In t(8;21) acute myeloid leukaemia (AML), the leukemogenesis is supposed to be promoted by interference with expression of AML1 target genes. Repressor complex associated with AML1-ETO fusion protein recruits class I histone deacetylases (HDAC). Valproic acid (VPA) was found to have an extensive effect on AML blasts, via inhibition of class I HDAC. We aimed to characterize the differentiation effect of VPA on AML1-ETO-positive leukemic cells and to determine the expression pattern of AML1 target genes. Kasumi-1 (M2 AML1-ETO-positive), Kasumi-6 (M2 AML1-ETO-negative), MV4-11 (MLL-AF4-positive) and K562 cells were treated with VPA and 12-0-tetra-decanoylphorbol-13-acetate (TPA) and examined by flow cytometry and qRT-PCR. Two AML1-ETO-positive and two negative patients' bone marrow diagnostic samples were treated with VPA and TPA to confirm in vitro findings. Valproic acid induced apoptosis in AML1-ETO-positive and MLL-AF4-positive cells in dose dependent manner. But changes of immunophenotype proving the differentiation were observed purely in AML1-ETO-positive cell line (decreased CD33/34/117 and increased CD11a/11b expression). However, differentiated cells exhibited positivity of AnnexinV; hence the relationship between cell death and differentiation had to be evaluated. Apoptosis was blocked by caspase inhibitor ZVAD, but the differentiation was still detected in the same extent. Conversely changes in immunophenotype were not detected in either of control cells. TPA was used to exclude incapability of cells to differentiate, induced monocytic differentiation in both AML1-ETO-positive and negative cells. As quantified by qRT-PCR, VPA treatment increased expression of genes PU.1, IGFBP7, BPI and C/EBPα in AML1-ETO-positive cells. No significant changes were detected in AML1-ETO-negative cells. Specific effect was confirmed in patients' bone marrow samples.