nature communications

Article

https://doi.org/10.1038/s41467-025-63137-3

Ataluren improves hematopoietic and pancreatic disorders in Shwachman-Diamond syndrome patients: a compassionate program case-series

Received: 20 January 2025

Accepted: 4 August 2025

Published online: 02 September 2025

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Valentino Bezzerri[1,2], Anna Pegoraro[1], Anca Manuela Hristodor[1], Genevieve M. Crane[3], Ilaria Meneghelli[1], Cecilia Brignole[1], Christian Boni[1], Elena Baldisseri[1], Antonio Vella[4], Giacomo Menichetti[1], Roberto Valli[5], Giovanni Porta[5], Giovanna D'Amico[6], Cristina Tecchio[7], Alice Parisi[8], Giuseppe Lippi[9], Angela Mercuri[10], Simone Cesaro[10], Seth J. Corey[11] & Marco Cipolli[1]

Shwachman-Diamond syndrome (SDS) is characterized by exocrine pancreatic insufficiency, neutropenia, and a high risk of myeloid malignancy. Most patients with SDS harbor nonsense mutations in Shwachman-Bodian-Diamond syndrome gene (*SBDS*), which encodes a ribosome assembly factor. We investigated the translational read-through effect of ataluren in three patients with SDS undergoing a compassionate use program for twelve months. The primary and secondary endpoints were restoring SBDS protein levels in hematopoietic cells and improving myelopoiesis, respectively. SBDS synthesis increased in hematopoietic cells, whereas the bone marrow showed improved cellularity with the maturation of myeloid progenitors. In parallel, absolute neutrophil count was improved in two out of three patients, whereas platelet count increased in all recruited patients. Ataluren treatment normalized mTOR phosphorylation in peripheral blood monocytes and lymphocytes, suggesting a reduction of ribosomal stress. The exocrine pancreatic function also improved. Although the reduced sample size may represent a major limitation of this work, our findings strongly encourages the further clinical development of ataluren to treat SDS.

Shwachman-Diamond syndrome (SDS) is a rare genetic disease that results from biallelic mutations in the Shwachman-Bodian-Diamond syndrome (*SBDS*) gene[1]. SDS involves exocrine pancreatic insufficiency, skeletal abnormalities, cognitive impairment, and neutropenia[2]. Affected individuals may develop bone marrow aplasia or, more frequently, myelodysplastic syndrome and acute myeloid leukemia (AML)[3].

SBDS interacts with elongation factor-like 1 (EFL1), a GTPase that facilitates the release of eukaryotic initiation factor (EIF6) and promotes the assembly of the large and small ribosomal subunits to form functional ribosomes[4]. Failure to produce full-length SBDS induces ribosomal stress, resulting in SDS. The two most common mutations in *SBDS* are c.258+2 T > C and c.183-184TA > CT. The first is a splicing mutation, and the second introduces an in-frame stop codon (K62X). International registries of patients with SDS report that more than 50% of these patients are compound heterozygotes consisting of c.258+2 T > C and c.183–184TA > CT mutations[5].

A full list of affiliations appears at the end of the paper. e-mail: marco.cipolli@aovr.veneto.it

Ataluren[6] is a translational read-through-inducing drug that received conditional approval in Europe for the treatment of Duchenne muscular dystrophy (DMD)[7]. Ataluren inhibits the activity of the termination complex of translation in ribosomes, forcing the suppression of nonsense mutations[8]. Cellular and animal models have demonstrated in vitro translational read-through efficacy of ataluren[9–11]. However, the clinical evaluation of ataluren remains understudied. In DMD, real-world treatment with ataluren delayed disease progression compared to patients undergoing standard care therapy[7,12]. We reported the beneficial effects of ataluren in ex vivo SDS cell models, showing a significant increase in neomorphic SBDS protein followed by an improved myelopoiesis, increased neutrophil differentiation, and reduced expression of biomarkers, including p53 and mTOR[13]. Based on these pre-clinical data, we initiated an ataluren compassionate program in three patients with SDS carrying the c.183-184TA > CT nonsense mutation in *SBDS*.

Results

Safety profile

During treatment, none of the three patients reported adverse events (Supplementary Table 1). The therapy was well-tolerated. Patients did not report any changes in the mutational status of *TP53* in BM cells after 6 months of ataluren treatment. In this context, UPN26 harbored c.701 A > G *TP53* variant in 29.4% of bone marrow cells before initiation of therapy, that however remained stable after treatment (Supplementary Table 2).

Rescue of SBDS protein synthesis and bone marrow cellularity

SBDS protein levels were normalized in bone marrow mononuclear cells (BM-MNC) isolated from UPN26 and UPN58 (Fig. 1a and Supplementary Fig. 1a). Given the decreased bone marrow cellularity of UPN74, western blot analysis was performed on PBMC in this case, showing an appreciable increase in SBDS protein synthesis after either 3 or 6 months of therapy (Supplementary Fig. 1b). Two study-blinded evaluators reviewed the embedded bone marrow tissues. Bone marrow biopsies obtained from UPN26, UPN58, and UPN74 before the initiation of ataluren therapy generally showed hypocellularity and decreased maturation of the hematopoietic lineages (Fig. 1b). Regarding UPN26, unremarkable megakaryocytes and the normal, heterogeneous pattern of trilineage hematopoiesis were better appreciated in the post treatment specimen (many cellular areas, almost 30% cellular), whereas in the pre-treatment, a slightly hyperchromatic megakaryocyte was appreciated with more patchy cellularity (5% to focally 30% cellular). A more normal, heterogenous maturation pattern was appreciated in the post treatment bone marrow of UPN58 (30–40% cellularity), as compared to the sparse cellularity of the pre-treatment (patchy cellularity, 5–20%). Histologic sections of the pre-treatment bone marrow of UPN74 showed only patchy cellularity (focally up to 10%), with the majority of the bone marrow being <5% cellular. In this case, post treatment cellularity was markedly increased (focally to 30%) with appropriate maturation (Fig. 1b). The colony assays consistently indicated a steady increase in myeloid colony formation (Fig. 1c, d and Supplementary Fig. 1c). No appreciable effect was observed on hemoglobin and reticulocytes (see Source Data), even though data from colony assays suggest a partial beneficial role of ataluren also for erythroid lineages (Fig. 1e).

Myeloid differentiation in bone marrow

Flow cytometry analysis confirmed the increased number of granulocytes in BM, parallelled with an improved maturation of myelocytes into neutrophils. UPN26 and UPN58 displayed 12% and 14% of granulocytes in BM before the initiation of therapy, increasing up to 28% and 20%, respectively, after 6 months of treatment. Neutrophil maturation rose from 22% (UPN26) and 13% (UPN58) to 64% and 62%, respectively (Fig. 2a, b). UPN74 showed an already elevated number of granulocytes (59%) in BM at the baseline, that was not affected by the treatment (Fig. 2c). Similarly, myelocyte maturation was stable before and after treatment in this patient.

Leukocytes in peripheral blood

The absolute neutrophil count (ANC) increased to within the normal range in two patients within 9 months of therapy (Fig. 3a, b). The platelet count improved in all three patients treated with ataluren, with values within the normal range in most measurements of UPN58 and UPN74 (Fig. 3c, d). No changes were observed in total leukocyte and monocyte counts after one year of treatment (see Source Data).

Patients with SDS have fewer B cells, mainly because of a severe deficiency in the B memory compartment[14]. In this study, we showed that ataluren restored the normal number of memory B cells ($CD19^+$ and $CD27^+$) in all patients who underwent therapy for 12 months (Supplementary Fig. 2a–c). Ataluren increased the number of non-switched ($CD27^+$ and IgD^+) B memory cells. In parallel, exhausted B memory cells ($CD27^-$, IgD^-) decreased after treatment. The total number of B cells and T lymphocytes did not show substantial changes after 12 months of therapy (see Source Data).

Pancreatic enzymes

All three patients reported exocrine pancreatic insufficiency at enrollment (Table 1). After one year of treatment, the release of pancreatic enzymes fecal elastase-1 and amylase increased. Fecal elastase-1 levels displayed 1.3-, 4.6-, and 2.5-fold increases in patients UPN26, UPN58, and UPN74, respectively (Fig. 4a). Serum amylase levels showed 3.6-, 4.4-, and 7.0-fold increase upon ataluren treatment in UPN74, UPN26, and UPN58, respectively (Fig. 4b).

Phosphorylation of mTOR as ribosomal stress marker

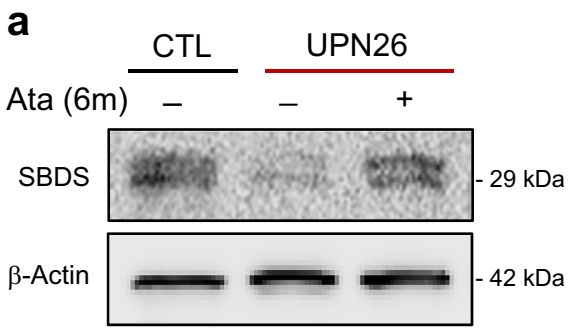
SDS is associated with a hyper-phosphorylation of mTOR Ser2448[15,16] with possible activation of both mTORC1 and mTORC2 complexes. Hyperactivation of mTOR in SDS may result from ribosomal stress. In fact, mTOR can inhibit the translational repressor 4EBP1 and enhance protein synthesis. Activation of mTOR also promotes ribosome biogenesis by inducing the transcription of ribosomal protein genes. This occurs through the activation of specific transcription factors as well as histone acetylation, which facilitates chromatin accessibility and gene expression. At the baseline, we observed a 2.4-fold increase in phospho-mTOR levels in SDS leukocytes compared to healthy donors (Fig. 5, Supplementary Figs. 3–5). After 6 months of treatment, we observed a 34% decrease in mean mTOR levels in white blood cell count (WBC) (Fig. 5a). Phospho-mTOR levels were significantly reduced by 22% in lymphocytes (Fig. 5b), and by 38% in monocytes (Fig. 5c), suggesting a reduction of ribosomal stress. We failed to observe a notable increase in phospho-mTOR levels in SDS neutrophils compared to those in healthy donors (Fig. 5d).

To assess whether the inhibition of mTOR activation could rescue myelopoiesis, we evaluated the effects of the mTOR inhibitors everolimus (RAD001) and dactolisib (NVP-BEZ-235) on myeloid differentiation in bone marrow progenitor cells isolated from patients with SDS (males 64%, mean age 18.3 years, range 7–44) using colony assays (Supplementary Table 3). Inhibition of mTOR did not improve granulopoiesis or erythropoiesis (Supplementary Fig. 6), suggesting that ataluren-induced mTOR activation was a secondary effect due to the restoration of SBDS protein synthesis, followed by improved overall cellular fitness.

Discussion

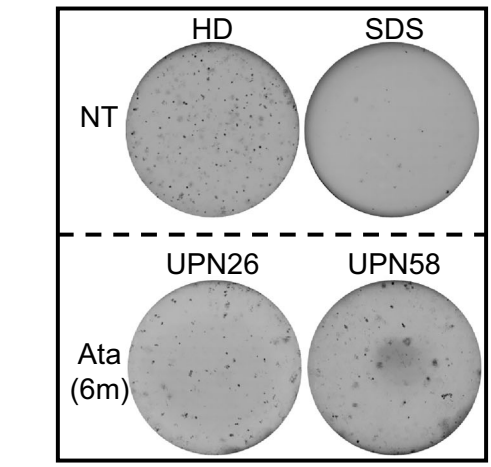
Currently, hematopoietic stem cell transplantation is the only effective therapy; however, it is not required for most patients. Herein, we report the first pharmacological treatment to improve hematopoiesis in SDS through nonsense suppression.

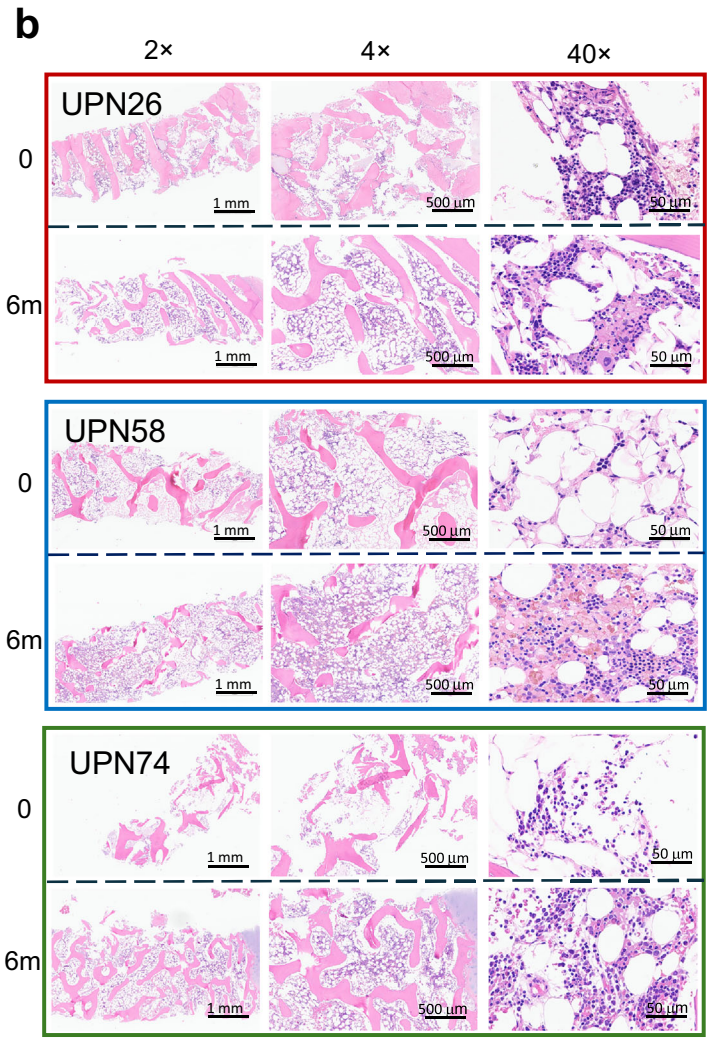
https://doi.org/10.1038/s41467-025-63137-3

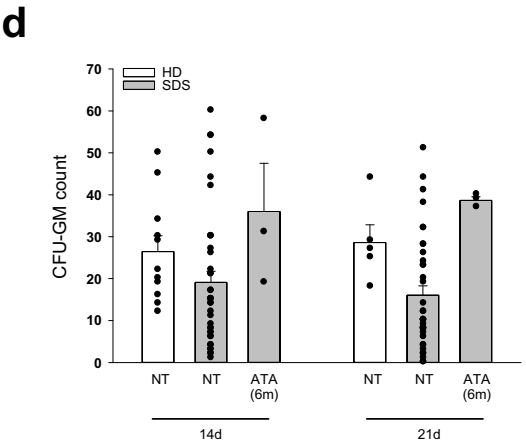


Densitometry analysis (% of β-actin)

	HD	SDS	SDS+Ata	
BM	109	51	95	UPN26
BM	42	14	41	UPN58
PB	265	6	18	UPN74







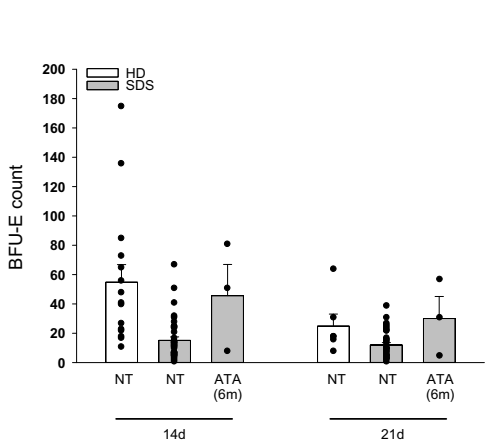


Fig. 1 | Ataluren-dependent restoration of SBDS protein synthesis was followed by improved bone marrow cellularity. Bone marrow biopsies and aspirates were performed in three male SDS patients aged 13 to 20 (mean 16.3) before and after 6 months of therapy (6 m). Total proteins were isolated from BM-MNC and paraffin-embedded bone marrow biopsies were processed for immunohistochemical analysis. Colony assays were performed, seeding 1×10^5 BM-MNC of each patient and healthy donor. **a** Western blot analyses (UPN26, representative) for SBDS before and after ataluren treatment with densitometry analysis. Other western blot analyses are included in Supplementary Fig. 1. **b** Immunohistochemical analysis of bone marrow biopsies collected from UPN26, UPN58, and UPN74 before (0) and after (6 m) therapy. **c** Representative colony assays demonstrating differences between patients with SDS and healthy donors (upper panel) and the effect of ataluren on SDS BM-MNC maturation after 6 months of treatment (lower panel). **d** Counts of myeloid (CFU-GM) colonies obtained from healthy donors (HD, white bars, $n = 11$, males 50%, mean age 38.3 years, range 15–52) and SDS patients at the baseline (NT, gray bars, $n = 22$, males 72%, mean age 18, range 7–44) and after 6 months of ataluren treatment (6 m, $n = 3$) after 14 days and 21 days of incubation. **e** Counts of erythroid (BFU-E) colonies from healthy donors (HD, white bars, $n = 15$) and SDS patients at the baseline (NT, gray bars, $n = 11$) and after 6 months of ataluren treatment (6 m, $n = 3$) after 14 days and 21 days of incubation. Data are mean ± SEM.

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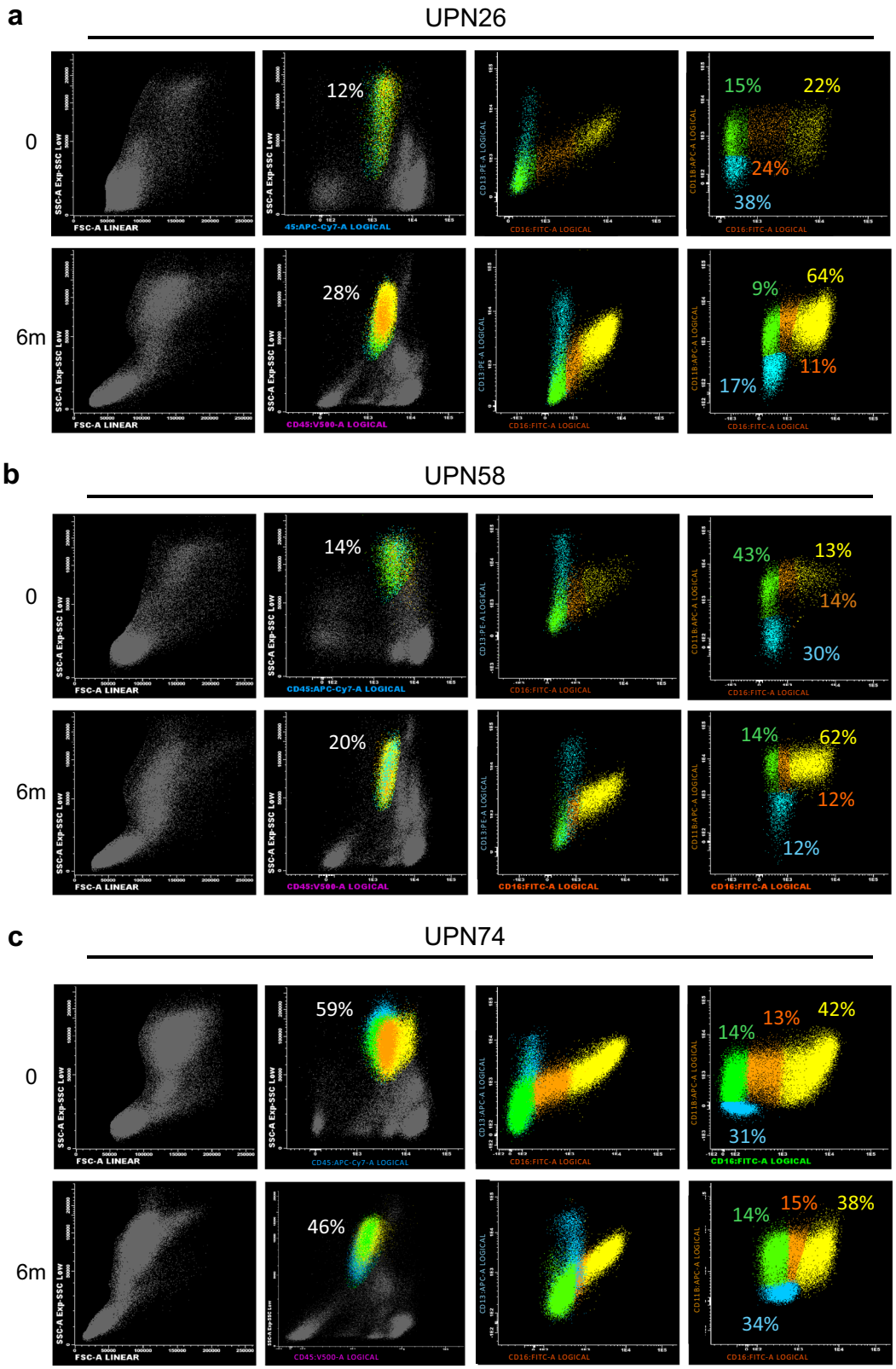

Fig. 2 | Ataluren treatment improved myeloid maturation in bone marrow. Immunophenotype of myeloid lineage was analyzed in whole bone marrow aspirates by flow cytometry ($n = 3$, mean age 16.3, range 13–20). Analysis of bone marrow aspirates obtained from UPN26 (**a**), UPN58 (**b**), UPN74 (**c**) before (0) and after 6 months of ataluren treatment (6 m). Granulocytes (PMN, white) were detected by morphological analyses using CD45 as leukocyte marker. PMN were gated, and promyelocytes (light blue), myelocytes (green), metamyelocytes (orange), and neutrophils (yellow) were detected, staining CD16 and CD11b.

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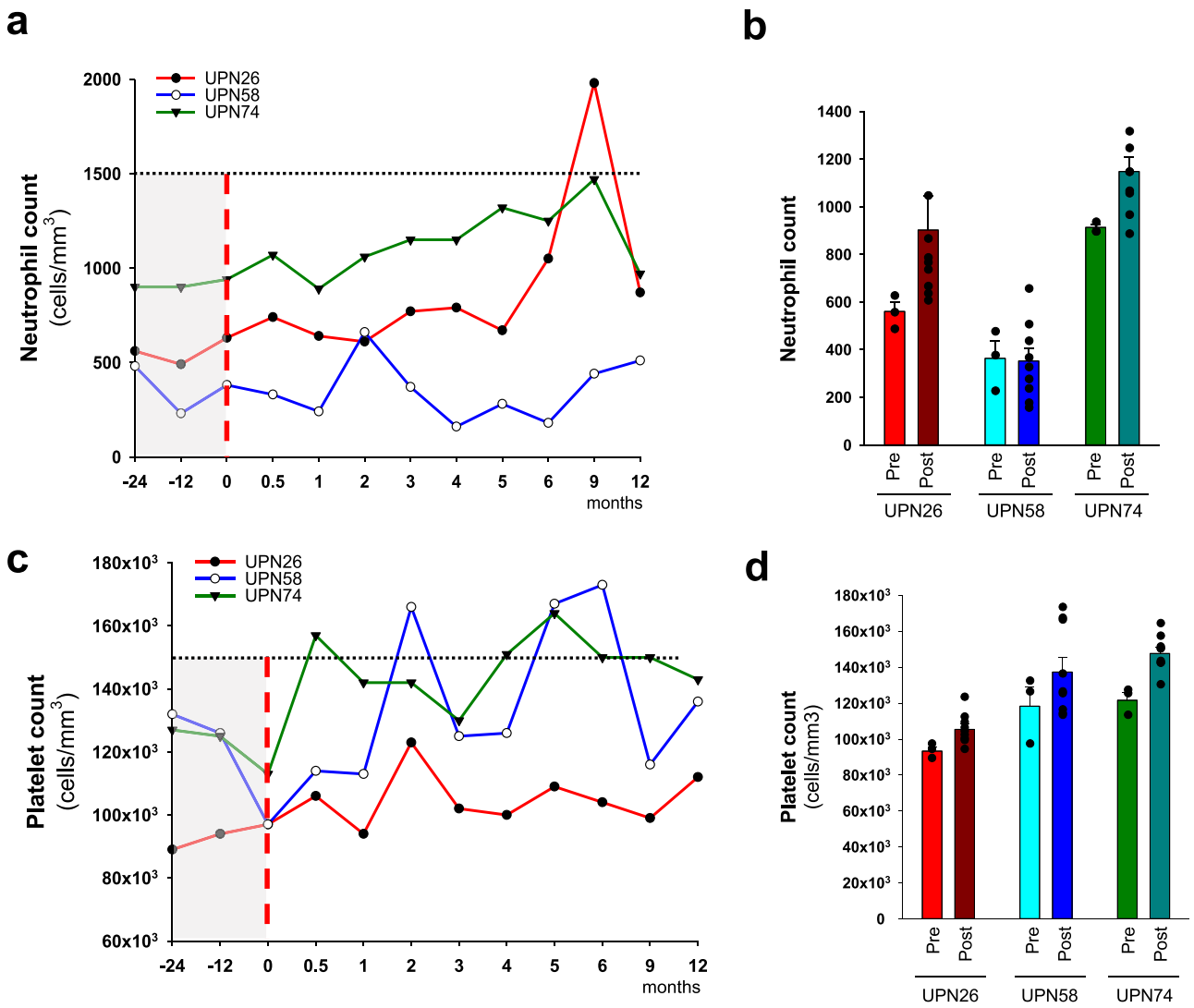

Fig. 3 | Ataluren treatment increased absolute neutrophil and platelet counts in peripheral blood of SDS patients. a Comparison of absolute neutrophil count before (from −24 to 0 months) and during treatment (from 0.5 to 12 months) in UPN26 (red, solid dot), UPN58 (blue, hollow dot), and UPN74 (green, inverted solid triangle). Initiation of therapy is indicated by the red dotted line. **b** Comparison of peripheral neutrophil number before (pre) and after (post) ataluren treatment. Data are mean ± SEM (pre $n = 3$, post $n = 9$). Student's t test has been indicated. **c** Comparison of platelet count before (from −24 to −12 months) and during treatment (from 0.5 to 12 months), as depicted in (**a**). **d** Comparison of peripheral platelet number before and after ataluren treatment. Data are mean ± SEM (pre $n = 3$, post $n = 9$). Mean age 16.3, range 13–20.

Over the past decade, several studies on ataluren in rare diseases caused by nonsense mutations have yielded ambivalent results. However, patients with SDS carry only one specific nonsense mutation (K62X), which reduces the variability of the mutation-dependent efficacy of treatment. Ataluren efficacy can vary in a tissue-dependent manner[17]. Potential determinants of ataluren's efficacy include variable SBDS transcript levels among individuals and mutations in genes involved in ribosome biogenesis. Based on real-world evidence from DMD indicating higher efficacy in younger patients[7], our study comprised three young SDS patients to control for age-dependent variations in drug response.

Here, we found an improvement in both hematopoietic and exocrine pancreatic tissues. Importantly, the primary endpoint of this study measured the direct effect of the drug (i.e., restoration of SBDS protein in the BM and improved hematopoiesis), whereas previous clinical trials for DMD and CF determined only surrogate endpoints, such as cardiopulmonary function measured by the six-minute walk test and respiratory function measured as forced expiratory volume in the first second (FEV1). Interestingly, although the levels of pancreatic enzymes did not normalize, experience from cystic fibrosis and SDS shows that 10–20% of pancreatic gland is enough to allow sufficient intestinal fat absorption. These data therefore suggest the possibility of a reduction, or a suspension, of pancreatic enzyme replacement therapy (PERT) in SDS patients undergoing chronic ataluren treatment, after the assessment of steatorrhea, even though the pancreatic enzymes were not within the normal range.

The major limitation of this study was its small sample size. Considering that SDS is ultra-rare, with an incidence of approximately 1:160,000 live births[18], and that ataluren treatment can be administered only to patients carrying nonsense mutations, three patients should be considered a reliable starting point for designing larger multicenter clinical trials. Furthermore, decreased ribosomal stress due to neomorphic SBDS might reduce the lifetime risk of myeloid malignancies in patients with SDS.

https://doi.org/10.1038/s41467-025-63137-3

Table 1 | Clinical and laboratory data of patients enrolled in the ataluren study

T	BMI	FE-1 (µg/g)	Conc. Med.	WBC (10^9/L)	RBC (10^{12}/L)	Hb (g/dL)	ANC (10^9/L)	PLT (10^9/L)	MCV (fL)	Cytogenetics BM [mitosis]	aCGH
T0	19.4	77.8	Vit, PERT	2.44	4.98	15.1	0.63	97	90	46, *XY* [1]	del(20)(q11.21–q11.23) ~19%
T9	19.4	97.2	Vit, PERT	2.27	4.81	14.9	0.87	112	92.1	46, *XY* [9]	del(20)(q11.21–q11.23) ~19%
T0	16.5	15	Vit, PERT	2.14	4.17	13.2	0.38	97	95.7	46, *XY* [6]	dup(1q)(q21.1–q44) ~16%
T9	16.2	68.5	Vit, PERT	2.33	4.09	13.3	0.51	136	98.8	N/A	dup(1q)(q21.1–q44) ~18%
T0	16.2	14	Vit, PERT	2.88	4.56	14.3	0.94	113	91.2	46, *XY*, del(20)(q11–q13)[2]/46, *XY*[16]	del(20)(q11.22-q13.1) ~10%
T9	17	34.6	Vit, PERT	3.07	4.73	14.9	0.97	143	92.4	46, *XY*, del(20)(q11–q13)[1]/46, *XY*[12]	del(20)(q11.22–q13.1) ~12%

T0 (initiation of therapy), *T9* (12 months of therapy), *M* male, *BMI* body mass index, *FE-1* fecal elastase-1, *Conc. Med.* concomitant medications, *WBC* white blood cell, *RBC* erythrocytes, *Hb* hemoglobin, *ANC* absolute neutrophil count, *PLT* platelet count, *MCV* mean corpuscular volume, *BM* bone marrow, *aCGH* array comparative genomic hybridization, *Vit* vitamin (A,D,E,K) supplementation, *PERT* pancreatic enzyme replacement therapy, *N/A* not available (unsuitable sample).

Thus, the results of this compassionate program case series suggest that further clinical development of ataluren to treat SDS in randomized clinical trials is warranted.

Methods

Patient recruitment

Three male patients aged 13 to 20 (mean 16.3) with SDS (Table 1) of the same genotype (c.183-184TA > CT/c.258+2 T > C) were enrolled for receiving ataluren for 12 months under a compassionate program. For the colony assays, 19 additional patients (Supplementary Table 3) and 15 healthy donors (Supplementary Table 4) were recruited. Written informed consent was obtained from the participants or their legal guardians for participation in the study and for publication of clinical data in agreement with the CARE guidelines and in compliance with the Declaration of Helsinki.

Physician guidance

Patients will be evaluated in the clinic before treatment initiation (0), after 2 weeks (2w), every month (3–6 m) for 6 months, then every 3 months until 12 months of treatment. In addition to routine monitoring (vital signs, height and weight, and physical examinations), hematology laboratory assessment, liver and renal function tests, serum electrolytes, urinalysis, and a review of concomitant medications will be performed at each clinic visit.

Purpose. The objective of this compassionate use is to offer drug access to ataluren to provide a potential clinical benefit to patients diagnosed with SDS who have a high unmet medical need according to the Italian Ministerial Directive (DM) 07.09.2017.

Eligibility. Patients (males, females) with SDS diagnosis carrying the c183-184TA > CT mutation in at least one allele, ≥ 6 years will be eligible to receive the drug (ataluren) for 6 months and depending on the medical judgment, treatment with ataluren may be extended for another 6 months. The patient will start treatment with ataluren only after informed consent has been provided (including the Information Sheet For The Processing Of Personal Information) and if he/she fulfills all inclusion/exclusion criteria.

Inclusion criteria. 1. Patients with a diagnosis of SDS carrying in at least one allele c183-184TA > CT mutation

2. Age ≥ 6 yrs

3. Neutrophil count <1000 PMN/mm^3 at T0 and at least one evaluation performed within 12 months before patient enrollment.

4. Bone marrow evaluation within 12 months of the patient's enrollment

5. Serum total bilirubin within normal limits; serum ALT, AST, or GGT ≤ 2.0 times the ULN, creatinine, BUN within normal limits

6. Evidence of signed and dated informed consent and assent documents (s). Note: If the patient is considered a child under local regulations, the parents or legal guardians must provide written consent, and the patient may be required to provide written consent.

7. In patients who are sexually active, willingness to abstain from sexual intercourse or employ a barrier or medical method of contraception during ataluren administration and the 60-day follow-up period.

8. Able to understand and comply with treatment requirements, restrictions, and instruction (as judged by the treating physician).

Exclusion criteria. 1. Presence of myelodysplasia or leukemia

2. Bone marrow transplantation

3. Blood transfusions in the past 3 months

4. High value (>4%) of HbF according to medical evaluation

6. Neutrophil count <300 PMN/mm^3

7. Serologic evidence of hepatitis B or C, or of HIV

8. Severe abnormalities of pulmonary or renal function

9. Ongoing intravenous (IV) aminoglycoside or IV vancomycin

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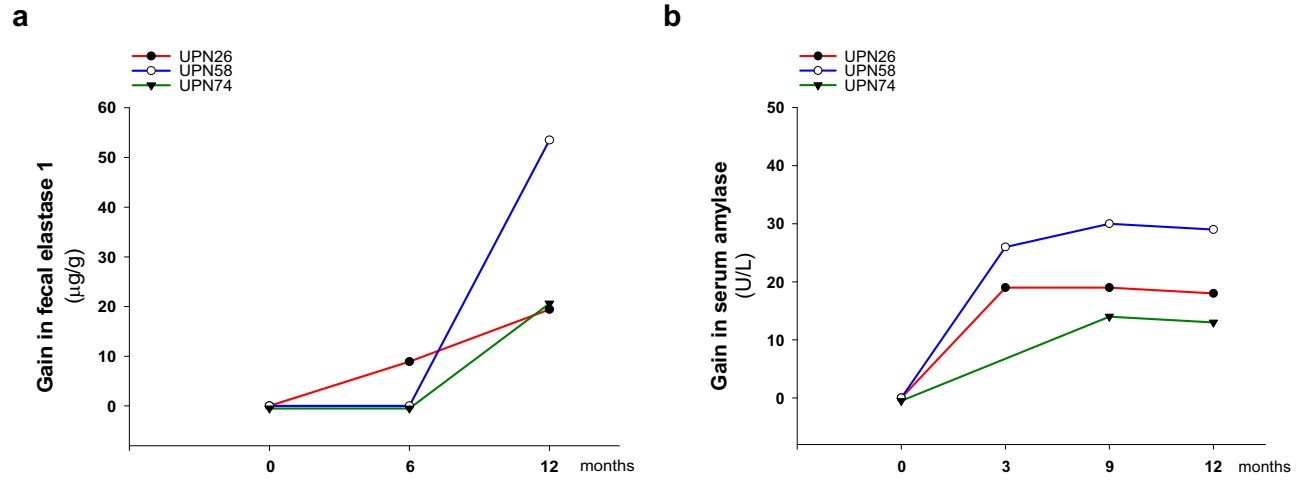

Fig. 4 | Ataluren treatment improved the secretion of pancreatic enzymes including fecal elastase-1 and amylase. a Fecal elastase-1 levels were measured by ELISA in stool samples before (0) and after 6 and 12 months of therapy in UPN26 (red, solid dot), UPN58 (blue, hollow dot), and UPN74 (green, inverted solid triangle) ($n = 3$, mean age 16.3, range 13–20). Data are represented as a gain of Fecal elastase-1 release relative to the baseline. **b** Amylase levels released into serum in each patient throughout 12 months of therapy ($n = 3$, mean age 16.3, range 13–20). Data are represented as a gain of serum amylase release relative to the baseline.

10. Pregnancy or breastfeeding.

11. Ongoing warfarin, phenytoin, or tolbutamide therapy.

12. Hypersensitivity to any of the ingredients or excipients of the study drug (polydextrose, polyethylene glycol 3350, poloxamer 407, mannitol 25C, crospovidone XL10, hydroxyethyl cellulose, vanilla, colloidal silica, or magnesium stearate).

13. History of solid organ or hematological transplantation

14. Prior or ongoing medical conditions (such as concomitant illness, alcoholism, drug abuse, and psychiatric condition), medical history, physical findings, ECG findings, or laboratory abnormalities that, in the investigator's opinion, could adversely affect the safety of the participant, making it unlikely that the course of treatment or follow-up would be completed or could impair the assessment of study results.

15. Inability to understand the informed consent

Eligibility assessments. Eligibility assessments should occur before the administration of ataluren.

The following eligibility assessments should be performed and documented by the treating physician:

1. Review of eligibility criteria

2. Collection of signed informed consent and assent (as applicable)

3. Review of medical history (including SDS genotype)

4. Liver and renal function tests and serum electrolytes (including ALT, AST, GGT, ALP, total bilirubin, cystatin C, BUN, urine protein, serum Na+, serum K+, serum Mg2+, serum Ca2+, serum phosphorous, and serum HCO3−)

5. Urine and serum pregnancy test for females of childbearing potential as judged by the treating physician

6. Complete blood count and peripheral blood immunophenotyping will be performed on the first day of treatment (before the treatment)

7. Review of prior and concomitant medications

Assessment and monitoring during the therapy with ataluren. Treating physicians should refer to the investigator's brochure to summarize the ataluren data. Patients should be evaluated in the clinic on the first day of treatment and after 2 weeks, 1, 2, 3, 4, 5, and 6 months. If treatment with ataluren is prolonged for another 6 months, patients should be evaluated at the third and 6th months of this extension period. In addition to routine monitoring (such as vital signs, height and weight, and physical examinations), additional monitoring may occur, if necessary, by the treating physician.

Ataluren administration and management. The ataluren regimen may be dispensed only under the supervision of the treating physician or an authorized designee and only for administration to the patient participating in this compassionate use. Ataluren will be provided as granules for oral suspension with a white to off-white powder appearance. Drug substances and products are manufactured under cGMP conditions. The formulation includes a matrix, suspending agents, surfactants, and various excipients that aid manufacturing. The granules for oral suspension are packaged in aluminum foil and child-resistant sachets (packets) and supplied with doses of 125, 250, or 1000 mg of the active drug substance. The powder in the sachet may be mixed with water, fruit juice, fruit punch, milk (skim milk, 1% fat, 2% fat, whole milk, chocolate milk, soy milk, or lactose-free milk), or semisolid food (yogurt, pudding, or apple sauce). PTC Therapeutics, Inc. will provide the drug.

Drug kits. Drug kits will be provided, each containing 30 sachets of one dose (125, 250, or 1000-mg). Sachets and cartons will be color coded to indicate the dosage strength (125 mg, brown; 250 mg, green; 1000 mg, dark blue).

Drug dispensation. Ataluren will be administered three times per day (TID). The dose level to be administered is as follows: 10 mg/kg in the morning, 10 mg/kg at mid-day, and 20 mg/kg in the evening

Storage and stability. Ataluren will be shipped and stored at room temperature (approximately 15–30 °C). The available stability data from representative samples supported using the drug product for 48 months when stored at room temperature.

Schedule of administration. Three doses should be administered per day—the 1st dose, 10 mg/kg, in the morning, the 2nd dose, 10 mg/kg, during the middle of the day (mid-day); and the 3rd dose, 20 mg/kg, in the evening. Ideally, each dose should be administered within approximately 30 min before or after a meal (e.g., at approximately 7:00 AM after breakfast, approximately 1:00 PM after lunch, and approximately 7:00 PM after dinner). Intervals for dosing should be ~6 h (±1 h) between morning and mid-day doses, approximately 6 h (±1 h) between mid-day and evening doses, and approximately 12 h (±1 h) between evening doses and the morning dose on the next day.

Instructions for delays in dosing. Dosing delays should be handled as follows: If dosing of ataluren is delayed by ≤1 h, the planned dose should be taken with no changes to the subsequent dose schedules. If ataluren dosing is delayed by >1 h but ≤3 h (or more than 6 h after the evening dose), the planned dose should be taken; however, all future doses for that day should be shifted later by an approximately

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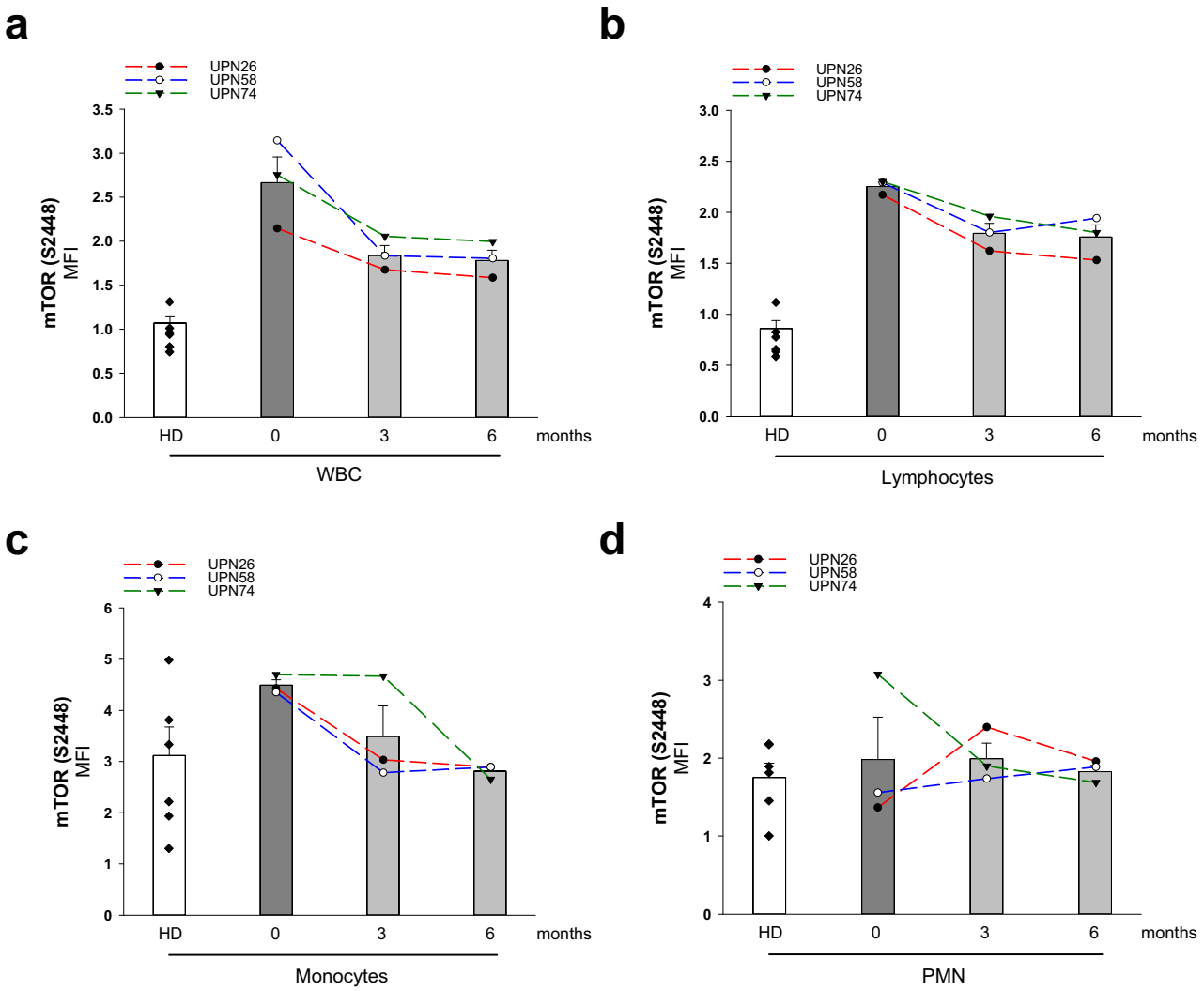

Fig. 5 | Ataluren reduced the levels of phospho-mTOR (S2248) in leukocytes from patients with SDS. Peripheral blood samples were collected from healthy donors (HD, white bars) and SDS patients (gray bars) before (0, dark gray), and after 3 and 6 months of therapy. Phospho-mTOR S2448 levels were analyzed by Phospho-Flow analysis in whole blood. Immunophenotypic markers were used to isolate different leukocyte populations. Data represent phospho-mTOR level in white blood cells (WBC) (**a**), lymphocytes (**b**), monocytes (**c**), and polymorphonuclear granulocytes (PMN) (**d**) of healthy donors (HD, $n = 6$) and SDS patients ($n = 3$, mean age 16.3, range 13–20) UPN26 (red lines, solid dot), UPN58 (blue lines, hollow dot), and UPN74 (green lines, inverted solid triangle). Data are mean ± SEM.

corresponding amount. If ataluren dosing is delayed by >3 h (or >6 h after the evening dose), the dose should not be taken. Ataluren administration may continue, but the missed dose should not be administered, and the planned timing of subsequent study drug dosing should not be altered.

Drug preparation and storage. Ataluren sachets should be stored at room temperature, away from the reach of the children, until reconstitution. They should only be opened at the time of dose preparation. The powder in the sachet may be mixed with water, milk (skim milk, 1% fat, 2% fat, whole milk, chocolate milk, soy milk, or lactose-free milk), or semi-solid food (yogurt, pudding, or apple sauce). The full contents of the sachets should be mixed with at least 30 mL of liquid (water, milk [skim, 1% fat, 2% fat, whole milk, chocolate milk, or lactose-free milk]), or three tablespoons of semi-solid food (yogurt, pudding, or apple sauce). The prepared dose should be thoroughly mixed before administration. The amount of liquid or semi-solid food can be increased based on patient preferences. Each prepared dose is best administered immediately after preparation.

Laboratory abnormalities and adverse events require evaluation and potential drug interruption/modification. During ataluren treatment, the participants will be closely monitored for adverse events or laboratory abnormalities. Renal abnormalities will be studied closely. For adverse events or laboratory abnormalities, treating physicians will use their judgment to determine whether the event or abnormality is clinically significant, whether a diagnostic evaluation is warranted, and whether potential interruption of the study drug treatment is appropriate. In general, life-threatening (grade 4) or severe (grade 3) adverse events or laboratory abnormalities should be considered clinically significant; however, recurrent or persistent moderate events (grade 2) may also be considered clinically significant in certain circumstances. The Common Terminology Criteria for Adverse Events is used to grade the severity of adverse events and laboratory abnormalities.

Concomitant and supportive therapy. Information regarding all concomitant medications will be collected and documented. Drugs metabolized by cytochrome P450 enzymes. As the primary route of ataluren metabolism is glucuronidation by UGT1A9, clinically significant interactions between ataluren and co-administered drugs metabolized by cytochrome P450 enzymes (CYPs) are unlikely. In particular, ataluren is not an inhibitor of CYP1A2, CYP2B6, CYP2C19, CYP2D6, or CYP3A4/5, and does not induce major CYP enzymes. Drugs that are metabolized by CYP2C8 or CYP2C9, which have low therapeutic indices (in particular, paclitaxel for CYP2C8 and coumarin

anticoagulants [such as warfarin], phenytoin, or tolbutamide for CYP2C9), may be of particular concern. Patients who require the use of these drugs will not be enrolled in the study. Coumarin anticoagulants are cleared by CYP2C9, and increases in their plasma concentrations of coumarin anticoagulants may have serious clinical consequences. For patients who require anticoagulation therapy, the use of an alternative form of anticoagulation (e.g., fractionated heparin) should be considered. Phenytoin is metabolized by CYP2C9, and its concomitant use with ataluren may be of potential concern. For patients who require anticonvulsant therapy during the study, the use of alternative anticonvulsant drugs should be considered. The metabolism of losartan to its active metabolites may be mediated, in part, by CYP2C9. However, the concomitant use of losartan and CYP2C9 inhibitors remains unexamined. Because this drug does not have a narrow therapeutic window, the potential for mild-to-moderate changes in activity does not require dose modification.

In vitro studies have shown that ataluren is a substrate for UGT1A9 and breast cancer-resistant protein (BCRP). Caution should be exercised when ataluren is co-administered with drugs that induce UGT1A9 (e.g., phenobarbital and rifampin) or inhibit BCRP (e.g., cyclosporine, eltrombopag, and gefitinib). For patients requiring systemic antibiotic therapy, intravenous aminoglycosides may be administered when necessary. Dietary restrictions. There are no specific dietary restrictions.

Hydration. Because of the potential risk of renal dysfunction during periods of dehydration in patients receiving ataluren, it is important to encourage them to maintain adequate hydration throughout treatment. Participants should be adequately hydrated before receiving any potentially nephrotoxic agent, and their hydration status should be carefully monitored throughout the administration of any agent with nephrotoxic characteristics. Treating physicians should be particularly vigilant of patients who experience nausea, vomiting, diarrhea, fever, or laboratory evidence of dehydration.

AE and SAE documentation and reporting. An AE is defined as any untoward medical occurrence in a patient during treatment; the event does not necessarily have a causal relationship with the treatment. This includes any newly occurring event or worsening of a preexisting condition (e.g., an increase in its severity or frequency) after the ICF and assent, if applicable, is signed. An SAE is any AE that meets any of the following criteria: Fatal (death, regardless of cause, that occurs during participation in compassionate use or death that occurs after participation in compassionate use and is suspected to be a delayed toxicity due to administration of ataluren). Life-threatening, such that the patient was at immediate risk of death from the reaction. Inpatient hospitalization or prolonged hospitalization. Persistent or significant disability/incapacity (disability is defined as a substantial disruption in a person's ability to perform normal life functions). Congenital anomalies or birth defects. An important medical event that, based on appropriate medical judgment, may jeopardize the patient or require medical or surgical intervention to prevent one of the outcomes listed above (e.g., allergic bronchospasm requiring intensive treatment in an emergency room or at home). If a patient had a hospitalization or procedure (e.g., surgery) for an event or condition that occurred before the patient signed the ICF and/or provided assent, and the hospitalization or procedure was planned before the patient signed the ICF and/or provided assent, the hospitalization or procedure should not be considered to indicate an SAE unless an AE caused the hospitalization or procedure to be rescheduled sooner or prolonged relative to what was planned. In addition, hospitalizations not clearly associated with an AE (e.g., social hospitalization for respite care) should not be considered indicative of an SAE.

Completion of safety information collection forms. The treating physician will complete the appropriate safety information collection form and submit it to PTC Therapeutics. Action taken with ataluren. The treating physician will classify the action taken with ataluren with regard to AE.

AE outcome. An AE will be followed up until the treating physician determines the final outcome.

Reporting procedures for SAEs. The institution (Azienda Ospedaliera Universitaria Integrata di Verona) will alert PTC PV via the mailbox pharmacovigilance@ptcbio.com when physicians have given products to patients, so they can track this internally. Institution will ensure that Physician notifies PTC Pharmacovigilance Department ("PTC PV") immediately via the mailbox pharmacovigilance@ptcbio.com within one business day of any serious adverse event (SAE), and to the extent permitted by applicable laws and regulations and to the extent able to do so under the circumstances, will reasonably cooperate with PTC in connection with any reports or filings to the competent authorities related to such SAE. Physicians and institutions will be responsible for submitting safety information according to the local EC requirements. The PTC and the physician shall manage all reports of suspicious adverse reactions according to the standards set out by the DM Salute, April 30, 2015, and manage all relevant information to the Ethics Committee. PTC shall promptly report to the Institution both during the Treatment Plan and for a reasonable period of time after the Treatment Plan is completed, any findings of PTC or its designees that could affect the safety or medical care of the patient, affect the willingness of the patient to continue participation in the Treatment Plan, influence the conduct of the Treatment Plan, or alter the EC's approval to continue the Treatment Plan.

Termination. PTC agrees to provide and deliver sufficient products for the treatment of the patients for 6 months at no cost to the physician and institution, for the sole purpose of treatment of the patients, following the terms and conditions of this agreement (the "Initial Supply"). The PTC shall have no further obligation to supply products and no other financial or payment obligations whatsoever in connection with the patients, the Treatment Plan or this Agreement, except to the extent expressly set forth herein. After the Initial Supply, PTC will supply a product free of charge for an additional 6 months to patients, showing a proven clinical benefit at the end of the observational period. Under no circumstances shall the PTC have any obligation to provide products beyond these additional 6 months. At the end of the 6-month period, the institution shall account for all quantities of product used for the patients and, unless otherwise agreed upon in writing by the parties, shall return or otherwise dispose of any remaining product per the PTC instructions. This compassionate use expires upon the completion of the Treatment Plan, except that any provisions would reasonably be expected to survive such a termination.

Compassionate use will terminate prematurely if:

- The patient withdraws consent and/or discontinues treatment with the product.

- The physician determines that treatment with the product should be withdrawn based on the patient's condition, inability to tolerate the Product or Physician's clinical judgment.

A Patient becomes eligible to participate in a clinical trial program initiated by the PTC in which the Product is the study drug.

- Access to the product is discontinued by competent authorities or the PTC

The product is commercially available in Italy under an approved label for treating SDS.

- Either party materially breaches any terms of the agreement and fails to cure such breach within 30 days of the written notice of the same.

- Physicians are no longer available at the institution to complete the Treatment Plan, and a replacement physician has not been agreed to by the parties within 30 days of notification to PTC that the Physician is no longer available at the institution;

- PTC determines at its sole discretion whether the product is safe or ineffective for the patient.

- The PTC no longer has access to the quantity or quality of products necessary to provide under the agreement.

Review of concomitant medications. The concomitant medication use of the patient should be assessed during each clinic visit.

Review of AEs and serious adverse events (SAEs). Treating physicians should review AEs and SAEs continuously throughout the participation of the patient (from signing the informed consent form [ICF] to assent, if applicable).

Hematological assessment

White blood cell counts with differential, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, total red cell counts with morphology, and platelet count were performed at the section of Clinical Biochemistry, AOUI Verona, using the validated diagnostic procedures. The total white blood cell count (WBC) was determined by Hematology Analyzer XN- 9000 (Sysmex Europe GmbH, Norderstedt, Germany).

Liver and renal function tests, serum electrolytes and urinalysis assessments

Levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (GGT), alkaline phosphatase (ALP), total bilirubin, cystatin C, nitrogenous products (BUN), urine protein, serum Na+, serum K+, serum Mg2+, serum Ca2+, serum phosphorous, and serum HCO3-, glucose, pH, specific gravity, ketones, blood, protein, creatinine, urobilinogen, bilirubin, nitrite, and leukocyte esterase were quantified using clinical chemistry and immunochemistry instruments (Architect C 16000 plus and Architect i2000sr plus; Abbott Diagnostics, Lake Forest, IL, USA).

Bone marrow biopsies

Bone marrow aspirates and biopsies were withdrawn after 6 months of treatment (approximately 12 months after the previous bone marrow biopsy) according to the standard follow-up for patients with SDS validated at AOUI Verona.

Western blot

Total proteins were extracted from the bone marrow (BM-MNC) and peripheral blood (PBMC) mononuclear cells, separated by 11% SDS-PAGE, and electroblotted onto Immobilon P filters (Millipore, Billerica, MA, USA) previously blocked with 5% non-fat milk in TBS (10 mM Tris-HCl pH 7.4, 150 mM NaCl) supplemented with 0.1% Tween-20 (TBS/T). The membranes were probed with: (i) anti-human SBDS rabbit polyclonal IgG antibody clone EPR7820 (Abcam, Cambridge, MA, dilution 1:1000); mouse monoclonal anti-β-Actin clone AC-15 (Sigma-Aldrich, dilution 1:10,000) in 5% non-fat milk TBS/T. Membranes were incubated overnight at 4 °C and then incubated with the secondary antibody horseradish peroxidase(HRP)-coupled goat anti-rabbit IgG (Cell Signaling Technologies, Danvers, MA, dilution 1:10,000) or HRP-conjugated goat anti-mouse IgG (Jackson ImmunoResearch, Cambridge, UK, dilution 1:10,000) for 1 h. Immunocomplexes were detected using the Clarity Max Western ECL Substrate (Biorad, California, USA) and ChemiDoc Imaging System (Biorad). Since we used scarce BM samples from cytopenic patients, we were able to perform only one run for each patient, separately. All uncropped blots are shown in the Source Data file.

mTOR phosphorylation assay

The phosphorylation level of mTOR (S2448) in peripheral blood leukocytes was tested using phospho-flow analysis. Briefly, total leukocytes from peripheral blood withdrawals were fixed and permeabilized using an Intracellular Fixation and Permeabilization Buffer Set (eBioscience, San Diego, CA, USA) following the manufacturer's protocol. After permeabilization, leukocytes were washed once in flow buffer and stained with 5µl monoclonal eFluor 450-conjugated anti-p-S2448-mTOR (eBioscience) or isotype control antibody for 30 min. A 13 colors, 4 lasers DX-Flex flow cytometer (Beckman Coulter) was employed. All acquired data files were analyzed using Kaluza software (Beckman Coulter).

Immunophenotypic analysis

BM cells were stained with the following monoclonal antibodies (mAbs), according to well-established immunophenotypic protocols[19]: CD11b-APC, CD13-PE, CD16-FITC, CD45-APC-Cy7/CD45-V500 (BD Bioscience, Franklin Lakes, NJ, USA). After collection, BM cells were incubated with mAbs combinations for 15 min at room temperature. After the staining, red blood cells were depleted using ammonium chloride solution. Samples were washed and then acquired by a FACSCanto cytometer (BD Bioscience). Data was analyzed with the FlowJo Software version 10 (Tree Star, Inc. Ashland, OR, USA). Blood samples were prepared according to the Clinical and Laboratory Standard Institute H42-A2 "Enumeration of Immunologically Defined Cell Populations by Flow Cytometry, 2nd Edition" Guidelines. Immunophenotyping was performed using a 13-color DX-Flex flow cytometer (Beckman Coulter). The UK Neqas External Quality Assessment "Leukocyte Immunophenotyping" internal quality control was used. The following fluorochrome-conjugated monoclonal antibodies were used: CD3-APC750, CD4-PC7, CD5-PC5, CD8-ECD, CD16-Pacific Blue, CD19-Krome-Orange, CD23-ECD, CD27-PC5.5, CD38-APC750, CD45-APC700, CD56-PC5, and HLA-DR-PE (Beckman Coulter). Peripheral blood lymphocytes were gated into side-scatter (SSlow) and CD45 positive area. Within the lymphocytes, B cells were gated as CD19 positive events and subsequently divided into CD19 + CD5- (B2 or conventional B cells) and CD19 + CD5+ (B1a cells) subpopulations. T cells were defined as CD3+ events and further gated into CD4+ and CD8 + T cells; NK cells were identified as CD3/CD19 double-negative events expressing CD56 and/or CD16. B-cell subsets were separated into CD27− (naive B cells) and CD27+ (memory B cells), or CD23+ (activated B cells). A total of 25,000 events were detected during each run. All acquired data files were analyzed using Kaluza software (Beckman Coulter).

Pancreatic activity test

Levels of fecal elastase 1 (FE-1) and amylase were analyzed in stool samples (500 µg/g of wet stools) and serum, respectively, at baseline and after 6 and 12 months by ELISA (ScheBo, Meridian Bioscience Europe, Milan, Italy). Stool samples were diluted in 50 µl assay buffer [0.1 M Tris·HCl (pH 8.0), 1 mM CaCl2, and 0.05% Tween 20] and added to the ELISA multi-wells containing the immobilized anti-elastase antibody. As blank, 50 µl of assay buffer were used. The plates were incubated at 22 °C for 30 min. After washes, 50 µl of a biotinylated anti-elastase antibody were complexed with peroxidase-conjugated streptavidin and added to the wells. The samples were incubated at 22 °C for 30 min in the dark. After washes, 100 µl of 2,2′-azino-bis (3-thylbenzothiazoline-6-sulfonic acid)-peroxidase substrate solution were added, and samples were incubated at 22 °C for 20 min in darkness. Stop solution (100 µl) was added into each well and incubated for 15 min. The absorbance was measured at 405 nm using a Victor Nivo plate reader (Perkin Elmer, Waltham, MA, USA). Values were expressed as µg/g of wet stool.

Colony assays

BM-MNC freshly isolated from bone marrow biopsies were seeded at a density of 10^5 cell/ml in methylcellulose medium (StemMacs HSC-CFU human, Miltenyi Biotec, Bergisch Gladbach, Germany) supplemented with 20 ng/ml human recombinant G-CSF (Filgrastim). The medium was then supplemented with everolimus (350 nM, Selleckchem, Houston, TX, USA), dactolisib (NVP-BEZ-235, 300 nM, Selleckchem), or DMSO as the vehicle control (1:10000, Merck, Rahway, NJ).

Spontaneous growth of CFU-GM and BFU-E was observed using an Axio Observer 7 (Zeiss, Oberkochen, Germany) inverted microscopy (magnification 5×) and colonies were counted after 7, 14, and 21 days of incubation at 37 °C.

TP53 sequencing

Diagnostic *TP53* gene sequencing and analysis were performed by NGS according to ERIC guidelines[20]. Genomic DNA was first extracted from patient samples using QIAamp® DNA Micro (Qiagen). DNA concentration was quantified using the Qubit™ fluorometric assay (Thermo Fisher Scientific). A total of 200 ng of high-quality genomic DNA was required for library preparation. Sequencing was performed on a MiSeq™ system (Illumina) using 251 × 2 cycles with MiSeq Reagent Kit V3 (600 cycles), according to manufacturer´s instructions. Alignment, base calling and variant annotation were performed with SOPHiA DDM software (SOPHiA Genetics, Saint-Sulpice, Switzerland). Single nucleotide variants, insertions/deletions (indels), copy number variations were detected. The limit of detection for the analysis was 2%.

Statistical analysis

The Shapiro–Wilk test was used to evaluate the normal distribution of samples, which enabled parametric or nonparametric tests to be selected. Independent group determination was performed using a two-tailed Student's *t* test for paired or unpaired data. A $p < 0.05$ was considered statistically significant. Statistical analyses were performed using Sigma Plot V14.0 (Systat Software Inc., San Jose, CA, USA).

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

De-identified patient data and source data are available in the Supplementary Information and the Source Data files. All original clinical data can be obtained by contacting the chief investigator (M.C.). Individual patient data will be made available, in de-identified and anonymized form, within three weeks of a reasonable request. Source data are provided with this paper.

References

1. Boocock, G. R. et al. Mutations in SBDS are associated with Shwachman-Diamond syndrome. *Nat. Genet.* **33**, 97–101 (2003).
2. Nelson, A. S. & Myers, K. C. Diagnosis, treatment, and molecular pathology of Shwachman-Diamond syndrome. *Hematol. Oncol. Clin. North Am.* **32**, 687–700 (2018).
3. Donadieu, J. et al. Classification of and risk factors for hematologic complications in a French national cohort of 102 patients with Shwachman-Diamond syndrome. *Haematologica* **97**, 1312–1319 (2012).
4. Warren, A. J. Molecular basis of the human ribosomopathy Shwachman-Diamond syndrome. *Adv. Biol. Regul.* **67**, 109–127 (2018).
5. Thompson, A. S. et al. Shwachman Diamond syndrome: narrow genotypic spectrum and variable clinical features. *Pediatr. Res.* **92**, 1671–1680 (2022).
6. Welch, E. M. et al. PTC124 targets genetic disorders caused by nonsense mutations. *Nature* **447**, 87–91 (2007).
7. McDonald, C. M. et al. Ataluren delays loss of ambulation and respiratory decline in nonsense mutation Duchenne muscular dystrophy patients. *J. Comp. Eff. Res.* **11**, 139–155 (2021).
8. Huang, S. et al. Ataluren binds to multiple protein synthesis apparatus sites and competitively inhibits release factor-dependent termination. *Nat. Commun.* **13**, 2413 (2022).
9. Sermet-Gaudelus, I. et al. Ataluren (PTC124) induces cystic fibrosis transmembrane conductance regulator protein expression and activity in children with nonsense mutation cystic fibrosis. *Am. J. Respir. Crit. Care Med.* **182**, 1262–1272 (2010).
10. Li, M., Andersson-Lendahl, M., Sejersen, T. & Arner, A. Muscle dysfunction and structural defects of dystrophin-null sapje mutant zebrafish larvae are rescued by ataluren treatment. *FASEB J.* **28**, 1593–1599 (2014).
11. Wang, X. et al. Efficacy of postnatal in vivo nonsense suppression therapy in a Pax6 mouse model of aniridia. *Mol. Ther. Nucleic Acids* **7**, 417–428 (2017).
12. Mercuri, E. et al. Safety and effectiveness of ataluren: comparison of results from the STRIDE registry and CINRG DMD natural history study. *J. Comp. Eff. Res.* **9**, 341–360 (2020).
13. Cipolli, M. et al. Ataluren improves myelopoiesis and neutrophil chemotaxis by restoring ribosome biogenesis and reducing p53 levels in Shwachman-Diamond syndrome cells. *Br. J. Haematol.* **204**, 292–305 (2023).
14. Bezzerri, V. et al. Peripheral blood immunophenotyping in a large cohort of patients with Shwachman-Diamond syndrome. *Pediatr. Blood Cancer* **66**, e27597 (2019).
15. Vella, A. et al. mTOR and STAT3 pathway hyper-activation is associated with evated interleukin-6 levels in patients with Shwachman-Diamond syndrome: further evidence of lymphoid lineage impairment. *Cancers* **12**, 597 (2020).
16. Mayer, C. & Grummt, I. Ribosome biogenesis and cell growth: mTOR coordinates transcription by all three classes of nuclear RNA polymerases. *Oncogene* **25**, 6384–6391 (2006).
17. Thada, V., Miller, J. N., Kovács, A. D. & Pearce, D. A. Tissue-specific variation in nonsense mutant transcript level and drug-induced read-through efficiency in the Cln1(R151X) mouse model of INCL. *J. Cell Mol. Med.* **20**, 381–385 (2016).
18. Minelli, A. et al. Incidence of Shwachman-Diamond syndrome. *Pediatr. Blood Cancer* **59**, 1334–1335 (2012).
19. Stetler-Stevenson, M. et al Clinical flow cytometric analysis of neoplastic hematolymphoid cells. Approved guideline-Second edition. *CLSI document H43-A2* (ISBN 1-56238-635-2) (Clinical and Laboratory Standards Institute, 2007).
20. Malcikova, J. et al. ERIC recommendations for TP53 mutation analysis in chronic lymphocytic leukemia-update on methodological approaches and results interpretation. *Leukemia* **32**, 1070–1080 (2018).

Acknowledgements

This work was partially supported by the Italian Association for Shwachman-Diamond Syndrome (AISS, to V.B., R.V., and M.C.), NIH R01DK132812 (to S.J.C.), Lisa Dean Moseley Foundation, VeloSano, and 2nd Chance 4 Kids. We thank PTC Therapeutics Inc. (NJ, USA) for providing the drug covering manufacturing costs and Fondazione Telethon (Milan, Italy), which contributed to the clinical development of ataluren. We are grateful to Ilaria Cavallari and Laura Bonaldi (Istituto Oncologico Veneto, Padua, Italy) for *TP53* sequencing, Federica Quiri (Cystic Fibrosis Center, Verona, Italy) for excellent technical assistance, Antonella Minelli, Cesare Danesino (University of Pavia, Italy), and Barouk Maurice Assael (Adult Cystic Fibrosis Center, IRCCS Ca' Granda, Milan, Italy) for helpful discussions, Francesca Buniotto and Sandra Perobelli (Cystic Fibrosis Center, Verona, Italy) for their contribution to the psychological assessment of patients during therapy.

Author contributions

V.B. conceived the idea, design the study, analyzed data, secured the funding, and wrote the original manuscript; A.P., G.D., G.M.C., C.T., and S.C. enrolled patients and healthy donors, performed bone marrow biopsies, and analyzed data; A.M.H., C.B., E.B., and G.M. performed ex vivo experiments, including cell cultures, colony assays, and western blots; I.M. contributed to clinical protocol set-up and submitted documents to the local Ethics Committee; C.B. performed nutritional

assessments and analyzed data; A.V. performed flow cytometry for mTOR analyses; R.V. and G.P. performed cytogenetic assays and analyzed data; A.P. performed histological analyses of bone marrow biopsies; G.L. analyzed data and secured funding; A.M. performed bone marrow immunophenotype; S.J.C. reviewed hematology assessments, analyzed data, secured the funding, proofread the English language in the text; M.C. conceived the idea, designed the study, contributed to clinical protocol set-up, supervised the study, secured the funding.

Competing interests

V.B. and M.C. are co-inventors of the patent US 11,207,300 B2 "Method of treatment of Shwachman–Diamond syndrome". All authors declare that they have no additional competing interests.

Ethical approval

The research included local and foreign researchers throughout the research process. All clinical data were analyzed by co-authors after de-anonymization of samples, according to pre-defined sharing measures between the involved research groups. Roles and responsibilities were agreed amongst collaborators ahead of the research. This study was approved by the Ethics Committee of the Azienda Ospedaliera Universitaria Integrata, Verona, Italy (approvals No. 4090 and 4182 CESC). Research did not result in stigmatization, incrimination, discrimination or otherwise personal risk to participants. Local and regional research relevant to this study was taken into account in citations. Participants received no compensation for their involvement in this study.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-025-63137-3.

Correspondence and requests for materials should be addressed to Marco Cipolli.

Peer review information *Nature Communications* thanks Timothy Olson and the other anonymous reviewer(s) for their contribution to the peer review of this work. A peer review file is available.

[1]Cystic Fibrosis Center, Azienda Ospedaliera Universitaria Integrata, Verona, Italy. [2]Department of Life Sciences, Health and Health Professions, Link Campus University, Rome, Italy. [3]Departments of Laboratory Medicine, Cleveland Clinic, Cleveland, OH, USA. [4]Unit of Immunology, Azienda Ospedaliera Universitaria Integrata, Verona, Italy. [5]Department of Medicine and Surgery, Universita' degli Studi dell'Insubria, Varese, Italy. [6]Centro Tettamanti, Fondazione IRCCS San Gerardo dei Tintori, Monza, Italy. [7]Department of Engineering for Innovation Medicine, University of Verona, Verona, Italy. [8]Department of Diagnostics and Public Health, Section of Pathology, University of Verona, Verona, Italy. [9]Department of of Engineering for Innovation Medicine, Section of Clinical Biochemistry, University of Verona, Verona, Italy. [10]Pediatric Hematology Oncology, Ospedale Donna Bambino, Azienda Ospedaliera Universitaria Integrata, Verona, Italy. [11]Departments of Cancer Biology and Pediatric Hematology/Oncology and Stem Cell Transplantation, Cleveland Clinic, Cleveland, OH, USA. ✉e-mail: marco.cipolli@aovr.veneto.it