SDC, Materials and Methods

Patient characteristics

Bone marrow (BM) and/or peripheral blood (PB) samples from 29 pedAML patients were evaluated for *TARP* expression (Table S2). Patients were selected based on sample availability (>$50x10^6$ cells) and CD34 expression (≥1%). Patients were treated according to the DB-AML01 protocol (n=9), NOPHO-DBH AML2012 protocol (n=15) or treated otherwise (n=5).

In addition, samples were collected from healthy subjects. Pediatric normal bone marrow (NBM, n=13, 12-18 yr.) was collected from posterior iliac crest during scoliosis surgery. Cord blood (CB, n=15) was obtained after full-term vaginal deliveries. Mobilized peripheral blood stem cells (mPBSCs) were collected from apheresis of adult donors pre-allotransplant (n=4). All healthy subjects and patients and/or their guardians gave informed consent. Approval was issued by the ethical committee in accordance with the declaration of Helsinki (EC2015-1443 and EC2019-0294).

Treatment protocols

De novo pediatric (< 18 yrs.) patients diagnosed with AML between 2010 and 2013 were included in the DB-AML01 trial (EudraCT 2009-014462-26). The treatment protocol of the DB-AML01 study is described elsewhere [1].

Patients diagnosed between 2015 and 2019 were included in the NOPHO-DBH AML2012 trial (EudraCT 2012-002934-35) unless they did not fulfill the inclusion criteria. NOPHO-DBH AML 2012-treated patients received two intensive induction courses, followed by risk-adapted consolidation with three courses of conventional chemotherapy for standard risk (SR) patients and allogeneic stem cell transplantation for high-risk (HR) patients. Patients with isolated central nervous system (CNS) or extramedullary leukemia, previous chemo- or radiotherapy, AML secondary to a previous bone marrow failure syndrome, myeloid leukemia in Down syndrome with age <5 or ≥5 yrs. with GATA1 mutation, acute promyelocytic leukemia (APL), juvenile myelomonocytic leukemia (JMML), myelodysplastic syndrome, Fanconi anemia and/or positive pregnancy test were excluded. Based on these exclusion criteria, 2/27 diagnostic patients i.e. 1 APL, 1 secondary AML evolved from JMML, were treated otherwise, and 1/27 patients (17 yrs.) was treated according to a similar adult protocol.

The definition of HR depends on the treatment protocol. In the DB-AML01 study [1], patients were considered as HR if ≥ 15% blasts persisted after the first induction course and ≤ 5% blasts after the second course (≥5% blasts after the second course was defined as refractory disease). In the NOPHO-DBH AML2012 study, patients were defined as HR if they achieved CR after two induction courses and had (i) *FLT3*-ITD/*NPM1* WT profiles, (ii) poor response after induction 1 (i.e. ≥15% leukemic cells at day 22 or at any subsequent evaluation prior to course 2) or (iii) intermediate response after induction 2 (i.e. 0.1%-4.9% leukemic cells before consolidation) [2].

Outcome definitions

Otherwise-treated patients (n=3), relapsed patients (n=2) and patients treated in the DB-AML01 study (n=9), were excluded from outcome analysis. For estimates of EFS, an event was defined as failure to achieve complete remission (CR), induction death, relapse, development of a second malignancy, or death due to any cause, whichever occurred first. EFS was calculated from date of diagnosis to the date of first event, with failure to achieve CR calculated as an event at t = 0. OS was calculated from date of diagnosis to the date of last follow-up or time of death due to any cause. Follow-up time was censored at the last follow-up visit if no failure was observed.

Cell-sorting of patients samples and healthy controls

Mononuclear cells (MNCs) were isolated from pedAML patients and healthy controls by Ficoll density gradient (Axis-shield). A CD34-enrichment procedure was performed if the number of CD34-positive cells was <50% (CD34 MicroBead Kit, Milteny). MNCs were spinoculated (5 min, 1500 rpm) followed by the addition of monoclonal antibodies (mAb) and incubated in the dark at room temperature (RT) for 20 min (Table S5). After incubation, cell pellets were washed with PBS+2% BSA (5 min, 1500 rpm), resuspended in 50%RPMI/50%FCS and used for cell-sorting on a FACSAria III (BD Biosciences).

All scatters were devoid of cell debris and doublets based on propidium iodide exclusion and FSC-H versus FSC-A plots, respectively. The immature myeloid compartment was defined by CD34, CD45 and scatter properties. CD34+/CD38+ and CD34+/CD38- compartments were gated to isolate control blasts (C-blasts) and hematopoietic stem cells (HSCs) from healthy controls, or leukemic blasts (L-blasts) and leukemic stem cells (LSC) from pedAML patients, respectively. Fluorescence-minus-one (FMO) controls were used to determine the CD38 expression cut-off. Lymphocytes were sorted based on high CD45 expression and low SSC-A properties. Delineated cell populations were backgated on FSC-A/SSC-A and CD45/SSC-A scatter plots to exclude non-specific events and other myeloid precursor populations. Post-sort purities exceeded 90%. Sorted cells were collected in 50%RPMI/50%FCS, spun down (10 min, 3000 rpm, 4° C) and either resuspended in 700 µL TRIzol for RNA extraction (LSCs and L-blasts from pedAML patients, HSCs and C-blasts from healthy controls), or cryopreserved in 90% FCS/10% dimethylsulfoxide (lymphocytes from pedAML patients).
Cell-sorting of CD34+/CD38+ and CD34+/CD38- cells from 29 pedAML patients yielded a total number of 29 L-blast and 24 LSC fractions, respectively. In addition, CD34+/CD38+ sorting of NBM (n=13) and CB (n=15) yielded a sufficient number of C-blasts for RNA isolation (n=28). The number of sorted HSCs was only sufficient in 13/15 CB and 7/13 NBM samples, and therefore complemented with four CD34+/CD38- fractions sorted from mobilized peripheral blood (mPBSC=4, total of 25 HSC fractions).

Tetramer staining

An in-house developed tetramer and membrane staining protocol was used, based on previous literature and in-house optimization. The volume of cells needed to stain 50 000 cells was isolated and washed with 500 µL Hank's Balanced Salt Solution buffer (HBSS, GIBCO®) supplemented with 1%FCS (1500 rpm, 5 min). After spinoculation, supernatant was removed, pellets were resuspended at a final dilution of 50 000 cells in 30 µL and stained with 5 µL 1/75 diluted PE-label tetramers, or 5 µL HBSS/1%FCS in case of FMO controls, for 30 min at RT in the dark. Surface staining immediately proceeded tetramer labeling, using an antibody cocktail of mAb against CD3, CD8, CD45 and HLA-A2 combined with a LIVE/DEAD (L/D) staining (Table S5). Samples were further incubated for 20 min at RT in the dark, washed with 1 mL HBSS/1%FCS medium (1500 rpm, 5 min) and subsequently measured on a FACSCanto II (BD Biosciences, San Jose, CA, USA) flow cytometer, equipped with three solid-state lasers, and instrument set-up performed strictly according to EuroFlow guidelines [3]. Retroviral transduced TARP-TCR transgenic T-cells, manufactured as previously described [4], were used as positive control.

Gating was performed using Infinicyt software v.1.8 (Cytognos, Salamanca, Spain). All scatters were devoid of doublets based on FSC-H/FSC-A, and living cells were selected based exclusion of the L/D marker. Lymphocytes were selected based on SSClow/FSClow/CD45++ expression, and cytotoxic T-cells (CTLs) were subsequently gated based on CD3+/CD8+ expression. Tetramer positivity was judged based on sample-specific FMO controls. Delineated tetramer-positive events were backgated

on FSC-A/SSC-A and CD45/SSC-A scatter plots to exclude non-specific events. All samples were analysed in duplicate (technical duplicates), and 5/12 samples (n=4/11 pedAML patients) were analysed in two different experiments, starting from the same batch of cryopreserved cells (biological duplicates).

mRNA sequencing experimental settings and data analysis

Wild-type (WT) cell lines MV4;11, HL-60, THP-1 and OCI-AML3 were purchased at ATCC or DMSZ, and grown in RPMI medium (Invitrogen) supplemented with 10% or 20% Fetal Calf Serum (FCS, Hyclone or ThermoFisher Scientific), according to supplier instructions, together with 100 U/mL Penicillin/Streptomycin (10000 U/ml, Invitrogen) and 100 µg/mL L-Glutamine (200 mM, Invitrogen). For THP-1, medium was additionally supplied with 0.05 mM β-mercaptoethanol. Cell lines were incubated at 37 °C in 5% CO_2 incubators.

Total RNA was extracted from $1x10^6$ wild-type and transgenic cell lines using the miRNeasy Mini or Micro Kit (Qiagen) in combination with on-column DNase I digestion (RNase-Free DNase set, Qiagen). For each condition (wild-type, knockdown and mock) and cell line (OCI-AML3, HL-60, THP-1 and MV4;11), three biological replicates were used, except for the mock where two biological replicates were used for technical reasons. RNA concentrations were measured by Nanodrop and fulfilled the following criteria to pass quality control: A260/A280 ratio [1.8 - 2.1] and A260/A230 ratio [1.8 -2.0], as measured by Nanodrop, and RNA integrity number (RIN) >8.0, as measured by Agilent 2100 Bioanalyzer. RIN ranged between 8.2 – 10.0, with a median value of 9.9 (95% CI 9.8 – 9.9).
Library prep and sequencing was performed by the VIB Nucleomics Core Leuven on a HiSeq 3000/HiSeq 4000 System, using the TruSeq and Illumina NextSeq500 High Output 75 kits, respectively. Pools were analysed in two NextSeq500 single-end 75bp runs, increasing the number of pass filtered (PF) reads (~800M PF reads + single-end 75bp reads), yielding very high quality data (>95% Avg%Q30). The data of raw counts were merged with the reference gene Homo sapiens Ensembl.GRCh38.88 annotations. Genes with less than one counts-per-million (absent genes) were removed, retaining 17585 genes. A within- and between-sample normalization was performed to avoid sample-specific effects. Within samples, GC-content was corrected by full quantile normalization on bins of GC-content (EDASeq package, Bioconductor). Between samples, a correction for library size and RNA composition was performed by full quantile normalization (EDASeq package, Bioconductor). Normalized counts were divided by the total number of counts (in millions), and the obtained scaled counts were divided by the gene length (in kbp) in order to obtain the number of Fragments Per Kilobase of gene sequence and per Million fragments of library size (FPKM).
Log2 fold change (FC) values were calculated for each pairwise comparison of interest based on the FPKM values. We adopted the criterion from the MAQC-I study and considered P-values <.001 as significant [5].

qPCR experimental settings and data analysis

RNA was extracted from sorted cells, from pedAML patients or from healthy controls, using the miRNeasy Mini or Micro Kit (Qiagen) in combination with on-column DNase I digestion (RNase-Free DNase set, Qiagen) according to manufacturer's instructions. RNA concentrations were measured by Nanodrop (ThermoFisher Scientific) or Qubit RNA HS Assay (Invitrogen). For patients' cell-sorted fractions, cDNA synthesis was performed after an additional in-solution gDNase elimination step (Heat&Run gDNA removal kit, ArcticZymes), using the 5x PrimeScriptTM RT Master Mix (Takara Bio Europe S.A.S.) in a final volume of 12.5 µL. For cell lines, cDNA synthesis was performed by Invitrogen

SuperScript III Reverse Transcriptase (Invitrogen) according to the supplier`s recommendations. cDNA from both patients and cell lines was diluted until a final concentration of 2.38 ng cDNA/µL. qPCR reactions were carried out in 96-well plates using 0.3 µM primers, 2x Takyon Low ROX SYBR 2X MasterMix (Eurogentec), 2.38 ng cDNA and H_2O (Sigma-Aldrich) in a 10 µL reaction. Samples were run in duplicate after a heat-activation step (3 min 95 °C) by a 2-step real-time protocol of 45 cycles (95 °C 15 sec, 60 °C 60 sec) on a Viia7 analyzer (ThermoFisher), combined with melting curve analysis (65 °C to 95 °C, gradually increasing with 0.5 °C/5 sec). Ct thresholds were automatically determined by the QuantStudio™ Real-Time PCR Software.
Primer sequences for *TARP* and housekeeping genes (*GAPD*, *HPRT1* and *TBP*) were previously described [4]. Ct values generated for each target were corrected for primer pair efficiency and expressed as relative quantities (RQ). Normalised relative quantities (NRQ) were calculated by normalising RQ values against the expression of housekeeping genes *GAPD*, *HPRT1* and *TBP* (NRQ). To allow inter-run comparison, calibrated NRQ values (CNRQ) were generated by taking into account the expression of a single inter-run calibrator (IRC), evaluated in each run by the respective primer pair. A cut-off for *TARP* overexpression was based on the average expression plus two standard deviations measured in the normal counterparts (HSC=25 and C-blast=28, respectively), and set to 0.59.

Statistical analysis

Graphics and statistical calculations were made in GraphPad Prism (version 5.04, La Jolla California USA) and SPSS (version 25.0.0.2, Inc., Chicago, IL). The Mann-Whitney U test was used to evaluate significant differential *TARP* expression between *FLT3*-ITD and *FLT3* WT pedAML patients. Association between *TARP* expression and dichotomous variables were evaluated by the Pearson's two-sided Chi-Square test if the expected count was ≥5, and the Fisher's exact test (two sided) otherwise.
The Kaplan Meier method was used to estimate the survival probabilities for EFS and OS. Univariate regression analysis was performed by the Kaplan-Meier log-rank test, and confirmed by the univariate COX regression log-rank test if significant, also used to calculate hazard ratios. Confirmation of significance in univariate models was performed by a multivariate Cox proportional hazard model, taking into account all aforementioned continuous and dichotomous variables.
P-values <.05 were considered significant, and the number of asterisks indicate the level of significance (one, P<.05; two, P<.01; three, P<.001 and four, P<.0001).

SDC, supplemental references

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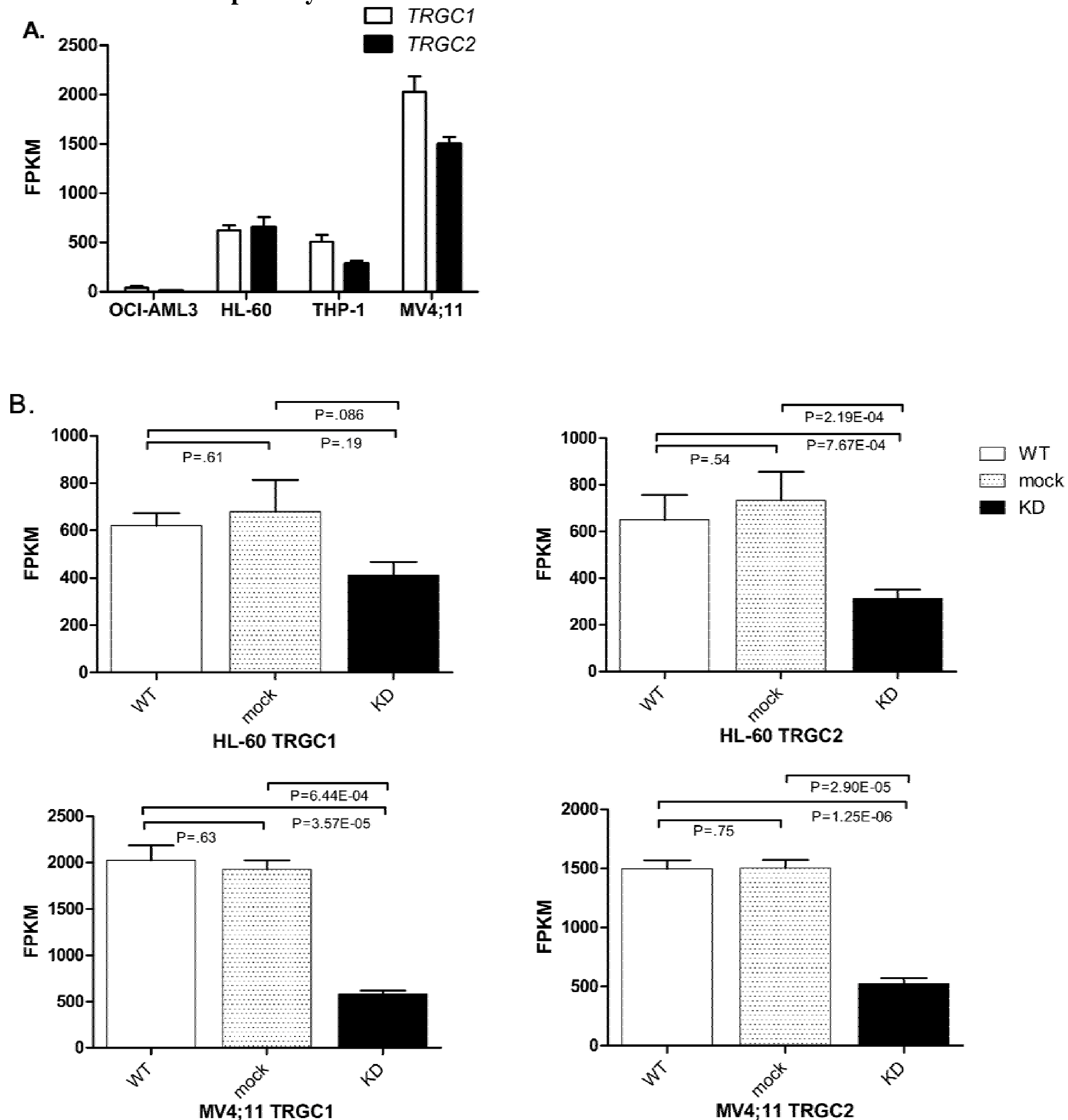
2. Chair) JAS: Research study for treatment of children and adolescents with acute myeloid leukaemia 0-18 years. . *NOPHO-DBH AML 2012 protocol v21* 2013, EUdract number 2012-002934-35.
3. Kalina T, Flores-Montero J, van der Velden VH, Martin-Ayuso M, Bottcher S, Ritgen M, Almeida J, Lhermitte L, Asnafi V, Mendonca A *et al*: EuroFlow standardization of flow cytometer instrument settings and immunophenotyping protocols. *Leukemia* 2012, 26(9):1986-2010.

4. Depreter B, Weening KE, Vandepoele K, Essand M, De Moerloose B, Themeli M, Cloos J, Hanekamp D, Moors I, I DH *et al*: TARP is an immunotherapeutic target in acute myeloid leukemia expressed in the leukemic stem cell compartment. *Haematologica* 2019.
5. Shi L, Reid LH, Jones WD, Shippy R, Warrington JA, Baker SC, Collins PJ, de Longueville F, Kawasaki ES, Lee KY *et al*: The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements. *Nat Biotechnol* 2006, 24(9):1151-1161.

SDC, supplemental figures and tables

SDC, Figure 1. Confirmation of *TRGC1*- and *TRGC2*-encoding *TARP* transcripts in AML cell lines and their susceptibility for knockdown.



TRGC1 and *TRGC2* transcript expression was measured by mRNA sequencing and expressed as Fragments Per Kilobase per Million fragments of library size (FPKM). The log2FC values between cell lines, with accompanying P-values (significant if <.001), are shown per *TRGC* transcript in Table S1. *TRGC1*, TCR γ chain constant domain 1; *TRGC2*, TCR γ chain constant domain 2; *TARP*, T-cell receptor

γ chain alternate reading frame protein; WT, wild type. KD, knockdown; FC, fold change; AML, acute myeloid leukemia.

(A) Differential *TRGC1* and *TRGC2* expression computed by mRNA sequencing in four WT AML cell lines, i.e. MV4;11, THP-1, HL-60 and OCI-AML3.

(B) Differential *TRGC1* and *TRGC2* expression upon TARP-knockdown (KD), compared to the respective WT and mock, for HL-60 (top) and MV4;11 (bottom).

SDC, Figure 2**. Differential *TARP* expression between leukemic and normal stem cell and blast populations.**
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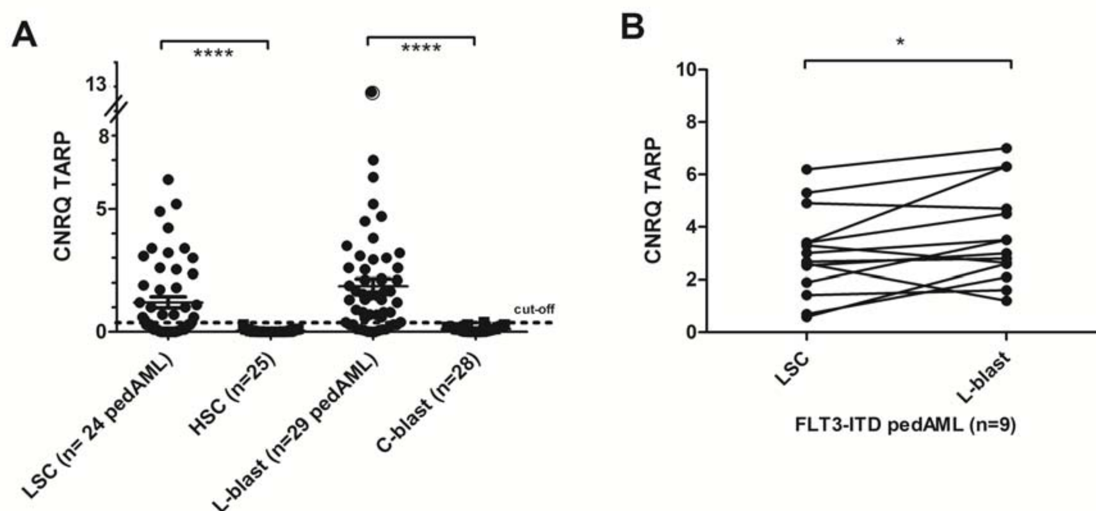

(A) Evaluation of *TARP* expression in 24 LSC versus 25 HSC (13 CB, 8 NBM and 4 mPBSC), and 29 L-blast versus 28 C-blast (15 CB, 13 NBM). Approximately half of the pedAML patients (n=13/29, 44.8%) showed a significant increased *TARP* expression (P<.0001) in LSC and L-blast compared to their healthy counterparts. Cut-off is indicated by a dotted line. Y-axis is interrupted between CNRQ values 8 and 13, indicated by double brackets, to allow better comparison with (B). **(B)** Paired comparison of *TARP* expression measured in LSC and L-blast sorted from 9/11 *FLT3*-ITD mutated pedAML demonstrated a marginal significant higher expression in the latter compartment (P=.041).

PedAML, pediatric acute myeloid leukemia; *FLT3*, fms-like tyrosine kinase receptor-3; ITD, internal tandem duplication; T-cell receptor γ chain alternate reading frame protein; CNRQ, calibrated normalised relative quantity; LSC, leukemic stem cell; HSC, hematopoietic stem cell; L-blast, leukemic blast; C-blast, control myeloblast; CB: cord blood; NBM, normal bone marrow; mPBSC, mobilised peripheral blood stem cells.

SDC, Table 1. Differential TRGC1 and TRGC2 transcript expression between WT and transgenic TARP-knockdown AML cell lines.

Pairwise comparison	*TRGC1*		*TRGC2*	
	log2FC	**P**	**log2FC**	**P**
MV4;11 WT vs HL-60 WT	**2.19**	**6.77E-05**	**1.34**	**4.41E-05**
MV4;11 WT vs OCI-AML3 WT	**6.27**	**5.06E-21**	**7.35**	**2.48E-58**
MV4;11 WT vs THP-1 WT	**2.43**	**1.21E-05**	**2.50**	**3.05E-13**
OCI-AML3 WT vs HL-60 WT	**-4.08**	**1.45E-11**	**-6.01**	**1.22E-42**
OCI-AML3 WT vs THP-1 WT	**-3.84**	**1.32E-10**	**-4.85**	**7.74E-30**
THP-1 WT vs HL-60 WT	-0.24	6.53E-01	**-1.16**	**4.65E-04**
MV4;11 TARP-KD vs mock	**-1.99**	**6.44E-04**	**-1.49**	**2.90E-05**
MV4;11 TARP-KD vs WT	**-2.28**	**3.57E-05**	**-1.61**	**1.25E-06**
MV4;11 mock vs WT	-0.29	6.30E-01	-0.11	7.54E-01
HL-60 TARP-KD vs mock	-1.00	8.58E-02	**-1.33**	**2.19E-04**
HL-60 TARP-KD vs WT	-0.70	1.86E-01	**-1.11**	**7.67E-04**
HL-60 mock vs WT	0.30	6.10E-01	0.22	5.44E-01

Four WT AML cell lines were subjected to mRNA sequencing, together with their respective TARP-KD and mock transgenic cell line variants for HL-60 and MV4;11. For each pairwise comparison of interest, log2FC values with accompanying P-values are shown. Significant differential log2FC values (P<.001) are indicated in bold. FC, fold change; KD, knockdown; WT, wild-type; TARP, T-cell receptor γ chain alternate reading frame protein.

SDC, Table 2. Demographics pedAML patients used for *TARP* expression evaluation by qPCR.

PedAML patient characteristics (n=29)	*TARP*-high (n=13)		*TARP*-low (n=16)		P*
	Mean (Range)		Mean (Range)		
Age, years	11.7 (7-17)		8.1 (1-16)		0,22
WBC count, x 10^9/L	109.5 (2.7-336)*		25.7 (3.1-118)		**0,02**
Morphological blast count					
BM, %	77.8 (31-96)†		61.8 (27-88)		**0,03**
PB, %	58.7 (9-95)		39.2 (1-78)		**0,04**
	N	%	N	%	
Gender					0,38
F	7	53,8%	6	37,5%	
M	6	46,2%	10	62,5%	
Time point					
Dx	12	92,3%	15	93,8%	
R	1	7,7%	1	6,3%	
Sample					
BM and PB couples	4	30,8%	7	43,8%	
Only BM	4	30,8%	8	50,0%	
Only PB	5	38,5%	1	6,3%	
Primary/secondary AML					
Primary	13	100,0%	15	93,8%	
Secondary	0	0,0%	1	6,3%	
Treatment protocol					
DB AML-01	4	30,8%	5	31,3%	
NOPHO-DBH AML2012	6	46,2%	9	56,3%	
Other (Dx)	2	15,4%	1	6,3%	
Other (R)	1	7,7%	1	6,3%	
CD34 positivity	11	84,6%	16	100,0%	0,19
WT1 overexpression					0,45
Yes	10	76,9%	10	62,5%	
No	3	23,1%	6	37,5%	
Translocation					**0,00**
Yes	3	23,1%	13	81,3%	
No	10	76,9%	3	18,8%	
Core-binding factor leukemia	0	0,0%	11	68,8%	**<.0001**
AML1-ETO + C-KIT^{WT}	0	0,0%	1	6,3%	
AML1-ETO + C-KIT^{MUT}	0	0,0%	2	12,5%	
AML1-ETO + C-KITND	0	0,0%	3	18,8%	
CBFB-MYH11	0	0,0%	5	31,3%	
FLT3					**<.0001**
ITD	11	84,6%	0	0,0%	
WT	2	15,4%	16	100,0%	
CEBPA					NA
Double mutated	0	0,0%	1	6,3%	
WT	12	92,3%	15	93,8%	
Unknown	1	7,7%	0	0,0%	
Karyotype					0,26
Normal	6	46,2%	4	25,0%	
Abnormal	6	46,2%	11	68,8%	
Unknown	1	7,7%	1	6,3%	
CNS involvement					1,00
Yes	2	15,4%	2	12,5%	
No	9	69,2%	14	87,5%	
Unknown	2	15,4%	0	0,0%	

Risk classification					**0,00**
SR	4	30,8%	14	87,5%	
HR	7	53,8%	0	0,0%	
Unknown	2	15,4%	2	12,5%	
FAB classification					0,30
Immature (M0 - M1)	3	23,1%	1	6,3%	
Mature (M2-M7)	10	76,9%	15	93,8%	

Characteristics of 29 pedAML patients used for *TARP* qPCR analysis. Patients were dichotomized as TARP-high (n=13/29, 44.8%) and -low (n=16/29, 55.2%), based on a cut-off calculated as described in Supplemental Materials. Two-sided P-values are representative for the significance of the differential characteristics between *TARP*-high and -low patients and indicated in bold if significant. Continuous variables (WBC count, age and percentage blasts in BM and PB) were dichotomized as described in Supplemental Methods. *WT1* overexpression was interpreted in regard to in-house or published (Cilloni et al. 2009) cut-offs. None of the patients harbored *NPM1* or *FLT3*-TKD mutations. Secondary AML implies AML after MDS/JMML. Superscripts indicate one (*) or two (†) missing data. NA indicates not applicable (number of positive cases too low).

PedAML, pediatric acute myeloid leukemia; qPCR, quantitative PCR; Dx, diagnosis; R, relapse; F, female; M, male; WBC, white blood cell; BM, bone marrow; PB, peripheral blood; FAB, French-British-American; *FLT3*, fms-like tyrosine kinase receptor-3; *NPM1*, nucleophosmin; *CEBPA*, CCAAT/enhancer-binding protein alpha; ITD, internal tandem duplication; WT, wild type; MT, mutated; *WT1*, Wilms' tumor 1; CNS, central nerve system; SR: standard risk; HR: high risk; MDS, myelodysplasia; JMML, juvenile myelomonocytic leukemia.

SDC, Table 3. PedAML *FLT3*-ITD physical characteristics correlated to *TARP* expression.
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FLT3-ITD+ pedAML (n=11) analysed by *TARP* qPCR	ITD characteristics			TARP expression (CNRQ)		
	No.	Length (bp)	AR (%)	Dichotomized	LSC	L-blast
pedAML2	4	21/30/33/39	6.6/19.1/5.5/15.2	High	3.3	2.6
pedAML5	1	33	47.0	High	ND	1.5
pedAML6	1	33	43.0	High	1.4	1.6
pedAML9	1	96	62.0	High	2.5	3.0
pedAML11	2	29/38	14.5/9.6	High	2.7	2.8
pedAML12	2	20/23	44.8/17.1	High	3.4	4.9
pedAML13	1	23	42.0	High	4.9	4.7
pedAML14	2	31/75	70.8/2.7	High	6.2	7.0
pedAML15	1	84	23.0	High	ND	1.4
pedAML20	1	78	3.0	High	0.5	2.4
pedAML29	1	23	15.4	High	3.0	2.9
median	1	33	17.7		3.0	2.8
95% CI median	1 - 2	23 - 39	9.7 - 43.0		1.6 - 4.7	1.6 - 3.7
range	1 - 4	20 - 96	2.7 - 70.8		0.5 - 6.2	1.4 - 7.0

Representation of ITD physical characteristics, i.e. number of clones, length of ITD(s) and AR, for 11 pedAML patients (numeric coded), with concomitant *TARP* expression values (CNRQ) measured in LSC and/or L-blast by qPCR. Median values with 95% confidence intervals (CI), and minimum-maximum values, are summarized at the bottom.

FLT3, fms-like tyrosine kinase receptor-3; ITD, internal tandem duplication; pedAML, pediatric AML; *TARP*, T-cell receptor γ chain alternate reading frame protein; no., number; bp, base pairs; AR, allelic ratio; CNRQ, calibrated normalised relative quantity; ND, not determined; LSC, leukemic stem cell; L-blast, leukemic blast.

SDC, Table 4. Multivariate analysis between dichotomous and continuous variables and EFS.

Variables in the Equation	B	SE	Wald	df	Sig.	Exp(B)	95,0% CI for Exp(B)	
							Lower	Upper
TARP	,000	38,130	,000	1	1,000	1,000	,000	2.9E+35
FLT3-ITD	,000	22,947	,000	1	1,000	1,000	,000	3.4E+22
Sexe	,000	2,891	,000	1	1,000	1,000	,003	2.9E+05
CD34	,000	24,284	,000	1	1,000	1,000	,000	4.7E+23
Age	,000	,326	,000	1	1,000	1,000	,527	1.9E+03
WBC count PB	,000	,079	,000	1	1,000	1,000	,857	1.2E+03
Percentage blasts BM	,000	,060	,000	1	1,000	1,000	,889	1.1E+03
Percentage blasts PB	,000	,096	,000	1	1,000	1,000	,828	1.2E+03
Translocation	,000	35,566	,000	1	1,000	1,000	,000	1.9E+33
Core-binding factor	,000	1,400	,000	1	1,000	1,000	,064	1.6E+04
WT1 overexpression	,000	2,190	,000	1	1,000	1,000	,014	7.3E+04
CEBPA	,000	11,609	,000	1	1,000	1,000	,000	7.6E+12
Karyotype			.	0a	.			
CNS invasion			.	0a	.			
Risk classification			.	0a	.			

Covariate Means	
Variables in the Equation	Mean
*TARP*_dich_final	,308
FLT3-ITD_presence_dich	,154
Sexe	,385
CD34_dich	,846
Age	9,077
WBC_count_PB	51,790
Perc_blast_BM	66,000
Perc_blast_PB	44,000
translocation_dich	,692
CBF	1,308
*WT1*_overexpression	,615
CEBPA	,231
Karyotype_normal	1,154
CNS_dich	,231
Risk_high_dich	,231

a Degree of freedom reduced because of constant or linearly dependent covariates

TARP expression (high versus low) did not significantly impacted EFS in a multivariate analysis model (SPSS version 25.0.0.2, Inc., Chicago, IL). *FLT3*, fms-like tyrosine kinase receptor-3; ITD, internal tandem duplication; pedAML, pediatric AML; TARP, T-cell receptor γ chain alternate reading frame protein; EFS, event-free survival; PB, peripheral blood; BM, bone marrow; CNS, central nerve system; *WT1*, Wilms' tumor 1.

SDC, Table 5. Overview of the used antibodies.

Antibody	Fluorochrome	Clone	Supplier	Cat. no.	dilution
CD34	PerCP-Cy5.5	8G12	BD Biosciences	333146	1/20
CD38	APC-H7	HB7	BD Biosciences	656646	1/80
CD45	PacO	HI30	Invitrogen	MHCD4530	1/100
CD8	PE-Cy7	SFCI21Thy2D3	Beckman Coulter	737661	1/50
CD3	APC	SK7	BD Biosciences	345767	1/100
LIVE/DEAD® Fixable Near-IR Dead Cell Stain	APC-H7	/	ThermoFisher Scientific	L10119	1/500
HLA-A2	FITC	BB7.2	BD Biosciences	343304	1/100
Anti-Mouse TCR β Chain (mTCRαβ)	PE	H57-597	BD Biosciences	561081	1/100