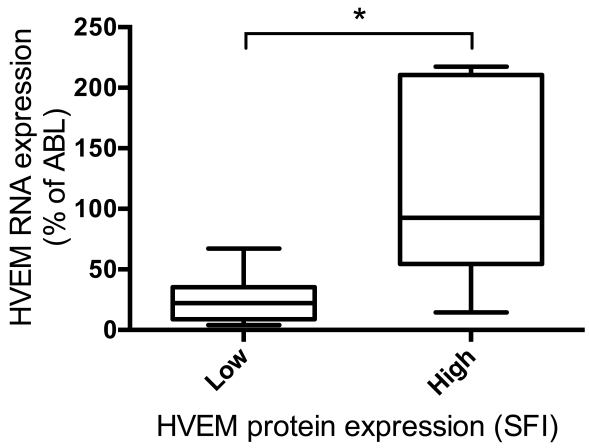
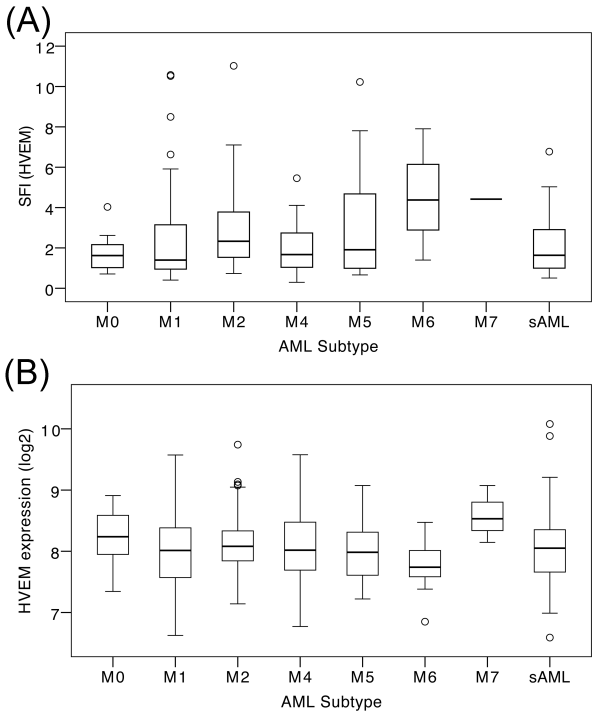
Cancer Immunology, Immunotherapy (submitted in 2015) – Felix Lichtenegger et al.

Supplementary Figure 1



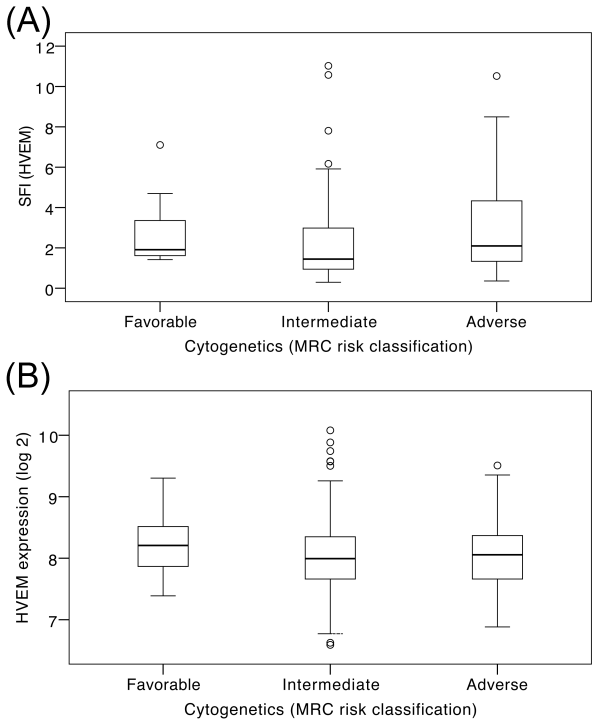
Supplementary Figure 1. Correlation between surface protein and RNA expression of HVEM in AML samples. HVEM RNA expression relative to ABL was determined for 8 samples with low and 6 samples with high HVEM surface protein expression. Significantly higher RNA expression (p=0.02) was found for the samples with high surface protein expression.

Supplementary Figure 2



Supplementary Figure 2. Correlation of HVEM expression with morphology. HVEM surface protein **(A)** and mRNA **(B)** expression levels were correlated with morphologic characteristics of the AML. No significant and consistent difference in expression levels was detected between the different FAB subtypes or in cases of secondary AML.

Supplementary Figure 3



Supplementary Figure 3. Correlation of HVEM expression with cytogenetics. HVEM surface protein **(A)** and mRNA **(B)** expression levels were correlated with the cytogenetic risk groups according to the refined MRC criteria. No significant and consistent difference in expression levels was detected between the three risk groups.

Supplementary Table 1

Gene	Description	p-value	Adjusted p-value	Log fold change	Biological function of protein	Significance of protein for AML
HOXA9	homeobox A9	<0.001	<0.001	-1.875	Transcription factor; key role in hematopoiesis	High expression has negative impact on prognosis [1,2]
MEIS1	Meis homeobox 1	<0.001	<0.001	-1.279	Transcription factor	Cofactor of HOXA9; increases transformation efficiency of HOXA9 [1]
HOXA5	homeobox A5	<0.001	<0.001	-1.268	Transcription factor	Overexpression often associated with HOXA9; high expression has negative impact on prognosis [2]
SEPP1	selenoprotein P, plasma, 1	<0.001	<0.001	-1.225	Extracellular glycoprotein; function as antioxidant	–
PBX3	pre-B-cell leukemia homeobox 3	<0.001	<0.001	-1.138	Transcription factor	Critical cofactor of HOXA9 in leukemogenesis [3]
HLA-DPA1	major histocompatibility complex, class II, DP alpha 1	<0.001	<0.001	1.024	HLA class II alpha chain paralogue	–
ST18	suppression of tumorigenicity 18 (breast carcinoma) (zinc finger protein)	<0.001	<0.001	1.046	Transcription factor	Part of a validated set of genes used for MRD monitoring in pediatric AML [4]
TGFBI	transforming growth factor, beta-induced, 68kDa	<0.001	<0.001	1.052	Cytokine; role in proliferation, differentiation and immune system	Pro-survival effect for AML cells in bone marrow niche [5]
MN1	meningioma (disrupted in balanced translocation) 1	<0.001	0.001	1.089	Transcriptional coactivator	High expression has negative impact on prognosis [6]
CD34	CD34 molecule	<0.001	<0.001	1.133	Cell surface glycoprotein; function in cell-cell adhesion	$CD34^{+}/CD38^{-}$ AML cells are enriched for leukemic stem cells [7]
HPGDS	hematopoietic prostaglandin D synthase	<0.001	<0.001	1.231	Sigma class glutathione-S-transferase; role in production of prostanoids in immune system and mast cells	–
LPAR6	lysophosphatidic acid receptor 6	<0.001	<0.001	1.309	G protein-coupled receptor	–
PROM1	prominin 1	<0.001	<0.001	1.528	Pentaspan membrane protein; role in organization of cell membrane topology	Expression on AML cells has negative impact on prognosis [8]

Supplementary Table 1. Genes significantly associated with *HVEM* expression. Differential gene expression analysis by Limma showed 13 probe sets with an expression highly significantly associated with the $HVEM^{high}$ group ($p \leq 0.001$ and fold change ≥ 1). A very brief description of the biological function of these genes as well as the significance for AML, if applicable, is provided.

1. Lawrence HJ, Rozenfeld S, Cruz C, Matsukuma K, Kwong A, Kömüves L, Buchberg AM, Largman C (1999) Frequent co-expression of the HOXA9 and MEIS1 homeobox genes in human myeloid leukemias. Leukemia 13:1993-1999

2. Zhao P, Tan L, Ruan J, Wei XP, Zheng Y, Zheng LX, Jiang WQ, Fang WJ (2015) Aberrant Expression of HOXA5 and HOXA9 in AML. Asian Pac J Cancer Prev 16:3941-3944

3. Li Z, Zhang Z, Li Y, Arnovitz S, Chen P, Huang H, Jiang X, Hong GM, Kunjamma RB, Ren H, He C, Wang CZ, Elkahloun AG, Valk PJ, Döhner K, Neilly MB, Bullinger L, Delwel R, Löwenberg B, Liu PP, et al (2013) PBX3 is an important cofactor of HOXA9 in leukemogenesis. Blood 121:1422-1431

4. Steinbach D, Bader P, Willasch A, Bartholomae S, Debatin KM, Zimmermann M, Creutzig U, Reinhardt D, Gruhn B (2015) Prospective validation of a new method of monitoring minimal residual disease in childhood acute myelogenous leukemia. Clin Cancer Res 21:1353-1359

5. Tabe Y, Shi YX, Zeng Z, Jin L, Shikami M, Hatanaka Y, Miida T, Hsu FJ, Andreeff M, Konopleva M (2013) TGF-β-Neutralizing Antibody 1D11 Enhances Cytarabine-Induced Apoptosis in AML Cells in the Bone Marrow Microenvironment. PLoS One 8:e62785

6. Metzeler KH, Dufour A, Benthaus T, Hummel M, Sauerland MC, Heinecke A, Berdel WE, Büchner T, Wörmann B, Mansmann U, Braess J, Spiekermann K, Hiddemann W, Buske C, Bohlander SK (2009) ERG expression is an independent prognostic factor and allows refined risk stratification in cytogenetically normal acute myeloid leukemia: a comprehensive analysis of ERG, MN1, and BAALC transcript levels using oligonucleotide microarrays. J Clin Oncol 27:5031-5038

7. Bonnet D, Dick JE (1997) Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. Nat Med 3:730-737

8. Tolba FM, Foda ME, Kamal HM, Elshabrawy DA (2013) Expression of CD133 in acute leukemia. Med Oncol 30:527

Supplementary Table 2

NAME	ES	NES	NOM p-val	FDR q-val
KEGG_PRIMARY_IMMUNODEFICIENCY	0.721	1.785	<0.001	0.105
KEGG_HEMATOPOIETIC_CELL_LINEAGE	0.688	1.703	<0.001	0.153
KEGG_LEUKOCYTE_TRANSENDOTHELIAL_MIGRATION	0.554	1.816	0.004	0.139
KEGG_NATURAL_KILLER_CELL_MEDIATED_CYTOTOXICITY	0.587	1.704	0.011	0.199
KEGG_NON_SMALL_CELL_LUNG_CANCER	0.438	1.596	0.014	0.348
KEGG_AXON_GUIDANCE	0.381	1.552	0.018	0.447
KEGG_T_CELL_RECEPTOR_SIGNALING_PATHWAY	0.516	1.637	0.025	0.271
KEGG_VIRAL_MYOCARDITIS	0.420	1.464	0.037	0.478
KEGG_GLYCOSPHINGOLIPID_BIOSYNTHESIS_GANGLIO_SERIES	0.539	1.510	0.039	0.446
KEGG_VASCULAR_SMOOTH_MUSCLE_CONTRACTION	0.363	1.472	0.040	0.492
KEGG_B_CELL_RECEPTOR_SIGNALING_PATHWAY	0.515	1.513	0.045	0.485
KEGG_LONG_TERM_POTENTIATION	0.365	1.507	0.052	0.413
KEGG_GLYCOSAMINOGLYCAN_BIOSYNTHESIS_CHONDROITIN_SULFATE	0.592	1.516	0.053	0.531
KEGG_REGULATION_OF_ACTIN_CYTOSKELETON	0.348	1.440	0.055	0.484

Supplementary Table 2. Gene sets significantly associated with *HVEM* expression.

Gene set enrichment analysis (GSEA) showed 11 gene sets enriched in the $HVEM^{high}$ group at $p<0.05$. Four gene sets were enriched at a false discovery rate <0.25.