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Panobinostat

Prop INN

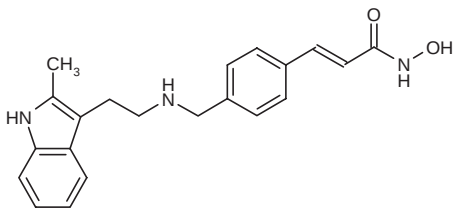
Histone Deacetylase (HDAC) Inhibitor
Apoptosis Inducer
Oncolytic

LBH-589
NVP-LBH-589

N-Hydroxy-3-[4-[2-(2-methyl-1*H*-indol-3-yl)ethylaminomethyl]phenyl]-2(*E*)-propenamide

3-[4-[2-(2-Methyl-1*H*-indol-3-yl)ethylaminomethyl]phenyl]-2(*E*)-propenohydroxamic acid

InChI=1/C21H23N3O2/c1-15-18(19-4-2-3-5-20(19)23-15)12-13-22-14-17-8-6-16(7-9-17)10-11-21(25)24-26/h2-11,22-23,26H,12-14H2,1H3,(H,24,25)/b11-10+



$C_{21}H_{23}N_3O_2$
Mol wt: 349.4263

CAS: 404950-80-7

EN: 397679

Abstract

Panobinostat (LBH-589) is a member of the hydroxamic acid group of histone deacetylase (HDAC) inhibitors that have been shown to impede multiple pathways implicated in cancer and reverse epigenetic events associated with cancer, thereby reducing survival and inducing apoptosis in cancer cells. Panobinostat is being investigated in various hematological malignancies, including chronic myelogenous leukemia (CML) and multiple myeloma, and in solid tumors. Preclinical data indicate efficacy against drug-resistant cancer cells, both as a single agent and in combination with other therapies. A phase I clinical study has demonstrated activity in treatment-experienced patients with cutaneous T-cell lymphoma (CTCL) and phase II/III trials for this condition and other hematological malignancies are ongoing.

Synthesis

Panobinostat can be synthesized as follows:

Reduction of 2-methylindole-3-glyoxylamide (I) with $LiAlH_4$ affords 2-methyltryptamine (II). 4-Formylcinnamic acid (III) is esterified with methanolic HCl, and the resulting aldehyde ester (IV) is reductively aminated with 2-methyltryptamine (II) in the presence of $NaBH_3CN$ to give (V). The title hydroxamic acid is then obtained by treatment of ester (V) with aqueous hydroxylamine under basic conditions (1). Scheme 1.

Background

Carcinogenesis and tumor progression are controlled by both genetic and epigenetic events. Epigenetic phenomena, evident in all biological processes, involve mitotic and meiotic heritable states of gene expression that are not due to alterations in DNA sequences. Unlike genetic changes where reversal is difficult or impossible, epigenetic aberrations can be reversed to reactivate epigenetically silenced tumor suppressor genes and possibly normalize malignant cell populations (Fig. 1). Researchers have therefore focused on epigenetic events as targets for effective cancer therapy and chemoprevention (2-6).

Several enzyme families are involved in epigenetic events. These include DNA methyltransferases (DNMTs), histone acetylases (HATs), histone deacetylases (HDACs), histone lysine methyltransferases (HMTs) and histone demethylases. All these enzymes can interact directly with DNA or histone tails, introducing modifications and thus changes in gene expression. DNA methylation and histone modification are the two epigenetic events that together intricately control the status of gene expression and ultimately determine the fate of a cell. Because human tumors commonly exhibit changes in DNA methylation and histone modifications, researchers have focused on the identification of epigenetic agents such as HDAC and DNMT inhibitors as potential anticancer agents (4-9).

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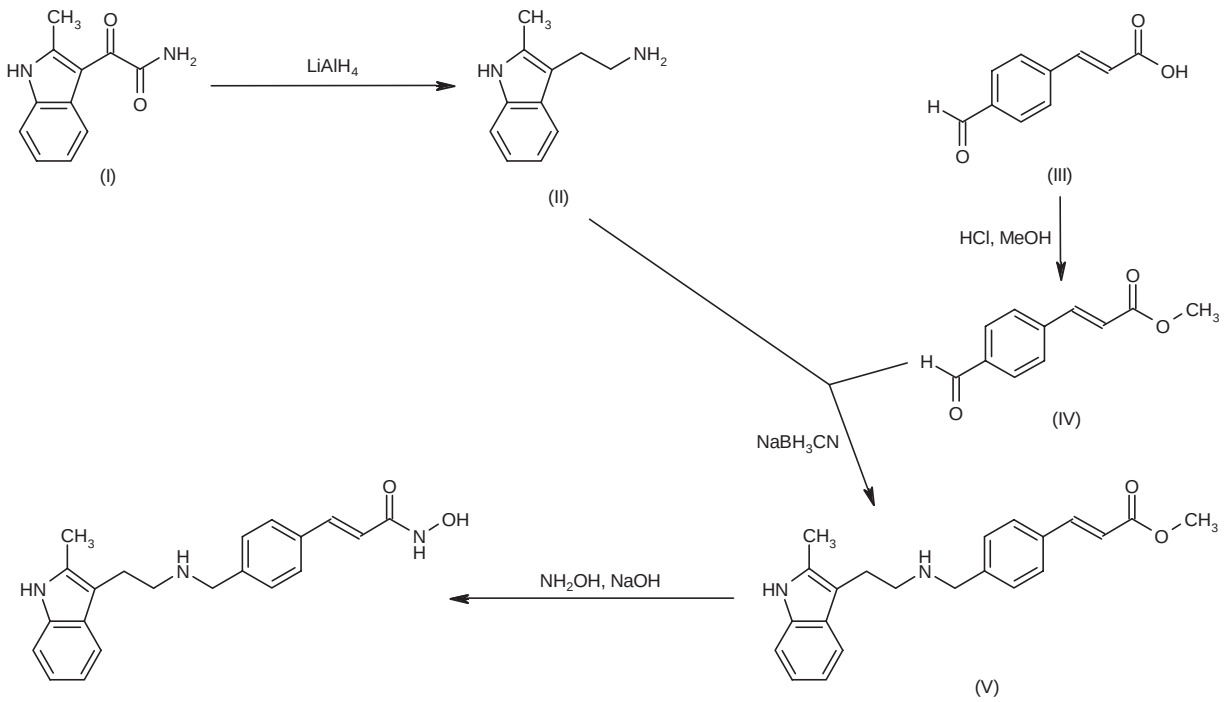
Scheme 1: Synthesis of Panobinostat



Histone is a highly conserved protein found in the nuclei of all eukaryotic cells, where it is complexed to DNA in chromatin and chromosomes. Histone can act as a nonspecific repressor of gene transcription, and histone acetylation in particular is one mechanism which regulates chromatin structure and its transcription. HDAC is the enzyme that removes an acetyl group from histones, allowing them to bind DNA and inhibit gene transcription. Inhibitors of HDAC can transcriptionally reactivate dormant tumor suppressor genes. In addition, these agents exhibit cell cycle-arresting and proapoptotic properties and induce chromatin remodeling and loss of fidelity during mitosis, although the exact mechanism of these actions is unknown (Fig. 1). Inhibitors of HDAC can be divided into four groups: short-chain fatty acids, hydroxamic acids, cyclic tetrapeptides and benzamides (10-16).

Researchers at Novartis and their collaborators designed a novel class of small-molecule hydroxamic acid-based HDAC inhibitors, exemplified by LAQ-824 and panobinostat (LBH-589), which inhibited class I and class II HDACs at nanomolar concentrations and selectively induced apoptosis in tumor cells, but not in normal cells. Both compounds were advanced to clinical development, although LAQ-824 was subsequently discontinued (17, 18). Panobinostat is currently in phase II/III trials for hematological cancers.

Preclinical Pharmacology

Panobinostat inhibited the proliferation of various drug-sensitive and -resistant multiple myeloma cell lines: MM.1S and MM.1R (dexamethasone-sensitive and -resistant, respectively), RPMI 8226, U266, U266LR7 and U266DOX4 (melphalan-sensitive, melphalan-resistant and doxorubicin-sensitive, respectively), OPM1 and KMS11 cells and melphalan-, doxorubicin- and mitoxantrone-resistant cell lines, with IC_{50} values of < 100 nM. Panobinostat retained its activity at low concentrations against MM.1S cells cultured in the presence of primary bone marrow stromal cells (BMSCs) obtained from patients with multiple myeloma. The cytotoxic effect on normal lymphocytes and bone marrow myeloid cells was markedly less than that on multiple myeloma cells. Molecular analysis showed that panobinostat concentration-dependently increased histone and tubulin hyperacetylation, and induced cell cycle arrest and apoptosis. Cell cycle arrest was marked by an accumulation of the cell cycle regulators p21, p53 and p57 and downregulation of c-myc. Apoptosis was confirmed by the release of cytochrome *c*, upregulation of apoptotic protease-activating factor-1 (Apaf-1) and cleavage of caspases-3, -8 and -9 and poly(ADP-ribose) polymerase (PARP). Apoptosis-inducing factor (AIF) was released, indicating that a caspase-independent apoptotic pathway was also induced. Panobinostat proved to be additive or synergistic in combination with the proteasome inhibitor bortezomib in samples obtained from patients with multiple myeloma. The combination was significantly less toxic against normal peripheral blood mononuclear cells (19-21).

Panobinostat induced cell cycle arrest and apoptosis in a concentration-dependent manner in the chronic myelogenous leukemia (CML) cell line K-562 (expressing the

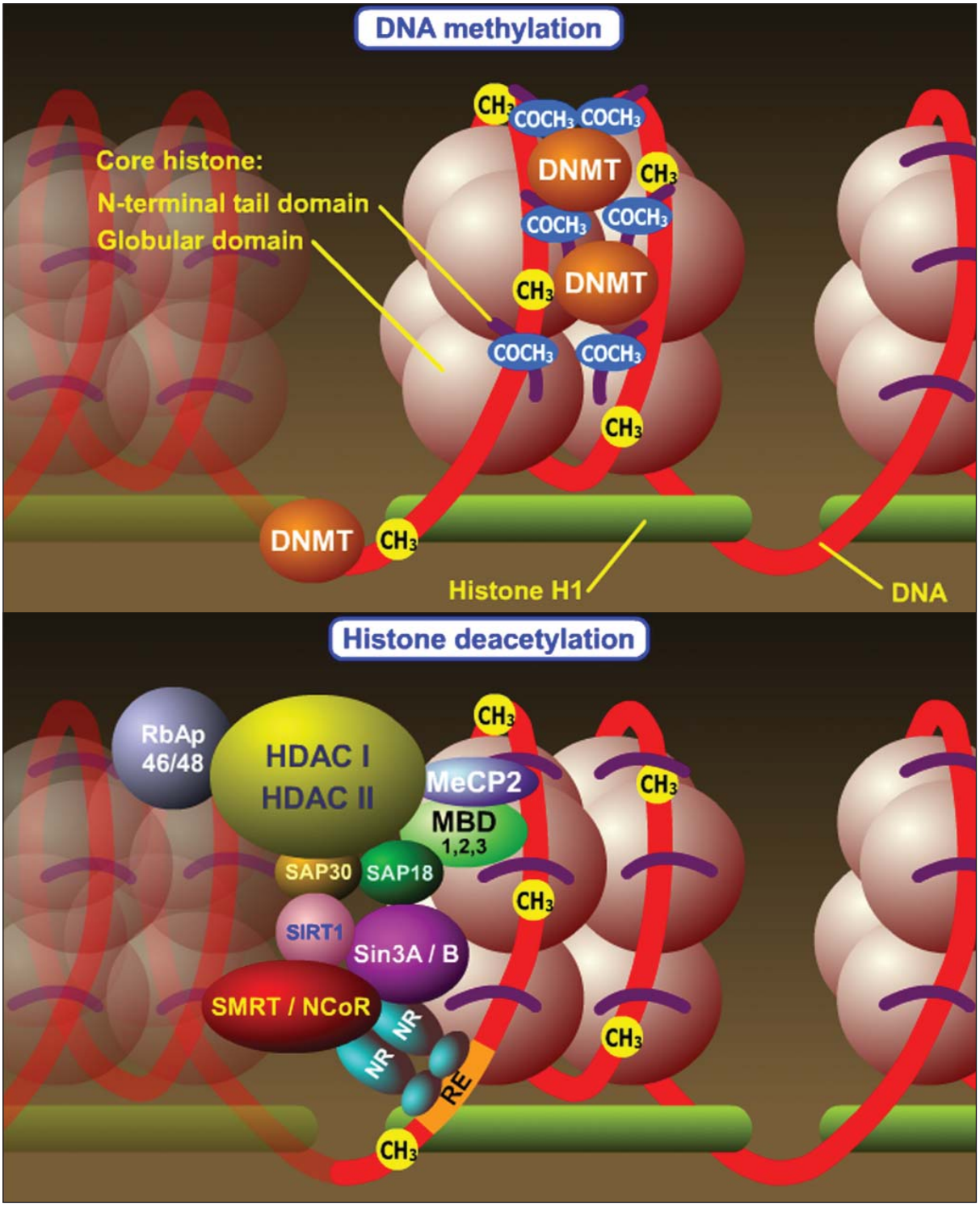


Fig. 1. A nucleosome octamer contains two copies of core histone proteins, H23A, H2B, H3 and H4, each of which has two distinct domains: the globular histone-fold domain and the lysine-rich, positively charged, *N*-terminal tail. Histone H1 fastens the DNA to the nucleosome core and helps pack nucleosomes together in the fiber. The silencing process of a gene begins with the recruitment of DNA methyltransferases (DNMTs) resulting in DNA methylation. After methylation, methyl-CpG-binding protein 2 (MeCP2) and methyl binding domain (MBD) proteins 1, 2 and 3 are recruited, which will recruit more silencing factors. In the absence of ligands, nuclear receptor dimers are associated with co-repressor complexes (SMRT/NCoR) that recruit histone deactylases (HDAC) either directly or indirectly through their interaction with enzymatic complexes. Deacetylation of the histone tail leads to chromatin compaction and transcriptional repression. Subscribers to the on-line version of *Drugs of the Future* and/or Integrity® can access the animation: Modulation of Transcriptional Activation and Nucleosome Remodeling by Histone Acetylation and DNA Methylation.

tyrosine kinase fusion protein BCR-ABL) and in the AML cell line MV-4-11 (expressing the activating internal tandem duplication in tyrosine kinase FLT-3). Treatment was associated with hyperacetylation of histones H3 and H4, upregulation of p21 and increased PARP cleavage. In the MV-4-11 cells, FLT-3 levels were downregulated, and in the K-562 cells, BCR-ABL was downregulated. In both cell lines, downstream signaling pathways (p-STAT5, p-Akt and p-ERK1/2) were also attenuated. Panobinostat produced synergistic apoptosis in combination with the heat shock protein HSP90 inhibitor geldanamycin in these cells, and the levels of BCR-ABL, FLT-3 and downstream effectors were also attenuated to a greater degree than by either agent alone; PARP cleavage was increased. The combination of panobinostat plus geldanamycin was also effective in an imatinib-refractory AML cell line expressing the BCR-ABL T315I mutation and in primary leukemia blasts from 5 patients with CML-blast crisis and 4 patients with relapsed AML with activating FLT-3 mutations (22-24).

Against cultured or primary BCR-ABL-expressing CML cells, the combination of panobinostat and nilotinib (AMN-107) was synergistic for apoptosis induction. In the K-562 and LAMA-84 cell lines expressing BCR-ABL, the combination attenuated p-STAT5, p-ERK1/2, c-myc and Bcl-x_L, and increased p27 and BIM levels to a greater extent than nilotinib alone. In mouse Ba/F3 cells transformed with the imatinib-resistant BCR-ABL mutants T315I or E255K, panobinostat depleted BCR-ABL levels and induced apoptosis. In three primary CML cell samples expressing the T315I mutant form of BCR-ABL, panobinostat was cytotoxic and the combination of the two drugs was synergistic (25, 26).

The EZH2 (enhancer of zeste homolog 2) protein is a component of the polycomb repressor complex PRC2, which is known to regulate the expression of Hox-A9 and Meis1 transcription factors involved in leukemogenesis. Incubation of leukemia cell lines K-562, LAMA-84, U-937 and HL-60 and primary AML and CML samples with panobinostat (10-100 nM) arrested the cells in the G1 phase of the cycle and induced apoptosis. This was associated with depleted levels of EZH2 protein and the other components of PRC2, SUZ12 and EED, followed by histone modification and downregulation of Hox-A9 and Meis1. Incubation of these cells with panobinostat in combination with short interfering RNA (siRNA) sequences targeting EZH2 expression led to further declines in the level of EZH2 expression and greater inhibition of clonogenic survival of the leukemia cells. Further experiments with panobinostat in CML-blast crisis cells demonstrated that it disrupted the interaction between EZH2 and DNA methyltransferase DNMT1 and between DNMT1 and HSP90. It appeared to downregulate DNMT1 by both transcriptional and post-transcriptional mechanisms (27-31).

In human umbilical vein endothelial cells (HUVEC) stimulated by vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), panobinostat caused cell cycle arrest in the G2/M phase and loss of cell viability. This was accompanied by an accumulation of acetylated histone H3 and α-tubulin, and by reduced expression of the angiogenesis-related genes hypoxia-inducible transcription factor-1 α (HIF-1 α), angiopoietin-2 (ANG-2), survivin and the chemokine receptor CXCR4 by VEGF-dependent and -independent pathways. At non-cytotoxic concentrations, panobinostat inhibited endothelial tube formation in the presence of high concentrations of VEGF-A (using both *in vitro* and *in vivo* Matrigel angiogenesis assays). The expression of ANG-2, survivin and CXCR4 genes (all downstream effectors of VEGF signaling) was reduced, as was the phosphorylation of Akt and ERK1/2. Panobinostat also inhibited the induction of CXCR4 gene expression under hypoxic conditions. Further experiments in human renal carcinoma cells in which HDAC6 expression was inhibited using siRNA showed that HIF-1 α expression was inhibited in parallel with an accumulation of acetylated α-tubulin, a marker of HDAC6 inhibition, in the presence of panobinostat or LAQ-824. This suggested a possible link between the inhibition of HDAC6 and the antiangiogenic action of panobinostat. In a human prostate cancer PC-3 xenograft model in mice, panobinostat (10 mg/kg i.p.) reduced angiogenesis and tumor growth over a 3-4-week period, in the absence of toxicity (32-34).

Exposure of primary chronic lymphocytic leukemia (CLL) cells from patients to panobinostat was found to result in induction of proapoptotic p73, independent of p53 status, leading to activation of PUMA (p53-upregulated modulator of apoptosis)-mediated cell death (35).

Panobinostat was associated with potent, concentration- and time-dependent cell cycle arrest and apoptosis induction in Philadelphia chromosome-negative (Ph^-) acute lymphoblastic leukemia (ALL) cell lines, which was correlated with induction of histone hyperacetylation and upregulation of genes involved in apoptosis, growth arrest and DNA repair (36).

In colon cancer cells, the HDAC inhibitors panobinostat and vorinostat (SAHA) produced concentration-dependent growth inhibition and suppression of epidermal growth factor receptor (EGFR) mRNA and protein expression, and synergistic growth inhibition was observed when they were used in combination with the EGFR-targeted monoclonal antibody cetuximab (37).

Human mesothelioma NCI-H252, NCI-H2052, MSTO-211H, ME13 and ME16 cells were treated with panobinostat (10-100 nM), cisplatin (0.5-5.0 μM) or a combination of the two agents (1:50) for 48 h. The combination exhibited synergistic antiproliferative and apoptotic activity in most cell lines tested but less activity in normal cells, suggesting an acceptable therapeutic index (38).

The combination of panobinostat and rapamycin (sirolimus) inhibited HIF-1 α expression in both HUVEC and the von Hippel Lindau-deficient renal carcinoma cell line UMRC2 to a greater extent than either agent alone. The combination was also effective against tumor growth and angiogenesis in mice bearing UMRC2 cells (39).

Using human lung cancer cell lines with defined EGFR status, panobinostat was found to induce apoptosis only in cells dependent on EGFR for survival, which

was associated with inhibition of proteins involved in survival signaling (40).

Combination of panobinostat plus irradiation was tested in a variety of human cancer cell lines expressing either the EGFR or the HER-2/neu epidermal growth factor receptor (erbB-2). Carcinoma cells lacking EGFR- or HER-2-dependent signaling were used as controls. Panobinostat abrogated the G2/M arrest caused by irradiation and enhanced the sensitivity of all cells to irradiation. This effect was particularly notable in those cells with increased HER-2 or EGFR signaling. Histone H3 acetylation increased and HSP90 levels decreased, and the levels of the HSP90 client proteins EGFR, HER-2, Raf-1, p-Akt and p-ERK also decreased (41).

Panobinostat (25-50 nmol/l) together with ionizing radiation (2-6 Gy) synergistically reduced survival and induced apoptosis in the non-small cell lung cancer cell (NSCLC) lines NCI-H23 and NCI-H460. Panobinostat increased the duration of γ-H2AX foci at DNA double-strand breaks seen following irradiation, indicating that the drug disrupts DNA repair in irradiated cells, and resulted in HDAC4 foci confined to the cytoplasm. In mice bearing NCI-H460 xenografts, irradiation (2 Gy x 5) or panobinostat (40 mg p.o. x 2) alone delayed tumor growth by 4 and 2 days, respectively, whereas in combination they delayed tumor growth by 20 days, with minimal toxicity (42, 43).

Approximately one-quarter of human breast cancers do not express the estrogen receptor (ER), rendering them insensitive to endocrine therapy. Panobinostat inhibited the growth of both the ER-positive human breast cancer cell line MCF7 and the ER-negative cell line MDA-MB-231 (IC_{50} = 30 and 100 nM, respectively). In MDA-MB-231 cells and another ER-negative human breast cancer cell line, MDA-MB-435, panobinostat restored ER gene expression and enhanced the sensitivity of the cells to tamoxifen. Treatment of MDA-MB-231 cells with panobinostat led to reduced HDAC1 and HDAC2 mRNA and protein expression. Molecular analysis of MDA-MB-231 and MDA-MB-435 cells suggested that panobinostat restored the silenced ER gene by accelerating the degradation of DNMT1 and reorganizing the chromatin structure (44, 45).

Panobinostat arrested cell growth at the G2/M phase and induced apoptosis in several human biliary tract cancer cell lines, with a mean IC_{50} of 0.04 μM for growth suppression. In a chimeric mouse model, panobinostat reduced tumor mass by 66% (bile duct cancer) and 87% (gallbladder cancer) over 28 days compared to placebo-treated animals. The agent also potentiated the effect of gemcitabine in these models (46).

The immunosuppressive properties of panobinostat were demonstrated in mouse and human mixed lymphocyte reactions (IC_{50} = 7 nM). In a heterotopic rat heart transplant (DA→Lewis) model, 1 mg/kg/day s.c. panobinostat increased survival to 28 days from 7 days in placebo-treated animals. The combination of subeffective doses of panobinostat (0.3 mg/kg/day) with subeffective doses of either of the immunosuppressants everolimus (0.3 mg/kg p.o.) or FTY-720 (0.1 mg/kg p.o.) resulted in > 28 days' survival with no or minimal graft rejection (47).

Pharmacokinetics and Metabolism

Following a single i.v. injection of panobinostat (10 mg/kg) in mice, the systemic plasma clearance was 18.3 l/h/kg, the volume of distribution was 36.1 l/kg and the estimated elimination half-life was 1.37 h. Comparison of pharmacokinetic values obtained after a single oral dose (50 mg/kg) indicated that the oral bioavailability was 4.62% (48).

Human pharmacokinetic data are discussed below in the Clinical Studies section.

Safety

Panobinostat inhibits the hERG channel with an IC_{50} of 3.9 μM (compared to 0.03 μM for HDAC inhibition), indicating the possibility of cardiac arrhythmia as a complication of drug administration. In two phase I studies, 45 patients with advanced solid tumors or hematological malignancies were treated with escalating doses of panobinostat (1.2-20 mg/m^2/day i.v.) on days 1-3 and 8-10 of a 21-day cycle, on days 1-3 and 15-17 of a 28-day cycle or on days 1-7 of a 21-day cycle. Pharmacokinetic analysis showed dose proportionality, a half-life of 6-26 h and 1.5-fold accumulation by day 3. Pharmacodynamic analysis revealed an increase in histone acetylation at doses of 4.8 mg/m^2 and above. Central tendency analysis of post-dose ECGs showed a dose-dependent increase in Q-T_{cF} of 20 ms or less on day 3. Twelve patients (28%) were outliers, with Q-T_{cF} > 500 ms and/or a > 60-ms change from baseline, which was again dose-dependent and occurred at doses of 4.8 mg/m^2 and above, mostly on days 3-5 (49).

A 60-year-old man with highly proliferative AML was treated with panobinostat (30 mg p.o. 3 times a week every other week) as part of a phase I/II trial. After 2 weeks, he presented with deteriorating renal function. Within 24 h of recommencing panobinostat therapy in conjunction with hydroxyurea, allopurinol and hydration, laboratory tumor lysis syndrome developed, with hypercalcemia, hyperphosphatemia and hyperuricemia. The patient recovered but the syndrome developed again within 24 h of recommencing panobinostat treatment. This case demonstrated the need for caution when treating patients with a high tumor burden with the agent (50).

Clinical Studies

In a phase I trial of panobinostat, 13 patients with solud tumors received escalating doses (1.2-7.2 mg/m^2 i.v.) on days 1-3 and 8-10 (arm 1), or on days 1-3 and 15-17 (arm 2) of a 28-day cycle. One case of dose-limiting toxicity, *i.e.*, prolonged grade 2 thrombocytopenia, was observed at the dose of 7.2 mg/m^2 in arm 1. Other toxicities included neutropenia, hypoglycemia and anemia. No abnormalities in ECGs were observed. Pharmacody-

namic analysis indicated histone acetylation in peripheral blood lymphocytes of some patients. Dose-proportional increases in AUC_{0-24h} were seen and the half-life was approximately 15-20 h (51).

In another phase I trial, 15 patients with refractory hematological malignancies received panobinostat (4.8-14 mg/m^2 i.v.) on days 1-7 of a 21-day cycle. Four dose-limiting toxicities of asymptomatic and reversible grade 3 Q-T_{cF} prolongations were observed at the highest dose and one at 11.5 mg. Other possibly drug-related toxicities included nausea, diarrhea, vomiting, hypokalemia, loss of appetite and thrombocytopenia. Eight of 11 patients with peripheral blasts had transient blast reductions during the 7-day treatment period. Bone marrow blast counts tended to increase during treatment. Pharmacodynamic analysis of bone marrow and peripheral blood cells showed increased histone H3 and H2B acetylation in $CD19^+$ B-cells and $CD34^+$ blasts, and apoptosis in $CD14^+$ cells. Pharmacokinetic analysis showed a dose-proportional increase in AUC and a terminal half-life of about 11 h (52, 53).

A phase I study investigated oral panobinostat in patients with advanced solid tumors or lymphoma. Nine patients were treated at two dose levels (15 and 30 mg/day 3 times a week on a 28-day cycle). Dose-limiting toxicities were not observed, and adverse events included diarrhea, thrombocytopenia, fatigue, weakness, anorexia, nausea and vomiting. Two patients at the higher dose had an increase in Q-T_{cF} of 50-60 ms from baseline. Pharmacodynamic analysis showed histone acetylation in 5 of 6 patients receiving 30 mg/day for at least 24 h after dosing. Pharmacokinetic analysis showed a t_{max} of 2 h, a terminal half-life of 16.5 h and 1.5-fold accumulation at steady state (3 days). At the higher dose, the C_{max} was 9.4 ng/ml and the AUC_{0-24h} was 153 ng.h/ml. By comparison with i.v. studies, the oral bioavailability was estimated to be 17% (54).

Preliminary results were reported from an open-label phase I trial of panobinostat (20 or 30 mg p.o. 3 times a week) in 10 evaluable patients with advanced-stage cutaneous T-cell lymphoma (CTCL). Two patients achieved a complete response, 4 a partial response, 1 had stable disease and 2 had disease progression. Serious adverse events that required discontinuations were grade 3 diarrhea (n=2) and grade 2 fatigue (n=1). However, 3 months later, a complete or partial response was achieved by 2 of the patients who had discontinued treatment, indicating continued disease regression. Gene expression profiling indicated an inverse relationship between the number of genes altered and response (55, 56).

A phase I trial in patients with advanced solid tumors or CTCL (expected enrollment = 18) is under way in Japan (57), and a phase II/III trial in adult patients with refractory CTCL (expected enrollment = 118) is also under way in the U.S. (58). Three open-label, nonrandomized phase II/III studies are investigating panobinostat in patients with hematological cancers: one in patients with refractory chronic-phase CML (59), another in patients with refractory accelerated- or blast-phase CML (60) and a third in patients with refractory multiple myeloma (61). An open-label phase I study of panobinostat alone or in combination with i.v. docetaxel and prednisone in patients with hormone-refractory prostate cancer was recently terminated (62).

Source

Novartis (CH, US).

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