LETTER TO THE EDITOR

Characterization of a novel *MEF2D-BCL9* fusion-positive acute lymphoblastic leukemia cell line

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CONTENT:

SUPPLEMENTAL TABLE

Supplemental table S1: Overview of used reagents.

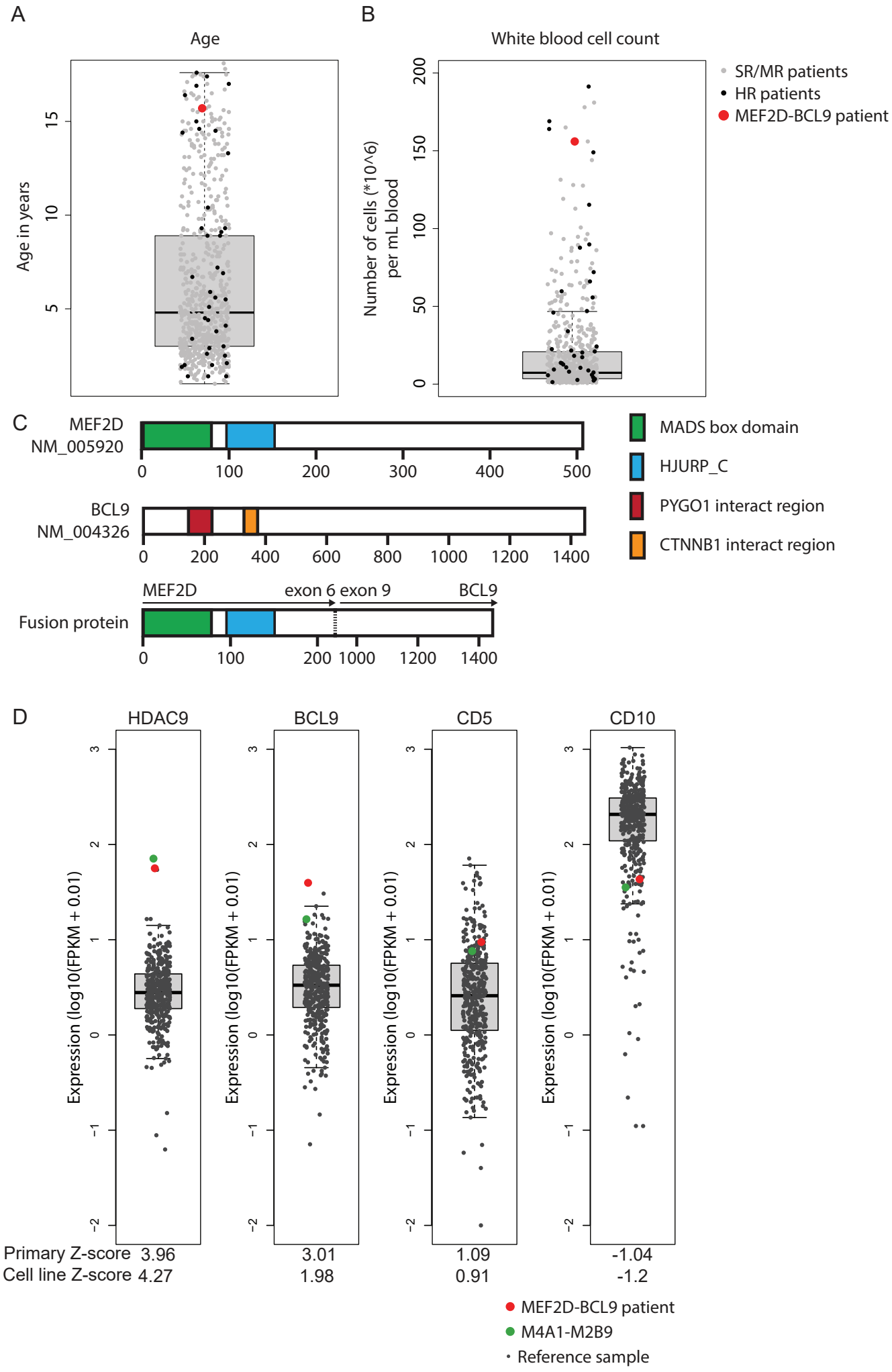
Product	Final concentration or dilution	Company	Category number	Application
TruSeq Stranded Total RNA Library Prep Kit with Ribo-Zero Human/Mouse/Rat	NA	Illumina	RS-122-2201	RNAseq
Novogene NGS DNA Library Prep Set	NA	Novogene	PT004	Whole exome sequencing
Agilent SureSelectXT Reagent Kit + Agilent SureSelect Human All ExonV6	NA	Agilent Technologies	G9611B	Whole exome sequencing
RPMI 1640 medium	NA	ThermoFisher	22409015	Primary medium
RPMI 1640 medium + GlutaMAX	NA	ThermoFisher	61870036	Cell line medium
heat-inactivated fetal calf serum	20% 17% 9%	Sigma Aldrich	130701	Primary medium cell line medium (high FCS) cell line medium (low FCS)
Recombinant human insulin, human transferrin and sodium selenite	NA	Merck	I1884-1VL	Primary medium
Gentamycin	0.2 mg/mL	ThermoFisher	15710049	Primary medium
L-Glutamine	200 mM	ThermoFisher	25030024	Primary medium
Penicillin/Streptomycin (10,000 U/mL)	100 U/mL	ThermoFisher	15140-122	Cell line medium
Amphotericin B (Fungizone) (250 ug/mL)	125 ng/mL	ThermoFisher	15290-026	Cell line medium
RNeasy Mini Kit	NA	Qiagen	74004	RNA isolation
SensiFAST™ cDNA Synthesis Kit	NA	Bioline	BIO-65053	cDNA synthesis
AmpliTaq Gold™ DNA Polymerase with Buffer II and MgCl2	NA	ThermoFisher	N8080241	PCR reaction
Forward primer (MEF2D_exon6) GCAGTTCAGCAATCCCAGC	10 uM	Integrated DNA technologies	*NA*	PCR reaction
Reverse primer (BCL9_exon9) TGGCCACAGTCTTGATAGCA	10 uM	Integrated DNA technologies	*NA*	PCR reaction
Forward primer (GADPH_FW) AAGCTTCCCGTTCTCAG	10 uM	Integrated DNA technologies	*NA*	PCR reaction
Reverse primer (GAPDH_RV) GTCGGAAACGGATT	10 uM	Integrated DNA technologies	*NA*	PCR reaction
TaqMan™ Fast Advanced Master Mix	NA	Thermo ischer	4444964	Q-PCR
Hs01081558_m1 (HDAC9) with FAM-MGB dye	NA	ThermoFischer	4453320	Q-PCR
Brilliant Violet 421™ anti-human CD5 Antibody	1:50	Biolegend	B326250	Immunophenotyping
Brilliant Violet 421™ anti-human CD10 Antibody	1:50	Biolegend	B259324	Immunophenotyping
APC anti-human CD22 Antibody	1:50	Biolegend	B289739	Immunophenotyping
Brilliant Violet 421™ anti-human CD45 Antibody	1:50	Biolegend	B303966	Immunophenotyping
PE anti-human CD19-PE Antibody	1:50	Miltenyi Biotec	5180517935	Immunophenotyping
Brilliant Violet 421™ anti-human CD19 Antibody	1:50	Biolegend	302234	Immunophenotyping ALL survival (ALL detection)
Alexa Fluor® 750 anti-human CD73 Antibody	1:100	R&D systems	FAB5795S	ALL survival (MSC detection)
Alexa Fluor® 750 anti-human CD146 Antibody	1:100	R&D systems	FAB932S	ALL survival (MSC detection)
Alexa Fluor® 750 anti-human Antibody	1:100	R&D systems	FAB6561S	ALL survival (MSC detection)
SytoxGreen	1:100	ThermoFisher	S34860	ALL survival (cell dead)
MTT	5 gr/L	Sigma Aldrich	M2128-5G	Drug and ligand exposure
2-propanol with 0,04 N HCl	NA			Drug and ligand exposure
FLT3L	32 ng/mL	ThermoFisher	PHC9414	Ligand exposure
TSLP	20 ng/mL	R&D systems	1398-TS-010	Ligand exposure
IL7	8 ng/mL	Miltenyi Biotec	130-093-937	Ligand exposure
Prednisolon	1:8 dilution	Hospital pharmacy	NA	Drug exposure
Dexamethasone	1:8 dilution	Hospital pharmacy	NA	Drug exposure
Panobinostat	1:8 dilution	MedChem Express	HY-10224	Drug exposure
PEG-Asparaginase	1:5 dilution	Oncospar	NA	Drug exposure
Vincristine	1:4 dilution	Hospital pharmacy	NA	Drug exposure
Cytarabine/Ara-C	1:4 dilution	Merck	PHR1787-500MG	Drug exposure
Daunorubicin	1:4 dilution	Sanofi	NA	Drug exposure
AR-42	1:4 dilution	Toronto Research Chemicals	735450	Drug exposure
Belinostat	1:10 dilution	MedChem Express	HY-10225	Drug exposure
Dacinostat	1:4 dilution	MedChem	HY-13606	Drug exposure

SUPPLEMENTAL FIGURES LEGENDS

Supplemental Figure S1: **Characteristics of *MEF2D*-fused patient and cell line compared to a representative B-ALL cohort.** (A) Comparison of the age and (B) the number of white blood cells at diagnosis for our *MEF2D-BCL9* fusion positive ALL patient versus the DCOG ALL10 cohort. Our *MEF2D* positive fusion patient is represented in red, the standard or medium (SR/MR) patients in light grey, and the high risk (HR) patients in black. (C) The protein structure of wild-type MEF2D (NM_005920) and the fusion partner BCL9 (NM_004326) as well as the protein structure of the fusion between MEF2D (exon 6, amino acid 222, chr1:156479290, hg38) and BCL9 (exon 9, amino acid 970, chr1:147622271, hg38). Numbers indicate the amino acid positions of the wild-type protein. (D) Boxplots showing the expression of *HDAC9*, *BCL9*, *CD5* and *CD10* in the *MEF2D-BCL9* fused patient and M4A1-M2B9 compared to the control B-ALL cohort (n = 424, derived from various treatment protocols). The red dots represent the expression of the *MEF2D-BCL9* fusion harboring primary material. Bars show IQR ± most extreme data point (max 1.5 x IQR).

Supplemental Figure S2: Sensitivity towards pan-HDAC inhibitors for primary cells and M4A1-M2B9. Primary material and M4A1-M2B9 were cultured for 4 days either in presence or absence of the drug starting at a cell concentration of 1.6x10^6 cells/mL for the primary material and 0.5x10^6 cells/mL for the M4A1-M2B9. Survival was measured using MTT and the metabolic activity was compared to untreated cells. X-axis, concentration of the drug, Y-axis, metabolic activity relative to the untreated cells, graphs show mean ± SD measured in duplo.

Supplemental Figure S1: Characteristics of *MEF2D*-fused patient and cell line compared to a representative B-ALL cohort.



Supplemental Figure S2: Sensitivity towards pan-HDAC inhibitors for primary cells and M4A1-M2B9.