iPSC line derived from a Bloom syndrome patient retains an increased disease-specific sister-chromatid exchange activity.

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Lab resource: Stem Cell Line

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Vincent Gatinois[a,b,d,g,1], Romain Desprat[a,c,g,1], Fabienne Becker[a,c,g], Lydiane Pichard[a,b,c,g], Florence Bernex[d,e,g], Bertrand Isidor[f,g], Franck Pellestor[a,b,d,g,*], Jean-Marc Lemaitre[a,b,c,g,*]

[a] IRMB, Univ Montpellier, INSERM, CHU Montpellier, Montpellier France
[b] Laboratory of Genome and Stem Cell Plasticity in Development and Aging, INSERM UMR1183, Montpellier, France
[c] SAFE-iPSC Facility INGESTEM, CHU de Montpellier, Montpellier, France
[d] Laboratory of Cytogenetics, ChromoStem Facility, Univ Montpellier, CHU de Montpellier, Montpellier, France
[e] Institut de Recherche en Cancérologie Montpellier, Univ Montpellier, INSERM, U1194, Montpellier, France
[f] Network of Experimental Histology, Univ Montpellier, BioCampus, CNRS, UMS3426, Montpellier, France
[g] Department of Medical Genetics, CHU Nantes, Nantes, France

A B S T R A C T

Bloom syndrome is characterized by severe pre- and postnatal growth deficiency, immune abnormalities, sensitivity to sunlight, insulin resistance, and a high risk for many cancers that occur at an early age. The diagnosis is established on characteristic clinical features and/or presence of biallelic pathogenic variants in the *BLM* gene. An increased frequency of sister-chromatid exchanges is also observed and can be useful to diagnose BS patients with weak or no clinical features. For the first time, we derived an induced pluripotent cell line from a Bloom syndrome patient retaining the specific sister-chromatid exchange feature as a unique tool to model the pathology.

Resource Table:

Unique stem cell line identifier	*REGUi004-A*
Alternative name(s) of stem cell line	*BLM iPSC*
Institution	*CHU Montpellier, Saint Eloi Hospital*
Contact information of distributor	*Dr. Lemaitre*
Type of cell line	*iPSC*
Origin	*human*
Additional origin info	*Age:18* *Sex: Woman* *Ethnicity: Caucasian*
Cell Source	*blood*
Clonality	*mixed*
Method of reprogramming	*Sendaï*
Genetic Modification	*NO*
Type of Modification	*Spontaneous mutation*
Associated disease	*Bloom syndrome*
Gene/locus	*Gene/locus (NM_00057) Bloom syndrome RecQ like helicase 15q26.*
Method of modification	*NA*
Name of transgene or resistance	*None*
Inducible/constitutive system	*None*
Date archived/stock date	*None*
Cell line repository/bank	*SAFE-iPSC facility IRMB*
Ethical approval	*CPP CHU MONTPELLIER. 2014-A00178-39*

1. Resource utility

Bloom syndrome (BS) is a heterogeneous genetic disease caused by mutations in the *BLM* gene encoding a RecQ-like (REQL) DNA helicase (Cunniff et al., 2017). The specific homozygous mutation of the patient c.2116del generates a reading-frame shift p.(Ser706Leu*11), resulting in an unstable mRNA or non-functional protein.

2. Resource details

Peripheral blood mononuclear cells (PBMC) were obtained from an 18-year-old woman with the specific *BLM* mutation c.2116del. This mutation is located in the gene region encoding the helicase functional domains of the Bloom protein (Fig. 1A). PBMCs were stimulated to proliferate and differentiate into the erythroid lineage with a specific set of human cytokines (StemSpan™ Erythroid Expansion medium). The four reprogramming factors OCT3/4, SOX2, KLF4, and C-MYC were

* Corresponding authors.
E-mail addresses: f-pellestor@chu-montpellier.fr (F. Pellestor), jean-marc.lemaitre@inserm.fr (J.-M. Lemaitre).
[1] Authors contribute equally: Vincent Gatinois and Romain Desprat

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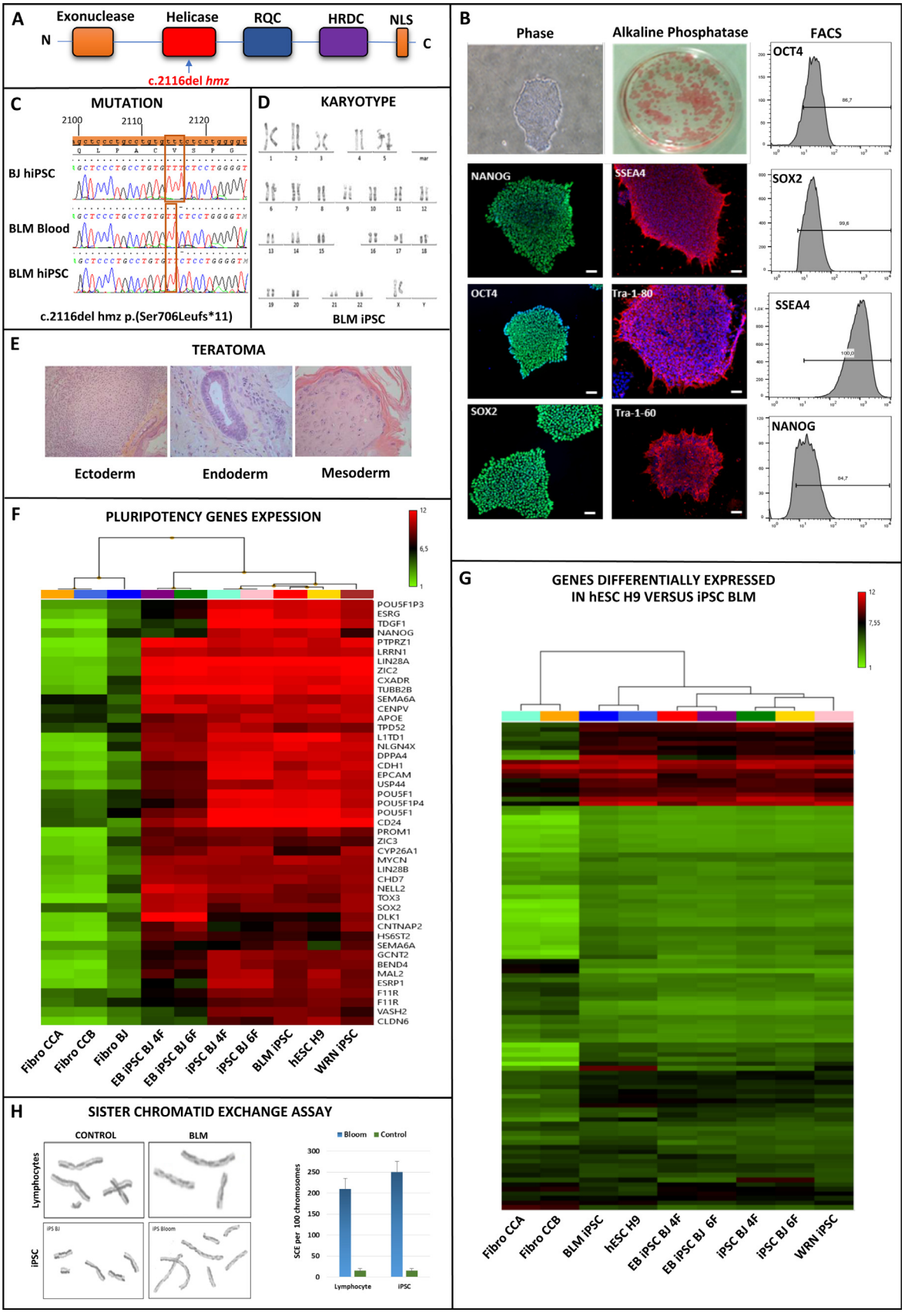

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Fig. 1. BLM iPSC line characterization. 1A. Functional domains of the Bloom protein. Predicted locus of mutation is indicated with an arrow. 1B. BLM iPSC line pluripotency characterization: morphology on light microscopy (phase), alkaline phosphatase essay, pluripotency markers expression revealed by immunocytochemistry staining on fluorescent microscopy (NANOG, OCT4, SOX2, SSEA4, Tra-1-80 and Tra-1-60, scale bar: 50 μm) and FACS analysis (OCT4, SOX2, SSEA4, and NANOG). 1C. BLM mutation locus sequenced by standard Sanger method in a control (BJ hiPSC), the parent cell line (BLM Blood) and the BLM iPSC line (BLM hiPSC). 1D. G-banding karyotyping of BLM iPSC line. 1E. Teratoma formation in immune-compromised mice. Histological sections stained with Hematoxylin-Eosin-Saffron. 1F. Supervised clustered heatmap of pluripotency gene expression. Colored scale from green to red shows the overexpression factor in comparison with the B2M housekeeping gene expression. 1G. Supervised clustered heatmap of upregulated and downregulated genes in hESC and BLM iPSC. Colored scale from green to red shows the overexpression factor in comparison with the B2M housekeeping gene expression. 1H. Sister chromatid exchange assay. Pictures of metaphasis captured with a light microscope of BLM lymphocytes and iPSC, and a gender- and age-matched lymphocytes and iPSC control. Number of SCEs counted per 100 chromosomes for each condition.

transiently expressed using the integration-free Sendai virus gene-delivery method. The resulting BLM iPSC line was alkaline phosphatase positive, showed an expected morphology and expressed pluripotency markers revealed by FACS analysis and by immunocytochemistry staining after a 10-passages in vitro culture (Fig. 1B, scale bar: 50 μm). The BLM iPSC line identity was confirmed by STR analysis in comparison with STR from the parent cells (provided as *Supplementary Table 1*) and by the presence of the homozygous *BLM* mutation (Fig. 1C). The BLM iPSC line exhibited a normal and stable diploid female karyotype 46,XX (Fig. 1D). Pluripotency was confirmed by the differentiation of BLM iPSCs into the three germ layer lineages and the teratoma formation in immune-compromised mice (Fig. 1E, histological sections stained with Hematoxylin-Eosin-Saffron**).** A transcriptomic analysis showed a high pluripotency gene expression (Fig. 1**F,** supervised clustering heatmap, list from Guenther et al., 2015**).** Gene expression profiles were performed on fibroblasts BJ (Fibro BJ), fibroblasts from GEO Samples GSM1566208 (Fibro CCA and CCB), iPSC derived from BJ fibroblasts with 4 reprogramming factors OSKM (IPSC BJ 4F) and 6 factors OSKMNL (iPSC BJ 6F) (Lapasset et al., 2011) and the corresponding Embryoids Bodies harvested at 15 days of differentiation (EB iPSC 4F and EB iPSC 6F), iPSC obtained from the BS patient (BLM iPSC), the human embryonic pluripotent stem cell line H9 (hESC H9) and a Werner syndrome iPSC (WRN iPSC) (Gatinois et al., 2019)). Based on the transcriptomic data, we performed a supervised clustering of genes upregulated and downregulated in hESC versus BLM iPSC. Strikingly, more than 67% of these genes are involved in DNA metabolism (Fig. 1G and *Supplementary Table 2*). Sister-chromatid exchange (SCE) is an allelic exchange of DNA content between two sister chromatids of a chromosome. The number of exchanges can be highlighted by BrdU incorporation during two cell cycles and Giemsa staining. A high rate of SCE was seen in BLM lymphocytes and BLM iPSC line (Fig. 1H) compared to normal lymphocytes control and BJ control iPSC line**.**

3. Materials and methods

3.1. Reprogramming PBMCs into iPSCs

PBMCs were isolated from blood using standard procedures and were cultured for 7 days with SFEM II medium (StemSpan® SFEM II Stemcell, cat#09605) and cytokines (StemSpan® Erythroid Expansion Supplement (100X) Stemcell, Catalog cat#02692) in a 5.10^5 cells/ml concentration. The obtained cells were transduced by Sendai-virus using the CytoTuneR-iPS 2.0 Sendai Reprogramming Kit (Thermo Fisher Scientific, cat#A34546) delivered at a Multiplicity-Of-Infection of 10–10–6 (KOS MOI = 10, hc-Myc MOI = 10, hKlf4 MOI = 6) without polybrene in a final volume of 1–1.5 mL. The incubating suspension was centrifuged at 1000 x g for 30 min at room temperature, resuspended in 1 mL of SFEM II medium with cytokines, and transferred in a 12-well plate. The plate was incubated overnight at 37 °C in a 5% CO_2, 5% O_2 humid atmosphere. Later the medium was modified according to the provider's instructions. After a 3 weeks culture, colonies with an ES-like appearance were manually isolated (Fig. 1B).

3.2. iPSC culture and genomic DNA extraction

iPSC line was maintained on an extracellular Matrigel® matrix (Fisher Scientific, no.354277) with Essential 8TM culture media (Thermo Fisher Scientific, no.A15169-01), according to the provider's instructions. DNA extraction was performed using the QIAamp DNA Mini Kit (Qiagen), following the provider's instructions.

3.3. Sister Chromatid Exchange study

On a 4-day cell culture, 10 μM 5-bromo-2′-deoxyuridine (BrdU) (Roche Applied Science, Mannheim, Germany) was added to the medium. After a 22 h incubation, cells were washed three times with phosphate-buffered saline (PBS, Invitrogen) and mitotic cells were arrested adding a 20 μM final colcemid (Invitrogen) solution for 4 h. Cells were harvested, fixed and spread. The number of SCEs was counted with a light microscope. 35 metaphasis were analyzed per condition. Results are presented as a number of SCE per 100 chromosomes (Fig. 1H).

Table 1

3.4. Flow cytometry analysis

A BD Stemflow Human Pluripotent Stem Cell Transcription Factor® Analysis was performed. The results are presented in Fig. 1B and the antibodies used are in Table 2.

3.5. Immunofluorescence

A StemLightTM Pluripotency Antibody® Kit (Cell Signaling, no.9656) was used. The antibodies panel included Oct-4A, Sox2, Nanog, SSEA4, TRA-1–60, and TRA-1–81. (Table 2)

3.6. Detection of disease-causing mutations in BLM gene

BLM mutations were confirmed by standard Sanger sequencing using the BigDye® Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) on a 3130xl system (Applied Biosystems) (Fig. 1C).

3.7. Karyotyping

Karyotyping was performed with the standard RHG-banding protocol at the ChromoStem® facility of Montpellier, France (Fig. 1D).

3.8. Teratoma formation

The differentiation potency was confirmed by in vivo teratoma production. Clusters of 3×10^6 iPSC cells were subcutaneously injected in the dorsolateral area of two 8 weeks old anesthetized NOD SCID gamma mice (NOD.CgPrkdcscidIl2rg tm1Wjl/SzJ) per iPSC line. After 4–8 weeks of latency, a 100% derivation efficiency was observed and teratomas were fixed, embedded in paraffin blocks, stained with Hematoxylin–Eosin–Saffron and analyzed by a pathologist for the presence of 3 embryonic germ layers structures (Fig. 1E).

Table 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Photography	*Visual record of the line: normal colonies*	*Fig. 1 panel B*
Phenotype	Qualitative analysis (Immunofluorescence and Flow Cytometry)	*Oct4, Nanog, Sox2, Nanog, SSEA4, Tra–1–81, Tra–1–60*	*Fig. 1 panel B*
	Quantitative analysis (Flow Cytometry)	*Oct3/4:100%, Nanog: 100%, SSEA-4: 100%, SOX-2: 100%*	*Fig. 1 panel B*
Genotype	- Karyotype (G-banding)	*46XX, Resolution 450*	*Fig. 1 panel D*
Identity		*DNA profiling*	*Not performed*
	STR analysis	*18 STR were sites tested, and matched between the original cell lines and the reprogramed one*	*Supplementary Table 1*
Mutation analysis (IF APPLICABLE)	Sequencing	*c.2116del hmz p.(Ser706Leufs*11)*	*Fig. 1 panel C*
	Southern Blot or WGS	*Not performed*	*Not available*
Microbiology and virology	Mycoplasma	*Tested by luminescence, as Negative*	*Supplementary Table 2*
Differentiation potential	Teratoma formation*]*	*Detection of the presence of the three-embryo germ layer by histochemistry (validated by a certified Anatomo-Histopathologist)*	*Fig. 1 panel E*
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	*Negative by Elisa analysis*	*Not shown but available with authors*
Genotype additional info (OPTIONAL)	Blood group genotyping	*Not performed*	Not available
	HLA tissue typing	*Not performed*	Not available

3.9. Transcriptomic analysis

Total RNA isolation was performed using the RNeasy mini kit (Qiagen) according to the provider's instructions. RNA was hybridized on an Affymetrix GeneChip Human Genome U133 Plus 2.0 array (Fig. 1F, 1G and *Supplementary Table 2*).

3.10. Short tandem repeat analysis (STR)

Analysis of 16 genetic loci was carried out with a PowerPlex® 16 kit (Promega Corporation) and the GeneMarker® V2.6.7 software (SoftGenetics) (*Supplementary Table 1*).

3.11. Mycoplasma detection

Mycoplasma assay was done with a MycoAlert® Detection Kit (Lonza) according to the provider's instructions (*Supplementary Table 3*).

Declaration of Competing Interest

None

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2019.101696.

Table 2
Reagents details-Bloom iPSC.

Antibodies used for immunocytochemistry/flow-Cytometry	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers Flow cytometry	*Oct-4A Rabbit mAb (Clone C30A3) IgG*	*1:200*	*Cell Signaling Technology Cat# 2840, RRID:AB_2167691*
Pluripotency Markers Flow cytometry	*Sox2 XP® Rabbit mAb (Clone D6D9) IgG*	*1:200*	*Cell Signaling Technology Cat# 3579, RRID:AB_2195767*
Pluripotency Markers Flow cytometry	*Nanog XP® Rabbit mAb (Clone D73G4) IgG*	*1:200*	*Cell Signaling Technology Cat# 4903, RRID:AB_10559205*
Pluripotency Markers Flow cytometry	*SSEA4 Mouse mAb (Clone MC813) IgG3*	*1:200*	*Cell Signaling Technology Cat# 4755, RRID:AB_1264259*
Pluripotency Markers Flow cytometry	*TRA–1–60(S) Mouse mAb (Clone TRA–1–60(S)) IgM*	*1:200*	*Cell Signaling Technology Cat# 4746, RRID:AB_2119059*
Pluripotency Markers Flow cytometry	*TRA–1–81 Mouse mAb (Clone TRA–1–81) IgM*	*1:200*	*Cell Signaling Technology Cat# 4745, RRID:AB_2119060*
Pluripotency Markers Immunostaining	*PE Mouse anti-human Nanog (Clone : N31-355)*	*1:5*	*BD Biosciences Cat# 560791, RRID:AB_1937305*
Pluripotency Markers Immunostaining	*PerCP-CyTM 5.5 Mouse anti-Oct3/4 (Clone : 40/Oct-3)*	*1:5*	*BD Biosciences Cat# 560794, RRID:AB_1937313*
Pluripotency Markers Immunostaining	*Alexa FluorR 647 Mouse anti-Sox2 (Clone : 245610)*	*1:5*	*BD Biosciences Cat# 560301, RRID:AB_1645308*
Pluripotency Markers Immunostaining	*Alexa FluorR 647 Mouse anti-SSEA-4 (Clone : MC813-70)*	*1:5*	*BD Biosciences Cat# 560796, RRID:AB_2033991*
Pluripotency Markers Immunostaining	*PE Mouse IgG1, κ Isotype Control (Clone MOPC-21)*	*1:5*	*BD Biosciences Cat# 554121, RRID:AB_395252*
Pluripotency Markers Immunostaining	*PerCP-Cy5.5 Mouse IgG1, κ Isotype Control (Clone: X40)*	*1:5*	*BD Biosciences Cat# 347202, RRID:AB_400265*
Pluripotency Markers Immunostaining	*Alexa Fluor® 647 Mouse IgG2a, κ Isotype Control (Clone: MOPC-173)*	*1:5*	*BD Biosciences Cat# 558020, RRID:AB_396989*
Pluripotency Markers Immunostaining	*Secondary Antibody Alexa Fluor® 488 conjugate Goat anti-Rabbit IgG*	*1:400*	*Invitrogen-Thermo Fisher Scientific Cat# A-11034, RRID:AB_2576217*
Pluripotency Markers Immunostaining	*Secondary Antibody Alexa Fluor® 555 conjugate Goat anti-Rabbit IgG*	*1:400*	*Invitrogen-Thermo Fisher Scientific Cat# A-21424, RRID:AB_141780*

Primers	Target	Forward/Reverse primer (5′ − 3′)
Bloom mutation (Sanger sequencing)	Bloom mutation	5′TGGCACCAGGGACAATATGC3′/5′ACTGCAAATTTAACTGCTGTGCT3′

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