Plitidepsin Inhibits the Growth of Cells Harboring JAK2V617F Mutation

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Introduction: Plitidepsin (Aplidin®) is a novel cyclic depsipeptide derived from the marine tunicate Aplidium albicans, currently obtained by chemical synthesis, that is under Phase II clinical development. Plitidepsin is effective against a large panel of tumor cells and corrects thrombocytopenia of myelofibrotic mice, reduces the frequency of megakaryocytes (Mk), normalizes angiogenesis in the bone marrow, and prevents extramedullary hematopoiesis.

Methods: Plitidepsin (Aplidin®) was kindly provided by PharmaMar (Madrid, Spain). Quantification of apoptotic cells was accomplished by Annexin-V-FLUOS Staining kit. Cell cycle distribution was analyzed by flow cytometry. The effects of Plitidepsin on cell lines were evaluated in liquid culture with WST1 and in Agar. MNCs were plated in Methylcellulose with cytokines. For the growth of CFU-Mk, CD34+ cells were plated in Megacult Collagen and Medium with lipids. P27 mRNA level was evaluated by RT-QPCR. Single colony genotyping was performed by JAK2 (ASO)-PCR. Protein targets were analysed by Western Blotting method.

Results: We assessed the effects of Plitidepsin on cell lines harboring homozygous (HEL and UKE-1) or heterozygous (SET2) JAK2V617F mutation and on cells from patients (pts) with myeloproliferative neoplasms (MPN). In 6 days clonogenic assay culture (Agar), we found that Plitidepsin prevented cell growth with IC50 values of 1.5±0.05 nM for HEL, 0.5±0.03 nM for UKE-1, and 0.8±0.02 nM for SET2, that were all lower than 2.7±0.3 nM for the BCR/ABL mutated K562 cell line (P<.001 in case of UKE-1 and SET2 cells). Similar results were obtained using a WST1 cell proliferation assay. We observed in SET2 cells that 5 and 10nM Plitideps in increase significantly apoptosis and cell cycle G0/G1 phase with a correspondent decrease of S phase. We then evaluated the effects of Plitidepsin on the growth of BFU-E, CFU-GM and CFU-Mk from MPN pts; PMF pts presented significantly lower IC50 value than controls (P<.002) and PV (P<.02). We then performed single colony genotyping in presence of 1 nM Plitidepsin. In 4/5 pts we observed a reduction of JAK2-mutated BFU-E and CFU-GM colonies, especially in one we found a decrease from 27% to 5%. Finally, since a correlation between levels of p27(Kip1) and the response of tumor cells to Plitidepsin has been described, we measured p27 levels in different cell lines after exposure to Plitidepsin. We observed that p27 mRNA levels increased 15-fold and 30-fold in UKE1 and HEL cells, respectively, compared to K562 cells after 24 hr with 10nM Plitidepsin; such an increase was mirrored by a protein content 1.2- to 2-fold greater than baseline in UKE-1 cells at 1.0 and 10 nM Plitidepsin, suggesting that JAK2V617F mutated cells responded to the drug by modulating their p27 levels.