Pass	Pass	
Mean TL (kb)	3.589	3.351	
SD	0.896	0.8061	
Median TL (kb)	3.463	3.262	

Figure S11. Representative SM-STELA images of the patient R02 CD34+ cells with (CD34 Tx+) or without (CD34 Tx-) EXG-001 treatment.

Telomeres of chromosome 17p were analyzed according to the established method[9] at TeloNostiX.

Figure S12

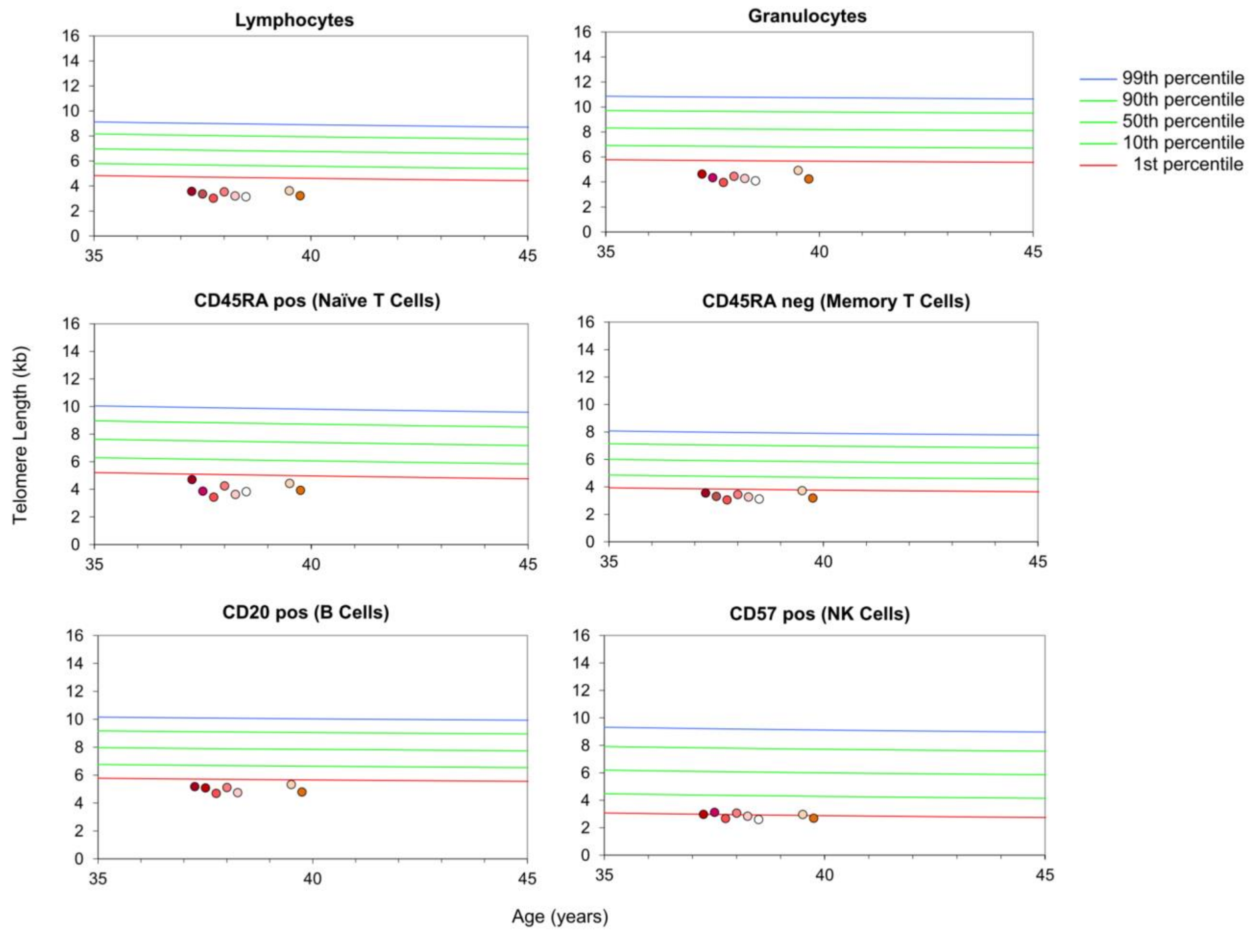


Figure S12. Nomograms of telomere flow-FISH results of patient R02's peripheral blood cells.

Telomere flow-FISH of lymphocytes, granulocytes, naïve T cells, memory T cells, B cells, and NK cells are shown based on the standard method (35-45 years range). The median values of the profiles are considered the telomere length (kb). Each circle represents a telomere length at each time point (from left to right): M0 (3 days before the EXG34217 infusion), M1 (1 month after the EXG34217 treatment), M3, M6, M9, M12, M20, and M24 (24 months after the EXG34217 treatment).

Figure S13

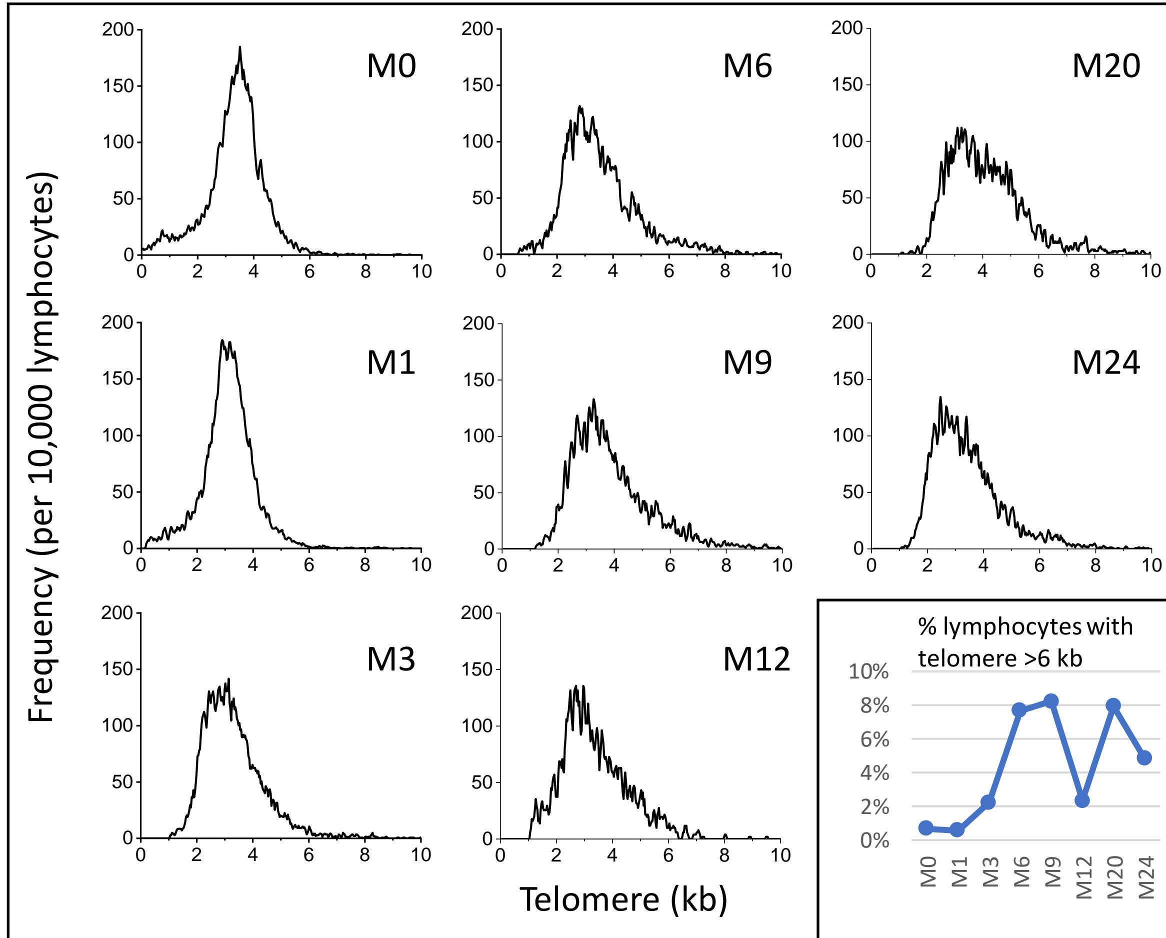


Figure S13. Telomere profiles of the patient R02's lymphocytes analyzed by telomere flow-FISH. An insert shows the fraction (%) of lymphocytes with telomeres longer than 6 kb.

Figure S14

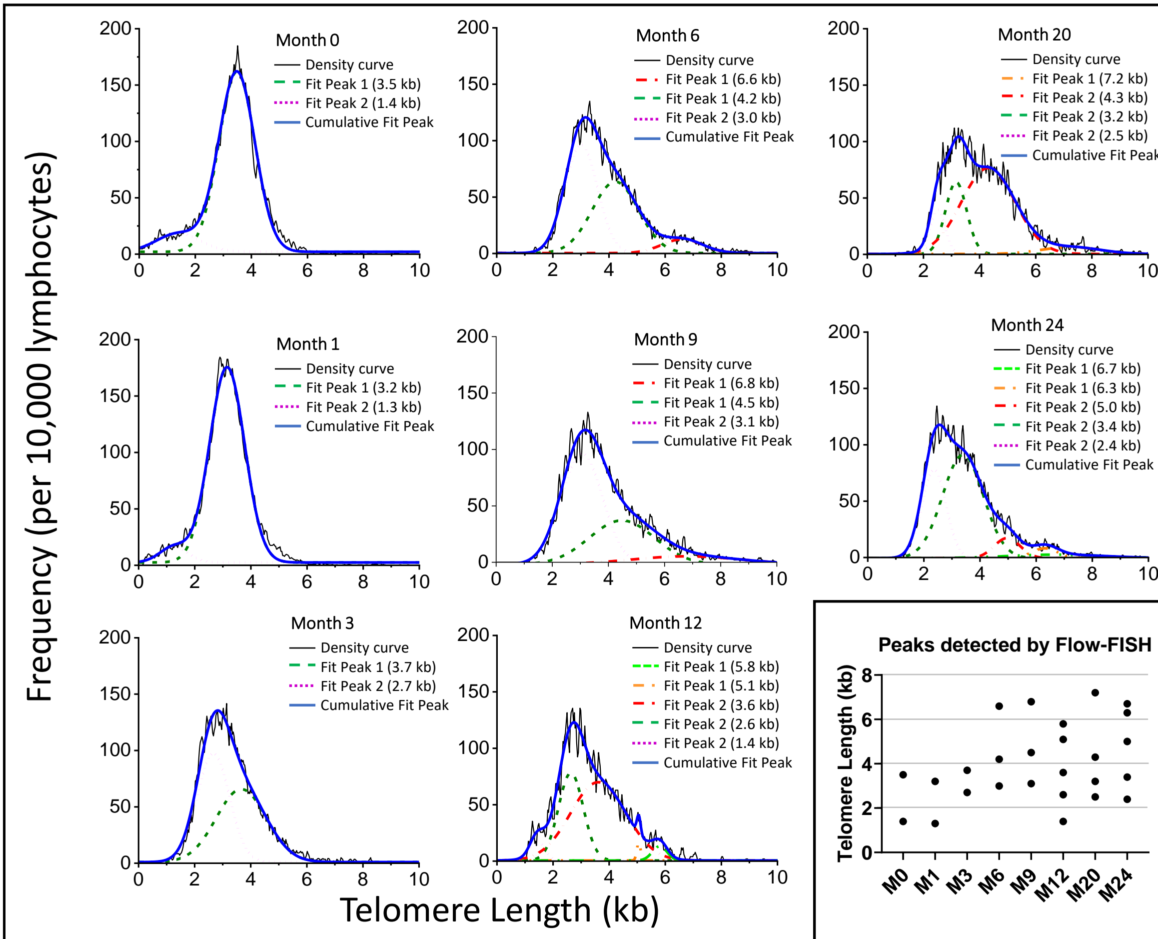


Figure S14. Deconvoluted telomere profiles of the patient R02's lymphocytes analyzed by telomere flow-FISH.

The telomere size distribution curves were deconvoluted into multiple Gaussian curves using OriginPro (Version 2024, OriginLab Corporation, Northampton, MA, USA). Assuming that each Gaussian curve represents cell populations with specific telomere lengths, each cell population's mean telomere length (kb) is shown. An insert shows the telomere lengths of the deconvoluted peaks.

Figure S15

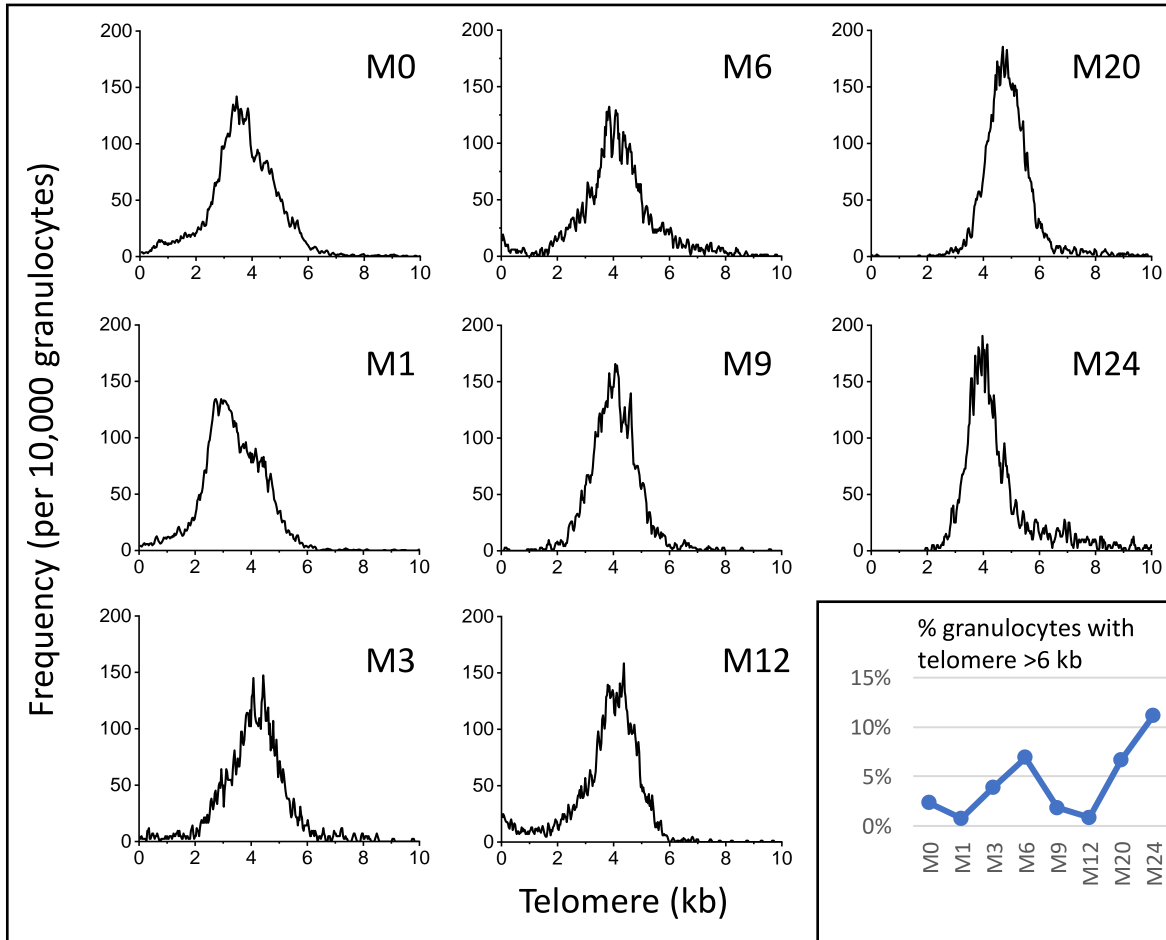


Figure S15. Telomere profiles of the patient R02's granulocytes analyzed by telomere flow-FISH. An insert shows the fraction (%) of granulocytes with telomeres longer than 6 kb.

Figure S16

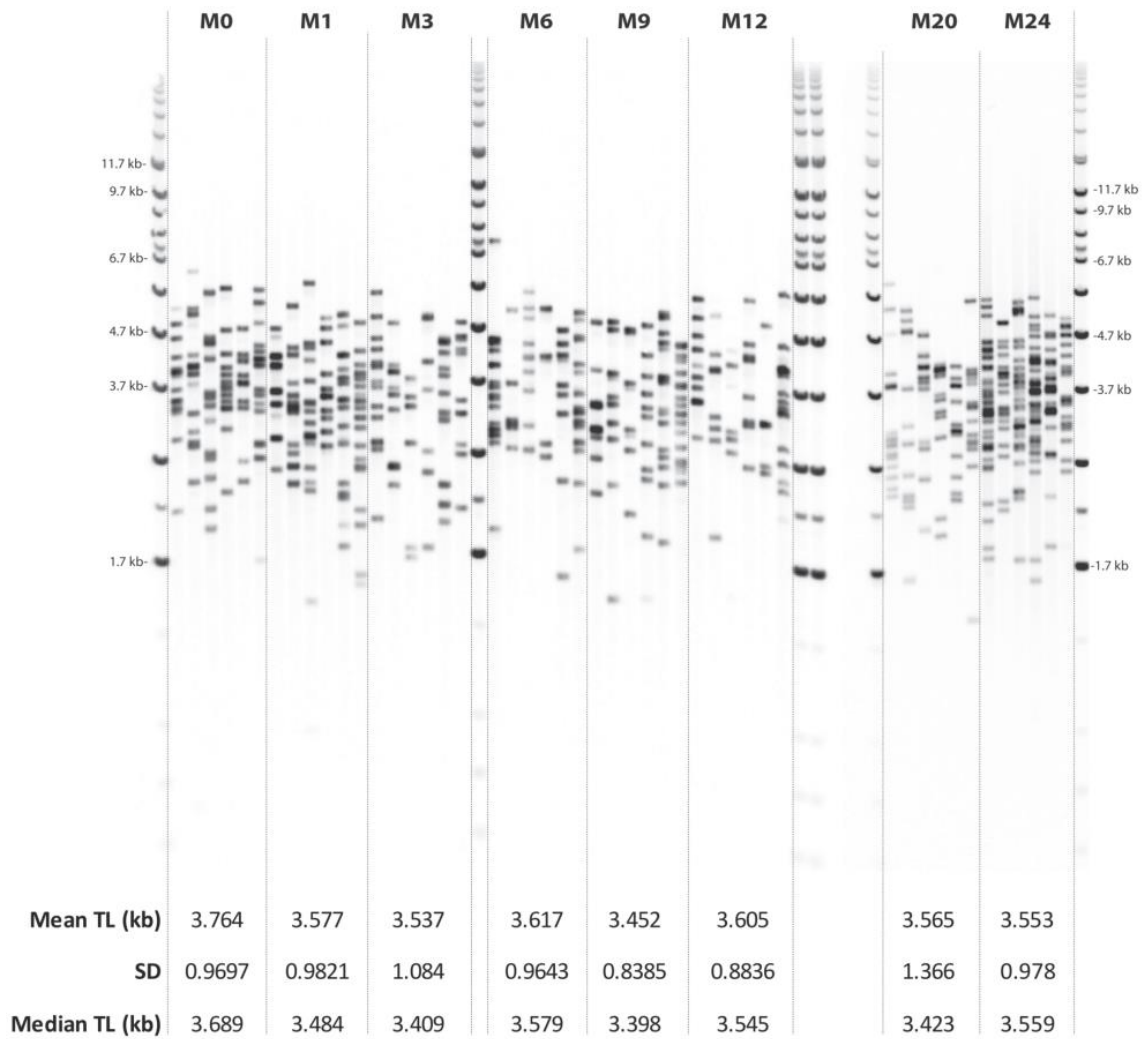


Figure S16. Representative images of the patient R02's MNCs analyzed by SM-STELA.

Telomeres of chromosome 17p were analyzed according to the established method[9] by TeloNostiX.

Figure S17

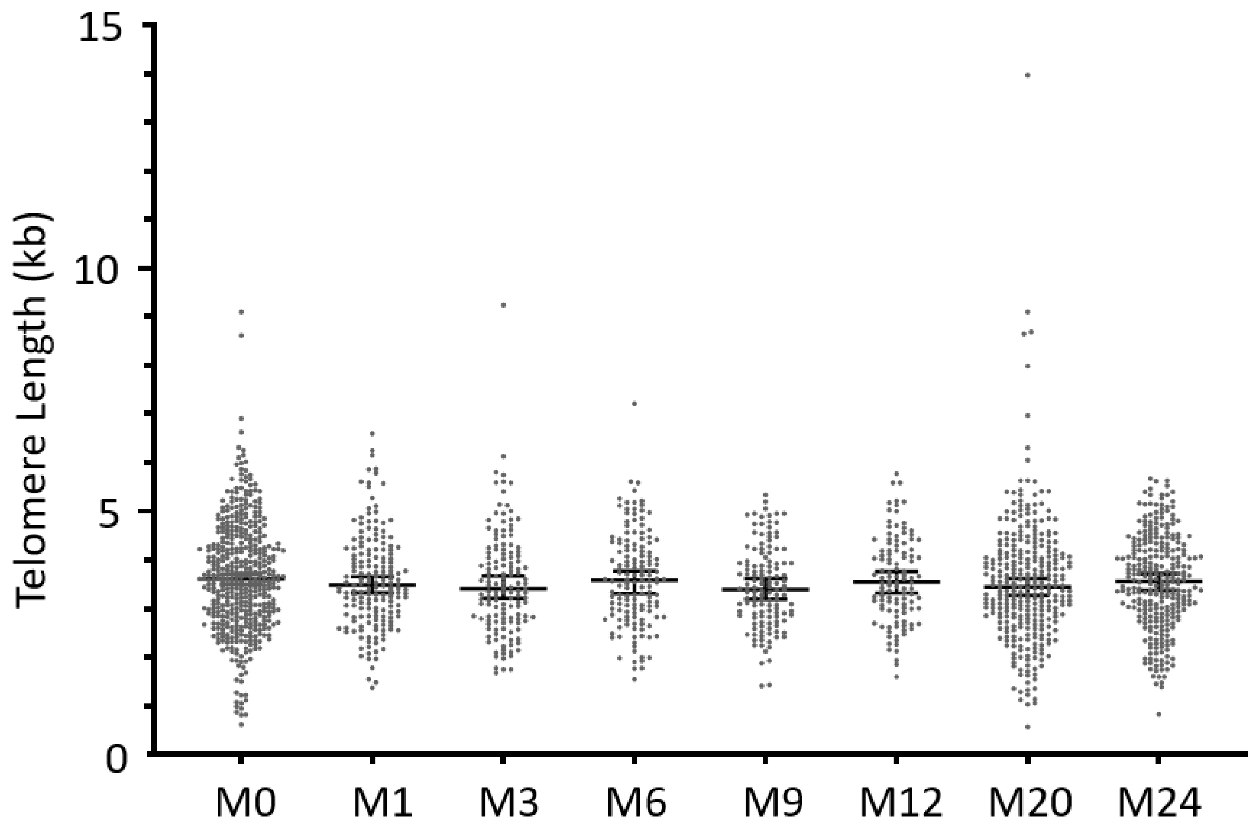


Figure S17. Scatter plots of the patient R02’s MNCs telomere length as analyzed by SM-STELA. The plots are derived from Figure S16. Each dot represents the telomere length of a single chromosome 17p. Bars indicate the mean with 95% CI.

Figure S18

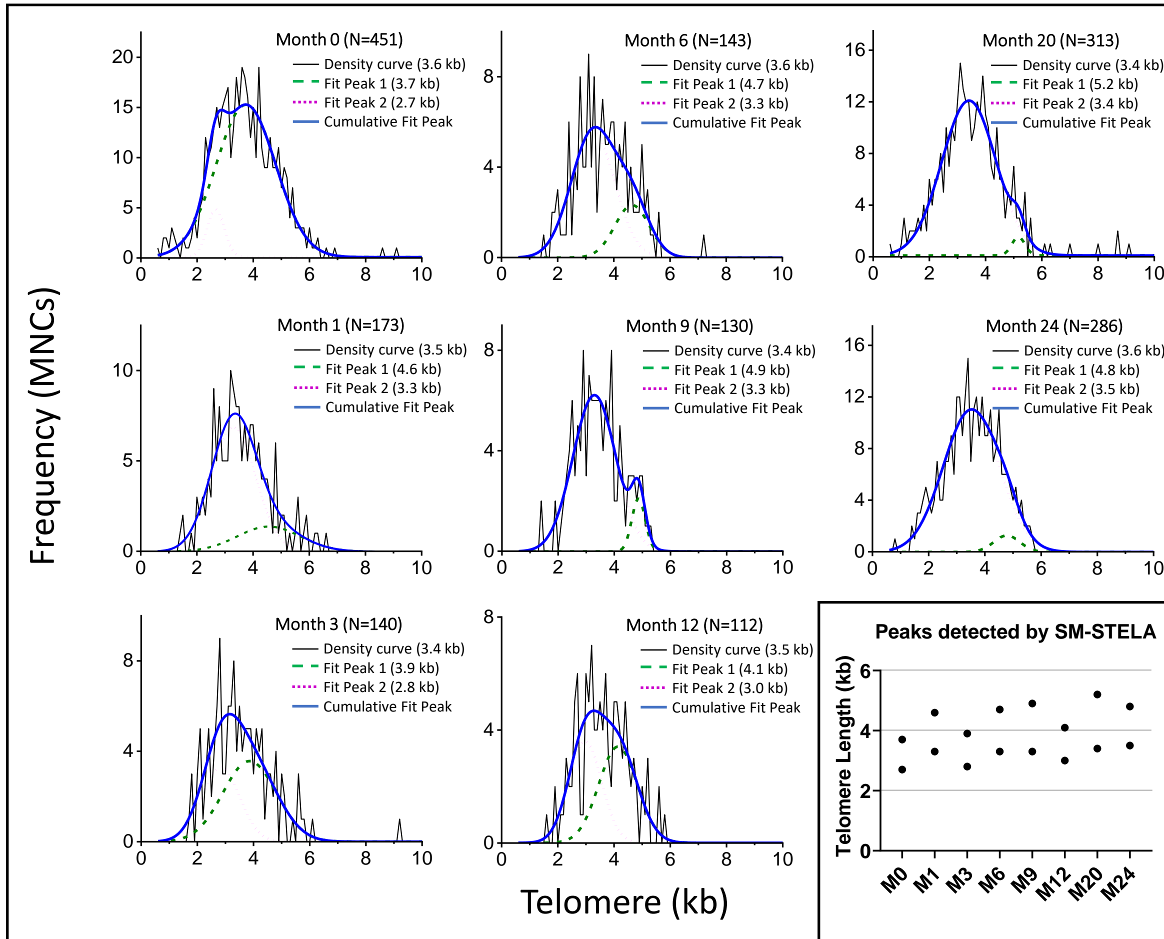


Figure S18. Deconvoluted telomere profiles of the patient R02's MNCs analyzed by SM-STELA.

The number of DNA fragments with specific telomere length (0.1 kb bin) (y-axis) were plotted against the telomere length of these DNA fragments (x-axis) (black curve). The telomere size distribution curves were deconvoluted into multiple Gaussian curves using OriginPro (Version 2024, OriginLab Corporation, Northampton, MA, USA). The deconvolution analyses of SM-STELA profiles (black curve) identified distinct telomere populations (pink and green curves). The standard telomere readouts based on median kb length are shown in brackets. An insert (bottom right) shows the telomere sizes of the deconvoluted peaks for different MNC mixtures.

Text S5. Patient R02's clinical data

Clinical Summary: 37-year-old man diagnosed with TBD (telomere length < 1st percentile) 10 years prior to enrollment in 2021. The patient is genetically undefined but has a family history of aplastic anemia and liver cirrhosis in a brother (deceased from liver transplant complications following HSCT) and pulmonary fibrosis and a lung transplant in his mother, consistent with TBD. Relevant medical history before the enrollment in 2021 included mild neutropenia and thrombocytopenia for 15 years, mild subclinical pulmonary fibrosis for 4 years, early greying, pulmonary arteriovenous malformations for 2 years, and recent-onset cardiomyopathy. Further genetic evaluation revealed a separate likely pathogenic mutation in the *TTN* gene leading to genetic susceptibility to cardiomyopathy.

Complete Blood Counts:

Figure S19 shows changes in blood counts over time before and after the EXG34217 treatment. The period between M0 and M6 may be influenced by the possible effects of G-CSF and Plerixafor that were administered to the patient immediately before the EXG34217 infusion for CD34+ cell mobilization. EXG34217 treatment showed an increase in ANC and WBC. However, RBC, hematocrit, and hemoglobin were not affected. Platelet numbers were slightly reduced. Lymphocyte subpopulations in peripheral blood were not evaluated.

Bone Marrow:

Table S1 shows the analyses of bone marrow cellularity, %CD34+ cells, karyotypes, MDS (myelodysplastic syndrome)-FISH, and somatic mutation panels before and after EXG34217 treatment. Figure S20 shows representative images of bone marrow biopsies. The patient has remained transfusion-independent without serious infections or evidence of myeloproliferation. Bone marrow cellularity is challenging to assess in patients with inherited BMF as it is often patchy, varying extensively throughout different locations in the bone marrow, as evidenced by the wide ranges in cellularity noted in morphologic examinations. The patient's cellularity overall remains stable to slightly improved since treatment within these limitations. CD34% is stable.

Pulmonary:

Pre-EXG34217 treatment: Given the underlying TBD diagnosis and family history of pulmonary fibrosis requiring a lung transplant in his mother, the patient was followed annually with spirometry and noted a clinically asymptomatic decline at -M40 (40 months before the EXG34217 treatment) in % predicted forced expiratory volume in one second (FEV1) from 106% to 88%, with the normal diffusing capacity of the lungs for carbon monoxide (DLCO) from 87% to 90%. The patient was subsequently referred to adult pulmonology and was noted to have mild fibrosis by CT imaging at -M13 prior to EXG34217 treatment.

Post-EXG34217 treatment: Clinical pulmonary monitoring demonstrated stable FEV1 (97% and 104%, respectively, at M12 and M22 after EXG34217 treatment), decreasing DLCO (68% and 78%, respectively, at M12 and M22 after EXG34217 treatment), and increased moderate fibrosis by CT at M12 after EXG34217 treatment. The patient started on Pirfenidone one year after the EXG34217 treatment.

Cardiac:

Pre-EXG34217 treatment: In -M4 (4 months before the EXG34217 treatment), a screening echo was obtained to look for evidence of shunting concerning pulmonary AVMs associated with liver disease. The patient was noted to have scant (less than 10) bubbles seen entering the left side of the heart after about 7 cardiac cycles, consistent with his underlying DC and risk of pulmonary AVMs, but also a new mild dilation of the left ventricle with mildly diminished systolic function at 45% EF. Of note, this was approximately 1 month after infection with COVID-19. Further cardiac evaluation, including cardiac

MRI, revealed no ongoing inflammation and was consistent with the echocardiogram. He was diagnosed with presumed post-viral cardiac dysfunction, leading to nonischemic cardiomyopathy.

Post-EXG34217 treatment: The patient has undergone clinical cardiac monitoring and care. The left ventricular ejection fraction was 37%, 36%, and 32% at M9, M21, and M23 after EXG34217 treatment. He is currently on Spironolactone, Sacubitril-valsartan, and Metoprolol with stable diminished function. Further genetic evaluation revealed a separate likely pathogenic mutation in the *TTN* gene leading to genetic susceptibility to cardiomyopathy.

Liver disease:

Pre-EXG34217 treatment: The patient clinically demonstrates no functional impairment of the liver. There is mild intermittent transaminitis, with persistence of mild AST elevation of 40-100 over many years (Figure S21). Bilirubin ranged from 0.3-0.6 mg/dl and remained stable with normal albumin, PT (prothrombin time), INR (international normalized ratio), and aPTT (activated partial thromboplastin time) (a slightly lower) values (Figure S21). Imaging showed some progression of liver disease with increasing median liver shear wave speed by abdominal ultrasound with ARFI and steatosis by MRI. Evidence of pulmonary shunting suggestive of liver disease by bubble echo first seen at -M4 (4 months before EXG34217 treatment).

Post-EXG34217 treatment: The patient clinically demonstrates no functional impairment of the liver. There is mild intermittent transaminitis, with persistence of mild AST elevation variably ranging from 28 to 118 units/l since EXG34217 treatment, most recently 62 units/l (Figure S21). He has also had mild intermittent ALT elevation variably ranging from 25 to 100 since EXG34217 treatment, most recently 50 units/l (Figure S21). Bilirubin (0.3-0.6 mg/dl), albumin (3.1-4.3 g/dl, except after apheresis 1.0 g/dl), total protein (6.1-8.1 g/dl), PT (10.7-12.1), and INR (0.95-1.09) remained within normal ranges and with slightly lower aPTT (24.6-28.2) (Fig. S21). Liver imaging has not been performed after EXG34217 treatment. Liver imaging assessments have not been performed after EXG34217 treatment.

Figure S19

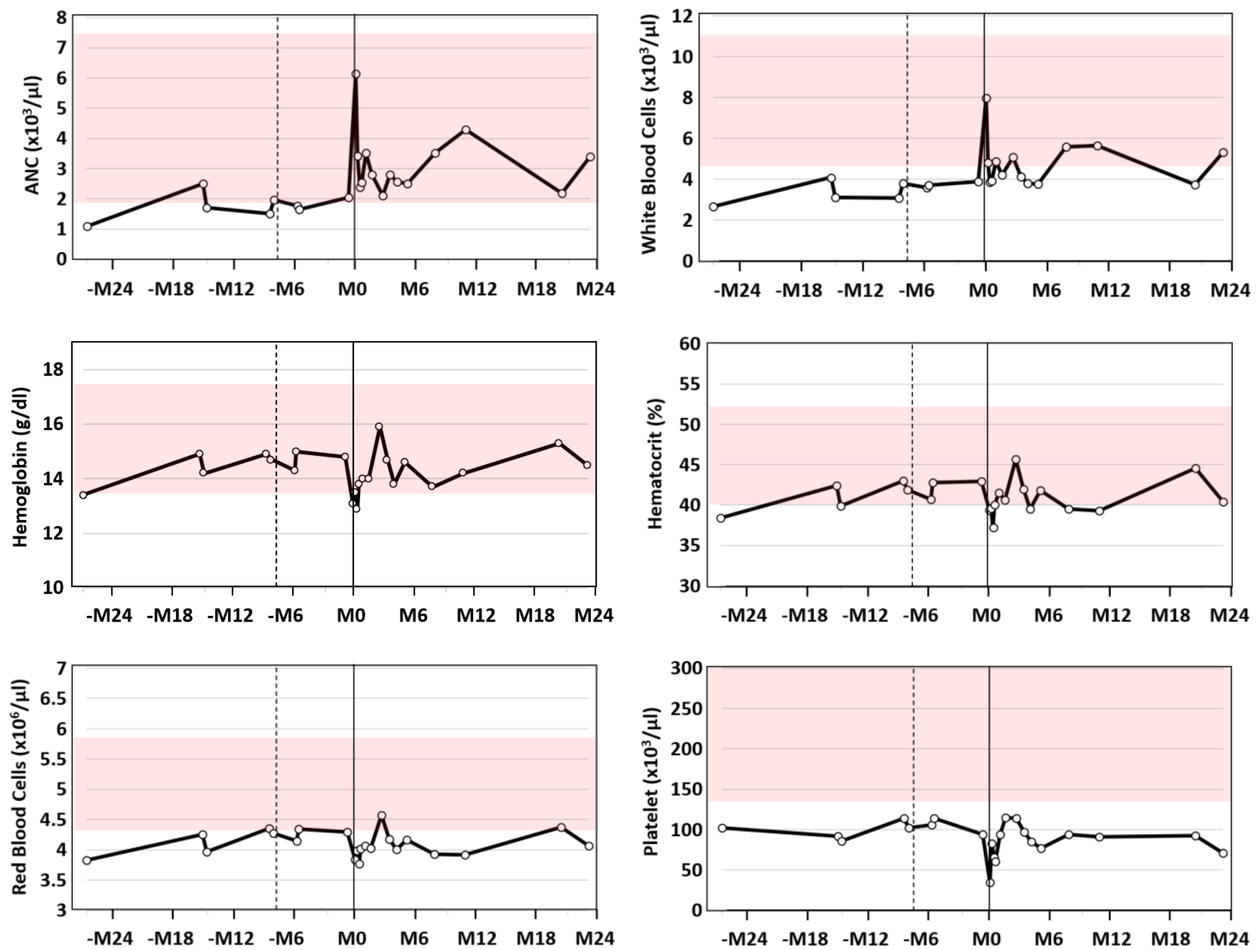


Figure S19. Changes in blood counts of the patient R02 over time before and after EXG34217 treatment. -M24, -M18, -M12, and -M6 denote months (M) before EXG34217 treatment. M0 denotes 3 days before the EXG34217 infusion. M6, M12, M18, and M24 denote months (M) after the EXG34217 infusion. Dotted vertical lines at -M8 (8 months before EXG34217 infusion) is the first mobilization attempt, resulting in no apheresis and no EXG34217 infusion. Vertical lines at M0 indicate the time of mobilization, apheresis, and EXG34217 infusion. Normal ranges are shown in pink: ANC (1.80-7.70 $x10^3$/µl), White Blood Cell (4.50-11.00 $x10^3$/µl), Red Blood Cell (4.40-5.90 $x10^6$/µl), Hematocrit (40.0-52.0%), Platelet (135-466 $x10^3$/µl), Hemoglobin (13.3-17.7 g/dl).

Table S1

Date	Marrow Cellularity	Fraction of CD34+ cells [*1]	Karyotype [No. of analyzed cells]	MDS FISH	somatic panel
-M27	0-35%, overall 10%	<0.1%	46 XY [20]	normal	not assessed
-M15	unable to assess due to sample quality	0.3% [*2]	46 XY [20]	normal	not assessed
-M4	20-25%	<0.1%	46 XY [18/20] [*3]	normal	not assessed
-M1	0-70%, overall 40%	0.1%	46 XY [20]	normal	not assessed
M10	0-50%, 30% overall	0.1%	46 XY [20]	normal	negative
M20	0-50%, 30-40% overall	0.2%	46 XY [20]	normal	negative

Table S1. Analyses of patient R02's bone marrow cells before and after EXG34217 treatment.

-M27, -M15, -M4, and -M1 indicate 27 months, 15 months, 4 months, and 1 month before the EXG34217 treatment. M10 and M20 indicate 10 months and 20 months after the EXG34217 treatment.

MDS FISH, myelodysplastic syndrome; somatic panel, somatic mutation panel.

*1. The fraction of CD34+ cells (%) in bone marrow was assessed by immunophenotyping.

*2. Low sample quality.

*3. Of the 20 cells analyzed, 18 featured a normal male karyotype or 46 XY. One cell featured a deletion of the long arm of chromosome 1, or del(1)(q12), while the second cell featured both material of unknown origin added on to the long arm of chromosome 2, or add(2)(q31), and a marker chromosome (+mar). These were not identified in an additional 19 cells examined. The significance of these two cells is unknown. Each may represent a single abnormal cell with no clinical significance. Alternatively, each may represent an abnormal clone present at a low level. These nonspecific changes can be seen transiently in patients with BMF, and in the absence of dysplasia or other concerning changes, it is often transient; repeat marrow was done clinically in three months for follow-up with was stable with normal testing including normal cytogenetics.

Figure S20

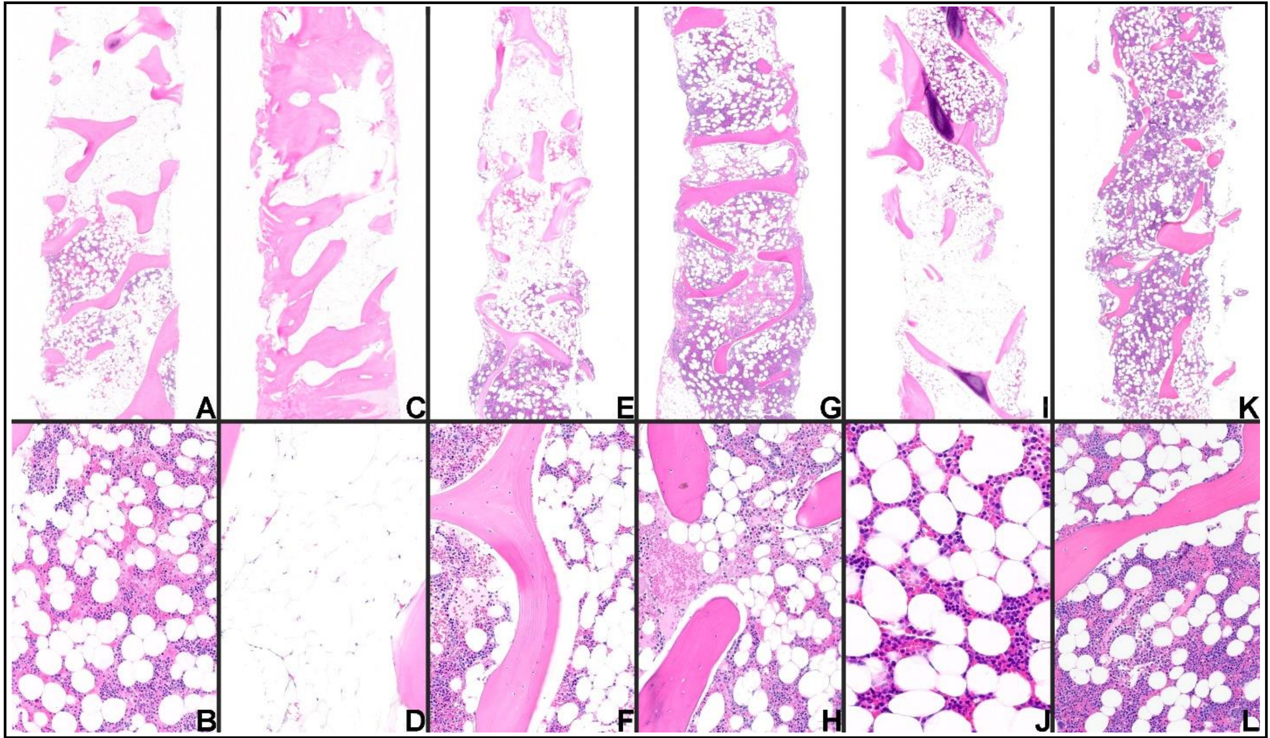


Figure S20. Representative images of bone marrow biopsies.

Representative fields of hematoxylin- and eosin-stained bone marrow core biopsies showing low power (40x magnification, top row) and high power (200x magnification, bottom row) views. The marrows show a range of marrow cellularity within the same biopsy with varying degrees of multi-lineage hematopoiesis. A, B (at -M27): 10% overall cellularity; C, D (at -M15): sample primarily consisting of bone and acellular marrow, most likely not representative of the marrow; E, F (at -M4): 20-25% overall cellularity; G, H (at -M1): 40% overall cellularity; I, J (at M10): 30% overall cellularity; K, L (at M20).: 30-40% overall cellularity. -M27, -M15, -M4, and -M1 denote 27 months, 15 months, 4 months, and 1 month before the EXG34217 treatment. M10 and M20 denote 10 months and 20 months after the EXG34217 treatment.

Figure S21

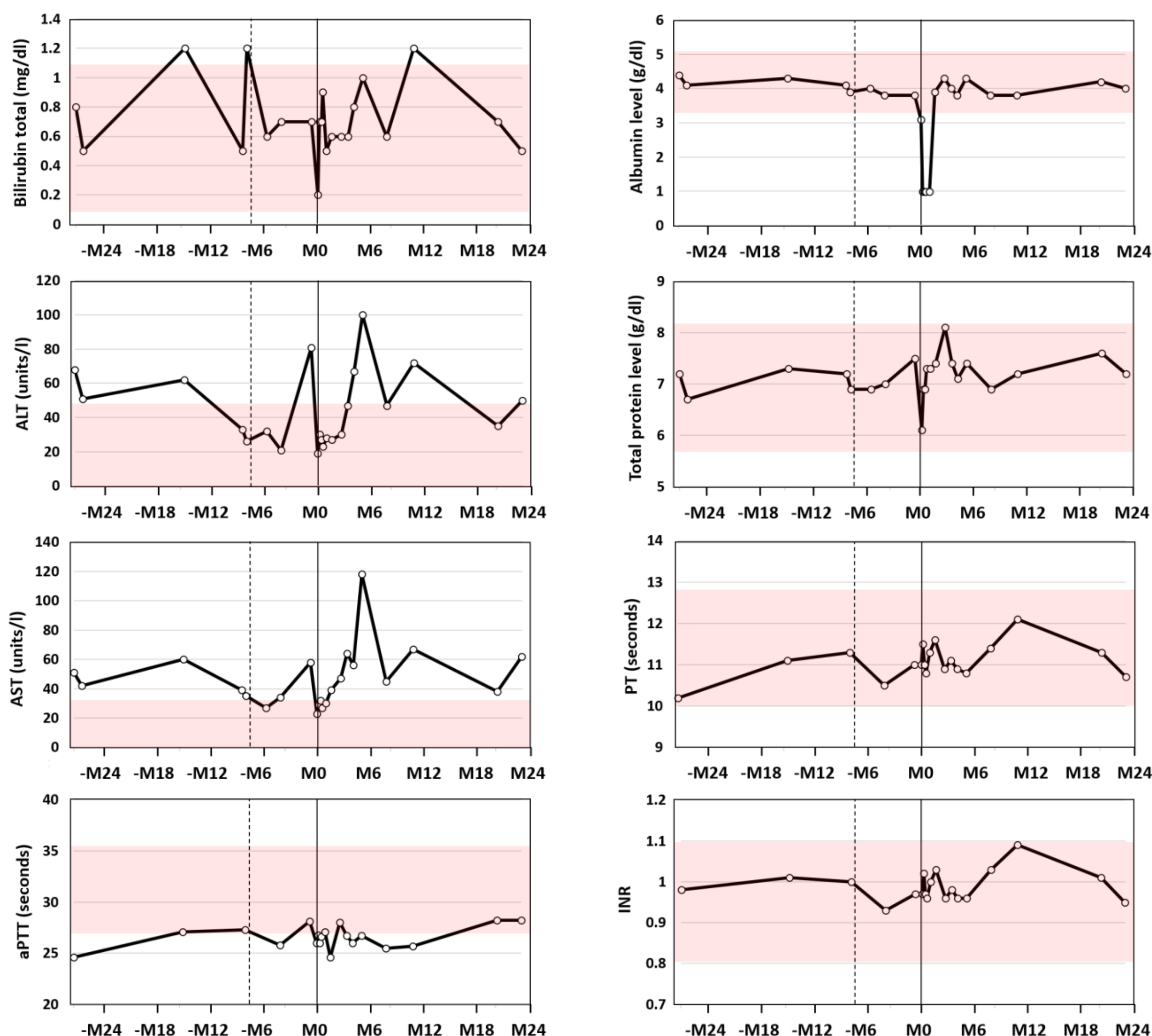


Figure S21. Changes in liver function markers of the patient R02 over time before and after EXG34217 treatment.

-M24, -M18, -M12, and -M6 denote months (M) before EXG34217 treatment. M0 denotes 3 days before the EXG34217 infusion. M6, M12, M18, and M24 denote months (M) after EXG34217 infusion. Dotted vertical lines at -M8 (8 months before EXG34217 infusion) is the first mobilization attempt, resulting in no apheresis and no EXG34217 infusion. Vertical lines at M0 indicate the time of mobilization, apheresis, and EXG34217 infusion.

Normal ranges are shown in pink: total bilirubin (0.1-1.1 mg/dl), albumin level (3.4-5.0 g/dl), total protein level (5.7-8.2 g/dl), ALT (≤ 49 units/l), AST (≤ 33 units/l), PT (9.9-12.7 seconds), INR (0.8-1.1), aPTT (27.3-35.3 seconds).

Section S3: Patient R05

Text S6. Patient R05's telomere data

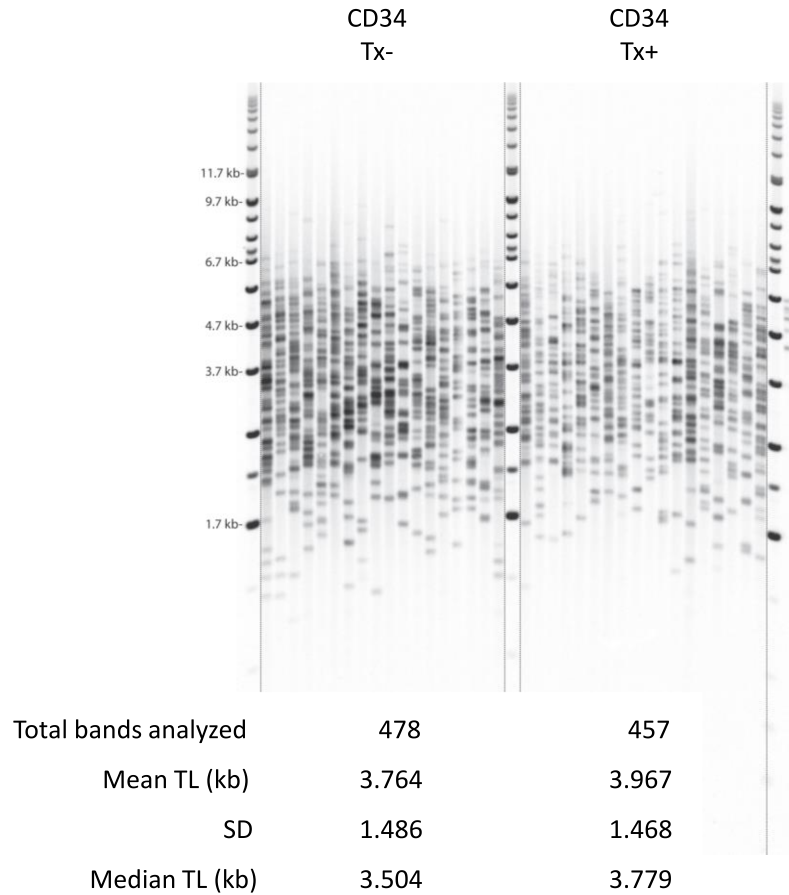
SM-STELA of the patient CD34+ cells ex vivo.
Figure S22 shows images of SM-STELA of the patient CD34+ cells, which are presented in Fig. 3D.

Nomograms of telomere flow-FISH results.
Nomograms of lymphocytes, granulocytes, naïve T cells, memory T cells, B cells, and NK cells from M0, M1, and M3 are based on the standard telomere flow-FISH analysis (x-axis: 30-40 years range) (Figure S23). The median values of the profiles are considered the telomere length (kb). Only the M0, M1, and M3 data are available as of September 31, 2024.

Changes in telomere profiles of the patient's lymphocytes measured by telomere flow-FISH.
Figure S24 (left) shows the telomere profiles of the patient's lymphocytes. Figure S24 (right) shows the deconvoluted peaks of telomeres in the patient's lymphocytes telomere flow-FISH. Only the M0, M1, and M3 data are available as of September 31, 2024.

Changes in telomere profiles of the patient's granulocytes measured by telomere flow-FISH.
Figure S25 (left) shows the telomere profiles of the patient's granulocytes. Figure S25 (right) shows the deconvoluted peaks of telomeres in the patient's lymphocytes telomere flow-FISH. Only the M0, M1, and M3 data are available as of September 31, 2024. The emergence of a granulocyte subpopulation with longer telomeres (5.8 kb) was noted at M3 by the deconvolution analysis.

Figure S22



	CD34 Tx-	CD34 Tx+
Total bands analyzed	478	457
Mean TL (kb)	3.764	3.967
SD	1.486	1.468
Median TL (kb)	3.504	3.779

Figure S22. Representative SM-STELA images of the patient R05 CD34+ cells with (CD34 Tx+) or without (CD34 Tx-) EXG-001 treatment.

Telomeres of chromosome 17p were analyzed according to the established method[9] at TeloNostiX.

Figure S23

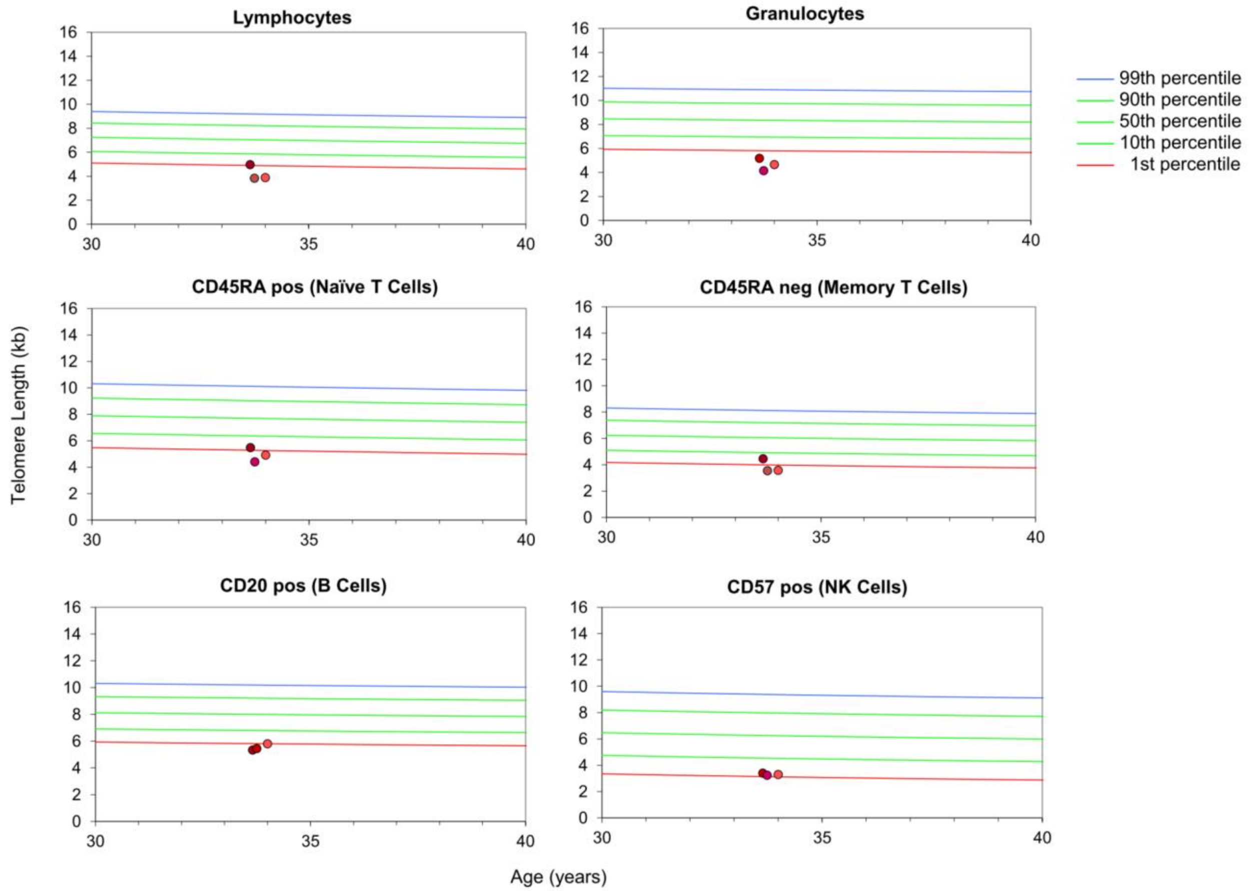


Figure S23. Nomograms of telomere flow-FISH results of patient R05's peripheral blood cells.

Telomere flow-FISH of lymphocytes, granulocytes, naïve T cells, memory T cells, B cells, and NK cells are shown based on the standard method (30-40 years range). The median values of the profiles are considered the telomere length (kb). Each circle represents a telomere length at each time point (from left to right): M0 (3 days before the EXG34217 infusion), M1 (1 month after the EXG34217 infusion), and M3 (3 months after the EXG34217 infusion).

Figure S24

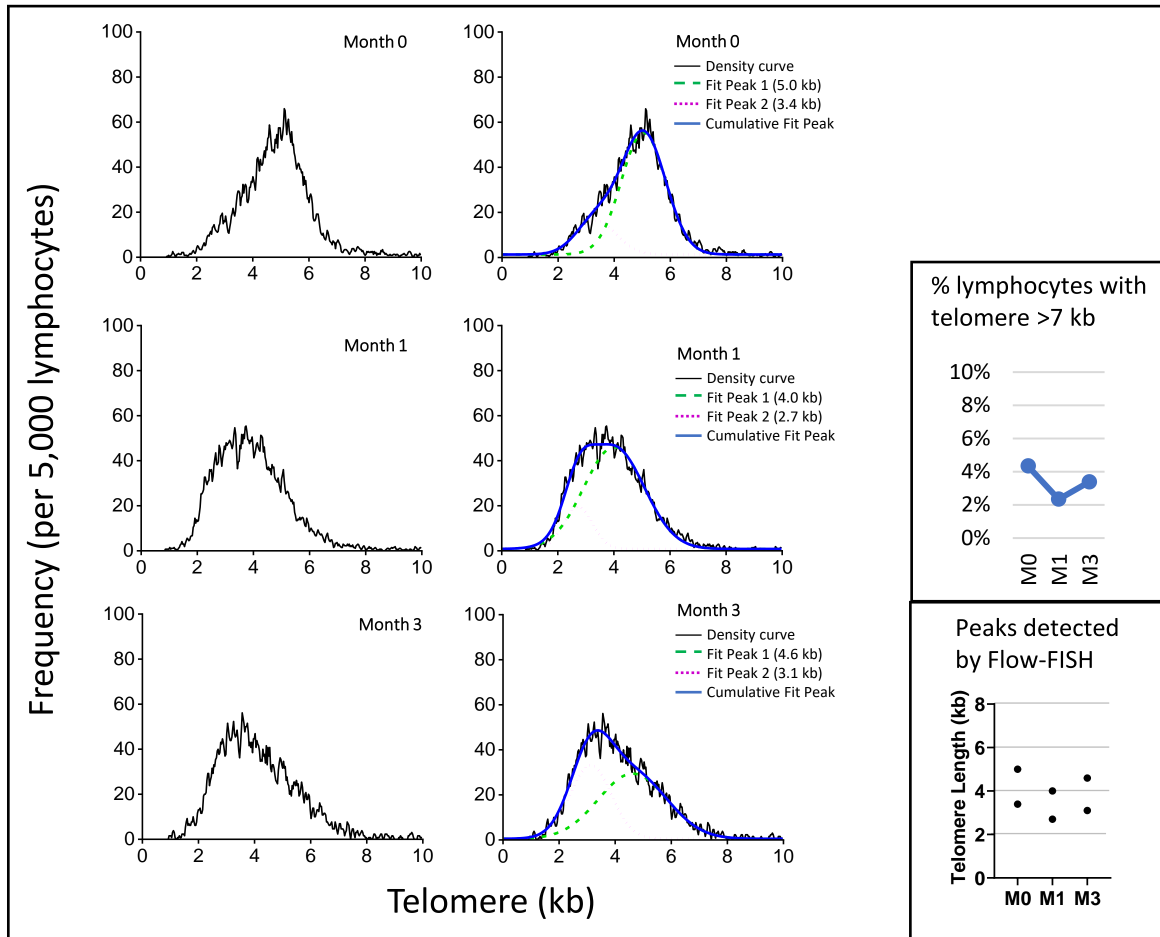


Figure S24. Telomere profiles and deconvoluted telomere profiles of the patient R05's lymphocytes analyzed by telomere flow-FISH.

Left: Telomere size distribution curves of month 0 (EXG34217 treatment), month 1 (1 month after the EXG34217 treatment), and month 3 (3 months after the EXG34217 treatment). Right: The telomere size distribution curves were deconvoluted to multiple Gaussian curves using OriginPro (Version 2024, OriginLab Corporation, Northampton, MA, USA). Assuming that each Gaussian curve represents cell populations with specific telomere lengths, each cell population's mean telomere length (kb) is shown.

The top insert shows the fraction (%) of lymphocytes with telomeres longer than 7 kb. A threshold was set to 7 kb for patient R05, whereas a threshold was set to 6 kb for patient R02 (Figure S13). The reason for this difference is that the telomere length of patient R05 was longer than that of patient R02. The telomeres of patient R05 are longer than the telomeres of patient R02. The bottom insert shows the telomere lengths of the deconvoluted peaks.

Figure S25

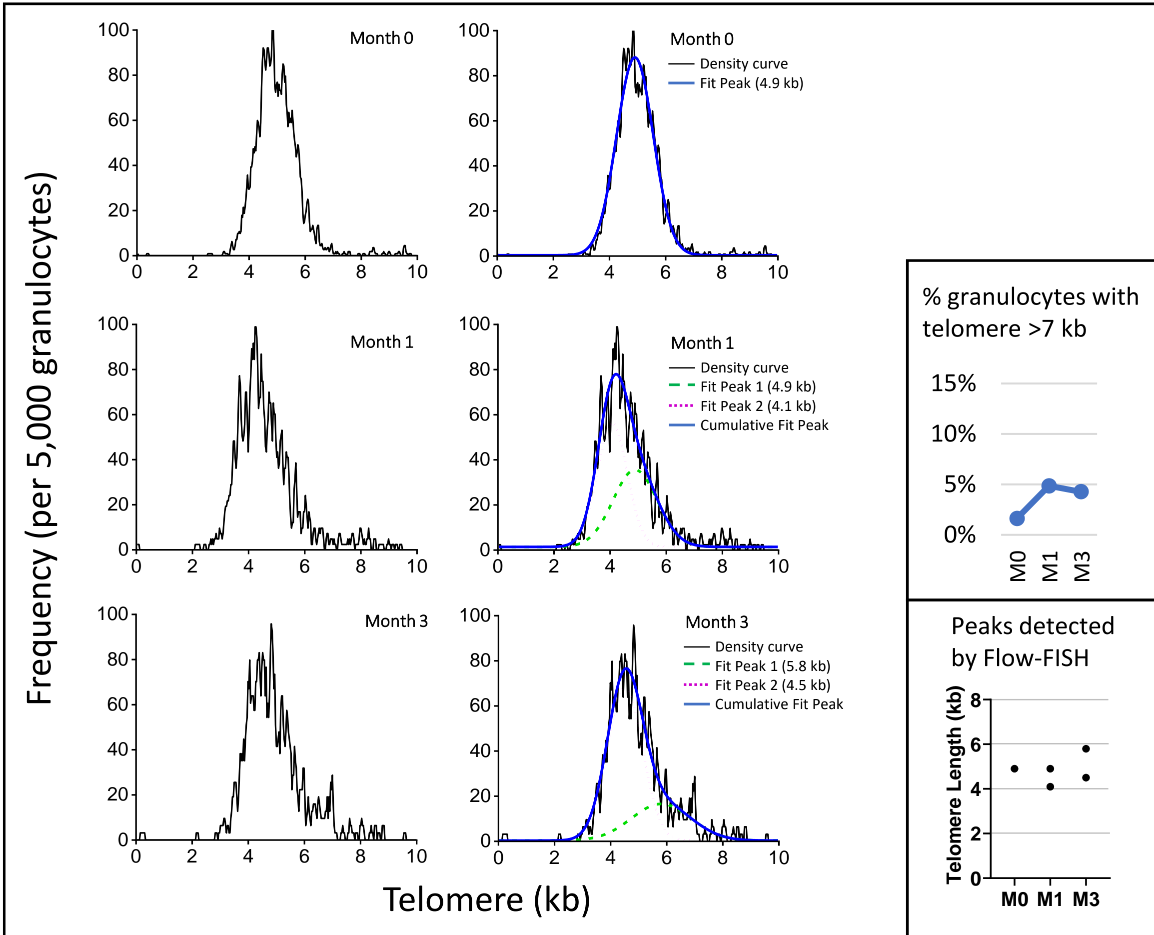


Figure S25. Telomere profiles and deconvoluted telomere profiles of the patient R05's granulocytes analyzed by telomere flow-FISH.

Left: Telomere size distribution curves of month 0 (EXG34217 treatment), month 1 (1 month after the EXG34217 treatment), and month 3 (3 months after the EXG34217 treatment). Right: The telomere size distribution curves were deconvoluted to multiple Gaussian curves using OriginPro (Version 2024, OriginLab Corporation, Northampton, MA, USA). Assuming that each Gaussian curve represents cell populations with specific telomere lengths, each cell population's mean telomere length (kb) is shown.

The top insert shows the fraction (%) of granulocytes with telomeres longer than 7 kb. A threshold was set to 7 kb for patient R05, whereas a threshold was set to 6 kb for patient R02 (Figure S15). The reason for this difference is that the telomere length of patient R05 was longer than that of patient R02. The bottom insert shows the telomere lengths of the deconvoluted peaks.

Text S7. Patient R05's clinical data

Clinical Summary: A 33-year-old woman was diagnosed with TBD (telomere length < 1st percentile) 5 years prior to initial enrollment in 2023 when she was noted to have mild intermittent neutropenia with hypocellular bone marrow. She was subsequently found to have a mutation in the *TERT* gene. Relevant medical history includes hypermobility syndrome with recurrent joint subluxations and endometriosis.

Complete Blood Counts:
Pre-EXG34217 treatment: Figure S25 shows changes in blood counts over time before and after the EXG34217 treatment. Intermittent low-dose (150-300 µg) G-CSF injection was first initiated 6 years prior to EXG34217 treatment for the treatment of fatigue, aphthous ulcers, and occasional grade 1-2 bone pain. G-CSF off was tried 9 months prior to the first mobilization attempt. The ANC declined over time as expected and was accompanied by a recurrence of mouth ulcers and fatigue. Therefore, intermittent (two to three times per week) G-CSF injection was restarted. After the first mobilization attempt, the patient was switched to intermittent G-CSF injection and monitored to maintain an ANC above $1 \times 10^3/\mu l$. She received a G-CSF injection approximately every 2-8 weeks for 1-2 days per dosing.

Post-EXG34217 treatment: The period between M0 and M6 may be influenced by the possible effects of G-CSF and Plerixafor that were administered to the patient immediately before the EXG34217 infusion for CD34+ cell mobilization (Figure S26). Assessment of peripheral blood counts after EXG34217 treatment is limited as the follow-up duration is 5 months and ongoing; however, the patient has not required G-CSF therapy since EXG34217 treatment and remains without the prior symptoms of fatigue, aphthous ulcers, or bone pain at the most recent (M5) follow-up.

Bone Marrow:
Pre-EXG34217 treatment: Table S2 shows the analyses of bone marrow cellularity, %CD34+ cells, karyotypes, MDS (myelodysplastic syndrome)-FISH, and somatic mutation panels before EXG34217 treatment. The marrow was slightly hypocellular (50%) before starting intermittent low-dose G-CSF injections but increased to normocellular (60-70%) or mildly hypercellular (90%) status after starting low-dose G-CSF, consistent with expected outcomes for G-CSF injection (Table S2). The percentage of bone marrow CD34+ cells was normal to slightly low (0.3%-1.0%) (Table S2). The karyotype was normal, and the MDS FISH and somatic mutation panel were negative.

Post-EXG34217 treatment: Bone marrow assessment has not yet been performed after EXG34217 treatment. The patient has remained G-CSF-independent and transfusion-independent without serious infections or evidence of myeloproliferation.

Pulmonary:
Pre-EXG34217 treatment: The patient's screening baseline high-resolution chest CT scan 6 years prior to EXG34217 treatment was normal without evidence of pulmonary fibrosis. The most recent serial pulmonary function testing at 17 months prior to EXG34217 treatment was normal. Quantitative measurements are not available.

Post-EXG34217 treatment: Pulmonary function assessment has not yet been performed after EXG34217 treatment. The patient has remained without shortness of breath or difficulty breathing.

Liver:
Pre-EXG34217 treatment: A screening MRI elastogram 6 years prior to EXG34217 treatment was negative for fibrosis of the liver. The patient clinically demonstrated no functional impairment of the liver with normal transaminases, bilirubin, and albumin levels, as well as normal PT (prothrombin time), INR (international normalized ratio), and aPTT (activated partial thromboplastin time) values (Figure S27).

Post-EXG34217 treatment: Liver imaging assessment has not yet been performed after EXG34217 treatment. The patient clinically demonstrates no functional impairment of the liver with normal transaminases, bilirubin, and albumin levels, as well as normal PT, INR, and aPTT values (Figure S27)

Joint hypermobility:
Pre-EXG34217 treatment: The patient has experienced hyperextensible joints since early childhood with recurrent joint subluxations requiring multiple joint surgeries.

Post-EXG34217 treatment: The patient has not had any additional joint injuries or concerns since EXG34217 treatment.

Endometriosis:
Pre-EXG34217 treatment: The patient has experienced endometriosis, pelvic congestion, and chronic pelvic pain requiring ablation for several years prior to EXG34217 treatment. Hysterectomy was planned for future treatment, however the patient elected to delay this intervention to explore alternative treatments.

Post-EXG34217 treatment: The patient underwent a hysterectomy as previously recommended approximately 5 months after EXG34217 treatment.

Figure S26

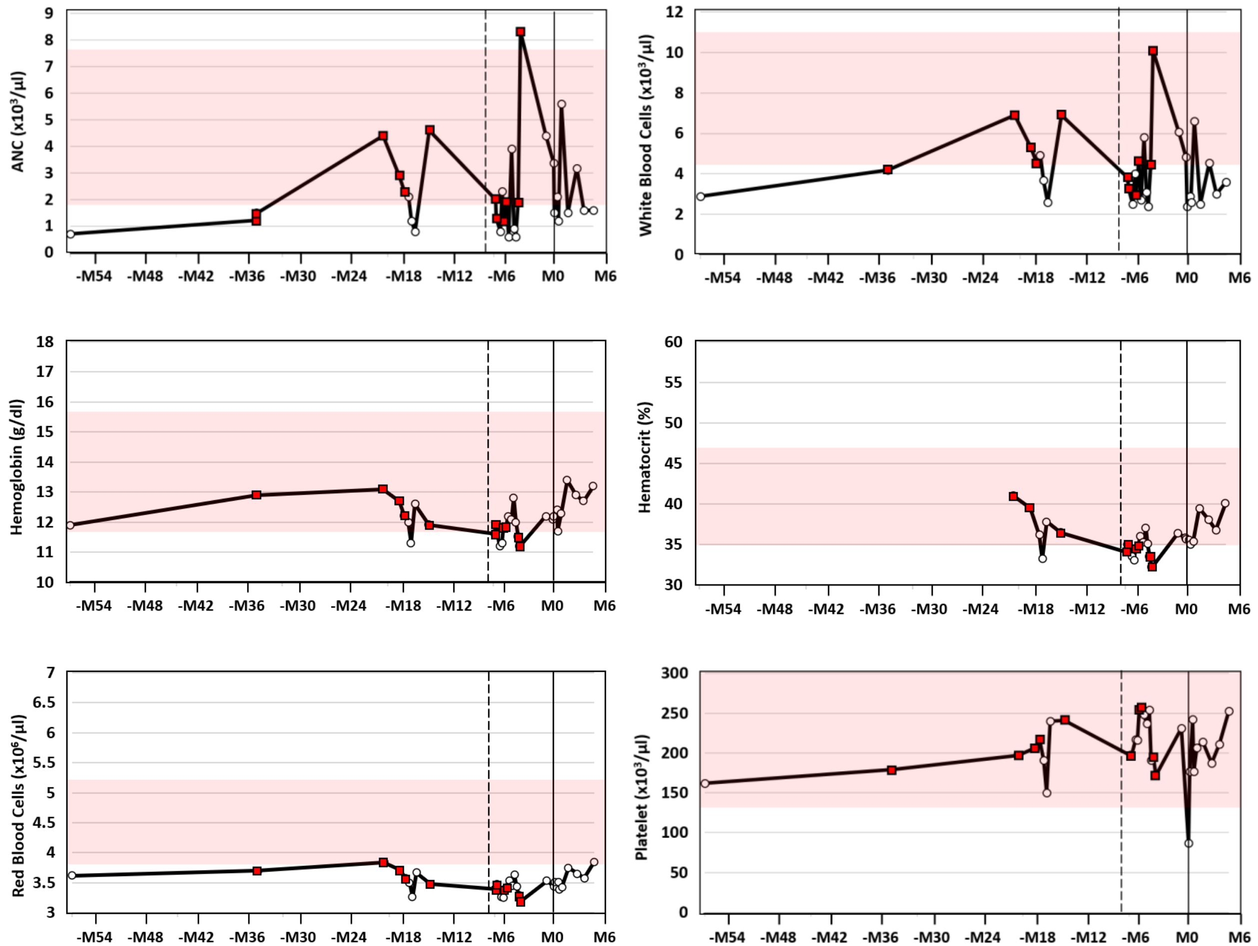


Figure S26. Changes in blood counts of the patient R05 over time before and after EXG34217 treatment.

-M54, -M48, -M42, -M36, -M30, -M24, -M18, -M12, and -M6 denote months (M) before EXG34217 treatment. M0 denotes 3 days before the EXG34217 infusion. M6 denotes 6 months after EXG34217 infusion. Dotted vertical lines at -M8 (8 months before EXG34217 infusion) is the first mobilization attempt, resulting in no apheresis and no EXG34217 infusion. Vertical lines at M0 indicate the time of mobilization, apheresis, and EXG34217 infusion. As described in Text S7, due to neutropenia, the patient received intermittent low-dose G-CSF treatments. Red squares denote data points when blood counts were performed while the patient received a low-dose G-CSF treatment.

Normal ranges are shown in pink: ANC (1.80-7.70 x10^3/µl), White Blood Cell (4.50-11.00 x10^3/µl), Red Blood Cell (3.80-5.20 x10^6/µl), Hematocrit (35.0-47.0%), Platelet (135-466 x10^3/µl), Hemoglobin (11.7-15.7 g/dl).

Table S2

Date	G-SCF*[1]	Marrow Cellularity	Fraction of CD34+ cells*[2]	Karyotype [No. of analyzed cells]	MDS FISH	Somatic panel
-M67	No	Slightly hypocellular (50%)	1.0%	46, XX [20]	not assessed	not assessed
-M51	Yes	Slightly hypercellular (90%)	1.0%	46, XX [20]	not assessed	not assessed
-M30	Yes	Normocellular (70%)	not assessed	46, XX [20]	not assessed	not assessed
-M13	Yes	Normocellular (60-70%)	not assessed	46, XX [20]	not assessed	negative
-M4	Yes	Normocellular (60%)	0.3%	46, XX [20]	not assessed (peripheral blood, negative in March 2024)	negative

Table S2. Analyses of patient R05's bone marrow cells before EXG34217 treatment.

No bone marrow biopsy data are available after the EXG34217 treatment as of September 30, 2024.

*1. Periods overlapped with intermittent low-dose G-CSF treatment for treatment of fatigue, aphthous ulcers, and occasional grade 1-2 bone pain.

*2. The fraction of CD34+ cells (%) in bone marrow was assessed by immunophenotyping.

-M67, -M51, -M30, -M13, and -M4 denote months (M) before the EXG34217 treatment. MDS FISH denotes myelodysplastic syndrome fluorescent in situ hybridization analysis; somatic panel, somatic mutation panel.

Figure S27

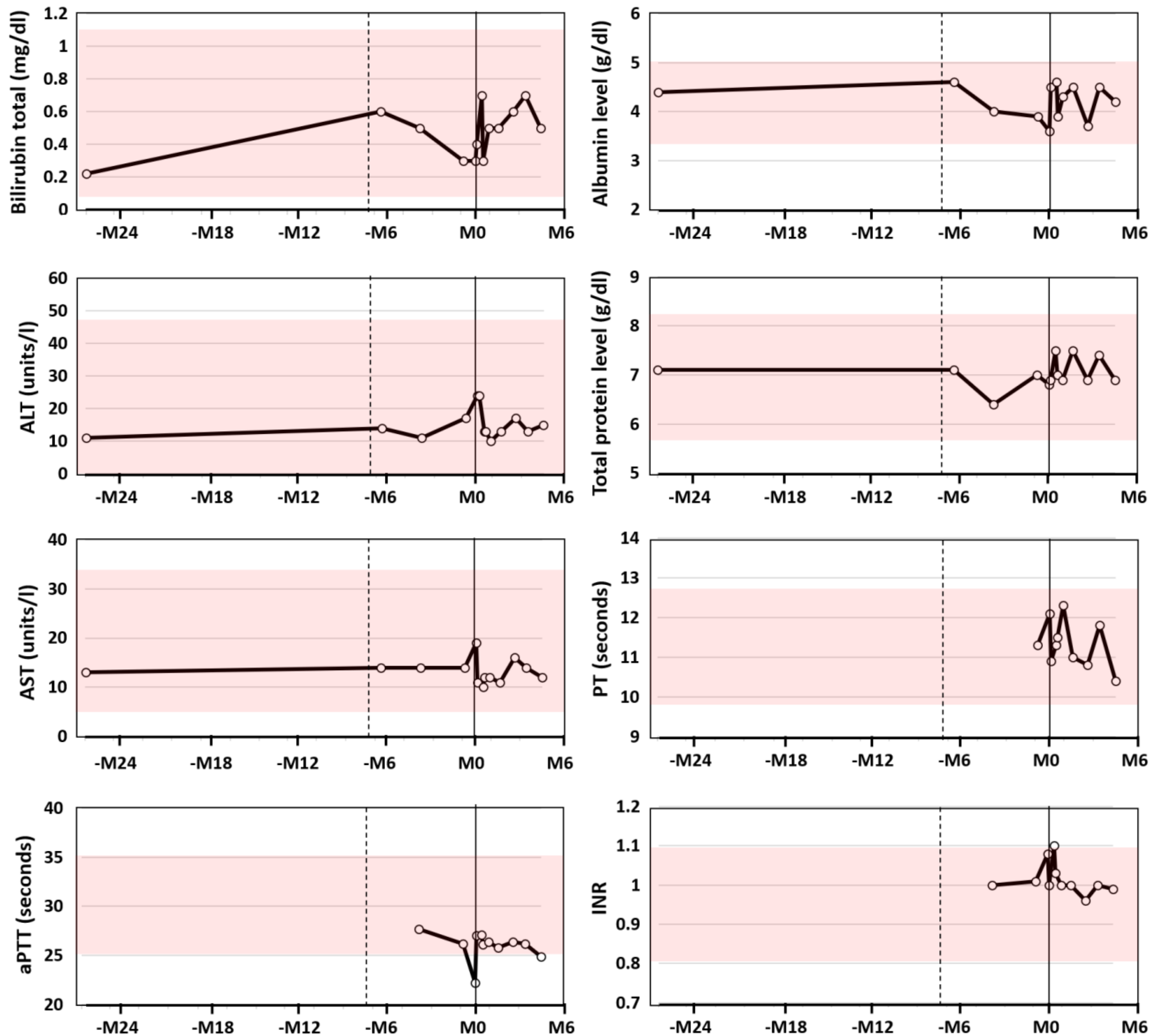


Figure S27. Changes in liver function markers of the patient R05 over time before and after EXG34217 treatment.

-M24, -M18, -M12, and -M6 denote months (M) before EXG34217 treatment. M0 denotes 3 days before the EXG34217 infusion. M6 denotes 6 months after EXG34217 infusion. Dotted vertical lines at -M8 (8 months before EXG34217 infusion) is the first mobilization attempt, resulting in no apheresis and no EXG34217 infusion. Vertical lines at M0 indicate the time of mobilization, apheresis, and EXG34217 infusion.

Normal ranges are shown in pink: total bilirubin (0.1-1.1 mg/dl), albumin level (3.4-5.0 g/dl), total protein level (5.7-8.2 g/dl), ALT (≤ 49 units/l), AST (≤ 33 units/l), PT (9.9-12.7 seconds), INR (0.8-1.1), aPTT (27.3-35.3 seconds).

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Table of Contents

	Page
Table of Contents	1
Study EXG-US-01: A Phase I/II Study to Evaluate the Safety and Tolerability of EXG34217 in Patients with Telomere Biology Disorders with Bone Marrow Failure	
Initial Protocol – Version 2.0, May 26, 2020	2
Current Protocol – Version 7.0, April 3, 2024	62
Summary of Amendments	122

CLINICAL TRIAL PROTOCOL

Study Title:	A Phase I/II Study to Evaluate the Safety and Tolerability of EXG34217 in Patients with Telomere Biology Disorders with Bone Marrow Failure
Study Number:	EXG-US-01
Study Phase:	Phase 1/2
Name of Investigational Product:	EXG34217
Name of Active Ingredient:	Autologous CD34+ cells contacted *ex vivo* with EXG-001 (Sendai virus vector encoding for human ZSCAN4)
IND Number:	19748
Sponsor:	Elixirgen Therapeutics, Inc.
Medical Monitor:	Kathleen Clarence-Smith, MD, PhD KM Pharmaceutical Consulting, LLC 1825 K Street NW, Suite 520 Washington, DC 20006 Office: 202-223-7001, Cell: 202-550-1133

	Version	Date
Original Protocol:	1.0	March 30, 2020
Amendment 1	2.0	May 26, 2020

Confidentiality Statement

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SYNOPSIS

Name of Sponsor/Company: Elixirgen Therapeutics, Inc.

Name of Test Product: EXG34217

Name of Active Ingredients: Autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]

Title of Study: A Phase I/II Study to Evaluate the Safety and Tolerability of EXG34217 in Patients with Telomere Biology Disorders with Bone Marrow Failure

Study Center(s): Single site

Principal Investigator:
Kasiani Myers, MD (Cincinnati Children's Hospital Medical Center, OH)

Studied Period (years): Estimated date first subject enrolled: June 2020 Estimated date last subject completed: TBD	**Phase of Development:** 1/2

Objectives:

Primary Objective:

- To assess the safety and tolerability of EXG34217 (autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]) in subjects with telomere biology disorders with bone marrow failure.

Secondary objectives:

- To assess the feasibility of *ex vivo* transduction and reinfusion of EXG34217 (autologous ZSCAN4 transduced CD34+ cells).
- To assess the feasibility of telomeres extension assessment.
- To assess clinical benefit by measuring complete blood count over time.

Study Design:

This is a Phase I/II, open label study in up to 12 subjects with telomere biology disorders with bone marrow failure. The study is open to all participants regardless of gender or ethnicity. Subjects who are enrolled but not evaluable will be replaced. Subjects will sign a consent form prior to any study related procedure and will complete baseline screening assessments. Subjects for this study will not require any preparative regimen such as chemotherapy or radiation.

The study will be conducted in four parts

1. Peripheral blood mononuclear cells (PBMNCs) collection; mobilization and apheresis,
2. *Ex vivo* cell processing
3. Processed cell infusion and post-infusion safety monitoring,
4. Follow-up (Weeks 2-5, Months 2, 3, 4, 5, 6, 9, and 12)

Name of Sponsor/Company: Elixirgen Therapeutics, Inc.
Name of Test Product: EXG34217
Name of Active Ingredients: Autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]

	Screening	outpatient
	↓	
Day 1-4	Pre-treatment of mobilization	outpatient
	↓	
Day 5	1 or 2 apheresis for PBMNC collections	
	↓	
	Ex Vivo Cell processing (approximately 36 hours)	
	↓	Inpatient
	Study drug infusion	
	↓	
	Post infusion Safety monitoring (up to 36 hours)	
	↓	
Day 8-9	Discharge	
	↓	
Week 2	Safety follow-up	outpatient*
	↓	
Week 3	Safety follow-up	outpatient*
	↓	
Week 4	Follow-up visit	outpatient
	↓	
Week 5	Follow-up visit	outpatient*
	↓	
Month 2	Follow-up visit	outpatient*
	↓	
Month 3	Follow-up visit	outpatient
	↓	
Month 4	Follow-up visit	outpatient*
	↓	
Month 5	Follow-up visit	outpatient*
	↓	
Month 6	Follow-up visit	outpatient
	↓	
Month 9	Follow-up visit	outpatient
	↓	
Month 12	Follow-up visit	outpatient*

*: This will not require clinical visit and can be done locally as an option.

Overall study design

Name of Sponsor/Company: Elixirgen Therapeutics, Inc.
Name of Test Product: EXG34217
Name of Active Ingredients: Autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]
All eligible subjects will start the mobilization with granulocyte colony stimulating factor (G-CSF) for 3 days in an outpatient setting and have the eligibility of the 1st apheresis assessed on Day 4. If eligible, the subject will be hospitalized and will be dosed with Plerixafor. On Day 5, subject will be dosed GCS-F and apheresis will start. After apheresis, the feasibility of the 2nd apheresis will be evaluated by Investigator. If the 2nd apheresis is not feasible, the subject will receive the study drug (processed cells) on Day 6. If the 2nd apheresis is feasible, subject will be dosed with G-CSF and the 2nd apheresis will occur on Day 6. Subject will receive the study drug EXG34217 (processed cells) on Day 7. Subjects will be monitored for safety up to 36 hours post-dosing and be discharged after Investigator has reviewed the safety lab results and determines discharge is appropriate. Subjects will be evaluated for safety weekly for the first month after infusion and then assessed once monthly for 6 months. After 6 months, subjects will be evaluated at Month 9 and Month 12. Week 2, 3, 5 and Month 2, 4, 5, 9 visits will not require a clinical visit and can be done locally as an option via telemedicine with Investigator (with the assistance of the local healthcare provider or home health). A 4-week staggering period between the first 3 subjects is planned. Specifically, 30 days must have elapsed from the infusion of the previous subject to the 1st G-CSF dosing of subsequent subject. After the first 3 subjects, no staggering period will be required. Collected PBMNC will be processed to separate CD34+ cells and to incubate with EXG-001 for 24 hours in a closed automated system at GMP facility (Cincinnati Children's Hospital Medical Center, Cell Processing Center). An independent Data and Safety Monitoring Board (DSMB) will be appointed to have responsibility for safeguarding the interests of the trial subjects, and assessing the safety and tolerability of the study treatments periodically throughout the trial.
Number of subjects (planned): Up to 12 subjects are planned.

Name of Sponsor/Company: Elixirgen Therapeutics, Inc.

Name of Test Product: EXG34217

Name of Active Ingredients: Autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]

Patient Population:

Adult males and females with telomere biology disorders with bone marrow failure.

Inclusion Criteria:

1) Signed an Institutional Review Board (IRB) approved informed consent document indicating that they understand the purpose of, and procedures required by the study and are willing to participate in the study and comply with all study procedures and restrictions. Informed consent must be obtained from the subject prior to initiating screening procedures to evaluate eligibility of the study.
2) Age ≥ 18 years.
3) Mild or moderate bone marrow failure defined by satisfying both conditions:
   - Peripheral blood neutrophils (ANC) < 1.5 × 10^9/L; or platelets < 100 × 10^9/L; or Hemoglobin <10 g/dL
   - Bone marrow hypocellular for age
4) Diagnosis of telomere biology disorders defined by one of the following:
   - age-adjusted mean telomere length < 1 percentile in all tested peripheral blood cells such as granulocytes, lymphocytes, B-cells, naïve T-cells, memory T-cells, and NK cells;
   - a pathogenic mutation in DKC1, TERC, TERT, NOP10, NHP2, TINF2, CTC1, PARN, RTEL1, ACD, USB1, or WRAP53

Exclusion Criteria:

1) Women of childbearing potential not willing to following the birth control as described in the informed consent or breastfeeding.
2) Subjects with cancer who are on active chemotherapeutic treatment.
3) Severe bone marrow failure defined by one of the following:
   - peripheral blood neutrophils (ANC) < 0.5 × 10^9/L;
   - platelets < 20 × 10^9/L
4) Clonal cytogenetic abnormalities associated with myelodysplastic syndrome or acute myeloid leukemia on bone marrow examination within the preceding 3 months.
5) Uncontrolled bacterial, viral or fungal infections.
6) Prior allogeneic marrow or stem cell transplantation.
7) Subjects who are not eligible for G-CSF and plerixafor dosing.
8) Subjects who are not eligible for the apheresis.
9) Subjects currently taking or have taken danazol and androgens within 60 days prior to Day 1.
10) Subjects with any other clinically relevant acute or chronic diseases which could interfere with the subjects' safety during the trial, expose them to undue risk, or which could interfere with study objectives.
11) Subjects who have participated in another clinical trial with an investigational drug within the previous 30 days.

Name of Sponsor/Company: Elixirgen Therapeutics, Inc.
Name of Test Product: EXG34217
Name of Active Ingredients: Autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]
Study Drugs: EXG34217; Autologous CD34+ cells contacted *ex vivo* with EXG-001 (Sendai virus vector encoding for human ZSCAN4)
Administration route: Single intravenous infusion at 3.3 mL/min infusion rate for 30 mins **Dose:** EXG34217 cells ($\leq 8.0 \times 10^6$ /kg) in suspended in 100 mL Plasma-Lyte A.
Study Stopping Rule: If any of the following events occur, the study will be suspended pending DSMB review: • Any SAE associated with the study procedure in one subject. • Allergic reaction, associated with infusion of EXG34217, Grade 3 in two subjects or Grade 4 in one subject. • Grade 4 infection within 1-month post infusion of EXG34217 uncontrolled for >7 days in one subject. • Any duration of grade 3 toxicity involving cardiac, pulmonary or neurologic systems. • Any other grade 3 toxicity that lasts >72 hours. • Any duration of grade 4 toxicity. • Any death that occurs within 30 days of receiving study product.
Criteria for Evaluation: **Safety/Tolerability Endpoint:** • Vital signs • Weight • Standard 12-lead electrocardiogram (ECG) • Clinical laboratory test (hematology, blood chemistry, and urinalysis), • Physical examination • Adverse events and serious adverse events • Immunogenicity of Sendai virus vector and hZSCAN4 protein **Exploratory Endpoint:** • Telomere length in any of the following: lymphocytes, granulocytes, B-cells, naïve T-cells, memory T-cells, and NK cells in peripheral blood. • Blood counts (neutrophils, platelets, or hemoglobin).
Statistical methods: Since this is an initial Phase 1/2 study, evaluation is intended to be hypothesis-raising rather than hypothesis-testing, it is not powered to reliably yield statistically significant conclusions. The data will be analyzed only by means of descriptive statistics (including means and standard deviations, medians and inter-quartile ranges, and tabulations as appropriate) to reveal possible trends that will help guide the design of future, more definitive, clinical trials.

Table 1a. Schedule of Events-1 (Screening to Discharge)

	Screening	Pre-treatment				Apheresis		EXG34217 dose
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
	-30 days	D1	D2	D3	D4	D5	D6	D7
Clinic visit	x	x	x	x				
Admission					x	x	x	x
Informed consent	x							
Demographics	x							
Medical history	x							
Inclusion/Exclusion criteria	x	x [4]						
Baseline disease status	x [2]							
G-CSF [1]		x	x	x	x	x	x	
Plerixafor [1]					x	x		
Apheresis [1][3]						x	x	
CD34+ cell counts					x	x	x	
Administration of EXG34217 [1]								x
Physical examination	x							
Vital signs	x					x	x	x
12- lead ECG	x							
Height	x							
Weight	x	x						
Safety laboratory panel	x							
Urinalysis	x							
Cytokine panel	x							
Pregnancy test	x							
Telomere length	x							
Immunogenicity	x	x						
Adverse events		x	x	x	x	x	x	x
Prior/Concomitant medications	x	x	x	x	x	x	x	x

1) Schedule for mobilization, apheresis, dosing and post dosing monitoring may vary by each patient
2) Pathogenic mutation information needs to be collected during the screening if patient does not have genetic information
3) Eligibility of apheresis will be determined by PI's clinical judgements
4) Confirm any changes by PI prior to infusion

Table 2b. Schedule of Events-2 (After Discharge Follow-up visits)

	Follow-up											
	Day 7-9	Week 2	Week 3	Week 4 (Month 1)	Week 5	Month 2	Month 3	Month 4	Month 5	Month 6	Month 9	Month 12
	D7-9	W2	W3	W4	W5	M2	M3	M4	M5	M6	M9	M12
Clinic visit		x[5)]	x[5)]	x	x[5)]	x[5)]	x	x[5)]	x[5)]	x	x[5)]	x
Admission	x											
Physical examination	x	x	x	x	x	x	x	x	x	x	x	x
Vital signs	x	x[6)]	x[6)]	x	x[6)]	x[6)]	x	x[6)]	x[6)]	x	x[6)]	x
12- lead ECG				x			x			x		x
Height												
Weight	x			x			x			x		x
Safety laboratory panel	x	x	x	x		x	x	x	x	x	x	x
Urinalysis												x
Cytokine panel	x			x			x			x		x
Pregnancy test												x
Telomere length				x			x			x		x
Immunogenicity	x			x			x			x		x
Adverse events	x	x	x	x		x	x	x	x	x	x	x
Prior/Concomitant medications	x			x			x			x		x

5) This will not require a clinic visit and can be done locally via telemedicine visits as an option
6) Vital sign will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health) is being performed.

Sponsor Signature Page

Protocol Title: A Phase I/II Study to Evaluate the Safety and Tolerability of EXG34217 in Patients with Telomere Biology Disorders with Bone Marrow Failure

Protocol Number: EXG-US-01

Chief Executive Officer: Akihiro C. Ko

May 26, 2020
Date:

Investigator Signature Page

I agree to conduct the study as outlined in the protocol entitled "A Phase I/II Study to Evaluate the Safety and Tolerability of EXG34217 in Patients with Telomere Biology Disorders with Bone Marrow Failure" in accordance with the guidelines and all applicable government regulations including US Title 21 Code of Federal Regulations (CFR) Part 54. I have read and understand all sections of the protocol.

____
Principal Investigator:

____
Date:

TABLE OF CONTENTS

SYNOPSIS 2
TABLE OF CONTENTS 11
LIST OF IN-TEXT TABLES 13
LIST OF IN-TEXT FIGURES 13
LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS 14
1 INTRODUCTION 16
1.1 Background 16
1.2 Study Rationale 21
2 STUDY OBJECTIVES 22
2.1 Primary Objective 22
2.2 Secondary Objectives 22
3 STUDY ENDPOINTS 22
3.1 Primary Endpoint 22
3.2 Secondary Endpoints 22
4 OVERALL STUDY DESIGN AND PLAN 23
5 STUDY POPULATION 26
5.1 Inclusion Criteria 26
5.2 Exclusion Criteria 26
5.3 Subject Identification 27
5.4 Randomization and Blinding 27
5.5 Prior, Concomitant, and Prohibited Medications 27
6 PROCEDURE FOR MOBILIZATION, APHERESIS AND STUDY DRUG INFUSION . 28
6.1 Detailed Procedure for Mobilization, Apheresis and Study Drug Infusion 30
6.2 Procedure of Study Drug (EXG34217) Administration 32
6.3 Re-infusion of Non-Cultured Collected Cells 32
7 STOPPING CRITERIA AND STUDY DURATION 33
7.1 During the Dosing Procedure 33
7.2 Subject Stopping Rules 33
7.3 Study Stopping Rules 33
7.4 Study Duration and Dates 33
8 LABELING, PACKAGING, STORAGE, DISPENSING, AND RETURN OF CLINICAL SUPPLIES 34
8.1 Product Description 34
8.2 Cell Processing 34
8.3 Treatment Administration 34
8.4 Primary Packaging and Labeling Information 34
8.5 Storage Requirements 34
8.6 Instructions for Dispensing 34
8.7 Assessment of Compliance 34
8.8 Drug Accountability/Return of Clinical Supplies 35
9 STUDY PROCEDURES 36
9.1 Informed Consent 36

9.2 Inclusion/Exclusion Criteria ... 36
9.3 Demographics ... 36
9.4 Medical History ... 36
9.5 Baseline Disease Characteristics ... 36
9.6 Body Weight ... 36
9.7 Physical Examination ... 36
9.8 Vital Signs ... 37
9.9 Electrocardiography ... 37
9.10 Clinical Laboratory Tests ... 37
9.11 CD34+ Cell Counts ... 38
9.12 Telomere Length ... 38
9.13 Immunogenicity ... 38
9.14 Cytokine Panel ... 38
9.15 Concomitant Medication Assessments ... 39
9.16 AE Assessments ... 39
10 STUDY ACTIVITIES ... 46
10.1 Screening ... 46
10.2 Day 1 ... 46
10.3 Day 2 ... 46
10.4 Day 3 ... 47
10.5 Day 4 ... 47
10.6 Day 5: Apheresis (1) ... 47
10.7 Day 6: Apheresis (2) ... 48
10.8 Day 7: Study Drug Administration ... 48
10.9 Day 7-9: Post Dose Safety Monitoring ... 48
10.10 Week 2 ... 49
10.11 Week 3 ... 49
10.12 Week 4/Month 1 ... 49
10.13 Week 5 ... 49
10.14 Month 2 ... 50
10.15 Month 3 ... 50
10.16 Month 4 ... 50
10.17 Month 5 ... 50
10.18 Month 6 ... 51
10.19 Month 9 ... 51
10.20 Month 12/Exit Visit ... 51
11 DATA SAFETY MONITORING BOARD (DSMB) ... 52
12 PLANNED STATISTICAL METHODS ... 53
12.1 General Considerations ... 53
12.2 Determination of Sample Size ... 53
12.3 Analysis Populations ... 53
12.4 Demographics and Baseline Characteristics ... 53
12.5 Safety Analysis ... 53
12.6 Exploratory Efficacy Analysis ... 53
12.7 Interim Analysis ... 54
13 ADMINISTRATIVE CONSIDERATIONS ... 55

13.1 Investigators and Study Administrative Structure 55
13.2 Regulatory Authority Approval 55
13.3 Ethical Conduct of the Study and IRB Approval 55
13.4 Subject Information and Consent 56
13.5 Confidentiality 56
13.6 Quality Control and Assurance 57
13.7 Data Management 57
13.8 Study Monitoring 57
13.9 Retention of Data 58
13.10 Financial Disclosure 58
14 REFERENCES 59

LIST OF IN-TEXT TABLES

Table 1a. Schedule of Events-1 (Screening to Discharge) 7
Table 1b. Schedule of Events-2 (After Discharge Follow-up visits) 8
Table 2. Study Administrative Structure 55

LIST OF IN-TEXT FIGURES

Figure 1. EXG-001 extends short telomeres of human primary fibroblast cells derived from a dyskeratosis congenita patient 20
Figure 2. EXG-001 extends telomeres of human CD34+ cells derived from healthy donors compared to untreated CD34+ cells 21
Figure 3. Overall study design 24
Figure 4. Study procedure algorithm 29

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	adverse event
ALT	alanine transaminase
AML	acute myeloid leukemia
ANC	absolute neutrophil count
APTT	activation partial thromboplastin time
AST	aspartate aminotransferase
BAR	bright, alert, responsive
BUN	blood urea nitrogen
CCHMC	Cincinnati Children's Hospital Medical Center
CFR	Code of Federal Regulations
CFU	colony forming unit
CPC	cell processing center
CTCAE	Common Terminology Criteria for Adverse Events
D	day
DC	dyskeratosis congenita
dL	deciliter
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DOB	date of birth
DSMB	Data and Safety Monitoring Board
ECG	electrocardiogram
ESC	embryonic stem cell
FISH	fluorescence in situ hybridization
g	gram
G-CSF	granulocyte colony stimulating factor
GFP	green fluorescent protein
GI	gastrointestinal
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplant
ICF	informed consent form
ICH	International Conference for Harmonisation
ID	identification
IFN	interferon
IL	interleukin
IRB	institutional review board
kg	kilogram
L	liter
M	month
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MDS	myelodysplastic syndrome
mg	milligram
mL	milliliter
MNC	mononuclear cells

MOI	multiplicity of infection
mRNA	messenger ribonucleic acid
NK cell	natural killer cell
NMAC	non-myeloablative conditioning
PBMNC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PI	principal investigator
PT/INR	prothrombic time/international normalized ratio
RBC	red blood cell
RIC	reduced intensity conditioning
RNA	ribonucleic acid
SAE	serious adverse event
SAP	statistical analysis plan
s.c.	subcutaneous
SOP	standard operating procedure
TT	thrombin time
μL	microliter
US	United States
W	week
WBC	white blood cell

1 INTRODUCTION

1.1 Background

1.1.1 Therapeutic Target Population: Patients with Telomere Biology Disorders

Telomeres are protective structures composed of DNA and proteins at the ends of chromosomes. Telomeres become shorter upon cell division and during aging; cell cycle arrest or cell death is activated upon reaching a critical shortness – a DNA damage response (Bessler, et al., 2010). This shortness is prevented in large part by the enzyme telomerase, which adds telomere repeat sequences on to the ends of existing telomeres (Bessler, et al., 2010).

Various mutations in telomere maintenance mechanisms cause severe telomere shortening, typically seen in patients with dyskeratosis congenita (DC) – a rare, inherited genetic disease (Agarwal 2018). Patients with dyskeratosis congenita usually have extremely short telomeres (below the 1st percentile of population in telomere lengths) (Alter et al., 2015). Originally, the disease was characterized by its "classic triad of abnormal skin pigmentation, nail dystrophy, and oral leukoplakia" (Fernández García and Teruya-Feldstein, 2014), but its typical presentation has since been expanded to include high rates of severe aplastic anemia, pulmonary fibrosis, and liver disease (Balakumaran et al., 2015).

The primary cause of death of those with dyskeratosis congenita is bone marrow failure – shortage of blood cells such as white blood cells, red blood cells and platelets, affecting 80-90% of all cases by 30 years old. The bone marrow failure is caused by deficiencies in the renewal abilities of the affected patient's hematopoietic stem cells (HSCs) due to severe telomere shortening (Balakumaran et al., 2015 and Goldman et al., 2008). In addition, patients are predisposed to cancers, and pulmonary fibrosis or abnormalities in pulmonary vasculature are seen.

It becomes increasingly clear that pathogenic mutations in genes involved in the telomere maintenance mechanism cause not only dyskeratosis congenita, but also a broad spectrum of diseases, which are now grouped under the name of "telomere biology disorders" (or telomeropathy, short telomere syndromes [Armanios and Blackburn, 2012]). Telomere biology disorders include dyskeratosis congenita, Revesz syndrome, Hoyeraal Hreidarsson syndrome, Coats plus, and can be associated with many cases of aplastic anemia, Myelodysplastic syndrome, Acute myeloid leukemia, Pulmonary fibrosis, Liver fibrosis, Familial melanoma, Familial lymphoproliferative disease, and Li-Fraumeni-like syndrome (Savage 2018).

Currently there are several treatment options for telomere biology disorders with bone marrow failure (Savage 2018 and Agarwal 2018).

- Supportive care
    - ✓ Transfusion: For patients with severe cytopenia, frequent transfusion support with red blood cells and platelets are required as rescue, but this treatment comes with various risks.
    - ✓ Stimulators of blood cell production: Commonly used erythropoietin and granulocyte colony stimulating factor (G-CSF) are not recommended, because they do not produce durable improvements. Also, patients do not respond to

Eltrombopag - an agonist of the thrombopoietin receptor.

- Androgens: Some patients respond to androgens, but only for a limited time (for months to years). Also, patients often do not tolerate the androgen therapy due to its severe side effects, such as impaired liver function, virilization, and behavioral problems. It is noteworthy that in one clinical trial in patients with shorter telomeres, Danazol – a synthetic steroid has been shown to extend telomeres, which are associated with the improvement of blood count (Townsley et al., 2016).
- Hematopoietic Stem Cell Transplantation (HSCT): Currently the only curative treatment available is HSCT, which can alleviate the hematologic manifestations of the condition. However, its use can be challenging with difficulties in finding well-matched donors and toxicities related to myeloablation (chemotherapy and radiation) and other treatment related complications, such as graft failure, graft versus host disease, sepsis, pulmonary fibrosis, cirrhosis, and veno-occlusive disease (Savage and Alter, 2009; Dietz et al., 2017). In fact, historically, patients undergoing myeloablation most commonly died from severe organ toxicity, as telomere maintenance is also important for tissue repair, particularly after exposure to DNA damaging agents in preparative regimens (Gadalla and Savage, 2011). Thus, the actual long-term survival of patients after myeloablative HSCT has been poor (Savage and Alter, 2009).

  To address this issue, a chemotherapy only (no radiation) lower intensity preparative regimen, i.e., a reduced-intensity conditioning (RIC) or non-myeloablative conditioning (NMAC) – has been tried. Nelson et al. (2016) showed improved overall survival of patients using a RIC regimen of alemtuzumab, fludarabine and melphalan. More recently Agarwal (2018) has utilized very low intensity conditioning with only alemtuzumab and fludarabine in an ongoing clinical trial (2018). There remain concerns regarding the use of HSCT as first-line therapy in dyskeratosis.

In sum, due to extremely poor prognosis and high mortality of telomere biology disorders with bone marrow failure, patients are in dire need of effective therapeutic options. However, currently only minimal treatment strategies (and no preventive strategies) are available for the patients. To address this unmet medical need, the development of innovative therapeutic options is urgently required.

1.1.2 Study Drug EXG34217

Study drug EXG34217 is autologous CD34+ cells contacted *ex vivo* with EXG-001 (Sendai virus vector encoding for human ZSCAN4).

Nonclinical data show:

(1) EXG-001 extension of telomeres of DC patient's fibroblast with short telomere in an *in vitro* study and,

(2) EXG34217 (human CD34+ cells contacted with EXG-001) engrafted and increased blood cells with normal proportional differentiation of cell types in an *in vivo* immunocompromised mice study.

Subject mononuclear cells (MNCs) are isolated from peripheral blood circulation by one or two apheresis after subcutaneous injections of G-CSF/Plerixafor. This process is a standard procedure for HSCT transplantation. CD34+ cells are isolated from collected MNCs and

incubated with EXG-001(Sendai virus vector encoding for human ZSCAN4 gene) for 24 hours in the GMP facility with appropriate quality control environment. Study drug EXG34217 cells are formulated in Plasma-Lyte A and dosed into patients intravenously.

Contacting EXG-001 will transiently produce human ZSCAN4 protein in patient's own (autologous) CD34+ cells, which will restore the damaged function of them by extending their abnormally short telomeres *ex vivo*. After dosing, EXG34217 (CD34+ cells with extended telomere) will be engrafted in patient's bone marrow and produce blood cells. Thus, the bone marrow failure of the patient expected to be ameliorated.

1.1.3 Safety of EXG34217

To assess the safety of EXG34217, GLP toxicology study was performed using NOG-EXL (immune-compromised mouse model that does not reject human cells and tissues). A total 210 mice (105 male and 105 female) were randomly assigned to three groups. Group 1 mice received the tail vein injection of a vehicle only (Phosphate Buffered Saline [PBS]). Group 2 mice received the tail vein injection of 4×10^7 cells/kg human CD34+ cells (untreated). Group 3 mice received the tail vein injection of 4×10^7 cells/kg human CD34+ cells treated with EXG-001 (i.e., EXG34217). The human CD34+ cells were obtained from three healthy volunteers. 60 mice were sacrificed one day after the injection to examine a possible acute toxicity. The remaining mice were sacrificed on Day 118/119 to examine the long-term toxicity.

- Clinical Chemistry/Hematology: No issues in clinical chemistry and hematology
- Clinical Observations: Overall no unexpected abnormalities detected all mice BAR (bright, alert, responsive) throughout experiment.
- No tumors detected.
- No differences in body weight and organ weight between CD34+ untreated (Group 2) vs CD34+EXG-001 (Group 3) except liver and spleen, which showed more enlargement in some CD34+ untreated mice (Group 2) than CD34+EXG-001 mice (Group 3).

In addition, i*n vitro* hematopoietic colony forming unit (CFU) assays were performed to determine whether EXG-001, temperature-sensitive Sendai virus vector encoding human ZSCAN4 gene, could affect the differentiation of human CD34+ hematopoietic stem cells isolated from mobilized peripheral blood from healthy donors. After 14 days in the standard CFU culture media, CD34+ hematopoietic stem cells formed colonies grouped into BFU-E, CFU-E, CFU-G, CFU-M, CFU-GM, CFU-GEMM. Based on the fraction of the colonies formed, both EXG-001-treated and untreated CD34+ cells showed similar broad differentiation potential. Therefore, these results indicate that the EXG-001 did not alter the differentiation potential of healthy CD34+ hematopoietic stem cells.

1.1.4 Safety of Sendai Virus Vector

EXG-001 is Sendai virus vector encoding for human ZSCAN4 gene manufactured at GMP facility (ID Pharma, Tsukuba, Japan). The Sendai virus vector is a derivative of Sendai virus - a

single-stranded RNA virus of the Paramyxovirus subfamily, which completes its entire infection cycle as RNA (i.e. without involvement of DNAs). In addition, Sendai virus is not pathogenic in humans.

Compared to other virus vectors used for this type of gene therapy (e.g., retrovirus, adenovirus, lentivirus), the Sendai virus vector proposed in our therapeutic scheme remains in the cytoplasm and is never converted to DNA. As the Sendai virus vector does not leave a footprint in the host cell genome, our therapeutic paradigm is considered much safer than traditional gene therapy using other vectors.

Bitzer et al. injected a Sendai virus vector intravenously (direct injection) in mice and did not observed any toxicity over a period of 22 days (Bitzer et al., 2003).

Also, the clinical trial using Sendai virus vector (direct injection) is currently conducted in patients with peripheral arterial disease (Phase I/IIa completed, currently Phase IIb). Phase I/IIa study was concluded that "single intramuscular injection of this Sendai virus vector up to 8.3×10^7 CIU/kg was safe and well tolerated" (Yonemitsu et al., 2013). This Sendai virus vector shares the same vector backbone as ours and lacks F (fusion) gene; however, unlike our Sendai virus vector, it is not temperature-sensitive. As expected, non-transmissible Sendai virus vector was not detected in circulation and tissues/organs other than the injection sites of the skeletal muscles, even injected virus vector itself.

The Sendai virus vector (SeV18/TS15ΔF) used for EXG-001 lacks F (fusion) gene, and thus, is not capable of producing the infectious virus particles. This safety feature prevents the EXG-001, once integrated into a cell, from spreading to neighboring cells.

Furthermore, the Sendai virus vector (SeV18/TS15ΔF) used for EXG-001 is temperature-sensitive, and thus, produces our therapeutic protein ZSCAN4 within the cytoplasm at 33 °C, but not at 37 °C (Ban et al., 2011; Amano et al., 2015). We will wash EXG34217 cells three times to minimize free EXG-001 in the dosing solution prior to dosing. Even if a few free EXG-001 are present after washing, the EXG-001 will never be active due to the non-permissive temperature – 37°C inside the patient's body after dosing.

We would thus expect that EXG-001 will stay in CD34+ cells contacted *ex vivo* and will not spread to other cells.

1.1.5 Safety of ZSCAN4 Protein

The transgene in EXG-001 is human ZSCAN4 (zinc finger and SCAN domain containing 4). The expression of this gene is transient and restricted to critical timings and cell types during development and adult life: specifically at the 2-cell stage of mouse preimplantation embryos (Falco et al., 2007) and 4- to 8-cell stage in human preimplantation embryos (Vassena et al., 2011); infrequent but high expression in tissue stem cells in mouse and human (Ko et al., 2013); expression in meiotic prophase I in both male and female mice (Ishiguro et al., 2016); and high and specific expression in 1-5% of mouse embryonic stem cells (ESCs) (Falco et al., 2007).

Previous results show that ZSCAN4 localizes to telomeres, extends telomeres by homologous

recombination (i.e., telomerase-independent mechanism), and increases the genome stability in mouse ESCs (Zalzman et al., 2010).

Our unpublished results show that ZSCAN4 extends telomeres in human fibroblast cells derived from a dyskeratosis congenita patient (from a 11-year-old male Caucasian with X-linked dyskeratosis congenita). As previously reported (Wong and Collins, 2006), telomeres of dyskeratosis congenita fibroblast cells are shorter than normal cells and quickly become even shorter in the cell culture. In the control experiments, transfections of synthetic mRNA encoding green fluorescent protein (GFP) did not change the telomere shortening patterns of cells. By contrast, transfection of synthetic mRNAs encoding human ZSCAN4 elongated the telomeres of the dyskeratosis congenita cells and prevented the telomeres from getting shorter.

Telomere extension was also observed by treating human fibroblast cells derived from a dyskeratosis congenita patient by the EXG-001 (see Figure 1 below).

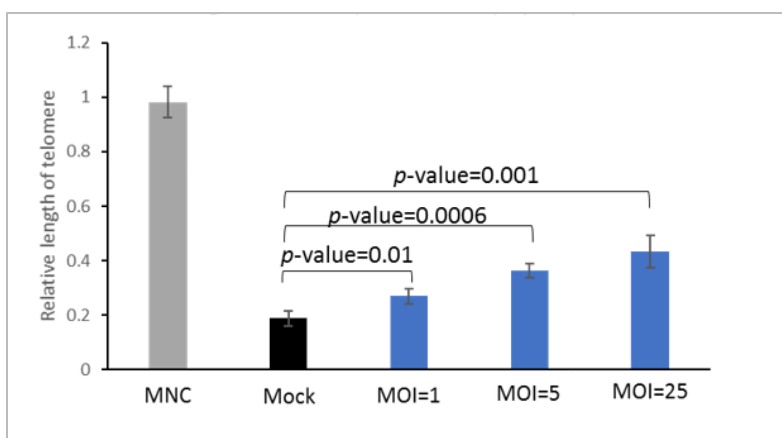

Figure 1. EXG-001 extends short telomeres of human primary fibroblast cells derived from a dyskeratosis congenita patient

DC fibroblast cells contacted with EXG-001 at three different MOI groups, MOI=1, MOI=5, and MOI=25, showed 1.4-fold, 1.9-fold, and 2.3-fold extension in their telomeres compared to the untreated DC fibroblast cells (Mock), respectively. Student's *t-test* for two paired samples of means was applied for statistical analysis. Compared to untreated DC cells (Mock), all EXG-001-treated groups showed statistically significant increase of telomere lengths: MOI=1 ($p<0.05$), MOI=5 ($p<0.005$), and MOI=25 ($p<0.005$). MNC (peripheral blood mononuclear cells) from a healthy donor was used as a control.

EXG-001 also increased telomere lengths in human CD34+ cells collected from healthy individuals after mobilization by G-CSF. CD34+ cells were contacted *ex vivo* with EXG-001 and incubated at 33 °C for 24 hours, subsequently cultured *in vitro* at 37 °C for 10 days. Compared to the untreated cells, EXG-001-treated cells showed statistically significant extension of telomeres (See Figure 2 below).

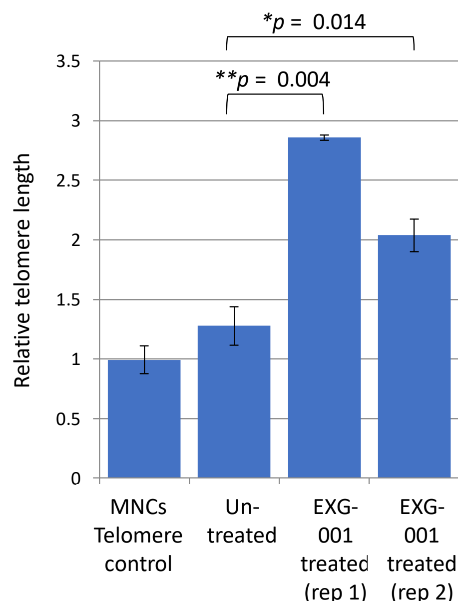

Figure 2. EXG-001 extends telomeres of human CD34+ cells derived from healthy donors compared to untreated CD34+ cells

MNC (peripheral blood mononuclear cells) from a healthy donor was used as a control.

ZSCAN4 proteins are released only within CD34+ cells after contacted to EXG-001. Therefore, it is not expected any free ZSCAN4 proteins will distributed in the body after dosing. Furthermore, ZSCAN4 and SeV-related proteins decrease rapidly at 37 °C (i.e., body temperature) due to the temperature-sensitivity of EXG-001. In this clinical trial, we plan to measure anti-ZSCAN4 antibody to be sure there is any unexpected safety concerns.

1.2 Study Rationale

Telomere biology disorders with bone marrow failure including dyskeratosis congenita is poor prognosis and high mortality disorder. Currently, HSCT is the only curative treatment, which can alleviate the hematologic manifestations of the condition. However, its use can be challenging with difficulties in finding well-matched donors and toxicities related to myeloablation (chemotherapy and radiation) and immune complications.

EXG34217, autologous CD34+ cells contacted *ex vivo* with EXG-001 (Sendai virus vector encoding for human ZSCAN4), has been shown to extend the telomeres in human CD34+ cells *in vitro* and *in vivo* nonclinical studies. This treatment does not require a well-matched donor and can use the patient's own cells. Contacting EXG-001 will transiently produce human ZSCAN4 protein in a patient's own (autologous) CD34+ cells, which will restore the damaged function of them by extending their abnormally short telomeres *ex vivo*. After dosing, EXG34217 (CD34+ cells with extended telomere) will be engrafted in patient's bone marrow and produce blood cells. Thus, the bone marrow failure of the patient may be ameliorated.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of the study is:

- To assess the safety and tolerability of EXG34217 (autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]) in subjects with telomere biology disorders with mild bone marrow failure.

2.2 Secondary Objectives

The secondary objective of the study is:

- To assess the feasibility of *ex vivo* transduction and reinfusion of EXG34217 (autologous ZSCAN4 transduced CD34+ cells).
- To assess the feasibility of telomeres extension assessment.
- To assess clinical benefit by measuring complete blood count over time.

3 STUDY ENDPOINTS

3.1 Primary Endpoint

Primary endpoints are safety and tolerability assessment by:

- Vital signs
- Weight
- Standard 12-lead electrocardiogram (ECG)
- Clinical laboratory test (hematology, blood chemistry, and urinalysis)
- Physical examination
- Adverse events (AEs) and serious adverse events (SAEs)
- Immunogenicity of Sendai virus vector and hZSCAN4 protein

3.2 Secondary Endpoints

Secondary endpoints of this study are exploratory efficacy assessments by.

- Increase of telomere length in any of the following: lymphocytes, granulocytes, B-cells, naïve T-cells, memory T-cells, and NK cells.
- Improvement of blood counts (neutrophils, platelets, or hemoglobin).

4 OVERALL STUDY DESIGN AND PLAN

This is a Phase I/II, open label study in up to 12 subjects with telomere biology disorders with bone marrow failure. The study is open to all participants regardless of gender or ethnicity. Subjects who are enrolled but not evaluable will be replaced.

Subjects will sign a consent form prior to any study related procedure and will complete baseline screening assessments.

Subjects for this study will not require any preparative regimen such as chemotherapy or radiation.

The study will be conducted in four parts

1. Peripheral blood mononuclear cells (PBMNCs) collection; mobilization and apheresis;
2. *Ex vivo* cell processing
3. Processed cells infusion and post-infusion safety monitoring;
4. Follow up Weeks 2-5, Months 2, 3, 4, 5, 6, 9 and 12)

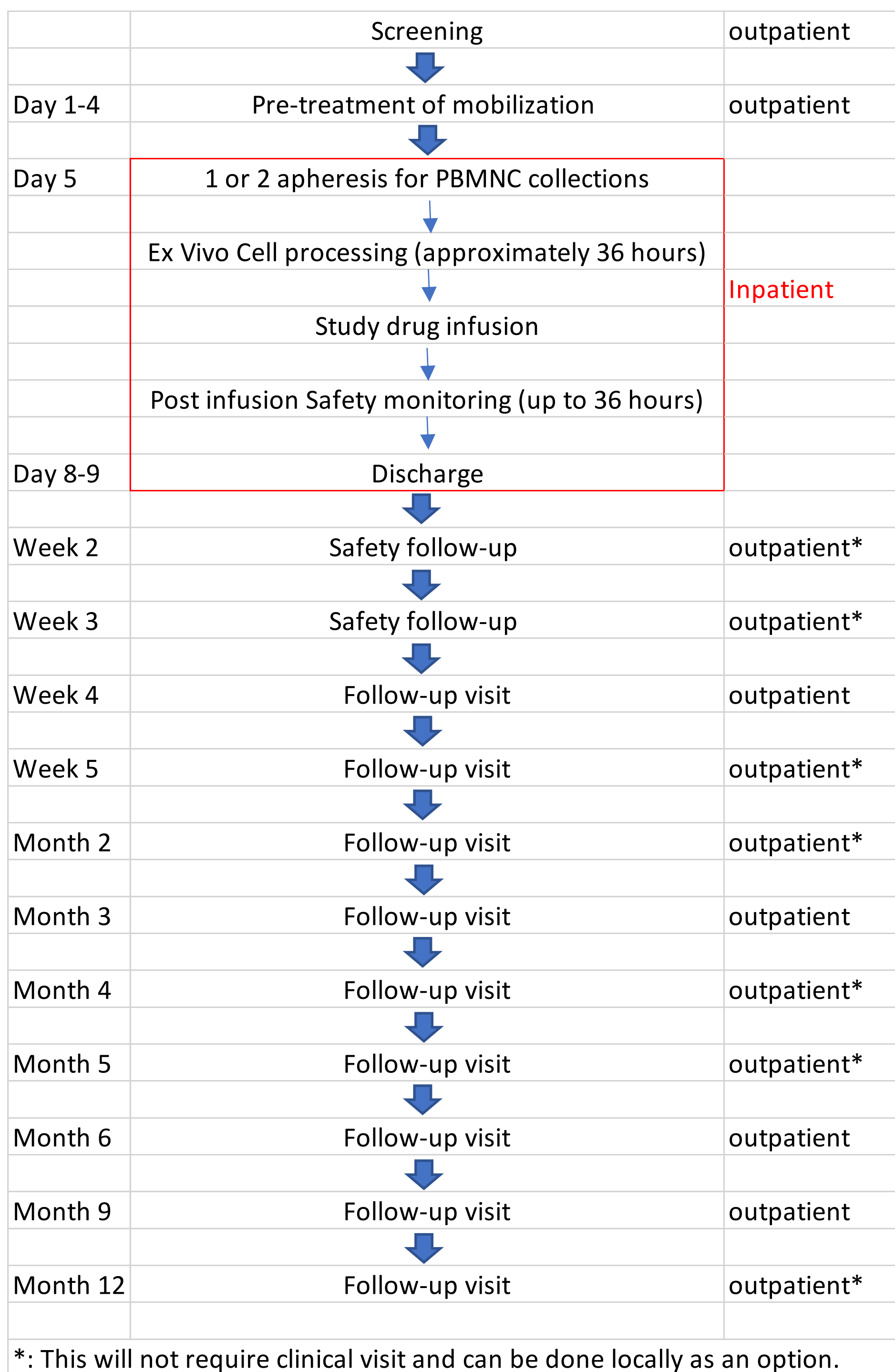

Figure 3. Overall study design

All eligible subjects will start the mobilization with G-CSF for 3 days in an outpatient setting and have the eligibility of the 1st apheresis assessed by PI's clinical judgment on Day 4. If eligible, the subject will be hospitalized and will be dosed with Plerixafor. On Day 5, subject will be dosed GCS-F and apheresis will start. After apheresis, the feasibility of the 2nd apheresis will be evaluated by Investigator.

If the 2nd apheresis proves not to be feasible, the subject will receive the study drug EXG34217 (processed cells) on Day 6. If the 2nd apheresis is feasible, subject will be dosed with G-CSF and the 2nd apheresis will occur on Day 6. On Day 7, Subjects will receive oral or IV doses of acetaminophen (650 mg) and diphenhydramine (50 mg) and an IV dose of hydrocortisone (100 mg) prior to the study drug dosing. The route of administration for acetaminophen and diphenhydramine will be at the investigator's discretion. Subject will receive the study drug EXG34217 (processed cells).

Subjects will be monitored for safety up to 36 hours post-dosing and be discharged after Investigator has reviewed the safety lab results and determines discharge is appropriate.

Subjects will be evaluated for safety weekly for the first month after infusion and then assessed once monthly for 6 months. After 6 months, subjects will be evaluated at Month 9 and Month 12. Week 2, 3, 5 and Month 2, 4, 5, 9 visits will not require a clinical visit and can be done locally as an option via telemedicine with Investigator (with the assistance of the local healthcare provider or home health).

Detailed study procedures are in Section 6 and Section 10.

A 4-week staggering period between the first 3 subjects is planned. Specifically, 30 days must have elapsed from the infusion of the previous subject to the 1st G-CSF dosing of subsequent subject. After the first 3 subjects, no staggering period will be required.

Collected PBMNC will be processed to separate CD34+ cells and to incubate with EXG-001 for 24 hours in a closed automated system at CCHMC cell processing center.

An independent Data and Safety Monitoring Board (DSMB) will be appointed to have responsibility for safeguarding the interests of the trial subjects, and assessing the safety and tolerability of the study treatments during the trial (see detail in Section 12).

5 STUDY POPULATION

Adult males and females with telomere biology disorders with bone marrow failure.

5.1 Inclusion Criteria

1) Signed an Institutional Review Board (IRB) approved informed consent document indicating that they understand the purpose of, and procedures required by the study and are willing to participate in the study and comply with all study procedures and restrictions. Informed consent must be obtained from the subject and/or a designated representative prior to initiating screening procedures to evaluate eligibility of the study.
2) Age > 18 years.
3) Mild or moderate bone marrow failure defined by satisfying both conditions:
   - Peripheral blood neutrophils (ANC) < 1.5×10^9/L; or platelets < 100×10^9/L; or Hemoglobin <10 g/dL
   - Bone marrow hypocellular for age
4) Diagnosis of telomere biology disorders defined by one of the following:
   - age-adjusted mean telomere length < 1 percentile in all tested peripheral blood cells such as granulocytes, lymphocytes, B-cells, naïve T-cells, memory T-cells, and NK cells;
   - a pathogenic mutation in DKC1, TERC, TERT, NOP10, NHP2, TINF2, CTC1, PARN, RTEL1, ACD, USB1, or WRAP53

5.2 Exclusion Criteria

1) Women of childbearing potential not willing to following the birth control as described in the informed consent or breastfeeding.
2) Subjects with cancer who are on active chemotherapeutic treatment.
3) Severe bone marrow failure defined by one of the following:
   - peripheral blood neutrophils (ANC) < 0.5×10^9/L;
   - platelets < 20×10^9/L
4) Clonal cytogenetic abnormalities associated with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML) on bone marrow examination within the preceding 3 months.
5) Uncontrolled bacterial, viral or fungal infections.
6) Prior allogeneic marrow or stem cell transplantation.
7) Subjects who are not eligible for G-CSF and plerixafor dosing.
8) Subjects who are not eligible for the apheresis.
9) Subjects currently taking or have taken danazol and androgens within 60 days prior to Day 1.
10) Subjects with any other clinically relevant acute or chronic diseases which could interfere with the subjects' safety during the trial, expose them to undue risk, or which could interfere with study objectives.
11) Subjects who have participated in another clinical trial with an investigational drug within the previous 30 days.

5.3 Subject Identification

All subjects who undergo Screening will be assigned a unique screening identification number at the time of Screening. Only subjects continuing to meet entry inclusion/exclusion criteria and be randomized will be assigned a unique subject number.

5.4 Randomization and Blinding

This study is open label study, not blinded.

5.5 Prior, Concomitant, and Prohibited Medications

Subjects currently taking or have taken danazol and androgens within 60 days prior to Day 1 cannot be enrolled in this trial.

All rescue treatment such as transfusion, treatment with danazol and androgens determine necessary by Investigator's clinical judgement will be allowed.

6 PROCEDURE FOR MOBILIZATION, APHERESIS AND STUDY DRUG INFUSION

All eligible subjects will start the mobilization with G-CSF for 3 days in an outpatient setting and have the eligibility of the 1st apheresis assessed by PI's clinical judgement on Day 4. If eligible, the subject will be hospitalized and will be dosed with Plerixafor. On Day 5, subject will be dosed GCS-F and apheresis will start. After apheresis, the feasibility of the 2nd apheresis will be evaluated by Investigator.

If the 2nd apheresis proves not to be feasible, the subject will receive the study drug (processed cells) on Day 6. If the 2nd apheresis is feasible, subject will be dosed with G-CSF and the 2nd apheresis will occur on Day 6. Subject will receive the study drug (processed cells) on Day 7.

Subjects will be monitored for safety up to 36 hours post-dosing and be discharged after Investigator has reviewed the safety lab results and feels discharge is appropriate.

See detailed procedures for mobilization, apheresis and study drug infusion in Section 6.1.

See detailed algorism of procedure for mobilization, apheresis and study drug infusion in Figure 4.

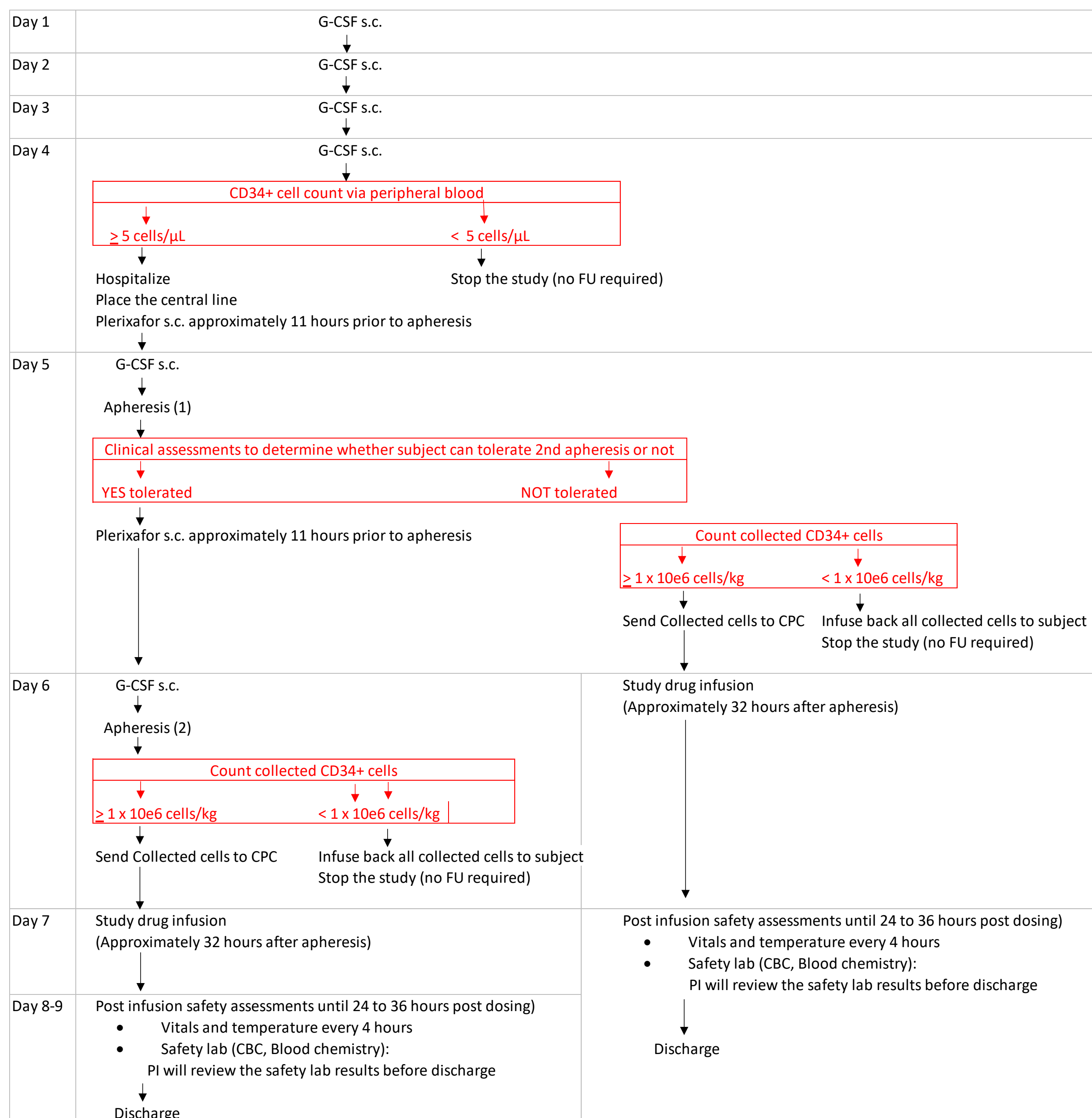

Figure 4. Study procedure algorithm

6.1 Detailed Procedure for Mobilization, Apheresis and Study Drug Infusion

Day 1 to 3

All eligible subjects will receive a daily G-CSF injection (10 µg/kg s.c.).

Day 4

After the G-CSF (10 µg/kg s.c.) injection, peripheral blood will be collected for CD34+ cell counts.

Subjects who have < 5 cells/µL of CD34+ cells in the peripheral blood will be withdrawn from the study. No follow-up will be required.

Subjects who have ≥ 5 cells/µL of CD34+ cells in the peripheral blood will continue on the study.

Subjects will be hospitalized, and access will be placed. Plerixafor (0.24 mg/kg s.c) will be administered in the evening, approximately 11 hours prior to apheresis.

Day 5

G-CSF (10 µg/kg s.c.) will be administered in the morning and apheresis initiated.

After apheresis, the Investigator will determine whether the subject is able to tolerate the 2nd apheresis or not by clinical assessments.

A. For any subjects judged to be unable to tolerate 2nd collection by the Investigator, no 2nd apheresis will be initiated.

The number of CD34+ cells in collected MNCs will be estimated.

- CD34+ cells $< 1.0 \times 10^6$ /kg: all collected MNCs will be infused back to the subject. Subjects will be withdrawn from study. No follow-up will be required.
- CD34+ cells $\geq 1.0 \times 10^6$ /kg: subjects will continue on the study.

Day 6-8

Subjects will receive oral or IV doses of acetaminophen (650 mg) and diphenhydramine (50 mg) and an IV dose of hydrocortisone (100 mg). The route of administration for acetaminophen and diphenhydramine will be at the investigator's discretion.

Subjects will receive the study drug (EXG34217, autologous CD34+ cells contacted *ex vivo* with EXG-001) on Day 6.

Subjects will be monitored for safety up to 36 hours post dosing. Subjects will be discharged on Day 7 or 8, after the Investigator has reviewed the safety lab results and determines discharge is appropriate.

B. *For any subject judged <u>to be able to</u> tolerate 2nd collection by the Investigator, the 2nd apheresis will be initiated.*

Post first apheresis, a CBC will be obtained, and the subject will be given RBC or platelet transfusion, if needed, to keep hemoglobin levels >10.5 g/dL and platelets >100K.

A bag containing mononuclear cells (MNCs) collected by the 1st apheresis will be kept overnight at controlled room temperature (+19 °C to +25 °C, [+67 °F to +77 °F]). The MNCs should not be kept longer than 24 hours before starting the labeling and selection procedure of CD34+ cells.

<u>Day 5 (continued)</u>

Plerixafor (0.24 mg/kg s.c) will be administered in the evening, approximately 11 hours prior to apheresis.

<u>Day 6</u>

G-CSF (10 μg/kg s.c.) will be administered in the morning and apheresis initiated.

The number of CD34+ cells in collected MNCs (total of 1st and 2nd apheresis) will be estimated.

- CD34+ cells $< 1.0 \times 10^6$ /kg: all collected MNCs will be infused back to the subject. Subjects will be withdrawn from study. No follow-up will be required.
- CD34+ cells $\geq 1.0 \times 10^6$ /kg: subjects will continue on the study.

Bags containing mononuclear cells (MNCs) collected by the 1st and 2nd apheresis will be transferred to CCHMC cell processing center.

<u>Day 7-9</u>

The study drug (EXG34217, autologous CD34+ cells contacted *ex vivo* with EXG-001) will be delivered to the inpatient unit for infusion.

Subjects will receive oral or IV doses of acetaminophen (650 mg) and diphenhydramine (50 mg) and an IV dose of hydrocortisone (100 mg). The route of administration for acetaminophen and diphenhydramine will be at the investigator's discretion.

Subjects will receive the study drug (EXG34217, autologous CD34+ cells contacted *ex vivo*

with EXG-001) on Day 7. See Section 6.2.

Subjects will be monitored for safety up to 36 hours post dosing. Subject will be discharged on Day 8 or 9, after Investigator has reviewed the safety lab results and determines discharge is appropriate.

6.2 Procedure of Study Drug (EXG34217) Administration

Subjects will receive oral or IV doses of acetaminophen (650 mg) and diphenhydramine (50 mg) and an IV dose of hydrocortisone (100 mg). The route of administration for acetaminophen and diphenhydramine will be at the investigator's discretion.

EXG34217 (autologous CD34+ cells contacted *ex vivo* with EXG-001) will be infused intravenously without filter from placed access at 3.3 mL/min infusion rate for 30 mins.

6.3 Re-infusion of Non-Cultured Collected Cells

If not enough CD34+ cells ($< 1.0 \times 10^6$ /kg) will be collected on Day 5 or Day 6, all collected cells will be reinfused back to the subject per institutional standard procedures.

7 STOPPING CRITERIA AND STUDY DURATION

7.1 During the Dosing Procedure

Before the infusion of EXG34217, Investigator will determine whether the subject is able to tolerate the infusion. If Investigator notes any AE which may jeopardize the subject's health in the investigator's judgment, then the subject will not receive the infusion of EXG34217, and the subject will be withdrawn from the study and will be replaced.

During the infusion of EXG34217, if Investigator notes any AE which may jeopardize the subject's health in the investigator's judgment, then the infusion will be stopped immediately. The follow-up of the subjects will continue.

7.2 Subject Stopping Rules

Subjects will be withdrawn from the study in the following circumstances:

- Subject wishes to withdraw;
- When the investigator judges that for any reason continuation of the study drug is inappropriate for the subject.

7.3 Study Stopping Rules

If any of the following events occur, the study will be suspended pending DSMB review:

- Any SAE associated with the study procedure in one subject.
- Allergic reaction, associated with infusion of EXG34217, Grade 3 in two subjects or Grade 4 in one subject.
- Grade 4 infection within 1-month post infusion of EXG34217 uncontrolled for >7 days in one subject.
- Any duration of grade 3 toxicity involving cardiac, pulmonary or neurologic systems.
- Any other grade 3 toxicity that lasts >72 hours.
- Any duration of grade 4 toxicity.
- Any death that occurs within 30 days of receiving study product.

7.4 Study Duration and Dates

The study will include a screening period of up to 30 days and a study period of 12 months.

8 LABELING, PACKAGING, STORAGE, DISPENSING, AND RETURN OF CLINICAL SUPPLIES

8.1 Product Description

EXG34217 is autologous CD34+ cells *ex vivo* contacted with EXG-001 suspended in 100 mL Plasma-Lyte A.

EXG-001 is a Sendai virus vector encoding for human ZSCAN4, manufactured at ID Pharma (Ibaragi, Japan).

8.2 Cell Processing

Cincinnati Children's Hospital Medical Center (CCHMC) Cell Processing Center (CPC) will manufacture EXG34217 from subjects' PBMNC cells collected by apheresis using a Miltenyi CliniMACS Prodigy instrument under GMP.

8.3 Treatment Administration

EXG34217 (autologous CD34+ cells contacted *ex vivo* with EXG-001) will be infused intravenously without filter from placed access at 3.3 mL/min infusion rate for 30 mins.

If CD34+ cells in the final product are $>8.0 \times 10^6$ cells /kg, only 8.0×10^6 cells/kg will be administered to subjects at the dosing in this study and the rest will be cryopreserved for the future research use.

Detailed instructions for dosing procedures are also provided in the Study Manual.

8.4 Primary Packaging and Labeling Information

EXG34217 are filled 100 mL in a CryoMACS Freezing Bag 250 (250 mL, Miltenyi Cat # 200-074-401).

Label contains manufacturer name, protocol number, product name, lot number, subject ID (with name) and DOB, cell numbers (cells/kg), total volume, expiration time, caution and instruction.

8.5 Storage Requirements

EXG34217 is fresh product. Study drug needs to be used before the time described in the label.

8.6 Instructions for Dispensing

Cell processing center will transfer the investigational product to clinic for infusion.

8.7 Assessment of Compliance

As the investigational product is administered in the clinic, compliance is not applicable. The start volume in the bag and the volume left after dosing should be recorded in the source document.

8.8 Drug Accountability/Return of Clinical Supplies

Under no circumstance will the Investigator(s) allow the study drug to be used other than as directed by this protocol. If the infusion is stopped mid-infusion and some product remains, the leftover product will be discarded per institutional practice and will be recorded accordingly.

9 STUDY PROCEDURES

9.1 Informed Consent

The informed consent form (ICF) must be executed prior to performing any study-related activities. The ICF must be approved by the reviewing Institutional Review Board (IRB). Informed consent will be obtained for all subjects participating in the study. Subjects may withdraw consent at any time.

Participation in the study may be terminated at any time without the subject's consent as determined by the Principal Investigator.

9.2 Inclusion/Exclusion Criteria

Eligibility screening of subjects will be completed prior to administration of the study drug and will be documented in the source documents. Confirmation of eligibility will be performed on Day 1 at the clinic to ensure no obvious changes in eligibility.

Screening failures and the reason for failure to meet the study eligibility requirements will be documented in the study site source documents.

9.3 Demographics

The following demographic parameters will be captured at screening: date of birth, sex, race and ethnicity.

9.4 Medical History

Relevant medical history, based on the opinion of the Investigator, will be obtained from the subject at Screening and recorded in the source documents. Medical history will capture the subject's health history, including history of hospitalization, and history of surgeries.

9.5 Baseline Disease Characteristics

The following baseline disease characteristics parameters will be captured at screening:
bone marrow cellularity and pathogenic mutation (if subject does not have genetic information it will be collected during the screening).

9.6 Body Weight

Body weight will be collected at screening, Day 1, prior to discharge, Month 1, Month 3, Month 6 and Month 12/Exit.

9.7 Physical Examination

A physical examination will be performed at the time of Screening, prior to discharge, weekly for the first month after infusion, and then once monthly for 6 months. After 6 months, subjects will be evaluated at Month 9 and Month 12/Exit. Weeks 2, 3, 5 and Month 2, 4, 5, 9 visits will not require a clinical visit and can be performed via telemedicine with Investigator (with the assistance of the local healthcare provider or home health) as an option. The findings of each

examination will be recorded on the source documents and clinically significant abnormalities will be recorded in the source documents. The physical examination will include:

- General appearance
- Head, eyes, ears, nose, and throat
- Respiratory
- Cardiovascular
- Musculoskeletal
- Abdomen
- Neurologic
- Extremities
- Dermatologic
- Lymphatic

For physical examinations, tattoos and piercings are not abnormal findings and are not required to be noted.

9.8 Vital Signs

Vital signs, including blood pressure, heart rate, respiratory rate and body temperature, will be measured through the trial, and those will be determined on Screening, after apheresis, day of dosing, prior to discharge, and weekly for the first month after infusion, and then once monthly for 6 months. After 6 months, subjects will be evaluated at Month 9 and Month 12/Exit. At inpatient unit after infusion of EXG34217, vital signs will be measured every 4 hours (per institution standard practice). After discharge, vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health) is being performed.

9.9 Electrocardiography

A standard digital 12-lead ECG will be recorded in singlicate at screening and at Month 1, Month 3, Month 6, and Month 12/Exit. Safety ECGs will be recorded and evaluated by a Cardiologist.

9.10 Clinical Laboratory Tests

Laboratory testing (hematology with differential, serum chemistry and urinalysis) will be performed using standard methods. Blood samples for the serum chemistry and hematology and coagulation listed below will be collected at Screening, prior to discharge, and weekly for the first month after infusion, and then once monthly for 6 months. After 6 months, subjects will be evaluated at Month 9 and Month 12/Exit. Week 2, 3, 5 and Month 2, 4, 5, 9 visits will not require a clinical visit and can be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the Investigator for assessment.

Hematology:	Hematocrit, Hemoglobin, Platelet count, Red blood cell (RBC) count, Reticulocyte count, White blood cell (WBC) count, WBC differentials, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), MCH concentration (MCHC)

Blood Chemistry:	Creatinine, Calculated Creatinine Clearance, blood urea nitrogen (BUN), Potassium (K+), Sodium (Na+), Chloride (Cl-), Magnesium (Mg++), Calcium, phosphate, Glucose, Urea, Bilirubin (Total), Bilirubin (direct), aspartate aminotransferase (AST), alanine transaminase (ALT), Alkaline phosphatase, Total Protein, Albumin
Coagulation:	Activation partial thromboplastin time (APTT), Prothrombin time/International normalized ratio (PT/INR), thrombin time (TT)
Urinalysis	Macroscopic examination routinely including specific gravity, pH, protein, glucose, ketones, blood and urobilinogen. A microscopic examination will be performed if warranted based on macroscopic results.

For all females, a serum pregnancy test will be performed at Screening, and at the Month 12/Exit Visit.

Urinalysis will be performed at Screening, and at the Month 12/Exit Visit.

Detailed instructions for laboratory sample collection, processing, and handling are provided in the Laboratory Manual.

9.11 CD34+ Cell Counts

On Day 4, CD34+ cell will be counted by peripheral blood samples. Detailed instructions for sample collection, processing, and handling are provided in the Laboratory Manual.

In addition, after each apheresis, collected CD34+ cells will be counted.

9.12 Telomere Length

Blood sample for telomere length assessment will be collected at Screening, Month 1, Month 3 Month 6, and Month 12/Exit.

Collected samples will be shipped to RepeatDx (North Vancouver, Canada) to measure telomere length by Flow FISH. Detailed instructions for sample collection, processing, and handling are provided in the Laboratory Manual.

9.13 Immunogenicity

Blood sample for Antibody against Sendai virus vector and hZSCAN4 protein will be collected at Screening, at Day 1, prior to discharge, Month 1, Month 3, Month 6 and Month 12/Exit.

Detailed instructions for sample collection, processing, and handling are provided in the Laboratory Manual.

9.14 Cytokine Panel

Blood sample for Plasma Cytokine Panel (GM-CSF, IFNg, IL-1 beta, IL-10, IL-2, IL-4, IL-5, IL-6, IL-8, TNF-α) will be collected at screening, prior to discharge, Month 1, Month 3, Month 6, and Month 12/Exit.

Detailed instructions for sample collection, processing, and handling are provided in the Laboratory Manual.

9.15 Concomitant Medication Assessments

The Investigator or designated qualified personnel will assess and record concomitant medication usage on the source documents at Screening and throughout the trial. Specific information regarding concomitant medication and prior therapy usage is provided in Section 5.5.

9.16 AE Assessments

9.16.1 Performing AE Assessments

The Investigator is responsible for promptly documenting and reporting all AEs observed during the study in the subject's source documents. If an AE is classified as "serious" as described in Section 9.16.6.1, it must be reported to Elixirgen Therapeutics or its designee no later than 24 hours after the Investigator recognizes/classifies the event as an SAE.

AEs will be collected from the time of the administration of G-CSF (Day 1) to the time of the Study Exit Visit or early termination from the study. Any events reported before administration of the study drug will be recorded as Medical History. For ongoing AEs at the time of the Exit Visit or early termination, additional data, such as AE resolution date, will be collected and reported to Elixirgen Therapeutics.

AEs will be classified according to the Common Terminology Criteria for Adverse Events (CTCAE), the most recent version available.

9.16.2 AE Definitions

The following definitions of terms are guided by the International Conference on Harmonisation (ICH) and the U.S. CFR [21 CFR 312.32] and are included herein.

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

AEs include, but are not limited to:

- Any symptom or condition not previously reported by the subject (medical history).
- An exacerbation of a pre-existing symptom or condition.
- A significant increase in frequency or intensity of a pre-existing episodic event or condition.
- A drug interaction.
- A condition first detected or diagnosed after study drug administration even though it

may have been present prior to the start of the study.

An AE does not include:

- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, blood transfusion); the condition that led to the procedure is an AE (e.g., bleeding esophageal varices, dental caries).
- Overdose of either study drug or concurrent medication without any clinical signs or symptoms.
- Non-clinically significant abnormal laboratory values. (If accompanied by signs/symptoms, the signs or symptoms are considered an AE).

AEs that occur between the time the subject signs the ICF for the study and the time when that subject is randomized will be summarized as medical history and not as study AEs unless the event meets the definition of a SAE as defined in Section 9.16.6.1.

9.16.3 Severity

The Investigator or designee will be asked to assess the severity of the AE using the CTCAE V4.03. These criteria assign a grade of 1 through 5 to indicate the severity of AEs. For AEs that are not listed in these criteria, the Investigator or designee will use medical judgment to assess the severity of the AE.

A general guideline to these grades of severity, taken from CTCAE V4.03, is:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (see CTCAE for more details).
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization of prolongation of hospitalization indicated; disabling; limiting self-care Activities of Daily Living.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

An AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as ‘serious’ when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

9.16.4 Relationship

The relationship of each AE to the study drug administration will be assessed by the Investigator after careful consideration, and according to the following guidelines:

Definitely Related:	There is clear evidence that the event is related to the use of the investigational product. An AE that follows a reasonable temporal sequence from administration of a drug (including the course after withdrawal of the drug); that follows a known or expected response pattern to the investigational product.
Reasonably or Possibly Related:	A reaction that follows a reasonable temporal sequence from administration of investigational product; that follows a known or expected response pattern to the investigational product; and/or that could not be reasonably explained by other factors such as underlying disease, complications, concomitant drugs, or concurrent treatments
Unlikely Related:	The temporal sequence from administration of the investigational product suggests that the relationship is unlikely; the response pattern is unlike that of the investigational product (if response pattern is previously known); could be reasonably explained by the subject’s clinical state
Not Related:	An AE that does not follow a reasonable temporal sequence from administration of a drug; for which sufficient data exist to indicate that the etiology is unrelated to the investigational product; and/or that can reasonably be explained by other factors, such as underlying diseases, complications, concomitant drugs, and concurrent treatments.

The investigator or designee may change his/her opinion of causality in light of follow-up information, amending the SAE data collection form accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

The Investigator or designee will also record the following for AEs:

- Duration: start and end date and time or continuing
- Action taken, if applicable
- Whether it is an SAE
- Outcome: resolved, continuing, death or unknown

9.16.5 Clinical Laboratory AEs

Many laboratory abnormalities observed during the course of a study will be included under a reported AE describing a clinical syndrome (e.g., elevated BUN and creatinine in the setting of an AE of renal failure, or decreased hemoglobin in a case of bleeding esophageal varices). In such cases, the laboratory abnormality itself (e.g., elevated creatinine in a setting of renal failure) does not need to be recorded as an AE. However, isolated laboratory abnormalities should be reported as AEs if they are considered to be clinically significant by the Investigator.

Criteria for a “clinically significant” laboratory abnormality are:

- A laboratory abnormality that leads to a dose-limiting toxicity (DLT) (e.g., an abnormality that results in study drug dose reduction, suspension or discontinuation).
- A laboratory abnormality that results in any therapeutic intervention (i.e., concomitant medication or therapy).
- Any other laboratory abnormality judged by the Investigator to be of any particular clinical concern (e.g., significant fall in hemoglobin not requiring transfusion).

For laboratory abnormalities that do not meet the above criteria but are outside of normal range (e.g., < or > normal reference range), the Investigator should indicate whether the value is clinically significant or not clinically significant for the subject.

9.16.6 SAEs

9.16.6.1 Definition

An SAE is defined by federal regulation as any AE occurring at any dose that results in any of the following outcomes:

- Death
- Life-threatening AE
- Hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the

outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Hospitalization for a pre-existing condition, including elective procedures, which has not worsened, does not constitute an SAE.

An AE is considered "life-threatening' if, in the view of the Investigator or Sponsor, its occurrence places the subject or subject at immediate risk of death. It does not include an adverse reaction or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

9.16.6.2 Reporting SAEs

In agreeing to the provisions of this protocol, the Investigator accepts all legal responsibilities for AE identification, documentation, grading, assignment of causality, and prompt notification of SAEs to Elixirgen Therapeutics or designee. All SAEs must be reported to Elixirgen Therapeutics no later than 24 hours after the Investigator recognizes/classifies the event as a SAE. At a minimum, a description of the event and the Investigator's judgment of causality must be provided at the time of the initial report using the appropriate form (e.g., SAE Report Form). After the initial report, as necessary, the Investigator must provide any additional information on a SAE to the Medical Monitor within two working days after he/she receives that information. This follow-up information will be a detailed written report that will include copies of hospital records, case reports, and autopsy reports, and other pertinent documents.

This verbal and faxed report must be followed no later than three working days by a written report **signed by the Investigator**. The information in both the initial report and follow-up report(s) will also be captured. The Sponsor is responsible for submitting the report to all applicable regulatory authorities.

Contact: Kathleen Clarence-Smith, MD, PhD
KM Pharmaceutical Consulting, LLC
1825 K Street NW, Suite 520
Washington, DC 20006
Office phone: 202-223-7001
Cell phone: 202-550-1133
Office FAX: 202-223-7004
Email address: kcsmith@kmphc.com

9.16.6.3 Supplemental Investigation of SAEs

The Investigator and supporting personnel responsible for subject care should discuss with the Medical Monitor any need for supplemental investigations of SAEs. The results of these additional assessments conducted must be reported to Elixirgen Therapeutics. If a subject expires during participation in the study and a post-mortem examination is performed, a copy of the autopsy report must be submitted to Elixirgen Therapeutics.

9.16.6.4 Post Study Follow-Up of AEs

All AEs, including a worsening of clinically significant laboratory values or physical examination findings compared with baseline values, must be followed until the event resolves, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up.

AEs ongoing at the Exit Visit will be followed for as long as necessary to adequately evaluate the subject's safety or until the event stabilizes or resolves. If resolved, a resolution date should be reported to Elixirgen Therapeutics. The Investigator is responsible to ensure that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals as is practical.

9.16.6.5 Notification of Post Study SAEs

Investigators are not obligated to actively follow subjects after the completion of the study. However, if the Investigator becomes aware of a post-study SAEs occurring up to 14 days following the last visit, it must be reported to Elixirgen Therapeutics, whether or not the event is attributable to study drug. All SAEs must be reported to Elixirgen Therapeutics no later than 24 hours after the Investigator recognizes/classifies the event as an SAE.

9.16.6.6 IRB Notification of SAEs

The Investigator is responsible for promptly notifying his IRB of all SAEs (as per institutional guidelines, including any follow- up information, occurring at his site and any SAE regulatory report, including any follow-up reports that he receives from Elixirgen Therapeutics. Documentation of the submission to the IRB must be retained for each safety report. The Investigator is also responsible for notifying Elixirgen Therapeutics if their IRB requires revisions to the ICF or other measures based on its review of an SAE report.

9.16.6.7 Health Authority Safety Reports

Elixirgen Therapeutics or its representatives will submit a safety report to the Food and Drug Administration (FDA) and/or any other appropriate regulatory agencies for any suspected adverse reaction that is both serious and unexpected within the appropriate time frame.

Elixirgen Therapeutics or its representatives will send copies of each safety report submitted to the FDA and/or other regulatory agencies to the Investigators who are actively participating in Elixirgen Therapeutics-sponsored clinical studies. Safety reports must be submitted to the appropriate IRB as soon as possible. Documentation of the submission to the IRB must be retained for each safety report.

9.16.7 Pregnancy

Any pregnancy that occurs from Screening until study completion must be reported to Elixirgen Therapeutics.

To ensure subject safety, each pregnancy must be reported to Elixirgen Therapeutics within 2

weeks of learning of its occurrence. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child.

9.16.8 Treatment-Emergent AEs

All AEs that occur at the time of and following the administration of study drug through the Final Follow-up visit will be considered as being treatment-emergent AEs.

10 STUDY ACTIVITIES

10.1 Screening

The following procedures and assessments will be performed during screening. Results will be documented in the source documents:

- Informed consent
- Demographics
- Medical history
- Baseline disease characteristics*
- Review and documentation of prior medications
- Physical examination
- Height and weight
- Vital signs
- Blood collection for safety labs (hematology, blood chemistry, and coagulation), cytokine panel and telomere length
- Urine collection for urinalysis
- Pregnancy test for females of childbearing potential
- 12-lead ECG
- Eligibility assessments

*: pathogenic mutation information needs to be collected during the screening if subject does not have genetic information on file

10.2 Day 1

Subjects meeting eligibility criteria will be administrated G-CSF dosing for mobilization.

The following procedures and assessments will be performed:

- Review eligibility and confirm if there is no change by the Investigator
- Blood collection for immunogenicity
- Weight
- G-CSF dosing
- Review and documentation of concomitant medications
- Review and documentation of AEs

10.3 Day 2

The following procedures and assessments will be performed. Results will be documented in the source documents:

- G-CSF dosing
- Review and documentation of concomitant medications
- Review and documentation of AEs

10.4 Day 3

The following procedures and assessments will be performed. Results will be documented in the source documents:

- G-CSF dosing
- Review and documentation of concomitant medications
- Review and documentation of AEs

10.5 Day 4

The following procedures and assessments will be performed. Results will be documented in the source documents:

- G-CSF dosing
- Plerixafor dosing
- Review and documentation of concomitant medications
- Review and documentation of AEs

After G-CSF injection, peripheral blood will be collected for CD34+ cell counts.

Subjects who have < 5 cells/µL of CD34+ cells will be withdrawn from the study. No follow-up will be required.

Subjects who have ≥ 5 cells/µL of CD34+ cells will continue on the study.

Subjects will be hospitalized, and access will be placed. Plerixafor will be administered in the evening, approximately 11 hours prior to apheresis.

10.6 Day 5: Apheresis (1)

The following procedures and assessments will be performed. Results will be documented in the source documents:

- G-CSF dosing
- Vital signs
- Apheresis to collect mononuclear cells
- Review and documentation of concomitant medications
- Review and documentation of AEs

After apheresis, Investigator will determine whether subject is able to tolerate the 2nd apheresis or not by clinical assessments.

For subjects who are judged to be intolerable by the Investigator, no 2nd apheresis will be initiated.

The collected CD34+ cells will be counted.

CD34+ cells < 1.0×10^6 /kg: all collected cells will be infused back to the subject. Subjects will

be withdrawn from study. No follow-up will be required.

CD34+ cells $\geq 1.0 \times 10^6$ /kg: subjects will continue on the study.

For any subject judged to be tolerable by the Investigator, the 2nd apheresis will be initiated. Plerixafor will be administered in the evening, approximately 11 hours prior to apheresis.

10.7 Day 6: Apheresis (2)

The following procedures and assessments will be performed. Results will be documented in the source documents:

- G-CSF dosing
- Vital signs
- Apheresis to collect mononuclear cells
- Review and documentation of concomitant medications
- Review and documentation of AEs

G-CSF will be administered in the morning and apheresis initiated.
After apheresis, the collected CD34+ cells (total of 1st and 2nd apheresis) will be counted.

CD34+ cells $< 1.0 \times 10^6$ /kg: all collected cells will be infused back to the subject. Subject will be withdrawn from study. No follow-up required.

CD34+ cells $\geq 1.0 \times 10^6$ /kg: subject will continue the study.

10.8 Day 7: Study Drug Administration

The following procedures and assessments will be performed. Results will be documented in the source documents:

- Subjects will receive oral or IV doses of acetaminophen (650 mg) and diphenhydramine (50 mg) and an IV dose of hydrocortisone (100 mg). The route of administration for acetaminophen and diphenhydramine will be at the investigator's discretion.
- Study Drug (EXG34217) Administration
- Vital signs every 4 hours (per institutional standard)
- Review and documentation of concomitant medications
- Review and documentation of AEs

10.9 Day 7-9: Post Dose Safety Monitoring

Subjects will be monitored for safety up to 36 hours post dosing. Subject will be discharged on Day 8 or 9, after Investigator has reviewed the safety lab results.

The following procedures and assessments will be performed. Results will be documented in the source documents:

- Vital signs
- Weight

- Blood collection for safety lab, cytokine panel and immunogenicity.
- Review and documentation of concomitant medications
- Review and documentation of AEs

10.10 Week 2

At Week 2, safety labs, vital signs measurement, and physical examination will be performed. This will not require a clinic visit and can be done at locally, as an option. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health). Vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health) is being performed.

10.11 Week 3

At Week 3, safety labs, vital signs measurement and physical examination will be performed. This will not require a clinic visit and can be done at locally, as an option. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health). Vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health) is being performed.

10.12 Week 4/Month 1

The following procedures and assessments will be performed. Results will be documented in the source documents:

- Physical Examination
- Vital signs
- Weight
- 12-lead ECG
- Blood collection for safety lab, cytokine panel, immunogenicity, and telomere length.
- Review and documentation of concomitant medications
- Review and documentation of AEs

In-between clinical visits, site personnel will contact to the subject at least once a month to ask the condition and AEs via phone.

10.13 Week 5

At Week 5, safety labs, vital signs measurement, and physical examination will be performed. This will not require a clinic visit and can be done at locally, as an option. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will

then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health). Vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health) is being performed.

10.14 Month 2

At Month 2, safety labs, vital signs measurement, and physical examination will be performed. This will not require a clinic visit and can be done at locally, as an option. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health). Vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health) is being performed.

10.15 Month 3

The following procedures and assessments will be performed. Results will be documented in the source documents:

- Physical Examination
- Vital signs
- Weight
- 12-lead ECG
- Blood collection for safety lab, cytokine panel, immunogenicity, and telomere length.
- Review and documentation of concomitant medications
- Review and documentation of AEs

In-between clinical visits, site personnel will contact to the subject at least once a month to ask the condition and AEs via phone.

10.16 Month 4

At Month 4, safety labs, vital signs measurement, and physical examination will be performed. This will not require a clinic visit and can be done at locally, as an option. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health). Vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health) is being performed.

10.17 Month 5

At Month 5, safety labs, vital signs measurement, and physical examination will be performed. This will not require a clinic visit and can be done at locally, as an option. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will

then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health). Vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health) is being performed.

10.18 Month 6

The following procedures and assessments will be performed. Results will be documented in the source documents:

- Physical Examination
- Vital signs
- Weight
- 12-lead ECG
- Blood collection for safety lab, cytokine panel, immunogenicity, and telomere length.
- Review and documentation of concomitant medications
- Review and documentation of AEs

10.19 Month 9

At Month 9, safety labs, vital signs measurement, and physical examination will be performed. This will not require a clinic visit and can be done at locally, as an option. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health). Vital sign measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health) is being performed.

10.20 Month 12/Exit Visit

The following procedures and assessments will be performed. Results will be documented in the source documents:

- Physical Examination
- Vital signs
- Weight
- 12-lead ECG
- Blood collection for safety lab, cytokine panel, pregnancy test (if applicable), immunogenicity, and telomere length.
- Urine collection for Urinalysis
- Review and documentation of concomitant medications
- Review and documentation of AEs

11 DATA SAFETY MONITORING BOARD (DSMB)

The DSMB is the primary data safety and advisory group for Sponsor. The membership includes two clinicians who is specialized in this disease population and the medical monitor.

The DSMB will review the data on the following schedule (minimal every 6 months when there are active subjects):

- The first 6 subjects completed 1-month assessments
- The first 6 subjects completed 6-month assessments
- All 12 subjects completed 1-month assessments
- If any of the following events occur:
  - Any study drug-related SAE in one subject,
  - Allergic reaction, associated with infusion of EXG34217, Grade 3 in two subjects or Grade 4 in one subject,
  - Grade 4 infection following infusion of EXG34217 uncontrolled for >7 days in one subject,
  - Any duration of grade 3 toxicity involving cardiac, pulmonary or neurologic systems,
  - Any other grade 3 toxicity that lasts >72 hours,
  - Any duration of grade 4 toxicity
- Any death that occurs within 30 days of receiving study product, or when determined to be necessary by the DSMB or the Sponsor

The DSMB will specifically review:

- All safety data include AEs, vital signs, weight, standard 12-lead ECG, clinical laboratory test (hematology, blood chemistry, and urinalysis), plasma cytokine panel, immunogenicity,
- Engraftment data by telomere length data

The DSMB will recommend one of the following actions to the Sponsor based on their data review:

a) Discontinue the study (with provisions for orderly discontinuation in accord with good medical practice).
b) Modifications to the study protocol. Modifications may include, but are not limited to, changes in inclusion/exclusion criteria, frequency of visits for safety monitoring, alterations in study procedures, changes in duration of observation and follow up.
c) Continue the study according to the protocol and any related amendments.

In addition to above, the DSMB will review the engraftment data with safety data after the first 3 patients and will discuss the futility analysis.

A detailed DSMB charter will be finalized prior to the initiation of the study.

12 PLANNED STATISTICAL METHODS

12.1 General Considerations

All collected study data will be presented in subject data listings. Statistical analyses will be performed using SAS® for Windows, version 9.4 or later.

A detailed Statistical Analysis Plan (SAP) will be finalized prior to database lock.

12.2 Determination of Sample Size

Since this study is an initial Phase 1/2 evaluation intended to be hypothesis-raising rather than hypothesis-testing, it is not powered to reliably yield statistically significant conclusions. The data will be analyzed only by means of descriptive statistics (including means and standard deviations, medians and inter-quartile ranges, and tabulations as appropriate) to reveal possible trends that will help guide the design of future, more definitive, clinical trials.

12.3 Analysis Populations

Two subject populations will be evaluated during this study and are defined as follows:

- **Safety Population**: All subjects who receive any study drug.
- **Efficacy Population:** All subjects who receive at least one dose of any study drug and have at least one follow-up efficacy assessment
- **Feasibility Population:** All subjects who enrolled (including subjects who do not received any study drug)

12.4 Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized using appropriate descriptive statistics.

12.5 Safety Analysis

The safety and tolerability of the study drugs will be assessed from;

- Vital signs,
- Weight,
- Standard 12-lead ECG,
- Clinical laboratory test (hematology, blood chemistry, and urinalysis),
- Plasma cytokine panel,
- Physical examination,
- AEs and SAEs,
- Immunogenicity of Sendai virus vector and hZSCAN4 protein

12.6 Exploratory Efficacy Analysis

The exploratory activities of the study drugs will be assessed from;

- Change of telomere length from baseline and in any of the following: lymphocytes, granulocytes, B-cells, naïve T-cells, memory T-cells, and NK cells

- Improvement of blood counts (neutrophils, platelets, or hemoglobin)

Prior to database lock, the methodology that will be used for the efficacy evaluations will be outlined in the final SAP.

12.7 Interim Analysis

No interim analysis is planned for this study.

13 ADMINISTRATIVE CONSIDERATIONS

13.1 Investigators and Study Administrative Structure

The study administrative structure is provided in Table 3.

Table 3. Study Administrative Structure

Sponsor Contact:	Elixirgen Therapeutics, Inc. 855 N. Wolfe Street, Suite 619 Baltimore, MD 21205
Sponsor Representative:	KM Pharmaceutical Consulting LLC 1825 K Street NW, Suite 520 Washington, DC 20006 Phone: 202-223-7001 Martine Francis: Cell 301-343-8894 Minako Koga: Cell 202-615-6004
Medical Monitor:	Kathleen Clarence-Smith, MD, PhD KM Pharmaceutical Consulting, LLC 1825 K Street NW, Suite 520 Washington, DC 20006 Office: 202-223-7001 Cell: 202-550-1133
Study Monitoring:	KM Pharmaceutical Consulting LLC 1825 K Street NW, Suite 520 Washington, DC 20006 Phone: 202-223-7001 Martine Francis: Cell 301-343-8894 Minako Koga: Cell 202-615-6004
Data Management & Statistical Analysis	Amarex Clinical Research 20201 Century Boulevard Germantown, MD 20874
Safety Clinical Laboratory Testing	Cincinnati Children's Hospital Medical Center Core Laboratories 3333 Burnet Avenue, MLC 7040 Cincinnati, OH 45229
Cell Processing Center	Cincinnati Children's Hospital Medical Center 3333 Burnet Avenue Cincinnati, OH 45229

13.2 Regulatory Authority Approval

Elixirgen Therapeutics will obtain approval to conduct the study from the appropriate regulatory agency in accordance with any applicable US regulatory requirements prior to a US site initiating the study.

13.3 Ethical Conduct of the Study and IRB Approval

The study will be conducted in accordance with Good Clinical Practice (GCP). These standards respect the following guidelines:

- Guideline for GCP E6(R2): Consolidated Guideline (International Conference on

Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use, November 2016).
- US CFR dealing with clinical studies (21 CFR parts 50, 54, 56, and 312).
- Declaration of Helsinki, concerning medical research in humans (Ethical Principles for Medical Research Involving Human Subjects) http://www.wma.net/en/10home/index.html.
- Any additional regulatory requirements.

The Investigator (or Elixirgen Therapeutics, where applicable) is responsible for ensuring that this protocol, the site's ICF, and any other information that will be presented to potential subjects (e.g., advertisements or information that supports or supplements the ICF) are reviewed and approved by the appropriate IRB. The Investigator agrees to allow the IRB direct access to all relevant documents. The IRB must be constituted in accordance with all applicable regulatory requirements.

Elixirgen Therapeutics will provide the Investigator with relevant document(s)/data that are needed for IRB review and approval of the study. If the protocol, the ICF, or any other information that the IRB has approved for presentation to potential subjects is amended during the study, the Investigator is responsible for ensuring the IRB reviews and approves these amended documents in accordance with regulatory requirements. The Investigator must follow all applicable regulatory requirements pertaining to the use of an amended ICF including obtaining IRB approval of the amended ICF before any new subject consents to take part in the study under the amended ICF. The IRB approval of the amended ICF/other information and the approved amended ICF/other information must be forwarded to Elixirgen Therapeutics promptly.

13.4 Subject Information and Consent

The study will be conducted in accordance with applicable subject privacy requirements. The proposed ICF, which must be in compliance with applicable regulations, must be reviewed and approved by the IRB and Elixirgen Therapeutics prior to initiation of the study.

The Investigator will be responsible for obtaining written informed consent from potential subjects prior to any study-specific screening for the study. A copy of the signed ICF will be provided to the subject. The original will be retained by the Investigator.

13.5 Confidentiality

13.5.1 Confidentiality of Data

By signing this protocol, the Investigator affirms to Elixirgen Therapeutics that information furnished to the Investigator by Elixirgen Therapeutics will be maintained in confidence and such information will be divulged to the IRB, or similar or expert committee, affiliated institution, and/or employees only under an appropriate understanding of confidentiality with such board or committee, affiliated institution, and/or employees. Data generated by this study will be considered confidential by the Investigator, except to the extent that it is included in a publication.

13.5.2 Confidentiality of Subject Records

By signing this protocol, the Investigator agrees that Elixirgen Therapeutics (or representative), IRB, or Regulatory Agency representatives may consult and/or copy study documents in order to verify worksheet/case report form data. By signing the consent form, the subject agrees to this process. If study documents will be photocopied during the process of verifying worksheet/case report form information, the subject will be identified by unique code only; full names/initials will be masked prior to transmission to Elixirgen Therapeutics. In addition, the Investigator agrees to treat all subject data used and disclosed in connection with this study in accordance with all applicable privacy laws (i.e., Health Insurance Portability and Accountability Act), rules and regulations.

13.6 Quality Control and Assurance

Elixirgen Therapeutics is responsible for implementing and maintaining quality control and quality assurance systems with written SOPs to ensure that trials are conducted, and data are generated, documented, and reported in compliance with the protocol, accepted standards of GCP, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.

13.7 Data Management

Data management procedures and information for this protocol will be provided by a vender selected by the Sponsor.

13.8 Study Monitoring

In accordance with applicable regulations, GCP, and Elixirgen Therapeutics procedures, clinical monitors will contact the site prior to subject enrollment to review the protocol and data collection procedures with site staff. In addition, the monitor will periodically contact the site, including conducting on-site visits. The extent, nature, and frequency of on-site visits will be based on such considerations as the study objective and/or endpoints, the purpose of the study, study design complexity, and enrollment rate.

During these contacts, the monitor will:

- Check the progress of the study.
- Review study data collected.
- Conduct source document verification.
- Identify any issues and address their resolution.

This will be done in order to verify that the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol (and any amendments), GCP, and all applicable regulatory requirements.

The Investigator agrees to allow the monitor direct access to all relevant documents and to

allocate his time and the time of his staff to the monitor to discuss findings and any relevant concerns. Upon completion of the study, the monitor will conduct the following activities in conjunction with the Investigator or site staff, as appropriate:

Return of all study data to Elixirgen Therapeutics.

- Data queries.
- Accountability, reconciliation, and arrangements for unused investigational product(s).
- Review of site study records for completeness.

After the final review of the study files, the files should be secured for the appropriate time period as specified in Section 13.9.

13.9 Retention of Data

Documents that individually and collectively permit evaluation of the conduct of the study and the quality of the data produced must be maintained for review by Elixirgen Therapeutics Quality Assurance auditors and by all applicable regulatory authorities. The period of time these documents must be maintained is governed by applicable regulations. Elixirgen Therapeutics or its designee will inform the Investigator when these documents may be destroyed. Elixirgen Therapeutics or its designee must be notified in writing **at least 6 months prior** to the intended date of disposal of any study record related to this protocol to allow Elixirgen Therapeutics to make alternate storage arrangements.

13.10 Financial Disclosure

The Principal Investigator or sub-Investigators named on the Form FDA 1572 will need to complete a financial disclosure form prior to study initiation, at any time during the study execution if new information needs to be disclosed, and for one year after study completion. Investigators should make the IRB aware of any financial interests that the Investigator has in the investigational product.

14 REFERENCES

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CLINICAL TRIAL PROTOCOL

Study Title:	A Phase I/II Study to Evaluate the Safety and Tolerability of EXG34217 in Patients with Telomere Biology Disorders with Bone Marrow Failure
Study Number:	EXG-US-01
Study Phase:	Phase 1/2
Name of Investigational Product:	EXG34217
Name of Active Ingredient:	Autologous CD34+ cells contacted *ex vivo* with EXG-001 (Sendai virus vector encoding for human ZSCAN4)
IND Number:	19748
Sponsor:	Elixirgen Therapeutics, Inc.
Medical Monitor:	Kathleen Clarence-Smith, MD, PhD KM Pharmaceutical Consulting, LLC 1825 K Street NW, Suite 520 Washington, DC 20006 Cell: 202-550-1133

	Version	Date
Original Protocol:	1.0	March 30, 2020
Amendment 1	2.0	May 26, 2020
Amendment 2	2.1	June 11, 2020
Amendment 3	2.2	September 4, 2020
Amendment 4	3.0	July 12, 2021
Amendment 5	4.0	September 27, 2022
Amendment 6	5.0	March 13, 2023
Amendment 7	6.0	January 23, 2024
Amendment 8	7.0	April 3, 2024

Confidentiality Statement

Property of Elixirgen Therapeutics, Inc.

This document is confidential and may not be used, divulged, published or otherwise disclosed without consent of Elixirgen Therapeutics, Inc.

SYNOPSIS

Name of Sponsor/Company: Elixirgen Therapeutics, Inc.

Name of Test Product: EXG34217

Name of Active Ingredients: Autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]

Title of Study: A Phase I/II Study to Evaluate the Safety and Tolerability of EXG34217 in Patients with Telomere Biology Disorders with Bone Marrow Failure

Study Center(s): Single site

Principal Investigator:
Kasiani Myers, MD (Cincinnati Children's Hospital Medical Center [CCHMC], OH)

Studied Period (years):	**Phase of Development:**
Estimated date first subject enrolled: June 2020 Estimated date last subject completed: TBD	1/2

Objectives:

Primary Objective:

- To assess the safety and tolerability of EXG34217 (autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]) in subjects with telomere biology disorders with bone marrow failure.

Secondary objectives:

- To assess the feasibility of *ex vivo* transduction and reinfusion of EXG34217 (autologous ZSCAN4 transduced CD34+ cells).
- To assess the feasibility of telomeres extension assessment.
- To assess clinical benefit by measuring complete blood count over time.

Study Design:

This is a Phase I/II, open label study in up to 12 subjects with telomere biology disorders with bone marrow failure. The study is open to all participants regardless of gender or ethnicity. Subjects who are enrolled but not evaluable will be replaced. Subjects will sign a consent form prior to any study related procedure and will complete baseline screening assessments. Subjects for this study will not require any preparative regimen such as chemotherapy or radiation.

The study will be conducted in four parts

1. Peripheral blood mononuclear cell (PBMNC) collection; mobilization and apheresis,
2. *Ex vivo* cell processing,
3. Processed cell infusion and post-infusion safety monitoring,
4. Follow-up (Weeks 2-5, Months 2, 3, 4, 5, 6, 9, and 12).

This is a brief description of the procedures. For full details on mobilization, apheresis, and dosing, see Section 6.)

All eligible subjects will start mobilization (with granulocyte colony stimulating factor [G-CSF] and Plerixafor). If eligible, subjects will undergo apheresis. Subjects may attempt to undergo apheresis 1-3 times. Subjects who successfully complete apheresis and continue to qualify will receive the study drug (processed cells) after apheresis.

Name of Sponsor/Company: Elixirgen Therapeutics, Inc.
Name of Test Product: EXG34217
Name of Active Ingredients: Autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]
Subjects will be monitored for safety up to 36 hours post dosing and be discharged after the Investigator has reviewed the safety lab results and determines discharge is appropriate. Subjects will be evaluated for safety weekly for the first month after infusion and then assessed once monthly for 6 months. After 6 months, subjects will be evaluated at Month 9 and Month 12. Week 4, Months 3, 6, and 12 will require a clinic visit at CCHMC. Weeks 2, 3, and 5 and Months 2, 4, 5, and 9 visits will not require a clinical visit and can be done locally via telemedicine with the Investigator (with the assistance of the local healthcare provider or home health nurse). Detailed study procedures are in Section 6 and Section 10. Any subject who does not have apheresis is eligible to re-enroll/re-attempt achieving adequate CD34+ cell (>5 cells/ µL) as long as the PI feels it is safe and in the best interest of the subject. On a case-by-case basis, for subjects who may experience difficulties traveling back to CCHMC, allowances to have assessments done by their local treating physician will be considered. There will be extensive and close communication between the Investigator and the local treating physician. For adult subjects (Age ≥ 18 years) A 4-week staggering period between the first 3 subjects in this population is planned. Specifically, 30 days must have elapsed from the infusion of the previous subject to the 1st G-CSF dosing of subsequent subject. After the first 3 subjects of this population, no staggering period will be required. For adolescent subjects (Age 12 to < 18 years) For the first 3 subjects, an independent Data and Safety Monitoring Board (DSMB) will review the 30 days of safety monitoring data following study drug administration for each subject before the 1st G-CSF dosing of the subsequent subject. After the first 3 subjects of this population, no staggering period will be required. An independent DSMB will be appointed to have responsibility for safeguarding the interests of the trial subjects and for assessing the safety and tolerability of the study treatments periodically throughout the trial.
Number of subjects (planned): Up to 12 subjects are planned.

Name of Sponsor/Company: Elixirgen Therapeutics, Inc.

Name of Test Product: EXG34217

Name of Active Ingredients: Autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]

Patient Population:

Adult males and females with telomere biology disorders with bone marrow failure.

Inclusion Criteria:

1) Signed an Institutional Review Board (IRB) approved informed consent document indicating that they understand the purpose of and procedures required by the study and are willing to participate in the study and comply with all study procedures and restrictions. Informed consent must be obtained from the subject prior to initiating screening procedures to evaluate eligibility of the study.
2) Age $\geq$ 12 years.
3) Mild or moderate bone marrow failure defined by satisfying both conditions:
   - Sometime within the past 4 months, peripheral blood neutrophils (ANC) $< 1.5 \times 10^9$/L; or platelets $< 100 \times 10^9$/L; or Hemoglobin <10 g/dL
   - Bone marrow hypocellular for age
4) Diagnosis of telomere biology disorders defined by one of the following:
   - Age-adjusted mean telomere length < 1 percentile in all tested peripheral blood cells such as granulocytes, lymphocytes, B-cells, naïve T-cells, memory T-cells, and NK cells;
   - A pathogenic mutation in DKC1, TERC, TERT, NOP10, NHP2, TINF2, CTC1, PARN, RTEL1, ACD, NAF1, ZCCHC8, or WRAP53

Exclusion Criteria:

1) Women of childbearing potential not willing to follow the birth control as described in the informed consent and/or who are breastfeeding.
2) Subjects with cancer who are on active chemotherapeutic treatment.
3) Persistence of severe bone marrow failure defined by one of the following for 3 times at least one month apart:
   - Peripheral blood neutrophils (ANC) $< 0.5 \times 10^9$/L (the use of G-CSF for intermittent neutropenia with associated infections or chronic G-CSF for prevention of recurrent ulcers/fatigue is not excluded);
   - Platelets $< 20 \times 10^9$/L
4) If a bone marrow examination within the preceding 3 months indicates clonal cytogenetic abnormalities associated with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML), or variances thought to be associated with higher risk of malignant transformation.
5) Uncontrolled bacterial, viral or fungal infections.
6) Prior allogeneic marrow or peripheral blood stem cell transplantation.
7) Subjects who are not eligible for G-CSF and plerixafor dosing.
8) Subjects who are not eligible for apheresis.
9) Subjects currently taking or have taken danazol or other androgens, or other growth factors within 60 days prior to Day 1.

Name of Sponsor/Company: Elixirgen Therapeutics, Inc.

Name of Test Product: EXG34217

Name of Active Ingredients: Autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]

10) Subjects with any other clinically relevant acute or chronic diseases which could interfere with the subjects' safety during the trial, expose them to undue risk, or which could interfere with study objectives.
11) Subjects who have participated in another clinical trial with an investigational drug within the previous 30 days.

Study Drugs:

EXG34217; Autologous CD34+ cells contacted *ex vivo* with EXG-001 (Sendai virus vector encoding for human ZSCAN4)

Administration route: Single intravenous infusion at 3.3 mL/min infusion rate for 30 mins

Dose: EXG34217 cells ($\leq 8.0 \times 10^6$ /kg) in suspended in 100 mL Plasma-Lyte A.

Study Stopping Rule:

If any of the following events occur, the study will be suspended pending DSMB review:

- Any SAE associated with any of the study procedures.
- Allergic reaction, associated with infusion of EXG34217, Grade 3 in two subjects or Grade 4 in one subject.
- Grade 4 infection within 1-month post infusion of EXG34217 which is uncontrolled for >7 days.
- Any duration of Grade 3 toxicity involving cardiac, pulmonary, or neurologic systems.
- Any other Grade 3 toxicity that lasts >72 hours.
- Any duration of Grade 4 toxicity.
- Any death that occurs within 30 days of receiving study product.

Criteria for Evaluation:

Safety/Tolerability Endpoint:

- Vital signs
- Weight
- Standard 12-lead electrocardiogram (ECG)
- Clinical laboratory test (hematology, blood chemistry, and urinalysis)
- Physical examination
- Adverse events (AEs) and serious adverse events (SAEs)
- Immunogenicity of Sendai virus vector and hZSCAN4 protein

Exploratory Endpoint:

- Telomere length in any of the following: lymphocytes, granulocytes, B-cells, naïve T-cells, memory T-cells, and NK cells in peripheral blood
- Blood counts (neutrophils, platelets, or hemoglobin)

Name of Sponsor/Company: Elixirgen Therapeutics, Inc.
Name of Test Product: EXG34217
Name of Active Ingredients: Autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]
Statistical methods: Since this is an initial Phase 1/2 study, evaluation is intended to be hypothesis-raising rather than hypothesis-testing. This study is not powered to reliably yield statistically significant conclusions. The data will be analyzed only by means of descriptive statistics (including means and standard deviations, medians and inter-quartile ranges, and tabulations as appropriate) to reveal possible trends that will help guide the design of future, more definitive, clinical trials.

	Screening	outpatient
	↓	
Day 1-4	Pre-treatment of mobilization	outpatient
	↓	Inpatient/outpatient may vary by subject
Day 5-7	1 to 3 apheresis for PBMNC collections	
	↓	
	Ex Vivo Cell processing (approximately 36 hours)	
	↓	
Day 7-9	Study drug infusion	
	↓	
	Post infusion Safety monitoring (up to 36 hours)	
	↓	
Day 7-10	Discharge	
	↓	
Week 2	Safety follow-up	outpatient*
	↓	
Week 3	Safety follow-up	outpatient*
	↓	
Week 4	Follow-up visit	outpatient
	↓	
Week 5	Follow-up visit	outpatient*
	↓	
Month 2	Follow-up visit	outpatient*
	↓	
Month 3	Follow-up visit	outpatient
	↓	
Month 4	Follow-up visit	outpatient*
	↓	
Month 5	Follow-up visit	outpatient*
	↓	
Month 6	Follow-up visit	outpatient
	↓	
Month 9	Follow-up visit	outpatient*
	↓	
Month 12	Follow-up visit	outpatient
*: This will not require clinical visit and can be done locally as an option.		

Table 1a. Schedule of Events-1 (Screening to Discharge)

	Screening	Pre-treatment				Apheresis			EXG34217 dose
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 7-9
	-30 days	D1	D2	D3	D4	D5	D6	D7	D7-9
Clinic visit	x	x	x	x					
Admission[1]					x	x	x	x	x
Informed consent	x								
Demographics	x								
Medical history	x								
Inclusion/Exclusion criteria	x	x[4]							
Baseline disease status	x[2]								
G-CSF[1]		x	x	x	x	x	x	x	
Plerixafor[1]						x	x	x	
Apheresis[1][3]						x	x	x	
CD34+ cell counts (research kinetics)[6]						x	x	x	
CD34+ cell counts [7][8]						x	x		
Administration of EXG34217[1]									x
Physical examination	x								
Vital signs	x					x	x	x	x
12- lead ECG	x								
Height	x								
Weight	x	x							
Safety laboratory panel	x								
Urinalysis	x								
Cytokine panel	x								
Pregnancy test[9]	x								
Telomere length	x[5]								
Immunogenicity		x							
Adverse events		x	x	x	x	x	x	x	x
Prior/Concomitant medications	x	x	x	x	x	x	x	x	x

1) Schedule for admission, mobilization, apheresis, dosing, and post dosing monitoring may vary with each patient.
2) If a subject does not have genetic information regarding pathogenic mutation and known driver mutations by somatic sequencing including U2AF1, TP53, PPM1D, DNMT3, and TET2, on file, these need to be collected during the screening.
3) Eligibility of apheresis will be determined by PI's clinical judgements. Subject may have 1 to 3 apheresis procedures.
4) Confirm any changes by PI prior to infusion.
5) The baseline telomere length sample can be obtained during mobilization any time prior to apheresis.
6) To evaluate the CD34+ kinetics after Plerixafor dosing, CD34+ cell count in peripheral blood will be measured every 2 hours after each dose of plerixafor up to apheresis.
7) Subjects who have ≥ 5 cells/μL of repeated CD34+ cells in the peripheral blood will continue on the study to start apheresis.
8) Subjects who have < 5 cells/μL of CD34+ cells in the peripheral blood will repeat the peripheral CD34+ cells count after mobilization.
9) Screening pregnancy test must be done or repeated within 7 days prior to GSF administration.

Table 1b. Schedule of Events-2 (After Discharge Follow-up visits)

	Follow-up[3]											
	Day 7-10	Week 2[3,4]	Week 3	Week 4 (Month 1)	Week 5	Month 2	Month 3	Month 4	Month 5	Month 6	Month 9	Month 12
	D7-10	W2	W3	W4 (M1)	W5	M2	M3	M4	M5	M6	M9	M12
Clinic visit		x[1]	x[1]	x	x[1]	x[1]	x	x[1]	x[1]	x	x[1]	x
Admission	x											
Physical examination	x	x	x	x	x	x	x	x	x	x	x	x
Vital signs	x	x[2]	x[2]	x	x[2]	x[2]	x	x[2]	x[2]	x	x[2]	x
12- lead ECG				x			x			x		x
Height												
Weight	x			x			x			x		x
Safety laboratory panel	x	x	x	x	x	x	x	x	x	x	x	x
Urinalysis												x
Cytokine panel	x			x			x			x		x
Pregnancy test												x
Telomere length				x			x			x	x[5]	x
Immunogenicity	x			x			x			x		x
Adverse events	x	x	x	x	x	x	x	x	x	x	x	x
Prior/Concomitant medications	x	x	x	x	x	x	x	x	x	x	x	x

1) This will not require a clinic visit and can be done locally via telemedicine visits.
2) Vital signs will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health nurse) is being performed.
3) Visit window: ±3 days for Week 2-5 visits, ± 7 days for Month 2 to Month 5 visits, and ± 14 days for Month 6 to Month 12 visits
4) Follow-up visit Week 2 is defined as 14-days (± 3 days) from Day 1.
5) Optional per PI's discretion

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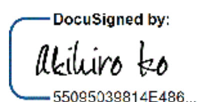
Sponsor Signature Page

Protocol Title: A Phase I/II Study to Evaluate the Safety and Tolerability of EXG34217 in Patients with Telomere Biology Disorders with Bone Marrow Failure

Protocol Number: EXG-US-01



4/19/2024

Chief Executive Officer: Akihiro C. Ko

Date:

Investigator Signature Page

I agree to conduct the study as outlined in the protocol entitled "A Phase I/II Study to Evaluate the Safety and Tolerability of EXG34217 in Patients with Telomere Biology Disorders with Bone Marrow Failure" in accordance with the guidelines and all applicable government regulations including US Title 21 Code of Federal Regulations (CFR) Part 54. I have read and understand all sections of the protocol.

Principal Investigator: ______________________ Date: ______________

TABLE OF CONTENTS

SYNOPSIS 2
TABLE OF CONTENTS 12
LIST OF IN-TEXT TABLES 14
LIST OF IN-TEXT FIGURES 14
LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS 15
1 INTRODUCTION 17
1.1 Background 17
1.2 Study Rationale 22
2 STUDY OBJECTIVES 24
2.1 Primary Objective 24
2.2 Secondary Objectives 24
3 STUDY ENDPOINTS 24
3.1 Primary Endpoint 24
3.2 Secondary Endpoints 24
4 OVERALL STUDY DESIGN AND PLAN 25
5 STUDY POPULATION 28
5.1 Inclusion Criteria 28
5.2 Exclusion Criteria 28
5.3 Subject Identification 29
5.4 Randomization and Blinding 29
5.5 Prior, Concomitant, and Prohibited Medications 29
6 PROCEDURE FOR MOBILIZATION, APHERESIS AND STUDY DRUG INFUSION . 30
6.1 Detailed Procedure for Mobilization, Apheresis and Study Drug Infusion 30
6.2 Procedure of Study Drug (EXG34217) Administration 32
6.3 Post Dose Safety Monitoring 32
7 STOPPING CRITERIA AND STUDY DURATION 33
7.1 During the Dosing Procedure 33
7.2 Subject Stopping Rules 33
7.3 Study Stopping Rules 33
7.4 Study Duration and Dates 33
8 LABELING, PACKAGING, STORAGE, DISPENSING, AND RETURN OF CLINICAL SUPPLIES 34
8.1 Product Description 34
8.2 Cell Processing 34
8.3 Treatment Administration 34
8.4 Primary Packaging and Labeling Information 34
8.5 Storage Requirements 34
8.6 Instructions for Dispensing 34
8.7 Assessment of Compliance 34
8.8 Drug Accountability/Return of Clinical Supplies 35
9 STUDY PROCEDURES 36
9.1 Informed Consent 36

9.2 Inclusion/Exclusion Criteria ... 36
9.3 Demographics ... 36
9.4 Medical History ... 36
9.5 Baseline Disease Characteristics ... 36
9.6 Body Weight ... 36
9.7 Physical Examination ... 36
9.8 Vital Signs ... 37
9.9 Electrocardiography ... 37
9.10 Clinical Laboratory Tests ... 37
9.11 CD34+ Cell Counts ... 38
9.12 Telomere Length ... 38
9.13 Immunogenicity ... 39
9.14 Cytokine Panel ... 39
9.15 Concomitant Medication Assessments ... 39
9.16 AE Assessments ... 39
10 STUDY ACTIVITIES ... 45
10.1 Screening ... 45
10.2 Day 1 ... 45
10.3 Day 2 ... 45
10.4 Day 3 ... 46
10.5 Day 4 ... 46
10.6 Apheresis Day(s) (5-7): Up to 3 days of apheresis ... 46
10.7 Study Drug Administration ... 47
10.8 Day 8-10: Post Dose Safety Monitoring ... 48
10.9 Week 2 (14 days [± 3 days] from Day 1) ... 48
10.10 Week 3 (± 3 days) ... 48
10.11 Week 4/Month 1 (± 3 days) ... 48
10.12 Week 5 (± 3 days) ... 49
10.13 Month 2 (± 7 days) ... 49
10.14 Month 3 (± 7 days) ... 49
10.15 Month 4 (± 7 days) ... 50
10.16 Month 5 (± 7 days) ... 50
10.17 Month 6 (± 14 days) ... 50
10.18 Month 9 (± 14 days) ... 50
10.19 Month 12/Exit Visit (± 14 days) ... 51
11 DATA SAFETY MONITORING BOARD (DSMB) ... 52
12 PLANNED STATISTICAL METHODS ... 53
12.1 General Considerations ... 53
12.2 Determination of Sample Size ... 53
12.3 Analysis Populations ... 53
12.4 Demographics and Baseline Characteristics ... 53
12.5 Safety Analysis ... 53
12.6 Exploratory Efficacy Analysis ... 53
12.7 Interim Analysis ... 54
13 ADMINISTRATIVE CONSIDERATIONS ... 55
13.1 Investigators and Study Administrative Structure ... 55

13.2 Regulatory Authority Approval 55
13.3 Ethical Conduct of the Study and IRB Approval 56
13.4 Subject Information and Consent 56
13.5 Confidentiality 56
13.6 Quality Control and Assurance 57
13.7 Data Management 57
13.8 Study Monitoring 57
13.9 Retention of Data 58
13.10 Financial Disclosure 58
14 REFERENCES 59

LIST OF IN-TEXT TABLES

Table 1a. Schedule of Events-1 (Screening to Discharge) 8
Table 1b. Schedule of Events-2 (After Discharge Follow-up visits) 9
Table 2. Study Administrative Structure 55

LIST OF IN-TEXT FIGURES

Figure 1. EXG-001 extends short telomeres of human primary fibroblast cells derived from a dyskeratosis congenita patient 21
Figure 2. EXG-001 extends telomeres of human CD34+ cells derived from healthy donors compared to untreated CD34+ cells 22
Figure 3. Overall study design 26

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

AE	adverse event
ALT	alanine transaminase
AML	acute myeloid leukemia
ANC	absolute neutrophil count
APTT	activation partial thromboplastin time
AST	aspartate aminotransferase
BAR	bright, alert, responsive
BUN	blood urea nitrogen
CBC	complete blood count
CCHMC	Cincinnati Children's Hospital Medical Center
CVC	central venous catheter
CFR	Code of Federal Regulations
CFU	colony forming unit
CML	Cell Manipulations Laboratory
CTCAE	Common Terminology Criteria for Adverse Events
D	day
DC	dyskeratosis congenita
dL	deciliter
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
DOB	date of birth
DSMB	Data and Safety Monitoring Board
ECG	electrocardiogram
ESC	embryonic stem cell
FISH	fluorescence in situ hybridization
g	gram
G-CSF	granulocyte colony stimulating factor
GFP	green fluorescent protein
GI	gastrointestinal
GLP	Good Laboratory Practice
GMP	Good Manufacturing Practice
HSC	hematopoietic stem cell
HSCT	hematopoietic stem cell transplant
ICF	informed consent form
ICH	International Conference for Harmonisation
ID	identification
IFN	interferon
IL	interleukin
IRB	institutional review board
kg	kilogram
L	liter
M	month
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MDS	myelodysplastic syndrome
mg	milligram

mL	milliliter
MNC	mononuclear cells
MOI	multiplicity of infection
mRNA	messenger ribonucleic acid
NK cell	natural killer cell
NMAC	non-myeloablative conditioning
PBMNC	peripheral blood mononuclear cell
PBS	phosphate buffered saline
PI	principal investigator
PICC	peripheral inserted central catheter
PIV	peripheral intravenous
PT/INR	prothrombin time/international normalized ratio
RBC	red blood cell
RIC	reduced intensity conditioning
RNA	ribonucleic acid
SAE	serious adverse event
SAP	statistical analysis plan
s.c.	subcutaneous
SOP	standard operating procedure
TT	thrombin time
µL	microliter
US	United States
W	week
WBC	white blood cell

1 INTRODUCTION

1.1 Background

1.1.1 Therapeutic Target Population: Patients with Telomere Biology Disorders

Telomeres are protective structures composed of DNA and proteins at the ends of chromosomes. Telomeres become shorter upon cell division and during aging; cell cycle arrest or cell death is activated upon reaching a critical shortness – a DNA damage response (Bessler, et al., 2010). This shortness is prevented in large part by the enzyme telomerase, which adds telomere repeat sequences on to the ends of existing telomeres (Bessler, et al., 2010).

Various mutations in telomere maintenance mechanisms cause severe telomere shortening, typically seen in patients with dyskeratosis congenita (DC) – a rare, inherited genetic disease (Agarwal 2018). Patients with dyskeratosis congenita usually have extremely short telomeres (below the 1st percentile of population in telomere lengths) (Alter et al., 2015). Originally, the disease was characterized by its "classic triad of abnormal skin pigmentation, nail dystrophy, and oral leukoplakia" (Fernández García and Teruya-Feldstein, 2014), but its typical presentation has since been expanded to include high rates of severe aplastic anemia, pulmonary fibrosis, and liver disease (Balakumaran et al., 2015).

The primary cause of death of those with dyskeratosis congenita is bone marrow failure – shortage of blood cells such as white blood cells, red blood cells and platelets, affecting 80-90% of all cases by 30 years old. The bone marrow failure is caused by deficiencies in the renewal abilities of the affected patient's hematopoietic stem cells (HSCs) due to severe telomere shortening (Balakumaran et al., 2015 and Goldman et al., 2008). In addition, patients are predisposed to cancers, and pulmonary fibrosis or abnormalities in pulmonary vasculature are seen.

It becomes increasingly clear that pathogenic mutations in genes involved in the telomere maintenance mechanism cause not only dyskeratosis congenita, but also a broad spectrum of diseases, which are now grouped under the name of "telomere biology disorders" (or telomeropathy, short telomere syndromes [Armanios and Blackburn, 2012]). Telomere biology disorders include dyskeratosis congenita, Revesz syndrome, Hoyeraal Hreidarsson syndrome, Coats plus, and can be associated with many cases of aplastic anemia, Myelodysplastic syndrome, Acute myeloid leukemia, Pulmonary fibrosis, Liver fibrosis, Familial melanoma, Familial lymphoproliferative disease, and Li-Fraumeni-like syndrome (Savage 2018).

Currently there are several treatment options for telomere biology disorders with bone marrow failure (Savage 2018 and Agarwal 2018).

- Supportive care
  - ✓ Transfusion: For patients with severe cytopenia, frequent transfusion support with red blood cells and platelets are required as rescue, but this treatment comes with various risks.
  - ✓ Stimulators of blood cell production: Commonly used erythropoietin and granulocyte colony stimulating factor (G-CSF) are not recommended, because they do not produce durable improvements. Also, patients do not respond to

Eltrombopag - an agonist of the thrombopoietin receptor.

- Androgens: Some patients respond to androgens, but only for a limited time (for months to years). Also, patients often do not tolerate the androgen therapy due to its severe side effects, such as impaired liver function, virilization, and behavioral problems. It is noteworthy that in one clinical trial in patients with shorter telomeres, Danazol – a synthetic steroid has been shown to extend telomeres, which are associated with the improvement of blood count (Townsley et al., 2016).
- Hematopoietic Stem Cell Transplantation (HSCT): Currently the only curative treatment available is HSCT, which can alleviate the hematologic manifestations of the condition. However, its use can be challenging with difficulties in finding well-matched donors and toxicities related to myeloablation (chemotherapy and radiation) and other treatment related complications, such as graft failure, graft versus host disease, sepsis, pulmonary fibrosis, cirrhosis, and veno-occlusive disease (Savage and Alter, 2009; Dietz et al., 2017). In fact, historically, patients undergoing myeloablation most commonly died from severe organ toxicity, as telomere maintenance is also important for tissue repair, particularly after exposure to DNA damaging agents in preparative regimens (Gadalla and Savage, 2011). Thus, the actual long-term survival of patients after myeloablative HSCT has been poor (Savage and Alter, 2009).

  To address this issue, a chemotherapy only (no radiation) lower intensity preparative regimen, i.e., a reduced-intensity conditioning (RIC) or non-myeloablative conditioning (NMAC) – has been tried. Nelson et al. (2016) showed improved overall survival of patients using a RIC regimen of alemtuzumab, fludarabine and melphalan. More recently Agarwal (2018) has utilized very low intensity conditioning with only alemtuzumab and fludarabine in an ongoing clinical trial (2018). There remain concerns regarding the use of HSCT as first-line therapy in dyskeratosis.

In sum, due to extremely poor prognosis and high mortality of telomere biology disorders with bone marrow failure, patients are in dire need of effective therapeutic options. However, currently only minimal treatment strategies (and no preventive strategies) are available for the patients. To address this unmet medical need, the development of innovative therapeutic options is urgently required.

1.1.2 Study Drug EXG34217

Study drug EXG34217 is autologous CD34+ cells contacted *ex vivo* with EXG-001 (Sendai virus vector encoding for human ZSCAN4).

Nonclinical data show:

(1) EXG-001 extension of telomeres of DC patient's fibroblast with short telomere in an *in vitro* study and,
(2) EXG34217 (human CD34+ cells contacted with EXG-001) engrafted and increased blood cells with normal proportional differentiation of cell types in an *in vivo* immunocompromised mice study.

Subject mononuclear cells (MNCs) are isolated from peripheral blood circulation by one to three apheresis after subcutaneous injections of G-CSF/Plerixafor. This process is a standard procedure for HSCT transplantation. CD34+ cells are isolated from collected MNCs and

incubated with EXG-001(Sendai virus vector encoding for human ZSCAN4 gene) for 24 hours in the GMP facility with appropriate quality control environment. Study drug EXG34217 cells are formulated in Plasma-Lyte A and dosed into patients intravenously.

Contacting EXG-001 will transiently produce human ZSCAN4 protein in patient's own (autologous) CD34+ cells, which will restore the damaged function of them by extending their abnormally short telomeres *ex vivo*. After dosing, EXG34217 (CD34+ cells with extended telomere) will be engrafted in patient's bone marrow and produce blood cells. Thus, the bone marrow failure of the patient expected to be ameliorated.

1.1.3 Safety of EXG34217

To assess the safety of EXG34217, a GLP toxicology study was performed using NOG-EXL (immune-compromised mouse model that does not reject human cells and tissues). A total of 210 mice (105 male and 105 female) were randomly assigned to three groups. Group 1 mice received the tail vein injection of a vehicle only (Phosphate Buffered Saline [PBS]). Group 2 mice received the tail vein injection of 4×10^7 cells/kg human CD34+ cells (untreated). Group 3 mice received the tail vein injection of 4×10^7 cells/kg human CD34+ cells treated with EXG-001 (i.e., EXG34217). The human CD34+ cells were obtained from three healthy volunteers. Sixty (60) mice were sacrificed one day after the injection to examine a possible acute toxicity. The remaining mice were sacrificed on Day 118/119 to examine the long-term toxicity.

- Clinical Chemistry/Hematology: No issues in clinical chemistry and hematology were found.
- Clinical Observations: Overall no unexpected abnormalities were detected all mice BAR (bright, alert, responsive) throughout experiment.
- No tumors were detected.
- No differences in body weight and organ weight between CD34+ untreated (Group 2) vs the CD34+EXG-001 treated (Group 3) except for the liver and spleen, which showed more enlargement in some CD34+ untreated mice (Group 2) than the CD34+EXG-001 treated mice (Group 3).

In addition, i*n vitro* hematopoietic colony forming unit (CFU) assays were performed to determine whether EXG-001, temperature-sensitive Sendai virus vector encoding human ZSCAN4 gene, could affect the differentiation of human CD34+ hematopoietic stem cells isolated from mobilized peripheral blood from healthy donors. After 14 days in the standard CFU culture media, CD34+ hematopoietic stem cells formed colonies grouped into BFU-E, CFU-E, CFU-G, CFU-M, CFU-GM, CFU-GEMM. Based on the fraction of the colonies formed, both EXG-001-treated and untreated CD34+ cells showed similar broad differentiation potential. Therefore, these results indicate that the EXG-001 did not alter the differentiation potential of healthy CD34+ hematopoietic stem cells.

1.1.4 Safety of Sendai Virus Vector

EXG-001 is Sendai virus vector encoding for human ZSCAN4 gene manufactured at a GMP facility (ID Pharma, Tsukuba, Japan). The Sendai virus vector is a derivative of Sendai virus - a single-stranded RNA virus of the Paramyxovirus subfamily, which completes its entire infection cycle as RNA (i.e. without involvement of DNAs). In addition, Sendai virus is not pathogenic in humans.

Compared to other virus vectors used for this type of gene therapy (e.g., retrovirus, adenovirus, lentivirus), the Sendai virus vector proposed in the EXG-001 therapeutic scheme remains in the cytoplasm and is never converted to DNA. As the Sendai virus vector does not leave a footprint in the host cell genome, the EXG-001 therapeutic paradigm is considered much safer than traditional gene therapy using other vectors.

Bitzer et al. injected a Sendai virus vector intravenously (direct injection) in mice and did not observe any toxicity over a period of 22 days (Bitzer et al., 2003).

Also, the clinical trial using Sendai virus vector (direct injection) is currently underway in patients with peripheral arterial disease (Phase I/IIa completed, Phase IIb is currently ongoing). Phase I/IIa study concluded that a “single intramuscular injection of this Sendai virus vector up to 8.3×10^7 CIU/kg was safe and well tolerated” (Yonemitsu et al., 2013). This Sendai virus vector shares the same vector backbone as EXG-001 and lacks F (fusion) gene; however, unlike the EXG-001 Sendai virus vector, it is not temperature-sensitive. As expected, non-transmissible Sendai virus vector was not detected in circulation or tissues/organs other than the injection sites of the skeletal muscles.

The Sendai virus vector (SeV18/TS15ΔF) used for EXG-001 lacks F (fusion) gene, and thus, is not capable of producing the infectious virus particles. This safety feature prevents the EXG-001, once integrated into a cell, from spreading to neighboring cells.

Furthermore, the Sendai virus vector (SeV18/TS15ΔF) used for EXG-001 is temperature-sensitive, and thus, produces the therapeutic protein ZSCAN4 within the cytoplasm at 33 °C, but not at 37 °C (Ban et al., 2011; Amano et al., 2015). EXG34217 cells are washed three times to minimize free EXG-001 in the dosing solution prior to dosing. Even if a few free EXG-001 are present after washing, the EXG-001 will never be active due to the non-permissive temperature – 37°C inside the patient’s body after dosing.

It is thus expected that EXG-001 will stay in CD34+ cells contacted *ex vivo* and will not spread to other cells.

1.1.5 Safety of ZSCAN4 Protein

The transgene in EXG-001 is human ZSCAN4 (zinc finger and SCAN domain containing 4). The expression of this gene is transient and restricted to critical timings and cell types during development and adult life: specifically at the 2-cell stage of mouse preimplantation embryos (Falco et al., 2007) and 4- to 8-cell stage in human preimplantation embryos (Vassena et al., 2011); infrequent but high expression in tissue stem cells in mouse and human (Ko et al., 2013); expression in meiotic prophase I in both male and female mice (Ishiguro et al., 2016); and high

and specific expression in 1-5% of mouse embryonic stem cells (ESCs) (Falco et al., 2007).

Previous results show that ZSCAN4 localizes to telomeres, extends telomeres by homologous recombination (i.e., telomerase-independent mechanism), and increases the genome stability in mouse ESCs (Zalzman et al., 2010).

Unpublished results show that ZSCAN4 extends telomeres in human fibroblast cells derived from a dyskeratosis congenita patient (from a 11-year-old male Caucasian with X-linked dyskeratosis congenita). As previously reported (Wong and Collins, 2006), telomeres of dyskeratosis congenita fibroblast cells are shorter than normal cells and quickly become even shorter in the cell culture. In the control experiments, transfections of synthetic mRNA encoding green fluorescent protein (GFP) did not change the telomere shortening patterns of cells. By contrast, transfection of synthetic mRNAs encoding human ZSCAN4 elongated the telomeres of the dyskeratosis congenita cells and prevented the telomeres from getting shorter.

Telomere extension was also observed by treating human fibroblast cells derived from a dyskeratosis congenita patient with the EXG-001 (see Figure 1 below).

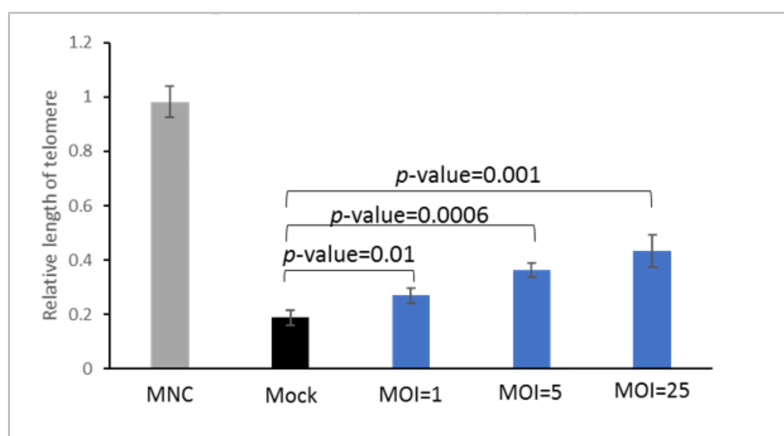


Figure 1. EXG-001 extends short telomeres of human primary fibroblast cells derived from a dyskeratosis congenita patient

DC fibroblast cells contacted with EXG-001 at three different MOI groups, MOI=1, MOI=5, and MOI=25, showed 1.4-fold, 1.9-fold, and 2.3-fold extension in their telomeres compared to the untreated DC fibroblast cells (Mock), respectively. Student's *t-test* for two paired samples of means was applied for statistical analysis. Compared to untreated DC cells (Mock), all EXG-001-treated groups showed statistically significant increase of telomere lengths: MOI=1 ($p<0.05$), MOI=5 ($p<0.005$), and MOI=25 ($p<0.005$). MNC (peripheral blood mononuclear cells) from a healthy donor was used as a control.

EXG-001 also increased telomere lengths in human CD34+ cells collected from healthy individuals after mobilization by G-CSF. CD34+ cells were contacted *ex vivo* with EXG-001 and incubated at 33 °C for 24 hours, subsequently cultured *in vitro* at 37 °C for 10 days. Compared to the untreated cells, EXG-001-treated cells showed statistically significant extension of telomeres (See Figure 2 below).

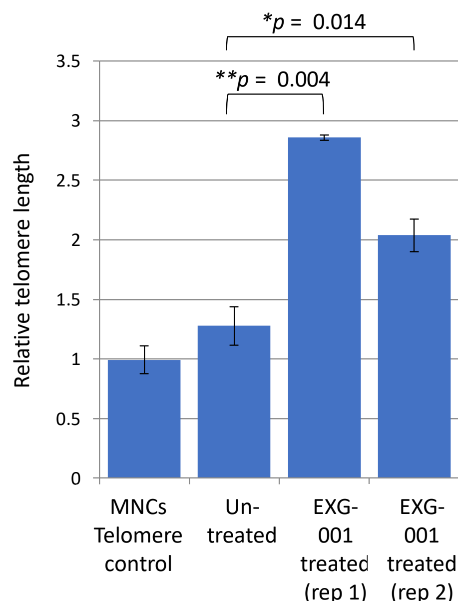


Figure 2. EXG-001 extends telomeres of human CD34+ cells derived from healthy donors compared to untreated CD34+ cells

MNC (peripheral blood mononuclear cells) from a healthy donor was used as a control.

ZSCAN4 proteins are released only within CD34+ cells after contact with EXG-001. Therefore, it is not expected any free ZSCAN4 proteins will be distributed in the body after dosing. Furthermore, ZSCAN4 and SeV-related proteins decrease rapidly at 37 °C (i.e., body temperature) due to the temperature-sensitivity of EXG-001. In this clinical trial, anti-ZSCAN4 antibody will be measured to ensure there are no unexpected safety concerns.

1.2 Study Rationale

Telomere biology disorders with bone marrow failure including dyskeratosis congenita have poor prognosis and high mortality rate. Currently, HSCT is the only curative treatment, which can alleviate the hematologic manifestations of the condition. However, its use can be challenging with difficulties in finding well-matched donors and toxicities related to myeloablation (chemotherapy and radiation) and immune complications.

EXG34217, autologous CD34+ cells contacted *ex vivo* with EXG-001 (Sendai virus vector encoding for human ZSCAN4), has been shown to extend the telomeres in human CD34+ cells *in vitro* and *in vivo* nonclinical studies. This treatment does not require a well-matched donor and can use the patient's own cells. Contacting EXG-001 will transiently produce human ZSCAN4 protein in a patient's own (autologous) CD34+ cells, which will restore the damaged function by extending their abnormally short telomeres *ex vivo*. After dosing, EXG34217 (CD34+ cells with extended telomere) will be engrafted in the patient's bone marrow and produce blood cells. Thus, the bone marrow failure of the patient may be ameliorated.

In this population it is often difficult to mobilize stem cells, by pre-dosing with granulocyte colony stimulating factor (G-CSF) and Plerixafor as a second mobilizing agent will significantly improve mobilization of CD34+ cells to achieve adequate cell collection (Rio et al., 2017;

Goldman et al., 2008; Uchida et al., 2020). It has been reported that the mobilization regimen of this protocol [the twice daily dosing of G-CSF (12 µg/kg s.c.) injection for up to 7 days and Plerixafor (240 mg/kg body weight/day) up to 3 days] improved mobilization and is well tolerated in the younger population (Sevilla et al., 2021; Koo et al., 2021).

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of the study is:

- To assess the safety and tolerability of EXG34217 (autologous CD34+ cells contacted *ex vivo* with EXG-001 [Sendai virus vector encoding for human ZSCAN4]) in subjects with telomere biology disorders with mild bone marrow failure.

2.2 Secondary Objectives

The secondary objective of the study is:

- To assess the feasibility of *ex vivo* transduction and reinfusion of EXG34217 (autologous ZSCAN4 transduced CD34+ cells).
- To assess the feasibility of telomeres extension assessment.
- To assess clinical benefit by measuring complete blood count over time.

3 STUDY ENDPOINTS

3.1 Primary Endpoint

Primary endpoints are safety and tolerability assessment by:

- Vital signs
- Weight
- Standard 12-lead electrocardiogram (ECG)
- Clinical laboratory test (hematology, blood chemistry, and urinalysis)
- Physical examination
- Adverse events (AEs) and serious adverse events (SAEs)
- Immunogenicity of Sendai virus vector and hZSCAN4 protein

3.2 Secondary Endpoints

Secondary endpoints of this study are exploratory efficacy assessments by.

- Increase of telomere length in any of the following: lymphocytes, granulocytes, B-cells, naïve T-cells, memory T-cells, and NK cells.
- Improvement of blood counts (neutrophils, platelets, or hemoglobin).

4 OVERALL STUDY DESIGN AND PLAN

This is a Phase I/II, open label study in up to 12 subjects with telomere biology disorders with bone marrow failure. The study is open to all participants regardless of gender or ethnicity. Subjects who are enrolled but are not evaluable will be replaced.

Subjects will sign an IRB approved informed consent document prior to any study related procedure and will complete baseline screening assessments.

Subjects for this study will not require any preparative regimen such as chemotherapy or radiation.

The study will be conducted in four parts:

1. Peripheral blood mononuclear cell (PBMNC) collection; mobilization and apheresis.
2. *Ex vivo* cell processing.
3. Processed cells infusion and post-infusion safety monitoring.
4. Follow up at Weeks 2-5, Months 2, 3, 4, 5, 6, 9 and 12.

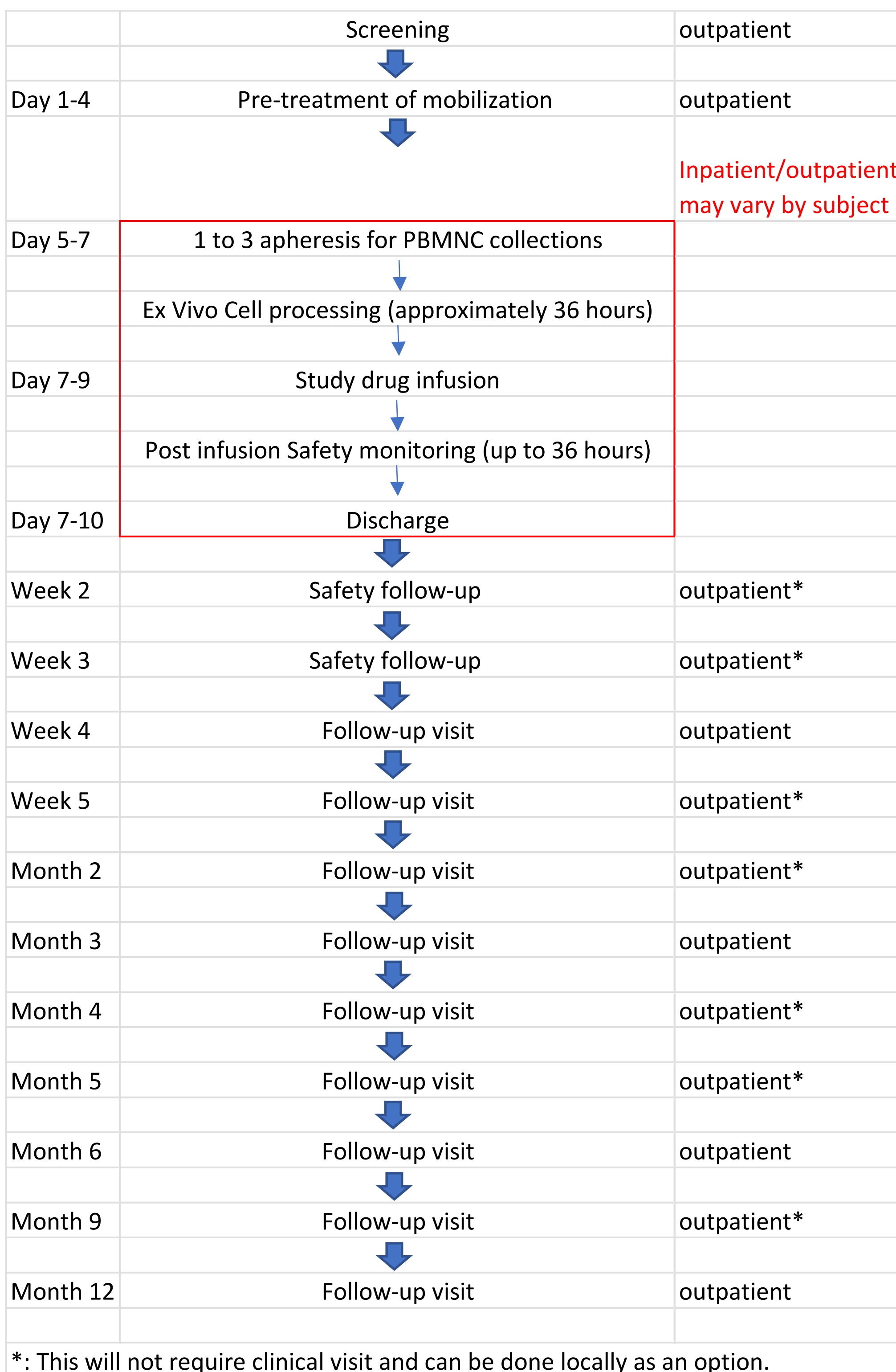


Figure 3. Overall study design

All eligible subjects will start mobilization (with G-CSF and Plerixafor). If eligible, subjects will undergo apheresis. Subjects may attempt to undergo apheresis 1-3 times. Subjects who successfully complete at least one apheresis and continue to qualify will receive the study drug

(processed cells) after apheresis. (See Section 6 for details on mobilization, apheresis, and dosing.)

Subjects will be monitored for safety up to 36 hours post dosing and will be discharged after the Investigator has reviewed safety lab results and determines discharge is appropriate.

Subjects will be evaluated for safety weekly for the first month after infusion and then assessed once monthly for 6 months. After 6 months, subjects will be evaluated at Month 9 and Month 12. Week 4, Months 3, 6, and 12 will require a clinic visit at Cincinnati Children's Hospital Medical Center (CCHMC). Weeks 2, 3, and 5 and Months 2, 4, 5, and 9 visits will not require a clinical visit and can be done locally via telemedicine with the Investigator (with the assistance of the local healthcare provider or home health nurse).

Detailed study procedures are in Section 6 and Section 10.

Any subject who does not have apheresis is eligible to re-enroll/re-attempt achieving adequate CD34+ cell (>5 cells/ µL) if the PI feels it is safe and in the best interest of the subject.

On a case-by-case basis, for subjects who may experience difficulties traveling back to CCHMC, allowances to have assessments done by their local treating physician will be considered. There will be extensive and close communication between the Investigator and the local treating physician.

For adult subjects (Age ≥ 18 years)

> A 4-week staggering period between the first 3 subjects in this population is planned. Specifically, 30 days must have elapsed from the infusion of the previous subject to the 1st G-CSF dosing of subsequent subject. After the first 3 subjects of this population, no staggering period will be required.

For adolescent subjects (Age 12 to < 18 years)

> For the first 3 subjects, an independent Data and Safety Monitoring Board (DSMB) will review the 30 days of safety monitoring data following study drug administration before the 1st G-CSF dosing of subsequent subject. After the first 3 subjects of this population, no staggering period will be required.

An independent DSMB will be appointed to have responsibility for safeguarding the interests of the trial subjects, and assessing the safety and tolerability of the study treatments during the trial (see detail in Section 12).

5 STUDY POPULATION

Adult males and females with telomere biology disorders with bone marrow failure.

5.1 Inclusion Criteria

1) Signed an Institutional Review Board (IRB) approved informed consent document indicating that they understand the purpose of and procedures required by the study and are willing to participate in the study and comply with all study procedures and restrictions. Informed consent must be obtained from the subject and/or a designated representative prior to initiating screening procedures to evaluate eligibility of the study.
2) Age ≥ 12 years.
3) Mild or moderate bone marrow failure defined by satisfying both conditions:
   - Sometime within the past 4 months, peripheral blood neutrophils (ANC) $< 1.5 \times 10^9$/L; or platelets $< 100 \times 10^9$/L; or Hemoglobin <10 g/dL
   - Bone marrow hypocellular for age
4) Diagnosis of telomere biology disorders defined by one of the following:
   - Age-adjusted mean telomere length < 1 percentile in all tested peripheral blood cells such as granulocytes, lymphocytes, B-cells, naïve T-cells, memory T-cells, and NK cells;
   - A pathogenic mutation in DKC1, TERC, TERT, NOP10, NHP2, TINF2, CTC1, PARN, RTEL1, ACD, NAF1, ZCCHC8, or WRAP53

5.2 Exclusion Criteria

1) Women of childbearing potential not willing to follow the birth control as described in the informed consent and/or who are breastfeeding.
2) Subjects with cancer who are on active chemotherapeutic treatment.
3) Persistence of severe bone marrow failure defined by one of the following for 3 times at least one month apart:
   - Peripheral blood neutrophils (ANC) $< 0.5 \times 10^9$/L (the use of G-CSF for intermittent neutropenia with associated infections or chronic G-CSF for prevention of recurrent ulcers/fatigue is not excluded);
   - Platelets $< 20 \times 10^9$/L
4) If a bone marrow examination within the preceding 3 months indicates clonal cytogenetic abnormalities associated with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML), or variances thought to be associated with higher risk of malignant transformation.
5) Uncontrolled bacterial, viral or fungal infections.
6) Prior allogeneic marrow or peripheral blood stem cell transplantation.
7) Subjects who are not eligible for G-CSF and plerixafor dosing.
8) Subjects who are not eligible for apheresis.
9) Subjects currently taking or have taken danazol or other androgens, or other growth factors within 60 days prior to Day 1.
10) Subjects with any other clinically relevant acute or chronic diseases which could interfere with the subjects' safety during the trial, expose them to undue risk, or which could interfere with study objectives.

11) Subjects who have participated in another clinical trial with an investigational drug within the previous 30 days.

5.3 Subject Identification

All subjects who undergo Screening will be assigned a unique screening identification number at the time of Screening. Only subjects continuing to meet entry inclusion/exclusion criteria will be assigned a unique randomization subject number.

5.4 Randomization and Blinding

This study is an open label study, not blinded.

5.5 Prior, Concomitant, and Prohibited Medications

Subjects currently taking or who have taken danazol or other androgens, or other growth factors within 60 days prior to Day 1 cannot be enrolled in this trial.

All rescue treatment such as transfusion, treatment with danazol and androgens determined necessary by the Investigator’s clinical judgement will be allowed.

6 PROCEDURE FOR MOBILIZATION, APHERESIS AND STUDY DRUG INFUSION

6.1 Detailed Procedure for Mobilization, Apheresis and Study Drug Infusion

Note: The day of the study procedures may change contingent on the day of the week subject presents at the hospital.

Days 1 to 4

All eligible subjects will receive twice daily dosing of G-CSF injection (12 µg/kg s.c. q12), one in the morning and one in the evening.

Subjects will be hospitalized and continue to receive the twice daily dosing of G-CSF (12 µg/kg s.c.) injection, one in the morning and again in the evening.

A peripherally inserted catheter for access will be placed (vein access will be via peripheral intravenous [PIV] line whenever possible for apheresis) according to the site parameters. The insertion of a central venous catheter (CVC) or peripheral inserted central catheter (PICC) will be secondary options when clinically necessary. This can be done on any day prior to Day 5.

Day 5

Up to Day 5, all subjects will follow the same mobilization schedule.

G-CSF (12 µg/kg s.c.) will be administered in the morning and Plerixafor (0.24 mg/kg s.c.) will be given as a second mobilizing agent 4-6 hours prior to apheresis. In this population it is often difficult to mobilize stem cells. The addition of Plerixafor as a second mobilizing agent will significantly improve mobilization of CD34+ cells to achieve adequate cell collection. Peripheral blood will be collected for CD34+ cell count to assess the eligibility of the 1st apheresis.

Subjects who do not qualify for apheresis will repeat Day 5. After 2 attempts, subjects who still have < 5 cells/uL of CD34+ cells in the peripheral blood will be withdrawn from the study. No follow-up will be required.

Subjects who have ≥ 5 cells/µL of CD34+ cells in the peripheral blood will continue the study.

A. Initial (1st) apheresis

The eligibility for the 1st apheresis will be assessed. If eligible, apheresis will start.

To evaluate the CD34+ kinetics after Plerixafor dosing, CD34+ cell count in peripheral blood will be measured every 2 hours after each dose of Plerixafor up to apheresis. Kinetics will not be assessed in real time. Only the final pre-apheresis CD34 count will be in real time.

After apheresis, the feasibility of the 2nd apheresis will be evaluated by the Investigator.

B. Subjects judged able to tolerate a second collection.

Post apheresis, a complete blood count (CBC) will be obtained, and the subject will be given RBC or platelet transfusion, per Institution's policy.

A bag containing MNCs collected by the first apheresis will be kept overnight in a monitored GMP refrigerator at 2-8 °C. The MNCs should not be kept for longer than 48 hours before starting the labeling and selection procedure of CD34+ cells.

The subject will continue to receive twice daily dosing of G-CSF (12 µg/kg s.c.), and Plerixafor (0.24 mg/kg s.c) will be administered approximately 4 to 6 hours prior to the initiation of the 2nd apheresis.

To evaluate the CD34+ kinetics after Plerixafor dosing, CD34+ cell count in peripheral blood will be measured every 2 hours after Plerixafor dosing up to apheresis. Kinetics will not be assessed in real time.

The number of CD34+ cells in collected MNCs (total of 1st and 2nd apheresis) will be estimated.

- CD34+ cells $< 1.0 \times 10^6$ /kg: subject will have a 3rd apheresis.
- CD34+ cells $\geq 1.0 \times 10^6$ /kg: subject will continue on study to receive drug dosing.

Bags containing MNCs collected by the 1st and 2nd apheresis will be transferred to CCHMC's Cell Manipulations Laboratory (CML).

C. Subjects judged unable to tolerate a second collection.

- CD34+ cells $< 1.0 \times 10^6$ /kg: all collected MNCs will be infused back to the subject per institutional standard procedures. Subjects will be withdrawn from study and will be followed up for safety by phone in 2-4 weeks later.
- CD34+ cells $\geq 1.0 \times 10^6$ /kg: subjects will continue on study to receive study drug dosing.

D. Subject judged (by the PI) able to tolerate a third collection.

Post apheresis, a CBC will be obtained, and the subject will be given RBC or platelet transfusion, per Institution's policy.

A bag containing MNCs collected by the second apheresis will be kept overnight in a monitored GMP refrigerator at 2-8 °C. The MNCs should not be kept for longer than 50-52 hours before starting the labeling and selection procedure of CD34+ cells.

The subject will continue to receive twice daily doing of G-CSF (12 µg/kg s.c.) and Plerixafor (0.24 mg/kg s.c) will be administered approximately 4 to 6 hours prior to initiation of the apheresis.

To evaluate the CD34+ kinetics after Plerixafor dosing, CD34+ cell count in peripheral blood will be measured every 2 hours after Plerixafor dosing up to apheresis. Kinetics will not be assessed in real time.

The number of CD34+ cells in collected MNCs (total of 1st, 2nd, and 3rd apheresis) will be estimated.

- CD34+ cells < 1.0×10^6 /kg: all collected MNCs will be infused back to the subject per institutional standard procedures. Subjects will be withdrawn from study and will be followed up for safety by phone in 2-4 weeks later.
- CD34+ cells ≥ 1.0×10^6 /kg: subjects will continue on study to receive drug dosing.

Bags containing MNCs collected by the 1st, 2nd, and 3rd apheresis will be transferred to CCHMC CML.

E. Subjects judged (by the PI) unable to tolerate a third collection.

The number of CD34+ cells in collected MNCs (total of 1st and 2nd apheresis) will be estimated.

- CD34+ cells < 1.0×10^6 /kg: all collected MNCs will be infused back to the subject per institutional standard procedures. Subjects will be withdrawn from study and will be followed up for safety by phone in 2-4 weeks later.
- CD34+ cells ≥ 1.0×10^6 /kg: subjects will continue to study drug dosing.

6.2 Procedure of Study Drug (EXG34217) Administration

Subjects will receive either oral or IV doses of acetaminophen (650 mg) and diphenhydramine (50 mg) and an IV dose of hydrocortisone (100 mg) prior to study drug dosing. The route of administration for acetaminophen and diphenhydramine will be at the Investigator's discretion.

EXG34217 (autologous CD34+ cells contacted *ex vivo* with EXG-001) will be infused intravenously without filter from placed access at 3.3 mL/min infusion rate for 30 mins.

6.3 Post Dose Safety Monitoring

Subjects will be monitored for safety up to 36 hours post dosing. Subjects will be discharged, after the Investigator has reviewed the safety lab results and determines discharge is appropriate.

7 STOPPING CRITERIA AND STUDY DURATION

7.1 During the Dosing Procedure

Before the infusion of EXG34217, the Investigator will determine whether the subject is able to tolerate the infusion. If Investigator notes any AE which may jeopardize the subject's health the subject will not receive the infusion of EXG34217, and the subject will be withdrawn from the study and will be replaced.

During the infusion of EXG34217, if the Investigator notes any AE which may jeopardize the subject's health, the infusion will be stopped immediately. The subject will continue to be followed for safety.

7.2 Subject Stopping Rules

Subjects will be withdrawn from the study in the following circumstances:

- Subject wishes to withdraw;
- When the Investigator judges that for any reason continuation of the study drug is inappropriate for the subject.

7.3 Study Stopping Rules

If any of the following events occur, the study will be suspended pending DSMB review:

- Any SAE associated with any of the study procedures.
- Allergic reaction, associated with infusion of EXG34217, Grade 3 in two subjects or Grade 4 in one subject.
- Grade 4 infection within 1-month post infusion of EXG34217 which is uncontrolled for >7 days.
- Any duration of Grade 3 toxicity involving cardiac, pulmonary or neurologic systems.
- Any other Grade 3 toxicity that lasts >72 hours.
- Any duration of Grade 4 toxicity.
- Any death that occurs within 30 days of receiving study product.

7.4 Study Duration and Dates

The study will include a screening period of up to 30 days and a study period of 12 months.

8 LABELING, PACKAGING, STORAGE, DISPENSING, AND RETURN OF CLINICAL SUPPLIES

8.1 Product Description

EXG34217 is autologous CD34+ cells *ex vivo* contacted with EXG-001 suspended in 100 mL Plasma-Lyte A.

EXG-001 is a Sendai virus vector encoding for human ZSCAN4, manufactured at ID Pharma (Ibaragi, Japan).

8.2 Cell Processing

Cincinnati Children's Hospital Medical Center (CCHMC) Cell Manipulations Laboratory (CML) will manufacture EXG34217 from subjects' PBMNC collected by apheresis using a Miltenyi CliniMACS Prodigy instrument under GMP.

8.3 Treatment Administration

EXG34217 (autologous CD34+ cells contacted *ex vivo* with EXG-001) will be infused intravenously without filter from placed access at 3.3 mL/min infusion rate for 30 mins.

If CD34+ cells in the final product are $>8.0 \times 10^6$ cells /kg, only 8.0×10^6 cells/kg will be administered to subjects at the dosing in this study and the rest will be cryopreserved for future research use.

Detailed instructions for dosing procedures are also provided in the Study Manual.

8.4 Primary Packaging and Labeling Information

EXG34217 is filled 100 mL in a CryoMACS Freezing Bag 250 (250 mL, Miltenyi Cat # 200-074-401).

Label contains manufacturer name, protocol number, product name, lot number, subject ID (with name) and DOB, cell numbers (cells/kg), total volume, expiration time, cautions and instruction.

8.5 Storage Requirements

EXG34217 is a fresh product. Study drug needs to be used before the time described on the label.

8.6 Instructions for Dispensing

CML will transfer the investigational product to clinical unit for infusion.

8.7 Assessment of Compliance

As the investigational product is administered in the clinical unit, compliance is not applicable. The start volume in the bag and the volume left after dosing should be recorded in the source document.

8.8 Drug Accountability/Return of Clinical Supplies

Under no circumstance will the Investigator(s) allow the study drug to be used other than as directed by this protocol. If the infusion is stopped mid-infusion and some product remains, the leftover product will be discarded per institutional practice and will be recorded accordingly.

9 STUDY PROCEDURES

9.1 Informed Consent

The informed consent form (ICF) must be executed prior to performing any study-related activities. The ICF must be approved by the reviewing Institutional Review Board (IRB). Informed consent will be obtained for all subjects participating in the study. Subjects may withdraw consent at any time.

Participation in the study may be terminated at any time without the subject's consent if it is determined necessary by the Principal Investigator.

9.2 Inclusion/Exclusion Criteria

Eligibility screening of subjects will be completed prior to administration of the study drug and will be documented in the source documents. Confirmation of eligibility will be performed on Day 1 at the clinic to ensure no obvious changes in eligibility.

Screen failures and the reason for failure to meet the study eligibility requirements will be documented in the study site source documents.

9.3 Demographics

The following demographic parameters will be captured at screening: date of birth, sex, race, and ethnicity.

9.4 Medical History

Relevant medical history, based on the opinion of the Investigator, will be obtained from the subject at Screening and recorded in the source documents. Medical history will capture the subject's health history, including history of hospitalization, and history of surgeries.

9.5 Baseline Disease Characteristics

The following baseline disease characteristics will be captured at screening: bone marrow cellularity, pathogenic mutation, and known driver mutations by somatic sequencing including U2AF1, TP53, PPM1D, DNMT3, and TET2 (if subject does not have genetic information, these will be collected during the screening).

9.6 Body Weight

Body weight will be collected at screening, Day 1, prior to discharge, Month 1, Month 3, Month 6 and Month 12/Exit.

9.7 Physical Examination

A physical examination will be performed at the time of Screening, prior to discharge, weekly for the first month after infusion, and then once monthly for 6 months. After 6 months, subjects will be evaluated at Month 9 and Month 12/Exit. Weeks 2, 3, and 5 and Months 2, 4, 5, and 9 visits will not require a clinical visit and can be performed via telemedicine with Investigator

(with the assistance of the local healthcare provider or home health nurse). The findings of each examination will be recorded on the source documents and clinically significant abnormalities will be recorded in the source documents. The physical examination will include:

- General appearance
- Head, eyes, ears, nose, and throat
- Respiratory
- Cardiovascular
- Musculoskeletal
- Abdomen
- Neurologic
- Extremities
- Dermatologic
- Lymphatic

For physical examinations, tattoos and piercings are not abnormal findings and are not required to be noted.

9.8 Vital Signs

Vital signs, including blood pressure, heart rate, respiratory rate, and body temperature, will be measured through the trial, and those will be measured on Screening, after apheresis, day of dosing, prior to discharge, and weekly for the first month after infusion, and then once monthly for 6 months. After 6 months, subjects will be evaluated at Month 9 and Month 12/Exit. At the inpatient unit after infusion of EXG34217, vital signs will be measured every 4 hours (per institution standard practice). After discharge, vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health nurse) is being performed.

9.9 Electrocardiography

A standard digital 12-lead ECG will be recorded in singlicate at screening and at Month 1, Month 3, Month 6, and Month 12/Exit. Safety ECGs will be recorded and evaluated by a Cardiologist.

9.10 Clinical Laboratory Tests

Laboratory testing (hematology with differential, serum chemistry and urinalysis) will be performed using standard methods. Blood samples for the serum chemistry and hematology and coagulation listed below will be collected at Screening, prior to discharge, and weekly for the first month after infusion, and then once monthly for 6 months. After 6 months, subjects will be evaluated at Month 9 and Month 12/Exit. Week 2, 3, 5 and Month 2, 4, 5, 9 visits will not require a clinical visit and can be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the Investigator for assessment.

Hematology:	Hematocrit, Hemoglobin, Platelet count, Red blood cell (RBC) count, Reticulocyte count, White blood cell (WBC) count, WBC differentials, Mean corpuscular volume (MCV), Mean corpuscular hemoglobin (MCH), MCH concentration (MCHC)
Blood Chemistry:	Creatinine, Calculated Creatinine Clearance, blood urea nitrogen (BUN), Potassium (K+), Sodium (Na+), Chloride (Cl-), Magnesium (Mg++), Calcium, phosphate, Glucose, Bilirubin (Total), Bilirubin (direct), aspartate aminotransferase (AST), alanine transaminase (ALT), Alkaline phosphatase, Total Protein, Albumin
Coagulation:	Activation partial thromboplastin time (APTT), Prothrombin time/International normalized ratio (PT/INR), thrombin time (TT)
Urinalysis	Macroscopic examination routinely including specific gravity, pH, protein, glucose, ketones, blood and urobilinogen. A microscopic examination will be performed if warranted based on macroscopic results.

For all females of childbearing potential, a serum pregnancy test will be performed at Screening, and at the Month 12/Exit Visit.

Urinalysis will be performed at Screening, and at the Month 12/Exit Visit.

Detailed instructions for laboratory sample collection, processing, and handling are provided in the Laboratory Manual.

9.11 CD34+ Cell Counts

On Day 5 or Day 6 (if apheresis is not completed on Day 5), CD34+ cells will be counted by peripheral blood samples. Detailed instructions for sample collection, processing, and handling are provided in the Laboratory Manual.

On the day of apheresis, CD34+ cell count in peripheral blood will be measured every 2 hours after Plerixafor dosing up to apheresis for research kinetic purposes only.

Any subject who enrolled, but did not have apheresis, is eligible to re-enroll/re-attempt achieving adequate CD34+ cell (>5 cells/µL) if the PI feels it is safe and in the best interest of the subject.

In addition, after each apheresis, collected CD34+ cells will be counted.

9.12 Telomere Length

Blood sample for telomere length assessment will be collected at Screening (the baseline telomere length sample can be obtained during mobilization (prior to apheresis) if not previously obtained), Month 1, Month 3, Month 6, Month 9 (optional per PI's discretion), and Month 12/Exit.

Collected samples will be shipped to RepeatDx (North Vancouver, Canada) to measure telomere length by Flow FISH. Detailed instructions for sample collection, processing, and handling are provided in the Laboratory Manual.

9.13 Immunogenicity

Blood sample for Antibody against Sendai virus vector and hZSCAN4 protein will be collected at Day 1, prior to discharge, Month 1, Month 3, Month 6 and Month 12/Exit.

Detailed instructions for sample collection, processing, and handling are provided in the Laboratory Manual.

9.14 Cytokine Panel

Blood sample for Plasma Cytokine Panel (GM-CSF, IFNg, IL-1 beta, IL-10, IL-2, IL-4, IL-5, IL-6, IL-8, TNF-α) will be collected at screening, prior to discharge, Month 1, Month 3, Month 6, and Month 12/Exit.

Detailed instructions for sample collection, processing, and handling are provided in the Laboratory Manual.

9.15 Concomitant Medication Assessments

The Investigator or designated qualified personnel will assess and record concomitant medication usage on the source documents at Screening and throughout the trial. Specific information regarding concomitant medication and prior therapy usage is provided in Section 5.5.

9.16 AE Assessments

9.16.1 Performing AE Assessments

The Investigator is responsible for promptly documenting and reporting all AEs observed during the study in the subject's source documents. If an AE is classified as "serious" as described in Section 9.16.6.1, it must be reported to Elixirgen Therapeutics or its designee no later than 24 hours after the Investigator recognizes/classifies the event as an SAE.

AEs will be collected from the time of the administration of G-CSF (Day 1) to the time of the Study Exit Visit or early termination from the study. Any events reported before administration of the study drug will be recorded as Medical History. For ongoing AEs at the time of the Exit Visit or early termination, additional data, such as AE resolution date, will be collected and reported to Elixirgen Therapeutics.

AEs will be classified according to the Common Terminology Criteria for Adverse Events (CTCAE), the most recent version available.

9.16.2 AE Definitions

The following definitions of terms are guided by the International Conference on Harmonisation (ICH) and the U.S. CFR [21 CFR 312.32] and are included herein.

An AE is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE (also referred to as an adverse experience) can be any

unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An AE can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

AEs include, but are not limited to:

- Any symptom or condition not previously reported by the subject (medical history).
- An exacerbation of a pre-existing symptom or condition.
- A significant increase in frequency or intensity of a pre-existing episodic event or condition.
- A drug interaction.
- A condition first detected or diagnosed after study drug administration even though it may have been present prior to the start of the study.

An AE does not include:

- Medical or surgical procedures (e.g., surgery, endoscopy, tooth extraction, blood transfusion); the condition that led to the procedure is an AE (e.g., bleeding esophageal varices, dental caries).
- Overdose of either study drug or concurrent medication without any clinical signs or symptoms.
- Non-clinically significant abnormal laboratory values. (If accompanied by signs/symptoms, the signs or symptoms are considered an AE).

AEs that occur between the time the subject signs the ICF for the study and the time when that subject is randomized will be summarized as medical history and not as study AEs unless the event meets the definition of a SAE as defined in Section 9.16.6.1.

9.16.3 Severity

The Investigator or designee will be asked to assess the severity of the AE using the latest version of the CTCAE. These criteria assign a grade of 1 through 5 to indicate the severity of AEs. For AEs that are not listed in these criteria, the Investigator or designee will use medical judgment to assess the severity of the AE.

A general guideline to these grades of severity, taken from CTCAE, is:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.
- Grade 2: Moderate; minimal, local or non-invasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (see CTCAE for more details).
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization of prolongation of hospitalization indicated; disabling; limiting self-care Activities of Daily Living.
- Grade 4: Life-threatening consequences; urgent intervention indicated.
- Grade 5: Death related to AE.

An AE that is assessed as severe will not be confused with an SAE. Severity is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets at least one of the pre-defined outcomes as described in the definition of an SAE.

9.16.4 Relationship

The relationship of each AE to the study drug administration will be assessed by the Investigator after careful consideration, and according to the following guidelines:

Definitely Related:	There is clear evidence that the event is related to the use of the investigational product. An AE that follows a reasonable temporal sequence from administration of a drug (including the course after withdrawal of the drug); that follows a known or expected response pattern to the investigational product.
Reasonably or Possibly Related:	A reaction that follows a reasonable temporal sequence from administration of investigational product; that follows a known or expected response pattern to the investigational product; and/or that could not be reasonably explained by other factors such as underlying disease, complications, concomitant drugs, or concurrent treatments
Unlikely Related:	The temporal sequence from administration of the investigational product suggests that the relationship is unlikely; the response pattern is unlike that of the investigational product (if response pattern is previously known); could be reasonably explained by the subject's clinical state
Not Related:	An AE that does not follow a reasonable temporal sequence from administration of a drug; for which sufficient data exist to indicate that the etiology is unrelated to the investigational product; and/or that can reasonably be explained by other factors, such as underlying diseases, complications, concomitant drugs, and concurrent treatments.

The investigator or designee may change his/her opinion of causality in light of follow-up information, amending the SAE data collection form accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

The Investigator or designee will also record the following for AEs:

- Duration: start and end date and time or continuing
- Action taken, if applicable
- Whether it is an SAE
- Outcome: resolved, continuing, death or unknown

9.16.5 Clinical Laboratory AEs

Many laboratory abnormalities observed during the course of a study will be included under a reported AE describing a clinical syndrome (e.g., elevated BUN and creatinine in the setting of an AE of renal failure, or decreased hemoglobin in a case of bleeding esophageal varices). In such cases, the laboratory abnormality itself (e.g., elevated creatinine in a setting of renal failure) does not need to be recorded as an AE. However, isolated laboratory abnormalities should be reported as AEs if they are considered to be clinically significant by the Investigator.

Criteria for a "clinically significant" laboratory abnormality are:

- A laboratory abnormality that leads to a dose-limiting toxicity (DLT) (e.g., an abnormality that results in study drug dose reduction, suspension or discontinuation).
- A laboratory abnormality that results in any therapeutic intervention (i.e., concomitant medication or therapy).
- Any other laboratory abnormality judged by the Investigator to be of any particular clinical concern (e.g., significant fall in hemoglobin not requiring transfusion).

For laboratory abnormalities that do not meet the above criteria but are outside of normal range (e.g., < or > normal reference range), the Investigator should indicate whether the value is clinically significant or not clinically significant for the subject.

9.16.6 SAEs

9.16.6.1 Definition

An SAE is defined by federal regulation as any AE occurring at any dose that results in any of the following outcomes:

- Death
- Life-threatening AE
- Hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered an SAE when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Hospitalization for a pre-existing condition, including elective procedures, which has not worsened, does not constitute an SAE.

An AE is considered "life-threatening' if, in the view of the Investigator or Sponsor, its occurrence places the subject or subject at immediate risk of death. It does not include an adverse reaction or suspected adverse reaction that, had it occurred in a more severe form, might have caused death.

9.16.6.2 Reporting SAEs

In agreeing to the provisions of this protocol, the Investigator accepts all legal responsibilities for AE identification, documentation, grading, assignment of causality, and prompt notification of SAEs to Elixirgen Therapeutics or designee. All SAEs must be reported to Elixirgen Therapeutics no later than 24 hours after the Investigator recognizes/classifies the event as a

SAE. At a minimum, a description of the event and the Investigator's judgment of causality must be provided at the time of the initial report using the appropriate form (e.g., SAE Report Form). After the initial report, as necessary, the Investigator must provide any additional information on a SAE to the Medical Monitor within two working days after he/she receives that information. This follow-up information will be a detailed written report that will include copies of hospital records, case reports, autopsy reports, and other pertinent documents.

This verbal and faxed report must be followed no later than three working days by a written report **signed by the Investigator**. The information in both the initial report and follow-up report(s) will also be captured. The Sponsor is responsible for submitting the report to all applicable regulatory authorities.

Contact: Kathleen Clarence-Smith, MD, PhD
KM Pharmaceutical Consulting, LLC
1825 K Street NW, Suite 520
Washington, DC 20006
Cell phone: 202-550-1133
Office FAX: 202-223-7004
Email address: kcsmith@kmphc.com

9.16.6.3 Supplemental Investigation of SAEs

The Investigator and supporting personnel responsible for subject care should discuss with the Medical Monitor any need for supplemental investigations of SAEs. The results of these additional assessments conducted must be reported to Elixirgen Therapeutics. If a subject expires during participation in the study and a post-mortem examination is performed, a copy of the autopsy report must be submitted to Elixirgen Therapeutics.

9.16.6.4 Post Study Follow-Up of AEs

All AEs, including a worsening of clinically significant laboratory values or physical examination findings compared with baseline values, must be followed until the event resolves, the condition stabilizes, the event is otherwise explained, or the subject is lost to follow-up.

AEs ongoing at the Exit Visit will be followed for as long as necessary to adequately evaluate the subject's safety or until the event stabilizes or resolves. If resolved, a resolution date should be reported to Elixirgen Therapeutics. The Investigator is responsible for ensuring that follow-up includes any supplemental investigations as may be indicated to elucidate the nature and/or causality of the AE. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals as is practical.

9.16.6.5 Notification of Post Study SAEs

Investigators are not obligated to actively follow subjects after the completion of the study. However, if the Investigator becomes aware of a post-study SAEs occurring up to 14 days following the last visit, it must be reported to Elixirgen Therapeutics, whether or not the event is attributable to study drug. All SAEs must be reported to Elixirgen Therapeutics no later than 24

hours after the Investigator recognizes/classifies the event as an SAE.

9.16.6.6 IRB Notification of SAEs

The Investigator is responsible for promptly notifying the PI's IRB of all SAEs (as per institutional guidelines, including any follow-up information, occurring at the PI's site and any SAE regulatory report, including any follow-up reports that he receives from Elixirgen Therapeutics. Documentation of the submission to the IRB must be retained for each safety report. The Investigator is also responsible for notifying Elixirgen Therapeutics if their IRB requires revisions to the ICF or other measures based on its review of an SAE report.

9.16.6.7 Health Authority Safety Reports

Elixirgen Therapeutics or its representatives will submit a safety report to the Food and Drug Administration (FDA) and/or any other appropriate regulatory agencies for any suspected adverse reaction that is both serious and unexpected within the appropriate time frame.

Elixirgen Therapeutics or its representatives will send copies of each safety report submitted to the FDA and/or other regulatory agencies to the Investigators who are actively participating in Elixirgen Therapeutics-sponsored clinical studies. Safety reports must be submitted to the appropriate IRB as soon as possible. Documentation of the submission to the IRB must be retained for each safety report.

9.16.7 Pregnancy

Any pregnancy that occurs from Screening until study completion must be reported to Elixirgen Therapeutics.

To ensure subject safety, each pregnancy must be reported to Elixirgen Therapeutics within 2 weeks of learning of its occurrence. The pregnancy must be followed up to determine outcome (including premature termination) and status of mother and child.

9.16.8 Treatment-Emergent AEs

All AEs that occur at the time of and following the administration of study drug through the Final Follow-up visit will be considered as being treatment-emergent AEs.

10 STUDY ACTIVITIES

10.1 Screening

The following procedures and assessments will be performed during screening. Results will be documented in the source documents:

- Informed consent
- Demographics
- Medical history
- Baseline disease characteristics*
- Review and documentation of prior medications
- Physical examination
- Height and weight
- Vital signs
- Blood collection for safety labs (hematology, blood chemistry, and coagulation), cytokine panel and telomere length
- Urine collection for urinalysis
- Pregnancy test for females of childbearing potential
- 12-lead ECG
- Eligibility assessments

*: pathogenic mutation and known driver mutation by somatic sequencing including U2AF1, TP53, PPM1D, DNMT3, and TET2 information needs to be collected during the screening if subject does not have genetic information on file (the baseline telomere length sample can be obtained during mobilization [prior to apheresis] if not previously obtained)

10.2 Day 1

Subjects meeting eligibility criteria will be administrated G-CSF dosing for mobilization.

The following procedures and assessments will be performed:

- Review eligibility and confirm if there is no change by the Investigator
- Blood collection for immunogenicity
- Weight
- G-CSF dosing (12 µg/kg s.c.) q12
- Pregnancy test, if applicable
- Review and documentation of concomitant medications
- Review and documentation of AEs

10.3 Day 2

The following procedures and assessments will be performed. Results will be documented in the source documents:

- G-CSF dosing (12 µg/kg s.c.) q12
- Review and documentation of concomitant medications
- Review and documentation of AEs

10.4 Day 3

The following procedures and assessments will be performed. Results will be documented in the source documents:

- G-CSF dosing (12 µg/kg s.c.) q12
- Review and documentation of concomitant medications
- Review and documentation of AEs

10.5 Day 4

The following procedures and assessments will be performed. Results will be documented in the source documents:

- Hospitalization (if not already hospitalized)
- G-CSF dosing (12 µg/kg s.c.) q12
- Review and documentation of concomitant medications
- Review and documentation of AEs
- The baseline telomere length sample can be obtained during mobilization (prior to apheresis) if not previously obtained

A peripherally inserted catheter for access will be placed. (Vein access will be via PIV whenever possible for apheresis.) The insertion of a CVC or PICC will be secondary options when clinically necessary) and apheresis initiated. This can be done on any day prior to Day 5.

10.6 Apheresis Day(s) (5-7): Up to 3 days of apheresis

See section 6.1 for detailed apheresis guidance.

The following procedures and assessments will be performed. Results will be documented in the source documents:

- G-CSF dosing (12 µg/kg s.c.) q12
- Plerixafor 4-6 hours prior to apheresis
- Peripheral CD34+ cell counts
- Assess eligibility for apheresis
- Vital signs
- Apheresis to collect mononuclear cells
- CBC after each apheresis
- Review and documentation of concomitant medications
- Review and documentation of AEs

The eligibility of the apheresis will be assessed. If eligible, apheresis will start.

To evaluate the CD34+ kinetics after Plerixafor dosing, CD34+ cell count in peripheral blood will be measured every 2 hours after each dose of Plerixafor up to apheresis. Kinetics will not be assessed in real time. Only the final pre-apheresis CD34 count will be in real time.

After each apheresis, the feasibility of an additional apheresis will be evaluated by the Investigator.

A. *Subject judged <u>able to</u> tolerate an additional collection.*

Bag(s) containing MNCs collected after each apheresis will be transferred to CCHMC's CML and will be kept in a monitored GMP refrigerator at 2-8 °C. The MNCs should not be kept for longer than 48 hours before starting the labeling and selection procedure of CD34+ cells.

The subject will continue to receive twice daily dosing of G-CSF (12 µg/kg s.c.) and Plerixafor (0.24 mg/kg s.c) will be administered approximately 4 to 6 hours prior to the initiation of an additional apheresis.

To evaluate the CD34+ kinetics after Plerixafor dosing, CD34+ cell count in peripheral blood will be measured every 2 hours after Plerixafor dosing up to apheresis. Kinetics will not be assessed in real time.

The number of CD34+ cells in collected MNCs (total all previously collected apheresis) will be estimated.

- CD34+ cells $< 1.0 \times 10^6$ /kg: subject will have an additional apheresis.
- CD34+ cells $\geq 1.0 \times 10^6$ /kg: subject will continue on study to receive drug dosing.

Post each apheresis, a CBC will be obtained, and the subject will be given RBC or platelet transfusion, per Institution's policy.

B. *Subjects judged <u>unable</u> to tolerate a second collection.*

- CD34+ cells $< 1.0 \times 10^6$ /kg: all collected MNCs will be infused back to the subject per institutional standard procedures. Subjects will be withdrawn from study and will be followed up for safety by phone in 2-4 weeks later.
- CD34+ cells $\geq 1.0 \times 10^6$ /kg: subjects will continue on study to receive study drug dosing.

10.7 Study Drug Administration

The following procedures and assessments will be performed. Results will be documented in the source documents:

- Subjects will receive either oral or IV doses of acetaminophen (650 mg) and diphenhydramine (50 mg) and an IV dose of hydrocortisone (100 mg) prior to study drug dosing. The route of administration for acetaminophen and diphenhydramine will be at the Investigator's discretion.
- Study drug (EXG34217) administration
- Vital signs every 4 hours (per institutional standard)
- Review and documentation of concomitant medications

- Review and documentation of AEs

10.8 Day 8-10: Post Dose Safety Monitoring

Subjects will be monitored for safety for up to 36 hours post dosing. Subject will be discharged on Day 8 or 9, after Investigator has reviewed the safety lab results.

The following procedures and assessments will be performed. Results will be documented in the source documents:

- Vital signs
- Weight
- Blood collection for safety lab, cytokine panel and immunogenicity
- Review and documentation of concomitant medications
- Review and documentation of AEs

After discharge, on a case-by-case basis, for subjects who may experience difficulties traveling back to CCHMC, allowances to have assessments done by their local treating physician will be considered. There will be extensive and close communication between the Investigator and the local treating physician.

10.9 Week 2 (14 days [± 3 days] from Day 1)

At Week 2, safety labs, vital signs measurement, and physical examination will be performed. Concomitant medications and adverse events will be recorded. This will not require a clinic visit and can be done locally. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health nurse). Vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health nurse) is being performed.

10.10 Week 3 (± 3 days)

At Week 3, safety labs, vital signs measurement and physical examination will be performed. Concomitant medications and adverse events will be recorded. This will not require a clinic visit and can be done locally. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health nurse). Vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health nurse) is being performed.

10.11 Week 4/Month 1 (± 3 days)

The following procedures and assessments will be performed. Results will be documented in the source documents:

- Physical Examination
- Vital signs
- Weight
- 12-lead ECG
- Blood collection for safety lab, cytokine panel, immunogenicity, and telomere length
- Review and documentation of concomitant medications
- Review and documentation of AEs

In-between clinical visits, site personnel will contact the subject via a phone call at least once a month to ask and document about any AEs.

10.12 Week 5 (± 3 days)

At Week 5, safety labs, vital signs measurement, and physical examination will be performed. Concomitant medications and adverse events will be recorded. This will not require a clinic visit and can be done locally. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health nurse). Vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health nurse) is being performed.

10.13 Month 2 (± 7 days)

At Month 2, safety labs, vital signs measurement, and physical examination will be performed. Concomitant medications and adverse events will be recorded. This will not require a clinic visit and can be done locally. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health nurse). Vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health nurse) is being performed.

10.14 Month 3 (± 7 days)

The following procedures and assessments will be performed. Results will be documented in the source documents:

- Physical Examination
- Vital signs
- Weight
- 12-lead ECG
- Blood collection for safety lab, cytokine panel, immunogenicity, and telomere length
- Review and documentation of concomitant medications
- Review and documentation of AEs

10.15 Month 4 (± 7 days)

At Month 4, safety labs, vital signs measurement, and physical examination will be performed. Concomitant medications and adverse events will be recorded. This will not require a clinic visit and can be done locally. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health nurse). Vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health nurse) is being performed.

10.16 Month 5 (± 7 days)

At Month 5, safety labs, vital signs measurement, and physical examination will be performed. Concomitant medications and adverse events will be recorded. This will not require a clinic visit and can be done locally. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health nurse). Vital signs measurement will be performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health nurse) is being performed.

10.17 Month 6 (± 14 days)

The following procedures and assessments will be performed. Results will be documented in the source documents:

- Physical Examination
- Vital signs
- Weight
- 12-lead ECG
- Blood collection for safety lab, cytokine panel, immunogenicity, and telomere length.
- Review and documentation of concomitant medications
- Review and documentation of AEs

In-between clinical visits, site personnel will contact the subject via a phone call at least once a month to ask and document about any AEs.

10.18 Month 9 (± 14 days)

At Month 9, safety labs, vital signs measurement, and physical examination will be performed. Concomitant medications and adverse events will be recorded. This will not require a clinic visit and can be done locally. The laboratory assessments (complete blood count, hepatic and renal panel) may be performed at local laboratories or at the local healthcare provider or by home health providers, and the results will then be forwarded to the study PI for assessment. The physical examination may also be performed via a telemedicine visit with the PI (with the assistance of the local healthcare provider or home health nurse). Vital sign measurement will be

performed unless a telemedicine visit (without any assistance of the local healthcare provider or home health nurse) is being performed. Blood will be collected for telomere length as optional per PI's discretion.

10.19 Month 12/Exit Visit (± 14 days)

The following procedures and assessments will be performed. Results will be documented in the source documents:

- Physical Examination
- Vital signs
- Weight
- 12-lead ECG
- Blood collection for safety lab, cytokine panel, pregnancy test (if applicable), immunogenicity, and telomere length
- Urine collection for urinalysis
- Review and documentation of concomitant medications
- Review and documentation of AEs

11 DATA SAFETY MONITORING BOARD (DSMB)

The DSMB is the primary data safety and advisory group for Sponsor. The membership includes two clinicians who are specialized in this disease population and the medical monitor.

The DSMB will review the data on the following schedule (minimum every 6 months when there are active subjects):

- Each of the first 3 adolescent subjects completed 1-month assessments
- The first 6 subjects completed 1-month assessments
- The first 6 subjects completed 6-month assessments
- All 12 subjects completed 1-month assessments
- If any of the following events occur:
  - Any study drug-related SAE in one subject,
  - Allergic reaction, associated with infusion of EXG34217, Grade 3 in two subjects or Grade 4 in one subject,
  - Grade 4 infection following infusion of EXG34217 uncontrolled for >7 days in one subject,
  - Any duration of grade 3 toxicity involving cardiac, pulmonary or neurologic systems,
  - Any other grade 3 toxicity that lasts >72 hours,
  - Any duration of grade 4 toxicity
- Any death that occurs within 30 days of receiving study product, or when determined to be necessary by the DSMB or the Sponsor

The DSMB will specifically review:

- All safety data include AEs, vital signs, weight, standard 12-lead ECG, clinical laboratory test (hematology, blood chemistry, and urinalysis), plasma cytokine panel, immunogenicity,
- Engraftment data by telomere length data

The DSMB will recommend one of the following actions to the Sponsor based on their data review:

a) Discontinue the study (with provisions for orderly discontinuation in accord with good medical practice).
b) Modifications to the study protocol. Modifications may include, but are not limited to, changes in inclusion/exclusion criteria, frequency of visits for safety monitoring, alterations in study procedures, changes in duration of observation and follow up.
c) Continue the study according to the protocol and any related amendments.

In addition to the above, the DSMB will review the engraftment data with safety data after the first 3 patients and will discuss the futility analysis.

A detailed DSMB charter will be finalized prior to the initiation of the study.

12 PLANNED STATISTICAL METHODS

12.1 General Considerations

All collected study data will be presented in subject data listings. Statistical analyses will be performed using SAS® for Windows, version 9.4 or later.

A detailed Statistical Analysis Plan (SAP) will be finalized prior to database lock.

12.2 Determination of Sample Size

Since this study is an initial Phase 1/2 evaluation intended to be hypothesis-raising rather than hypothesis-testing, it is not powered to reliably yield statistically significant conclusions. The data will be analyzed only by means of descriptive statistics (including means and standard deviations, medians and inter-quartile ranges, and tabulations as appropriate) to reveal possible trends that will help guide the design of future, more definitive, clinical trials.

12.3 Analysis Populations

Two subject populations will be evaluated during this study and are defined as follows:

- **Safety Population**: All subjects who receive any study drug.
- **Efficacy Population:** All subjects who receive at least one dose of any study drug and have at least one follow-up efficacy assessment
- **Feasibility Population:** All subjects who enrolled (including subjects who do not received any study drug)

12.4 Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized using appropriate descriptive statistics.

12.5 Safety Analysis

The safety and tolerability of the study drugs will be assessed from:

- Vital signs
- Weight
- Standard 12-lead ECG
- Clinical laboratory test (hematology, blood chemistry, and urinalysis)
- Plasma cytokine panel
- Physical examination
- AEs and SAEs
- Immunogenicity of Sendai virus vector and hZSCAN4 protein

12.6 Exploratory Efficacy Analysis

The exploratory activities of the study drugs will be assessed from:

- Change of telomere length from baseline and in any of the following: lymphocytes, granulocytes, B-cells, naïve T-cells, memory T-cells, and NK cells

- Improvement of blood counts (neutrophils, platelets, or hemoglobin)

Prior to database lock, the methodology that will be used for the efficacy evaluations will be outlined in the final SAP.

12.7 Interim Analysis

No interim analysis is planned for this study.

13 ADMINISTRATIVE CONSIDERATIONS

13.1 Investigators and Study Administrative Structure

The study administrative structure is provided in Table 2.

Table 2. Study Administrative Structure

Sponsor Contact:	Elixirgen Therapeutics, Inc. 855 N. Wolfe Street, Suite 619 Baltimore, MD 21205
Sponsor Representative:	KM Pharmaceutical Consulting LLC 1825 K Street NW, Suite 520 Washington, DC 20006 Phone: 202-223-7001 Martine Francis: Cell 301-343-8894 Minako Koga: Cell 202-615-6004
Medical Monitor:	Kathleen Clarence-Smith, MD, PhD KM Pharmaceutical Consulting, LLC 1825 K Street NW, Suite 520 Washington, DC 20006 Cell: 202-550-1133
Study Monitoring:	KM Pharmaceutical Consulting LLC 1825 K Street NW, Suite 520 Washington, DC 20006 Phone: 202-223-7001 Martine Francis: Cell 301-343-8894 Minako Koga: Cell 202-615-6004
Data Management & Statistical Analysis	Amarex Clinical Research 20201 Century Boulevard Germantown, MD 20874
Safety Clinical Laboratory Testing	Cincinnati Children’s Hospital Medical Center Core Laboratories 3333 Burnet Avenue, MLC 7040 Cincinnati, OH 45229
Cell Manipulations Laboratory	Cincinnati Children’s Hospital Medical Center 3333 Burnet Avenue Cincinnati, OH 45229
Analytical Laboratory	Syneos 2500, rue Einstein Quebec, QC G1P 0A2 Canada
Analytical Laboratory	Repeat Diagnostics 267 W Esplanade, Suite 309 North Vancouver, BC V7N 1A5 Canada

13.2 Regulatory Authority Approval

Elixirgen Therapeutics will obtain approval to conduct the study from the appropriate regulatory agency in accordance with any applicable US regulatory requirements prior to a US site initiating the study.

13.3 Ethical Conduct of the Study and IRB Approval

The study will be conducted in accordance with Good Clinical Practice (GCP). These standards respect the following guidelines:

- Guideline for GCP E6(R2): Consolidated Guideline (International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use, November 2016).
- US CFR dealing with clinical studies (21 CFR parts 50, 54, 56, and 312).
- Declaration of Helsinki, concerning medical research in humans (Ethical Principles for Medical Research Involving Human Subjects)
- Any additional regulatory requirements.

The Investigator (or Elixirgen Therapeutics, where applicable) is responsible for ensuring that this protocol, the site's ICF, and any other information that will be presented to potential subjects (e.g., advertisements or information that supports or supplements the ICF) are reviewed and approved by the appropriate IRB. The Investigator agrees to allow the IRB direct access to all relevant documents. The IRB must be constituted in accordance with all applicable regulatory requirements.

Elixirgen Therapeutics will provide the Investigator with relevant document(s)/data that are needed for IRB review and approval of the study. If the protocol, the ICF, or any other information that the IRB has approved for presentation to potential subjects is amended during the study, the Investigator is responsible for ensuring the IRB reviews and approves these amended documents in accordance with regulatory requirements. The Investigator must follow all applicable regulatory requirements pertaining to the use of an amended ICF including obtaining IRB approval of the amended ICF before any new subject consents to take part in the study under the amended ICF. The IRB approval of the amended ICF/other information and the approved amended ICF/other information must be forwarded to Elixirgen Therapeutics promptly.

13.4 Subject Information and Consent

The study will be conducted in accordance with applicable subject privacy requirements. The proposed ICF, which must be in compliance with applicable regulations, must be reviewed and approved by the IRB and Elixirgen Therapeutics prior to initiation of the study.

The Investigator will be responsible for obtaining written informed consent from potential subjects prior to any study-specific screening for the study. A copy of the signed ICF will be provided to the subject. The original will be retained by the Investigator.

13.5 Confidentiality

13.5.1 Confidentiality of Data

By signing this protocol, the Investigator affirms to Elixirgen Therapeutics that information furnished to the Investigator by Elixirgen Therapeutics will be maintained in confidence and such information will be divulged to the IRB, or similar or expert committee, affiliated

institution, and/or employees only under an appropriate understanding of confidentiality with such board or committee, affiliated institution, and/or employees. Data generated by this study will be considered confidential by the Investigator, except to the extent that it is included in a publication.

13.5.2 Confidentiality of Subject Records

By signing this protocol, the Investigator agrees that Elixirgen Therapeutics (or representative), IRB, or Regulatory Agency representatives may consult and/or copy study documents in order to verify worksheet/case report form data. By signing the consent form, the subject agrees to this process. If study documents will be photocopied during the process of verifying worksheet/case report form information, the subject will be identified by unique code only; full names/initials will be masked prior to transmission to Elixirgen Therapeutics. In addition, the Investigator agrees to treat all subject data used and disclosed in connection with this study in accordance with all applicable privacy laws (i.e., Health Insurance Portability and Accountability Act), rules and regulations.

13.6 Quality Control and Assurance

Elixirgen Therapeutics is responsible for implementing and maintaining quality control and quality assurance systems with written SOPs to ensure that trials are conducted, and data are generated, documented, and reported in compliance with the protocol, accepted standards of GCP, and all applicable federal, state, and local laws, rules and regulations relating to the conduct of the clinical study.

13.7 Data Management

Data management procedures and information for this protocol will be provided by a vender selected by the Sponsor.

13.8 Study Monitoring

In accordance with applicable regulations, GCP, and Elixirgen Therapeutics procedures, clinical monitors will contact the site prior to subject enrollment to review the protocol and data collection procedures with site staff. In addition, the monitor will periodically contact the site, including conducting on-site visits. The extent, nature, and frequency of on-site visits will be based on such considerations as the study objective and/or endpoints, the purpose of the study, study design complexity, and enrollment rate.

During these contacts, the monitor will:

- Check the progress of the study.
- Review study data collected.
- Conduct source document verification.
- Identify any issues and address their resolution.

This will be done in order to verify that the:

- Data are authentic, accurate, and complete.

- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol (and any amendments), GCP, and all applicable regulatory requirements.

The Investigator agrees to allow the monitor direct access to all relevant documents and to allocate his time and the time of his staff to the monitor to discuss findings and any relevant concerns. Upon completion of the study, the monitor will conduct the following activities in conjunction with the Investigator or site staff, as appropriate:

Return of all study data to Elixirgen Therapeutics.

- Data queries.
- Accountability, reconciliation, and arrangements for unused investigational product(s).
- Review of site study records for completeness.

After the final review of the study files, the files should be secured for the appropriate time period as specified in Section 13.9.

13.9 Retention of Data

Documents that individually and collectively permit evaluation of the conduct of the study and the quality of the data produced must be maintained for review by Elixirgen Therapeutics Quality Assurance auditors and by all applicable regulatory authorities. The period of time these documents must be maintained is governed by applicable regulations. Elixirgen Therapeutics or its designee will inform the Investigator when these documents may be destroyed. Elixirgen Therapeutics or its designee must be notified in writing **at least 6 months prior** to the intended date of disposal of any study record related to this protocol to allow Elixirgen Therapeutics to make alternate storage arrangements.

13.10 Financial Disclosure

The Principal Investigator or sub-Investigators named on the Form FDA 1572 will need to complete a financial disclosure form prior to study initiation, at any time during the study execution if new information needs to be disclosed, and for one year after study completion. Investigators should make the IRB aware of any financial interests that the Investigator has in the investigational product.

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Protocol EXG-US-01
Summary of changes made from initial protocol (Version 2.0, May 26,2020)
to current version (Version 7.0, April 3, 2024)

- Revised the study stopping rules.
- Extended follow-up duration from 6 months to 12 months.
- Additional safety assessments (physical examination, vital signs, and clinical laboratory tests) added – weekly for the first month, monthly for 6 months, at 9 months, and at 12 months/exit visit.
- Added pretreatment for allergic reactions.
- Changed the timing of Go/No Go decision for apheresis based on peripheral CD34+ cell count
- Updated the list of pathogenic mutations of telomere biology disorders in Inclusion #4 per the most recent consensus
- Added safety follow-up to early withdrawn subjects after apheresis before study drug administration
- Allowed re-enrollment subjects who withdrew before apheresis
- On a case-by-case basis, for subjects who may experience difficulties traveling back to CCHMC, allowances to have assessments done by their local treating physician were considered. There will be extensive and close communication between the Investigator and the local treating physician.
- Blood sample for telomere length assessment may be collected at Month 9 (optional per PI's discretion).
- Updated exclusion criterion #3 to exclude patients with persistence of severe bone marrow failure defined by one of the following for 3 times at least one month apart:
  - peripheral blood neutrophils (ANC) < 0.5×10^9/L (the use of G-CSF for intermittent neutropenia with associated infections is not excluded);
  - platelets < 20×10^9/L
- Updated exclusion criterion #4 to exclude patients with bone marrow examination within the preceding 3 months indicates clonal cytogenetic abnormalities associated with myelodysplastic syndrome or acute myeloid leukemia, or variances thought to be associated with higher risk of malignant transformation.
- Collected genetic information regarding known driver mutations by somatic sequencing including U2AF1, TP53, PPM1D, DNMT3, and TET2 at the screening visit.
- Allowed up to 3 apheresis procedures to obtain adequate number of CD34+ cells to be collected for subject to be eligible to receive study drug
- Kpetgcued G-CSF dose prior to apheresis to increase mobilization of CD34+ cells
- Ej cpi gf "I /EUH"cpf "r ngtkzchqt "f quci g"\ko kpi "cpf "cdded measurement of CD34+ cell count every 2 hours after plerixafor dosing up to apheresis for CD34+ cell kinetic research purposes only
- Added the time window of sometime within 4 months prior to participating in trial to inclusion criterion #3 for patients meeting specified laboratory values indicative of bone marrow failure
- Updated exclusion criterion (#3) that chronic G-CSF for prevention of recurrent ulcers/fatigue are not excluded
- Updated inclusion criterion (#2) to enroll patients ≥ 12 years of age
- Added the following procedures for adolescent subject (12 to < 18 years of age) cohort
  "For the first 3 subjects, an independent Data and Safety Monitoring Board (DSMB) will review the 30 days of safety monitoring data following study drug administration for each subject before the 1st G-CSF dosing of the subsequent subject. After the first 3 subjects of this population, no staggering period will be required."