nature portfolio

Corresponding author(s): Marco Cipolli

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Reporting Summary

Nature Portfolio wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Portfolio policies, see our Editorial Policies and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☐	☒	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☒	☐	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Microsoft 365 Excel
Data analysis	SigmaPlot v. 14.0 (SYSTAT, Palo Alto, CA)

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors and reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Portfolio guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A description of any restrictions on data availability
- For clinical datasets or third party data, please ensure that the statement adheres to our policy

De-identified patient data and source data are available in supplementary information. All original clinical data can be obtained by contacting the chief investigator (M.C.). Individual patient data will be shared in a de-identified and anonymized format, following our data-sharing process.

Research involving human participants, their data, or biological material

Policy information about studies with human participants or human data. See also policy information about sex, gender (identity/presentation), and sexual orientation and race, ethnicity and racism.

Reporting on sex and gender	Sex and gender were not considered in the study design. Sex and/or gender of participants statement was not required. Data reported disaggregated for sex and gender.
Reporting on race, ethnicity, or other socially relevant groupings	Only Italian subjects (Causcasian ethnicity) were recruited in this study.
Population characteristics	Patients (age>6 years) were recruited on the basis of clinical condition (SDS) and genetic analysis (nonsense mutations in SBDS gene), with ANC<1000 PMN/mm3 at the baseline and at least one hematological evaluation performed within twelve months before patient enrollment, and a bone marrow evaluation within 12 months of the patient's enrollment, serum total bilirubin within normal limits; serum ALT, AST, or GGT <=2.0 times the ULN, creatinine, BUN within normal limits.
Recruitment	Three adolescent male patients with SDS carrying the same genotype (c.183-184TA>CT/c.258+2T>C) were enrolled for receiving ataluren for 12 months under a compassionate program. Patients were enrolled on a voluntary basis, without any discrimination based on ethnicity, sex, or religion. Informed consent was obtained from the local Ethics Committee (approval No. 4090 CESC) in agreement with the Declaration of Helsinki. For the colony assays, 22 additional patients were randomly recruited on the basis of scheduled follow-up visits sustained during this study. Informed consent was obtained from the local Ethics Committee (approval No. 4182 CESC).
Ethics oversight	All the clinical protocols were approved by the Ethics Committee of the Azienda Ospedaliera Universitaria Integrata, Verona, Italy.

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Three patients were recruited during this compassionate program. SDS is an ultra-rare disease. In addition, we could recruit only patients carrying nonsense mutations (56%). A statistical analysis to calculate sample size was not applicable. For colony assays, all available SDS patients (n=38, a large cohort considering the rareness of SDS) harboring nonsense mutations, followed at the Cystic Fibrosis Center, Azienda Ospedaliera Universitaria Integrata, Verona, were analyzed.
Data exclusions	No data were excluded from analyses.
Replication	Excluding colony assays, for which multiple tests have been performed on human samples derived from a cohort of SDS patients, all clinical data deriving from the compassionate use program were not replicable and cannot be reproduced. Given the scarce bone marrow and hypocellular samples, we could not replicate western blot analyses before and after treatment. We specified this limitation in the manuscript.
Randomization	Not applicable. A compassionate program was conducted in all three patients enrolled. There was no possibility of randomization.
Blinding	A compassionate program was conducted. All three patients underwent ataluren treatment, with no blinded design.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
[ ]	[x] Antibodies
[ ]	[x] Eukaryotic cell lines
[x]	[ ] Palaeontology and archaeology
[x]	[ ] Animals and other organisms
[ ]	[x] Clinical data
[x]	[ ] Dual use research of concern
[x]	[ ] Plants

Methods

n/a	Involved in the study
[x]	[ ] ChIP-seq
[ ]	[x] Flow cytometry
[x]	[ ] MRI-based neuroimaging

Antibodies

Antibodies used

Anti-SBDS antibody, clone EPR7820, lot GR97873-7, Abcam, cod. Ab128946, dilution 1:1000. Mouse monoclonal anti-beta-actin antibody, clone AC-15, Sigma-Aldrich, cod. A5441, dilution 1:10,000. HRP-conjugated goat anti-rabbit IgG antibody, cod. 7074, Cell Signaling Technologies, Danvers, MA, dilution 1:10,000. HRP-conjugated goat anti-mouse IgG antibody, cod. 115-035-062, Jackson ImmunoResearch, Cambridge, UK, dilution 1:10,000. Mnoclonal antibody (MRRBY), eFluor 450 conjugated anti p-mTOR (S2448) (cat. 48-9718-42, eBioscience). Mouse IgG2a kappa Isotype Control (eBM2a), eFluor 450 conjugated antibody (cat. 48-4724-82, eBioscience). The following high-quality in vitro diagnostic (IVD) and analyte specific reagent (ASR) certified antibodies were used for peripheral blood immunophenotyping: CD3-APC750 (cod. A94680), CD4-PC7 (cod. 737660), CD5-PC5 (cod. A07754), CD8-ECD (cod. 737659), CD16-Pacific Blue (cod. B36292) CD19-Krome-Orange (cod. A96418), CD23-ECD (cod. IM3609U), CD27-PC5.5 (cod. B21444), CD38-APC750 (cod. B49200), CD45-APC700 (cod. A79390), CD56-PC5 (cod. A07789), and HLA-DR-PE (cod. IM1639) (Beckman Coulter, Brea, CA). The following IVD and ASR grade antibodies were used for BM immunophenotyping: anti-CD45-APC-Cy7 (clone 2D1, cod. 348815), anti-CD45-V500 (clone 2D1, cod. 655873), anti-CD16-FITC (clone NKP15, cod. 335035), anti-CD13-PE (clone L138, cod. 347406), anti-CD11b-APC (clone D12, cod. 340936), all purchased from BD Biosciences and used according to the manufacturer's specifications.

Validation

Anti-SBDS antibody was internally validated in KO cells as previously reported (Cipolli et al, Br J Haematol, 2024, doi.org/10.1111/bjh.19134).

Mouse monoclonal anti-beta-actin peroxidase-conjugated antibody clone AC15 was validated by the manufacturer (Sigma Aldrich, St. Louis, MO) and tested in human fibroblasts and chicken fibroblasts (Cross reactivity: sheep, carp, feline, chicken, rat, mouse, Hirudo medicinalis, rabbit, canine, pig, human, bovine, guinea pig (Ref: Eschenburg G. et al. Cancer Res 2012. doi: 10.1158/0008-5472.CAN-11-4072).

The eFluor 450 Mouse IgG2a, K Isotype Control has been validated for flow cytometric analysis in normal human peripheral blood cells by the manufacturer (eBioscience).

eFluor-conjugated anti-mTOR MRRBY antibody was pre-titrated and tested by intracellular staining followed by flow cytometric analysis of stimulated normal human peripheral blood cells by the manufacturer (eBioscience).

All Beckman Coulter and BD antibodies for immunophenotyping were high-quality and IVD/ASR certified (https://www.bdbiosciences.com/en-us/products/reagents/flow-cytometry-reagents/clinical-diagnostics/ASR-single-color-antibodies) (https://www.beckman.it/en/reagents/coulter-flow-cytometry/antibodies-and-kits/single-color-antibodies)

Eukaryotic cell lines

Policy information about cell lines and Sex and Gender in Research

Cell line source(s)	Primary bone marrow mononuclear cells (BM-MNC) were freshly isolated fthrough Ficoll-Plus gradient centrifugation from bone marrow aspirates obtained from SDS patients.
Authentication	Not appicable
Mycoplasma contamination	BM-MNC were mycoplasma free as for internal control by qPCR and IF mycoplasma detection.
Commonly misidentified lines (See ICLAC register)	Not available

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	Not applicable (Compassionate use program).
Study protocol	CRCFC-SDSAUT48
Data collection	Data were collected from June 2023 to September 2024. Data have been collected at the Cystic Fibrosis Center, Azienda Ospedaliera Universitaria Integrata, Verona, Italy.
Outcomes	The primary endpoint of this study was to evaluate the restoration of SBDS protein in the BM that was assessed by western blot. The secondary endpoint was to assess ataluren-dependent improvement of myelopoiesis in bone marrow by immunohistochemistry and increased number of neutrophils and platelets in peripheral blood by clinical biochemistry assessments.

Plants

Seed stocks	*Report on the source of all seed stocks or other plant material used. If applicable, state the seed stock centre and catalogue number. If plant specimens were collected from the field, describe the collection location, date and sampling procedures.*
Novel plant genotypes	*Describe the methods by which all novel plant genotypes were produced. This includes those generated by transgenic approaches, gene editing, chemical/radiation-based mutagenesis and hybridization. For transgenic lines, describe the transformation method, the number of independent lines analyzed and the generation upon which experiments were performed. For gene-edited lines, describe the editor used, the endogenous sequence targeted for editing, the targeting guide RNA sequence (if applicable) and how the editor was applied.*
Authentication	*Describe any authentication procedures for each seed stock used or novel genotype generated. Describe any experiments used to assess the effect of a mutation and, where applicable, how potential secondary effects (e.g. second site T-DNA insertions, mosiacism, off-target gene editing) were examined.*

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	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. The UK Neqas External Quality Assessment “Leucocyte Immunophenotyping” internal quality control (IQC) was used. The following fluorochrome-conjugated monoclonal antibodies were used: CD3-APC750, CD4-PC7, CD5-PC5, CD8-ECD, CD16-Pacific Blue, CD19-Chrome-Orange, CD23-ECD, CD27-PC5.5, CD38-APC750, CD45-APC700, CD56-PC5, and HLA-DR-PE (Beckman Coulter). Whole bone marrow aspirates were depleted from RBC by hypotinic buffer, fixed with Cytofix Fixation Buffer (BD, cod. 554655), then stained with antibodies for surface markers including CD45, CD11b, CD13, and CD16, washed and then analyzed. Phospho-flow analysis of mTOR phosphorylation (S2448) was conducted in whole peripheral blood depleted from RBC by hypotonic buffer. Leukocytes were fixed and permeabilized using the Intracellular Fixation & Permeabilization Buffer Set (ThermoFisher, Waltham, MA), following the manufacturer’s instructions. Subsequently, cells were washed twice in flow buffer (PBS, pH 7.2, with 0.2% BSA and 0.09% sodium azide) and then stained with monoclonal antibody (MRRBY), eFluor 450 conjugated anti p-mTOR (S2448) (cat. 48-9718-42, eBioscience) or mouse IgG2a kappa Isotype Control (eBM2a), eFluor 450 conjugated antibody (cat. 48-4724-82, eBioscience) for 30 min.
Instrument	Peripheral blood: cells were acquired on a 13-color DxFLEX flow cytometer (Beckman Coulter). Bone marrow: Samples were washed and then acquired by a FACSCanto cytometer (BD Bioscience).
Software	Peripheral blood: all acquired data files were analyzed using Kaluza software, version 2.2 (Beckman Coulter). Bone marrow: data were analyzed with the FlowJo Software version 10 (Tree Star, Inc.).
Cell population abundance	A total of 25,000 events were analyzed in each run for BM aspirates and PB samples. A total of 10,000 events were analyzed in each run for Phospho-flow analyses (mTOR).
Gating strategy	Doublets were discriminated by plotting FSC height vs FCS area, before isolating cells on the basis of their forward and side

scatter properties. BM PMN were gated into side-scatter (SShigh) and CD45 positive area. PB lymphocytes were gated into side-scatter (SSlow) and CD45 positive area. Within the PB 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).

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.