**Structure-function analysis of vitamin D2 and D3 analogs as potential inducers of differentiation in   
acute myeloid leukemia cells.**

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The primary biological function of 1,25-dihydroxyvitamin D3 (1,25 D) is to maintain the calcium and phosphorus homeostasis of the organism. In addition, 1,25D is involved in immunomodulation, cell proliferation and differentiation of many cell types. 1,25D mediates its biological effects by binding to the nuclear receptor (VDR) which acts as a ligand- dependent transcription factor. VDR initiates different intracellular signaling pathways, although the exact mechanism of 1,25D action in target cells is not yet fully understood. It has been postulated for years to use pro-differentiating compounds to induce differentiation of myeloid leukemia cells. Because of maturation block during hematopoiesis, differentiation is blocked resulting the accumulation of immature cells and consequently, the occurrence of acute myeloid leukemia (AML). Side effects of 1,25D are manifested by the ability to mobilize calcium from bones, intestine and kidney to blood serum, resulting in bones decalcification and the formation of calcium deposits in soft tissues. Therefore, 1,25D semi-selective analogs, with reduced calcemic activity and with maintained or increased ability to induce cell differentiation, have been synthesized.

In this work, 1,25D and eight synthetic, semi-selective analogs with reduced calcemic activity were tested in their potency to induce differentiation of leukemic cells isolated from peripheral blood of patients and AML cell lines: HL60, THP-1, NB-4, U-937, MV4-11, MOLM-13. Moreover, it was tried to define a group of patients for whom therapy with differentiating agents will be effective and the most active analogs were selected. The results obtained suggested to examine the gene expression and protein level of the main   
1,25D catabolic enzyme – 24-hydroxylase (CYP24A1).

It was shown that three of the analogs studied proved to be particularly effective: analog of vitamin D3 – PRI-2191 and analogs of vitamin D2 – PRI-1906 and PRI-1907. The two groups of patients, whose cells responded to 1,25D and analogs differently were defined:   
the group with a Flt3 mutated cells responded to pro-differentiating compounds worse than the others, and the group of patients with NPM1 gene mutated cells better than the others. However, MV4-11 and MOLM-13 cell lines, having mutations in a Flt3 gene, differentiate under the influence of 1,25D or its analogs, thus Flt3 mutations are not the only ones, and the direct cause of weaker cell differentiation in AML patients.

PRI-1907 analog, having an ethyl group in the side chain of the molecule, has a very high activity at low concentrations. It was observed both, in experiments with cells of patients with AML and in the cell lines. High activity of PRI-1907 in comparison to the other compounds, prompted that this may be related to its different catabolism. In all examined cell lines, constitutive levels of CYP24A1 were very low, but after exposure to 1,25D, PRI-1906, PRI-1907 or PRI-2191 a systematic, slow, but very large increase of CYP24A1 mRNA level occurred. Although it was found that PRI-1907 analog exhibits the highest activity to induce differentiation of cell lines and causes the weakest induction of CYP24A1 mRNA synthesis in HL60 cells, it was not in the case of other cell lines. Therefore, catabolism isn’t responsible for the high activity of PRI-1907, but presumably some other mechanisms. Attempts to strengthen the differentiation through the specific inhibitor of CYP24A1 – CTA09 and non-specific inhibitors have not shown a significant increase in the percentage of CD11b or CD14 positive cells.

Additionally, the results obtained show, that there is a correlation between the induction of the expression of VDR and increased expression of C/EBPβ factors in the cell nucleus, and the ability of analogs to induce cell differentiation.

VDR FokI polymorphism in various cell lines and in cells isolated from peripheral blood of patients with AML did not explain differences in their sensitivity or resistance to the differentiation-inducing treatment.