Supplementary Methods

Cell separation and cryopreservation

Peripheral blood mononuclear cells (PBMCs) from the AML patients were isolated from freshly obtained EDTA-anticoagulated blood by means of Histopaque (Sigma-Aldrich) gradient centrifugation**.** The cells were then counted and cryopreserved in freshly prepared freezing medium (10% DMSO (Sigma-Aldrich) in FBS (Life Technologies), and stored in liquid nitrogen for further analysis. Moreover AML plasma was isolated by centrifugation and freezed.

ELISA

BAFF and BCMA levels in EDTA-plasma samples were quantified by means of commercially available enzyme-linked immunosorbent assays (ELISA, R&D Biosystems). According to the manufacturer's instructions. The protein levels in the specimens were calculated from a reference curve generated by using reference standards. The samples were analyzed with automated light absorbance reader (LEDETEC 96 system). Results were calculated by MicroWin 2000 software.

Extracellular and Intracellular staining for flow cytometry

Multicolor flow cytometry method was used in order to analyze frequencies of CD34+ AML cells expressing BAFF, APRIL and their receptors (BAFF-R, TACI and BCMA), as previously described (1-3) . Briefly, the PBMCs were incubated with Fc-block for 15 min at 4ºC in the dark. For extracellular staining, the cells were labeled with primary: anti-BAFF-R (clone 8A7) (eBioscience) or anti-TACI (clone 165609) or anti-BCMA (clone 335004) (R&D Systems) mAbs (30 min, 4ºC in the dark) in stain buffer (Militenyi Biotec). Thereafter cells were washed and incubated with FITC- (e-Bioscience) or APC- (BD) conjugated anti-IgG secondary antibodies (30 min, 4ºC in the dark), washed again and stained directly with anti-CD33 PE mAbs (30 min, 4ºC in the dark). Finally, the cells were washed and fixed with Cellfix (BD) and analyzed with FACSCalibur flow cytometer.

For intracellular staining PBMCs were fixed/permeabilized with PermBuffer II (BD) for 5 min, in room temeperature. Fixed/permeabilized cells were washed with stain buffer and labeled with anti-BAFF FITC (clone 1D6, eBioscience), anti-APRIL APC conjugated (clone REA347, Miltenyi Biotec) and anti CD33 PE conjugated mABS (30 min, 4ºC in the dark). Finally, cells were washed and fixed with Cellfix (BD) and analyzed with FACSCalibur flow cytometer. For all stainings fluorescence-minus-one (FMO) controls were used for setting compensation and to assure correct gating. Collected data were processed by FlowJo ver 7.6.5 software (Tree Star, gating strategy is presented on Supplementary Figure S1)

Cell cytotoxicity

CD33+ AML blasts were seeded at the density of 1×10^5 cells/ml in 24-well plate (Corning) and incubated for 24h in the presence or absence Cytarabine (AraC) and 200ug rhBAFF or 200ug rhAPRIL (R&D Biosystem). Next, the viability of cells was determined by flow cytometry (FACSCalibur, BD) using Propidium Iodide (PI, Becton Dickinson).

CD33+ AML blast separation

CD33+ AML blast isolation from PBMCs was performed by magnetic cell sorting (MACS, Miltenyi Biotec, positive selection), with the use of LS columns and MidiMACS (Miltenyi Biotec), following the manufacturer's instructions. Purity of the cells was > 95%, as determined by FACS analysis (FACSCalibur, BD). Obtained CD33+ AML cells were used for further analysis.

qPCR

The mRNA was purified from CD33+ AML cells by means of RNeasy Micro kit (QIAGEN), according to the manufacturer's instructions. For cDNA synthesis, 1µg of mRNA from each sample was reverse transcribed using a high-capacity cDNA reverse-transcription kit (Thermoscientific), according to the manufacturer's instructions. Expression level of BAFF, APRIL, TACI, BAFF-R and BCMA was quantified with TaqMan Real-Time PCR (all primer sets were designed by and purchased from Lifetechnologies) by using StepOnePlus real-Time PCR System (LifeTechnologies). The relative expression of BAFF, APRIL, TACI, BAFF-R and BCMA (by the change-in-cycling-treshold ($2^{-\Delta Ct}$) method) were calculated and normalized to β-actin or Elongation Factor 1-α (EF1α) expression.

Immunofluorescence staining

One hundred thousand CD33+ AML cells (isolated as described above) were immobilized onto glass slides by centrifugation (4) and fixed with 4% paraformaldehyde (Sigma Aldrich). Immunofluorescence staining was performed as previously described (5) with modifications. Briefly, cells on glass slides were washed ones with PBS and fixed with absolute methanol for 5 minutes at room temperature and washed 3 times with PBS. Thereafter cells were incubated in 2 ml block solution (PBS containing 10% FBS) for 30 min at room temperature. The block solution was replaced by 250 µl staining buffer (10% BSA in PBS) containing primary antibody anti-BCMA (1:100 dilution, R&D System) or isotype control antibody. Cells were incubated for 3h at room temperature. Thereafter, cells were washed 3 times with PBS and incubated with Alexa Fluor 488 conjugated secondary antibodies in staining buffer (1:2000 dilutions, Invitrogen) for 1h at room temperature in the dark. After 3 washes with PBS one drop of ProLong Gold with DAPI was added (Life Technologies) and slide has been covered with coverglass and stored at room temperature in the dark. Images were obtained with laser scanning confocal microscopy LSM800 (Zeiss).

Combined bisulfite-restriction analysis (COBRA)

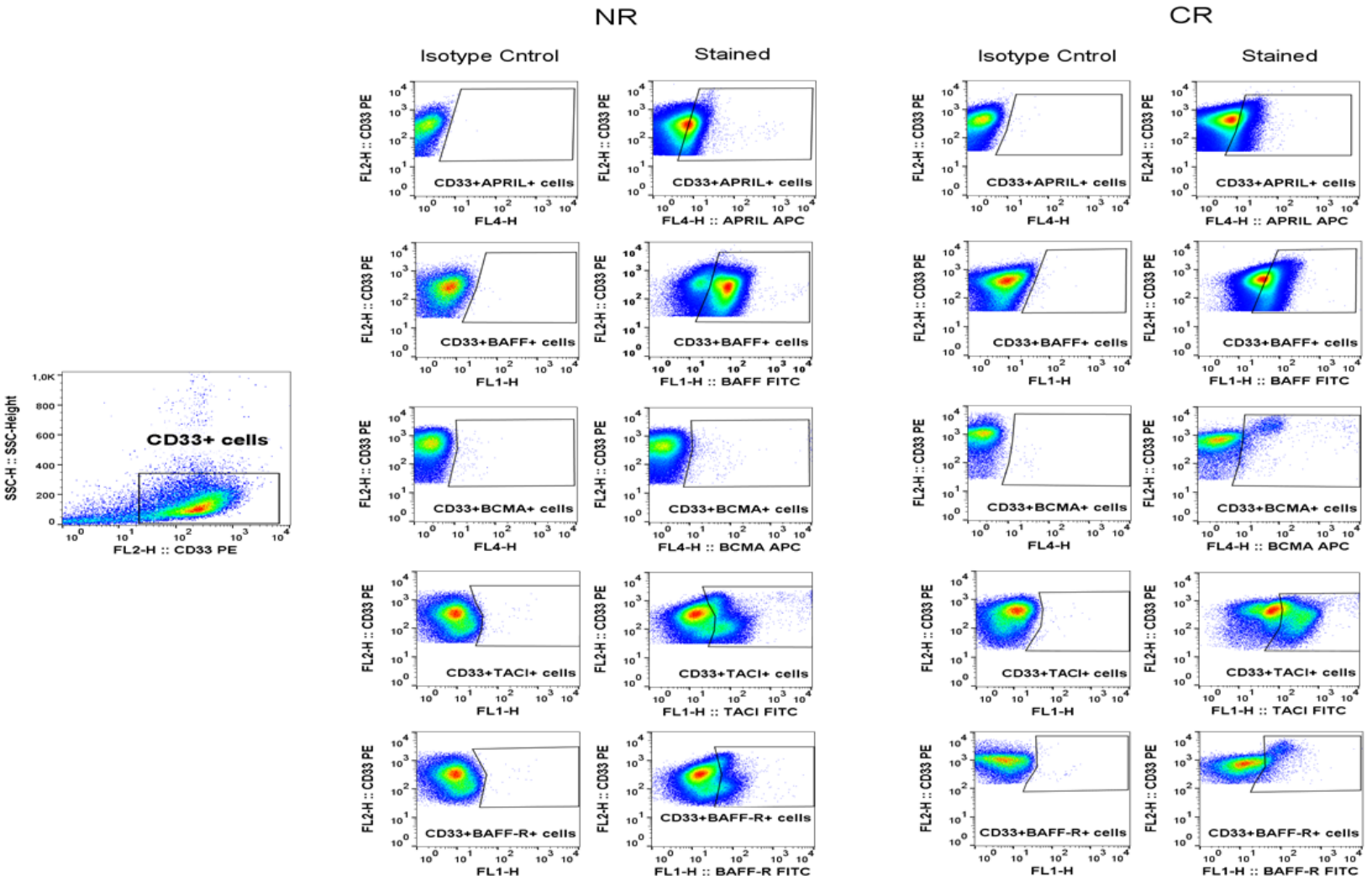
The DNA methylation status of promotor sequences of APRIL, BAFF, BAFFR, BCMA and TACI were examined by COBRA analysis. Briefly, 1 µg of genomic DNA was used for bisulfite modification using the EpiTect Fast Bisulfite Conversion Kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. Promotor sequences were amplified by nested PCR using bisulfite-treated gDNA as template, AmpliTaq Gold polymerase (Applied Biosystems, Foster City, CA) and specific primers. Both first-and second-round PCR were performed with 8 min at 95°C; 2 cycles of 2 min at 95°C, 1 min annealing and 1 min at 72°C; 35-50 cycles of 30 sec at 95°C, 1 min annealing and 1 min at 72°C; and 1 cycle of 10 min at 72°C. As a negative control water was used instead

of genomic DNA. The COBRA assay was performed by incubation of the final PCR products with appropriate restriction enzyme for 3 h and subsequent agarose gel electrophoresis to assess the cutting pattern. Densitometry analysis was performed using ImageJ (6). Sequences of primers, annealing temperatures and restriction enzymes used during COBRA analysis are listed in Supplementary Table S1.

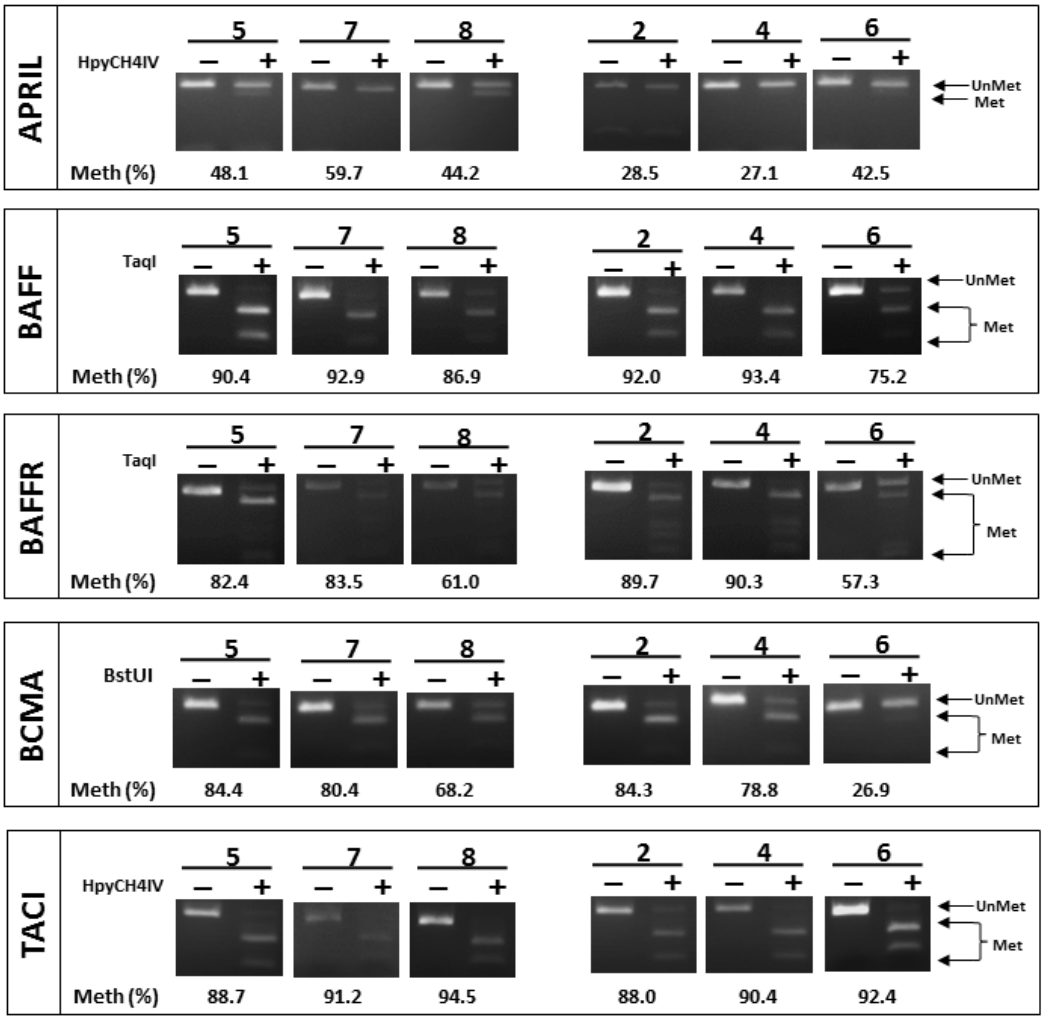
Supplementary Table S1
Sequences of primers employed for bisulfite sequencing and enzymes used for COBRA analysis

Promotor region	EPD promotor ID	Sequence		Annealing temp.	COBRA
APRIL	TNFSF13_1	1st PCR	F: TTTTATTGGGGTTTAGGGGATAT R: CCAACCAAAACCTATACCAACAC	56°C	HpyCH4IV
		2nd PCR	F: 1st PCR forward was used R: 1st PCR reverse was used	56°C	
BAFF	TNFSF13B_1	1st PCR	F: GGAATTAGTTATTAGGATAAGGTAGG R: CTTTTTCCTTCTAAAACTCATCA	53°C	TaqI
		2nd PCR	F: 1st PCR forward was used R: 1st PCR reverse was used	53°C	
BAFFR	TNFRSF13C_1	1st PCR	F: GGTTGAATTGGGGAATTATAGGTA R: AAAAAAACACTCTACCCTACCCC	56.5°C	TaqI
		2nd PCR	F: 1st PCR forward was used R: 1st PCR reverse was used	56.5°C	
BCMA	TNFRSF17_1	1st PCR	F: TAAGATTTAAATTTAGAAATTTGAATTAGA R: AAACAAAAAAACTACAAAAAAAACAAC	54.5°C	BstUI
		2nd PCR	F: 1st PCR forward was used R: 1st PCR reverse was used	54.5°C	
TACI	TNFRSF13B_1	1st PCR	F: TAATGGAGTTTTGAAGGAAGAGTAG R: CCTAAATTTTCCCCTCAACCTATTA	55°C	HpyCH4IV
		2nd PCR	F: ATATTTAGGAAGAGGGAAAGGTTTG R: 1st PCR reverse was used	55°C	

EPD, The Eucariotic promotor database; F, forward; R, Reverse



Supplementary Figure S1. Gating strategy applied for flow cytometry analysis.



Supplementary Figure S2. DNA methylation status of promoter sequences of gene. No differences in methylation levels with regard to the type of clinical response to the therapy

References:

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6. Schneider CA, Rasband WS, Eliceiri KW. NIH Image to ImageJ: 25 years of image analysis. Nat Methods. 2012; 9(7):671-675