Depsipeptide (FK228) as a Novel Histone Deacetylase Inhibitor: Mechanism of Action and Anticancer Activity

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Abstract: Depsipeptide (FK228), a new histone deacetylase inhibitor, has been recently introduced into clinical trials. This agent shows interesting metabolic properties, novel mechanism of action, and is undergoing phase I-II clinical studies in hematopoietic malignancies and solid tumors. Mechanism of action, pharmacokinetics and anticancer activity of depsipeptide is the subject of this review.

Key Words: Epigenetic therapy, histone deacetylase inhibitors, depsipeptide (FK228), cutaneous T-cell lymphoma, solid tumors.

INTRODUCTION

Alteration of gene expression due to epigenetic modifications is one of the mechanisms involved in tumorigenesis. The two epigenetic changes, promoter methylation and histone acetylation, have been recognized and intensively studied. These modifications are not dependent on changes in DNA sequence itself [1, 2]. Histones and DNA form the nucleosomes, structural units of chromatin that are essential in packaging eukaryotic DNA [3]. The post-translation modifications of the tail region of the histones, including acetylation, methylation, phosphorylation, ADP-ribosylation, and ubiquitination, are implicated in the regulation of the gene transcription. These modifications may also occur within the globular domain of histones that contacts with DNA [3, 4]. It has been suggested that histone acetylation is particularly important among them since results in the loosening of the chromatine structure, thereby enabling gene transcription [5]. Acetylation of core histones has been also correlated with other genome functions such as DNA repair and recombination [6]. Additionally, proteins other than histones, such as p53 and GATA-1, have been identified as targets for acetylation [7, 8].

The level of histone acetylation depends on the balance between histone acetyltransferase (HAT) and histone deacetylase (HDAC) activity, and defines the status of transcription of most eukaryotic genes [4, 9]. HAT enzyme regulates gene expression by transfer of acetyl moiety from acetyl-CoA to the lysine residues of histones and triggers the initiation of gene transcription by recruiting chromatin remodeling factors and the general transcription machinery to promoter regions [10, 11]. In contrast, HDAC catalyzes the hydrolysis of acetyl groups from the amino-terminal lysine residues of nucleosomal core histones contributing to the formation of transcriptionally inactive heterochromatin [10, 11]. It is hypothesized that the balance between the actions of HAT and HDAC leads to a decreased level of acetylation and decreased expression of genes that regulate cell growth, and to the development of various neoplasms [12].

HDAC proteins are divided into 3 families, based on homology to yeast HDAC proteins. All HDACs share a zinc-dependent catalytic domain with a high degree of homology and less conserved accessory domains which show regulatory functions [2]. In mammals, class 1 HDACs, including HDACs 1, 2, 3, and 8, are related to the yeast RPD3 HDAC, and localized in the nucleus. HDAC class II, including HDACs 4-7, 9, and 10, are related to the yeast HDA1HDAC, and are found in both the cytoplasm and nucleus. HDAC11 is the unique member of class IV and localized in the nucleus. Class I HDACs are widely expressed, whereas classes II and IV show various degrees of tissue specificity [2, 13]. It has been reported that altered HDAC activity plays an important role in the development of acute myelogenous leukemia (AML). The formation of abnormal fusion protein AML1-ETO in AML patients carrying the specific translocation t(8; 21) or PML/RARA protein in patients with acute promyelocytic leukemia with the translocation t(15,17) has been shown to cause abnormal recruitment of HDAC to the promoter regions of transcription genes involved in myeloid differentiation, leading to the repression of expression of these genes [14-16]. These observations and other preclinical studies revealed that the inhibition of HDAC activity may be important in the treatment of certain tumor types [12, 14-17].

HDAC inhibitors are a new class of antineoplastic agents identified by their ability to reverse the malignant phenotype of transformed cells, and currently being evaluated in clinical trials. HDAC inhibitors consist of several different chemical families: short chain fatty acids, including sodium n-butyrate and valproic acid; organic hydroxamic acids, including trichostatin A (TSA), suberoylanilide hydroxamic acid (SAHA), LAQ824; cyclic tetrapeptides, including trapoxin (TPX); benzamides, including CI-994, MS-275; and the bicyclic depsipeptide, specifically depsipeptide (FK228) [1, 17].

PHYSICAL, CHEMICAL AND PHARMACEUTICAL PROPERITIES OF DEPSIPEPTIDE (FK228)

Depsipeptide (FK228, FR 901228, NSC630176) is a unique bicyclic pentapeptide isolated as a fermentation

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product of *Chromobacterium violaceum* [18-20]. It originally was developed as an anti-ras compound [21, 22], later was found to interfere with mitogen-induced signaling pathways [23-25], and recently has been shown to be a HDAC inhibitor [26-28]. FK228 has a novel chemical structure composed of four amino acids (D-valine, D-cysteine, dehydrobutyrine, and L-valine) and a novel acid (3-hydroxy-7-mercapto-4-heptenoic acid) configured in a cage-shaped bicyclic depsipeptide (Fig. **1**). Molecular formula of depsipeptide is $C_{24}H_{36}N_4O_6S_2$, molecular weight of 540,71, and chemical name is (E)-(1S,4S,10S,21R)-7-[(Z)-ethylidene]-4,21-diisopropyl-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo-[8,7,6]-tricos-16-ene-3,6,9,19,22-pentanone. FK228 forms non-hygroscopic white crystals, soluble in dehydrated ethanol, slightly soluble in water and insoluble in ether [20].

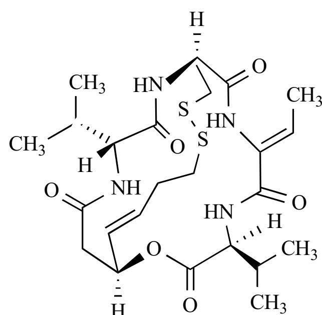

Fig. (1). Chemical structure of depsipeptide (FK228).

MECHANISM OF ACTION

FK228 is converted to its active form by reduction of an intramolecular disulfide bond by intracellular antioxidants involving glutathione (Fig. **2**). It has been suggested that one of the sulfhydryl groups of the reduced form of depsipeptide interacts with zinc ions of the active site of various enzymes, thus preventing the access of the substrate [29]. The mechanism of depsipeptide action is the inhibition of HDAC activity [1]. Reduced FK228 (redFK228) more strongly inhibited HDAC1 and HDAC2 class I enzymes (the concentration of drug necessary to obtain 50% inhibition (IC_{50}) of HDAC activity was 1.6±0.9nM and 3.9±2.7 nM, respectively) than HDAC4 and HDCA6 class II enzymes (IC_{50} was 25±7.3nM and 790±110 nM, respectively). RedFK228 is less active than the parent form in inducing *in vivo* acetylation due to rapid inactivation in serum. Thus, FK228 serves as a stable prodrug to inhibit HDAC class I enzymes and is activated by reduction after uptake into cells [26, 29]. Precisely, it inhibits the removal of acetyl groups from lysine tails of histones, and helps to maintain DNA in the more transcriptionally active open chromatin state. This chromatin conformation may facilitate access to DNA-binding transcription factors, thereby increasing gene transcription [1, 10]. It is hypothesized that increased acetylation of histones promotes a loosening of chromatine structure, allowing transcription factors increased access to bind to specific promoter regions, thereby increasing gene transcription [30]. The cellular action of depsipeptide does not only result in increasing histone acetylation, but also in regulation of other nuclear or cytoplasmic proteins by acetylation. The mechanism of action of FK228 and other HDAC inhibitors is presented in Fig. **3**. However, the precise pathways by which FK228 affects cell cycle, apoptosis and angiogenesis have not been completely defined [1].

Mechanism of Cell Cycle Arrest

The induction of growth arrest with cellular differentiation or induction of apoptosis by FK228 depends on cell line tested and drug concentration [1]. In some cases, growth arrest is induced at low doses of depsipeptide, and apoptosis is induced at higher doses; in other cases, growth arrest precedes apoptosis. However, cells may undergo apoptosis without significant changes in their cell-cycle profile [2]. The cell cycle arrest is predominantly exhibited in the cell lines in which FK228 induces the expression of p21 protein. Cell lines with low p21 expression do not undergo growth arrest and preferentially undergo apoptosis when exposed to depsipeptide [24, 25]. Interestingly, normal cells are more resistant to FK228 than tumor cells [2].

It has been reported that FK228 induces both a p53-independent/p21-dependent G1 and a p21-independent G2/M arrest, with the G2 arrest appearing more important than the G1 arrest [24-26]. The G1 arrest is accompanied by decreased phosphorylation of the mitogen-activated protein (MAP) kinase and the retinoblastoma protein (Rb) and decreased cyclin D expression and cyclin-dependent kinase (CDK) 2 activity. Though FK228 does not directly inhibit

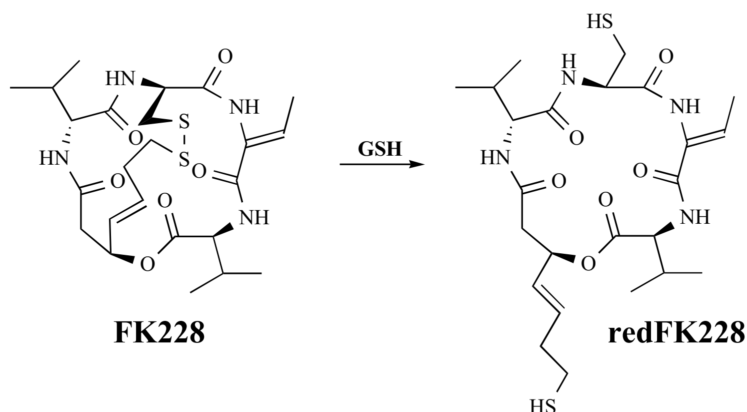

Fig. (2). The formation of FK228 active metabolite (redFK228) following reduction by glutathione (GSH).

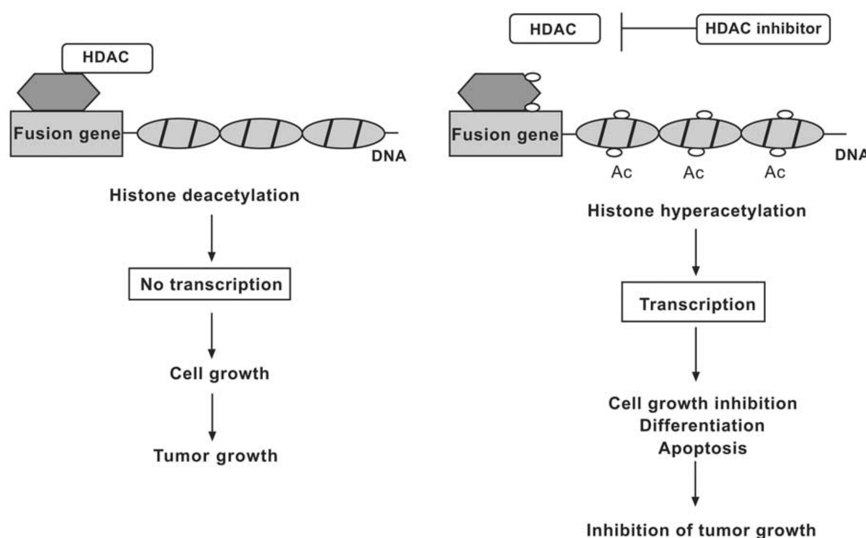

Fig. (3). The mechanism of action of FK228 and other HDAC inhibitors.

kinases in cell extracts, exposure of the whole cells to this agent causes downregulation of cyclin D1 and c-myc but p53-independent p21WAF1/Cip1 induction. This p21 induction leads to inhibition of CDK and dephosphorylation of Rb, resulting in cell cycle arrest in the early G1 phase [25, 31]. Other possible mechanisms involved in the growth cell inhibition include the altered expression of cycline A, cyclin D and p27/Kip1, resulting in a reduction in CDK2 and CDK4 activities [25]. FK228 has been also reported to block the ras-mediated signaling transduction pathway leading to the growth inhibition and G0/G1 arrest that correlates with the morphological reversion of the transformed cells [22].

Mechanism of Apoptosis

Chemotherapeutic agents can induce cell death by activating two major apoptotic pathways, the death receptor pathway and the intrinsic pathway [32]. The precise changes that lead to apoptosis after the exposure to depsipeptide are not well known. It has been reported that HDAC inhibitors induce hyper-acetylation of the promoters of death receptors, including TRAIL (tumor-necrosis factor-related apoptosis-inducing ligand) and its receptor, death receptor 5 (DR5), FAS ligand (FASL) and FAS. The acetylation of the transcription factors SP1 or SP3, and subsequent recruitment of CBP seem to be involved in this process [33, 34]. However, the effect on the death receptor pathway is not universal since it has been observed that some leukemic cells do not undergo apoptosis in response to HDAC inhibitors and do not show the induction of TRAIL or FAS pathway [34]. The second mechanism of apoptosis, known as the intrinsic pathway, includes the perturbation of mitochondrial membranes which results in the release of cytochrome c and subsequent activation of caspase-9, modulation of the expression of Bcl-2 family proteins, and the generation of reactive oxygen species (ROS) [2, 35, 36]. The activation of the proapoptotic Bcl-2 protein Bid upstream of mitochondrial disruption was observed in the cells exposed to FK228, however, depsipeptide-induced Bid cleavage was significantly attenuated by polycaspase inhibitor zVAD-fmk [35]. The accumulation of pro-apoptotic ROS seems to be selective to HDAC inhibitors-induced apoptosis in transformed cells. After the exposure to HDAC inhibitors, normal cells do not accumulate ROS due to thioredoxin (TXN) expression, in contrast to transformed cells that do not express TXN, accumulate ROS and undergo apoptosis [37]. Since direct acetylation of non-histone proteins may also trigger growth arrest or apoptosis, the investigators have studied this question in several model systems [1]. FK228 has been found to cause hyper-acetylation of the chaperone protein HSP90 leading to the degradation of specific proteins, including some oncoproteins as mutant p53, Raf-1, and bcr-abl, that require the chaperone function of HSP90 [38, 39]. Additionally, the HSP90 inhibitor geldanamycin antagonizes the activity of the HDAC inhibitor [40], while proteasome inhibitors act synergistically when combined with FK228 [36]. The chemical structure of geldanamycin is shown in Fig. (**4**).

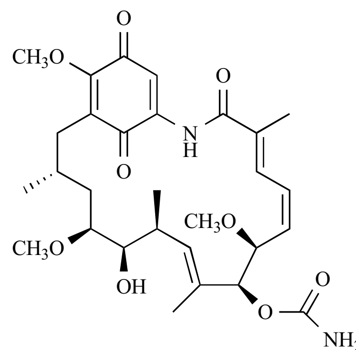

Fig. (4). The chemical structure of geldanamycin.

Inhibition of Angiogenesis

The *in vitro* and *in vivo* studies revealed that depsipeptide inhibits angiogenesis. It blocks the hypoxia-stimulated proliferation, invasion, migration, adhesion and tube formation of bovine aortic endothelial cells, as well as inhibits the neovascularisation in mice. FK228 decreases expression of angiogenic-stimulating factors, such as vascular endothelial growth factor (VEGF) and kinase insert domain receptor,

and induce angiogenic-inhibiting factors, such as von Hippel Lindau and neurofibromin 2. In addition, FK228 may suppress tumor expansion by inhibition of neovascularization [41, 42].

IN VITRO AND *IN VIVO* ANTIPROLIFERATIVE ACTIVITY

FK228 showed antiproliferative activity against human tumor cell lines, including non-small cell lung cancer, small cell lung cancer, stomach cancer, breast cancer, and colon cancer. The cells were exposed to FK228 for 4 days and antiproliferative activity was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolinum bromide (MTT) assay. The concentration of depsipeptide required to reduce cell survival by 50% (IC_{50}) ranged from 0.3 to 3.2 ng/mL (0.56 to 5.9 nM), depending upon the tumor cell line. FK228 was less potent against normal human or murine cell lines than tumor cells [20]. Other *in vitro* studies have revealed the antiproliferative FK228 effect against human B-cell chronic lymphocytic leukemia cells [43], T-cell lymphoma cells [44], esophageal cancer cells [45], pancreatic [46], and multiple myeloma cells [47] with the IC_{50} value ranged from 1 to 500 nM. FK228 showed also antitumor activity in several human tumor xenograft models [17]. It is known that cancers become resistant to anticancer agents *via* the mechanism associated with the upregulation of P-glycoprotein (P-gp) or other genes of the ATP-binding cassette (ABC) transporter family [48]. FK228 is a substrate for P-glycoprotein and induces the multidrug resistance gene (MDR1). Cell lines that overexpress P-gp are resistant to depsipeptide, and this resistance is reversed by P-gp inhibitors [49, 50]. FK228 shows no cross-resistance with the cytotoxic agents such as vincristine, 5-fluorouracil, mitomycin C, and cyclophosphamide, whereas it shows cross-resistance to adriamycin [17].

PHARMACOKINETICS AND PHARMACODYNAMICS

In vivo preclinical studies performed at the National Cancer Institute (NCI) demonstrated that higher doses of depsipeptide FK228 could be given and greater antitumor efficacy achieved with an intermittent administration schedule than with daily treatment because of greater host tolerance for this agent. It was also observed that short (from 30 s to 4 min) and prolonged infusions (more than 24 h) caused the greater toxicity than infusions of 1 to 4 h [19, 51]. Thus pharmacokinetic profiles were assessed in patients with advanced or refractory neoplasms who received FK228 by a 4-hour intravenous (i.v.) infusion on days 1 and 5 of a 21-day cycle at doses of 1.0 to 24.9 mg/m^2. Dose-limiting toxicity (DLT) was observed and included grade-3 fatigue, grade-3 nausea and vomiting, grade-4 thrombocytopenia and grade-4 cardiac arrhythmia. The maximum tolerated dose (MTD) was defined as 17.8 mg/m^2 of depsipeptide given over 4 h. The mean volume of distribution (V_C), distribution half-life ($T_{1/2}\alpha$), and elimination half-life ($T_{1/2}\beta$) with a dose of 17.8 mg/m^2 were 8.6 liters/m^2, 0.42 h, and 8.1 h, respectively used a two-compartment model [51]. The mean maximum plasma concentration (C_{max}) at the MTD dose was 472.6 ng/mL (range, 249- 577.8 ng/mL). The mean FK228 clearance (CL) and the area under the plasma FK228 concentration-time curve from 0 to infinity ($AUC_{0-\infty}$) was 11.6 (±5.8) L/h/m^2 and 2.27 (±1.34) ng/mL/h, respectively [51]. The phase I study conducted in pediatric patients with refractory solid tumors, who received depsipeptide as a 4-hour i.v. infusion on days 1, 5 and 8 of a 28-day cycle, determined the MTD as 17 mg/m^2. At the MTD, the median CL of FK228 was 6.8 L/h/m^2 with $AUC_{0-\infty}$ of 2.414 ng/mL/h, similar to that in adult patients [52]. The pharmacokinetics of FK228 was linear across the 1.0 to 24.9 mg/m^2 dose range with terminal half-life ($T_{1/2}$) of 8.1 ±5.1 hours [51]. The pharmacokinetics profile did not change with repeated administration [51, 52]. FK228 is rapidly distributed to virtually all tissues following administration. It is extensively metabolized with CYP3A4 being identified as the principal cytochrome P-450 isoform involved in depsipeptide metabolism. More than 90% of the administered dose is excreted into urine and feces within 48 hours after administration in rats. The primary route of FK228 elimination is through bile and its subsequent excretion in feces [20, 51, 53].

Pharmacodynamic studies have revealed the ability of serum from patients treated with FK228 to induce cell cycle arrest and confirmed the histone acetylation increase in circulating mononuclear cells (PBMCs) [51, 52]. The maximal accumulation of acetyl H3 histones in PBMCs occurred at 4 hours after the end of FK228 infusion [52]. Other studies have also reported that in patients with acute myelogenous leukemia (AML) and chronic lymphocytic leukemia (CLL) treated with 13 mg/ m^2 depsipeptide i.v. on days 1, 8, and 15, HDAC inhibition and histone acetylation of at least 100% were observed and the levels of H3 and H4 histones acetylation were higher at the 4-hour time point than 24 hours. Additionally, an increase in p21 expression occurs concurrently with H4 histone acetylation of the p21 promoter [54]. The recent pharmacodynamic studies on *scid* mice bearing childhood tumors have shown that increased acetylation state of core histones (H2A, H2B, H3, and H4) in depsipeptide-sensitive and resistant tumors followed the same pattern; maximal increases in histone acetylation occurred at 8 hours and were elevated for up to 96 hours. The p53 expression was increased in sensitive tumor lines (wild-type p53), being maximal at 8 hours and associated with induction of p21^{cip1}, whereas p53 expression was stable in tumors with mutant p53 [55].

CLINICAL ACTIVITY IN NEOPLASTIC DISEASES

Recent phase I and phase II studies have demonstrated that depsipeptide has a favorable anticancer activity particularly in patients with cutaneous T-cell lymphoma (CTCL) and peripheral T-cell lymphoma (PTCL), as well as in solid and hematologic tumor types in children and adults [51, 52, 54, 56-59, [*1, *2]] (Table **1**).

In a phase I study conducted at the NCI, 4 patients with T-cell lymphoma were enrolled and treated with FK228 at the dose of 12.7 or 17.8 mg/m^2. Three patients with CTCL achieved a partial response (PR) and one patient with PTCL, unspecified, had a complete response (CR) [56]. Subse-

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Table 1. Activity of Depsipeptide FK228 in Hematologic Malignancies and Solid Tumors

Study	Treatment Schedule	Patients	No. of pts.	Response			Comments
				CR	PR	SD	
Piekarz *et al.* 2001 [52] A case report	12.7 or 17.8 mg/m^2 d1 and d5 of a 21-day cycle	T-cell lymphoma	4	1	3	0	First report demonstrated high activity of depsipeptide in T-cell lymphomas.
Sandor *et al.* 2002 [47] Phase I	1-24.9 mg/m^2 d1 and d5 of a 21-day cycle	Refractory solid tumors	37	0	1	8	MTD was defined as 17.8 mg/m^2
Marshal *et al.* 2002 [55] Phase I	1-17.7 mg/m^2 d1, d5 and d8 of a 28-day cycle	Advanced incurable cancer	33	NA	NA	NA	MTD was defined as 13.3 mg/m^2.
Byrd *et al.* 2005 [50] Phase I	9-16 mg/m^2, d1, d5 and d8 of a 28-day cycle	AML and CLL	20	0	0	0	Progressive onset of constitutional symptoms and minimal clinical activity
Fouladi *et al.* 2006 [48] Phase I	10-22 mg/m^2 d1, d5 and d8 of a 28-day cycle	Children with refractory or recurrent solid tumors	18	0	0	3	MTD was defined as 17 mg/m^2.
Odenike *et al.* 2003 [*2] Phase II	13 mg/m^2 d1, d5 and d8 of a 28-day cycle	Relapsed or refractory AML	8	0	0	1	Rapid clearance of bone marrow blasts in 1 patient with t(8; 21).
Stadler et al. 2006 [54] Phase II	13 mg/m^2 d1, d5 and d8 of a 28-day cycle	Refractory metastatic renal cancer	29	1	1	0	The drug activity at this dose and schedule is not sufficient.
Whittaker *et al.* 2006 [*1] Phase II	14 mg/m^2, d1, d5 and d8 of a 28-day cycle	Cutaneous T-cell lymphoma	17	1	5	9	Response duration 2-6 months. Toxicity was manageable.

Abbreviations: CR-complete response; PR- partial response; SD- stable disease; AML- acute myeloid leukemia; CLL – chronic lymphocytic leukemia; MTD – maximum-tolerated dose; NA- not assessed, d-day.

quently to these observations similar findings were reported in the interim report from the international multicenter phase II study. Thirty patients with CTCL who had failed at least one prior systemic treatment received up to 6 cycles of depsipeptide as a 4-hour i.v. infusion on days 1, 8 and 15 of a 28-day cycle. Of 17 patients evaluable for response assessment, one patient achieved CR and 4 patients had PR lasting from 2 to 6 months. Stable disease (SD) was observed in 9 patients [*1]. FK228 has been also investigated in patients with refractory solid tumors [51, 52, 57-59]. Sandor *et al.* performed phase I trial in patients with refractory neoplasms. One PR after two cycles was observed at a dose of 9.1 mg/m^2 in a patient with renal carcinoma and 8 patients achieved SD [51]. The phase I study in patients with refractory CLL and refractory or relapsed AML did not reveal any objective response to FK228 and only minimal evidence of clinical activity was observed [54]. In the phase I study conducted in pediatric patients with refractory solid tumors no objective responses were observed, however 3 patients experienced prolonged disease stabilization [52]. Recently, the results of a multi-institutional, single-arm, phase II study of FK228 in refractory metastatic renal cell cancer have been published. Of 29 patients, 2 experienced an objective response including one CR, for an overall response rate of 7% [58].

The above data demonstrate that FK228 has some clinical activity in heavily pretreated patients with refractory or relapsed malignant disease, especially in T-cell lymphoma.

Toxicity and Side Effects

A major concern during phase I and phase II trials of FK228, based on the preclinical studies, was to identify any occurrence of cardiac toxicity [51, 57, 60]. Cardiac toxicities recognized in these trials consisted mainly of asymptomatic arrhythmias and nonspecific ST/T-wave changes on the electrocardiograms (ECGs). Recently, Piekarz *et al.* summarized the cardiac monitoring of 42 patients with T-cell lymphoma treated with FK228 in a phase II trial [60]. T-wave flattening (grade-1) or ST segment depression (grade-2) was observed in more than half of the ECGs obtained post-treatment. However, these ECG abnormalities were not associated with elevation of cardiac troponin or with altered left ventricular function. Additionally, post-treatment ECGs had a mean heart rate-corrected QT interval prolongation of 14.4 milliseconds compared with baseline. These data indicate that FK228 can be given without high frequency of acute cardiac toxicities [60]. Moreover, the long term follow-up extending beyond 3 years provides some assurance that the various laboratory abnormalities were not associated with the late onset of cardiac events [1, 59]. It should be noted however,

that in patients with metastatic neuroendocrine tumors treated with depsipeptide, a high number of potentially serious cardiac adverse events was observed [57]. In one patient a sudden death, attributed to possible fatal ventricular arrhythmia, occurred within 24 hours after the fifth FK228 dose. Furthermore, asymptomatic grade-2 ventricular tachycardia in 2 patients and prolonged corrected QT interval (QTc), probably related to depsipeptide, in 3 patients were observed. Grade-4 cardiac arrhythmia (atrial fibrillation) was also noted in phase I trial in one patient with refractory cancer [51].

Hematological toxicity was observed in some patients receiving FK228 in phase I or phase II studies [51, 52]. Grade-3 or -4 thrombocytopenia was observed after the dose of 13.5 mg/m^2 and 24.9 mg/m^2, respectively [59]. Grade-2 or -3 neutropenia was seen in some patients after the doses of 6.5-24.9 mg/m^2 [51]. Episodes of neutropenia and thrombocytopenia had a distinct time course unlike that of chemotherapeutic agents in clinical use. There was a rapid drop in both platelets and neutrophils count with rapid recovery and no evidence of cumulative toxicity was observed [51]. Since thrombocytopenia and neutropenia were maximal at 10 and 5 days, respectively, similar to the life span in blood, these results may suggest that FK228 affect mature cells. Although, rapid recovery from neutropenia and thrombocytopenia occurred, mechanisms other than those of myelosuppresive cytotoxic agents may be involved [51].

The most common nonhematological toxicities associated with FK228 treatment were fatigue, nausea, vomiting and anorexia [51, 52, 56, [*1]]. At a dose of 24.9/m^2 (DLT) almost all patients had some degree of fatigue, and one third of patients experienced fatigue that was profound, functionally limiting, and lasted up to 1 week [51]. The fatigue was not associated with anemia or other biochemical abnormalities. In some patients receiving FK228 at the dose of 24.9/m^2 hypophosphatemia was observed. It may indicate that depsipeptide could induce cellular changes in ATP levels or availability. Hypocalcemia was also noted but it was not associated with symptoms or significant clinical findings [51].

OTHER HISTONE DEACETYLASE INHIBITORS

HDAC inhibitors are a heterogenous group of compounds with different chemical structure. The structural classes of HDAC inhibitors are presented in Table **2**.

The first generation of HDAC inhibitors are the short chain fatty acids (SCFAs). Butyric acid derivatives have undergone clinical investigations for several years, initially for non-malignant indications and more recently for the treatment of cancer. Of the butyric acid derivatives, sodium phenylbutyrate has been investigated intensively [61, 62]. This compound has been evaluated in patients with AML and myelodysplastic syndrome (MDS) in phase I trials [63, 64]. While no responses were noted, hematological improvements were observed. Administration of phenylbutyrate by i.v. or oral routes is well tolerated at concentrations which effect acetylation of histones *in vitro* [62-64]. Although valproic acid is in clinical use as an anticonvulsant, it has been recently recognized as a HDAC inhibitor and is being investigated in clinical trials [1, 2]. A pilot study demonstrated that valproic acid combined with retinoic acid caused partial and complete responses in 30% of patients with AML and MDS, however, an extended phase II study has shown only hematological improvements [2, 65, 66]. SCFAs are not ideal agents because of the high concentration required (millimolar) to achieve inhibition of HDAC activity and multiple effects on other enzymes [67, 68].

The first described hydroxamic acid was trichostatin A (TSA). TSA, originally developed as an antifungal agent, is a potent HDAC inhibitor that is active at nanomolar concentrations but has limited use due to its extremely short half-life

Table 2. Histone Deacetylase (HDAC) Inhibitors

Structural class	Name
Short chain fatty acids	Sodium n-butyrate Valproic acid
Hydroxamic acids	Trichostatin A (TSA) Suberoylanilide hydroxamic acid (SAHA) Oxamflatin CBHA Scriptaid Pyroxamide
Cyclic tetrapeptides containing the AOE moiety	Trapoxin A Chlamydocin Diheteropeptin
Cyclic tetrapeptides not containing the AOE moiety	Depsipeptide (FK228, FR901228) Apicidin CHAPs
Benzamides	MS-275 (MS-27-275) CI-994

AOE- 2-amino-8-oxo-9,10-epoxy-decanoyl

[67, 69, 70]. Suberoylanilide hydroxamic acid (SAHA) is the prototype of a family of hydroxamic acid-based hybrid polar compounds (HPCs) that induce growth arrest in transformed cells and have antineoplastic activity [71, 72]. SAHA inhibits HDACs at micromolar concentrations or lower *in vitro* and *in vivo* [67, 72]. This agent is currently being tested in both parenteral and oral formulations in phase I and II clinical trials. It has induced complete and partial responses in patients with refractory solid tumors, AML and MDS [73, 74].

Among benzamides, in phase I/II trials, CI-994 (4-acetylamino-N-(2'aminophenyl)-benzamide; acetyldinaline) has been tested alone and in combination with conventional chemotherapy in patients with solid tumors [1, 75, 76]. MS-275 is still in phase I clinical studies. This compound has a good tolerability and the long half-life time (39-80 hours) in patients with refractory solid tumors, leukemias and lymphomas [1, 2, 77].

CONCLUSIONS

In recent years, a novel class of HDAC inhibitors that target epigenetic silencing mechanisms has been developed. Depsipeptide is the most promising HDAC inhibitor in current clinical studies. It has novel metabolic properties and the mechanism of action with acceptable toxicity profile. Depsipeptide has demonstrated some clinical activity in heavily pretreated patients with refractory or relapsed malignant disease, especially in T-cell lymphoma.

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ABBREVIATIONS

AML = Acute myeloid leukemia
AUC = Area under the concentration-time curve
CL = Clearance
CLL = Chronic lymphocytic leukemia
C_{max} = Maximum plasma concentration
CR = Complete response
CTCL = Cutaneous T-cell lymphoma
DLT = Dose limiting toxicity
ECG = Electrocardiogram
GSH = Glutathione
HAT = Histone acetyltransferase
HDAC = Histone deacetylase
i.v. = Intravenous
IC_{50} = Concentration that due to 50% inhibition of cell proliferation
MDS = Myelodysplastic syndrome
MTD = Maximum tolerated dose
NCI = National Cancer Institute
OR = Overall response
Pgp = P-glycoprotein
PR = Partial response
PTCL = Peripheral T-cell lymphoma
Qtc = Corrected QT interval
redFK228 = Reduced form of FK228
ROS = Reactive oxygen species
SAHA = Suberoylanilide hydroxamic acid
SCFAs = Short chain fatty acids
TPX = Trapoxin
TSA = Trichostatin A
$T_{1/2}$ = Half life time
VEGF = Vascular endothelial growth factor
V_d = Volume of distribution

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