Histone deacetylase inhibitors and pancreatic cancer: Are there any promising clinical trials?

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Abstract
Pancreatic cancer, although not very frequent, has an exceptionally high mortality rate, making it one of the most common causes of cancer mortality in developed countries. Pancreatic cancer is difficult to diagnose, allowing few patients to have the necessary treatment at a relatively early stage. Despite a marginal benefit in survival, the overall response of pancreatic cancer to current systemic therapy continues to be poor, and new therapies are desperately needed. Histone deacetylase (HDAC) enzymes play an important role in the development and progression of cancer and HDAC inhibitors (HDACIs) have been shown to induce differentiation and cell cycle arrest, activate the extrinsic or intrinsic pathways of apoptosis, and inhibit invasion, migration and angiogenesis in different cancer cell lines. As a result of promising preclinical data, various HDACIs are being tested as either monotherapeutic agents or in combination regimens for both solid and hematological malignancies. Vorinostat was the first HDACI approved by the Food and Drug Administration for patients with cutaneous T-cell lymphoma. The use of HDACIs in clinical trials, in pretreated and relapsed patients suffering from advanced pancreatic cancer is discussed. Unfortunately, clinical data for HDACIs in patients with pancreatic cancer are inadequate, because only a few studies have included patients suffering from this type of neoplasm and the number of pancreatic cancer patients that entered HDACIs phase II/III trials, among others with advanced solid tumors, is very limited. More studies recruiting patients with pancreatic cancer remain to determine the efficiency of these therapies.

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Key words: Pancreatic cancer; Histone deacetylases; Histone deacetylase inhibitors; Clinical trials

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INTRODUCTION

Worldwide, over 200 000 people die annually of pancreatic cancer. The incidence of pancreatic cancer varies greatly across regions, with the highest incidence and mortality rates found in developed countries, which suggests roles for lifestyle and environmental factors. Deaths from pancreatic cancer rank fourth among cancer-related deaths in the United States. Risk factors for pancreatic cancer include, among others, high-fat diet, smoking, chronic pancreatitis, primary sclerosing cholangitis, hereditary pancreatitis, family history of pancreatic cancer, and diabetes mellitus. Age seems to be a significant risk factor, with incidence increasing with age.

Due to few early illness indicators and lack of screening tests, pancreatic cancer is difficult to diagnose and often at the time of presentation, the cancer has already become advanced. The overall 5-year survival rate among
patients with pancreatic cancer is < 5%. Approximately 20% of patients present with localized, potentially curable tumors. The majority (95%) of cases of pancreatic cancer are adenocarcinomas, resembling the pancreatic ductal cell. Metastasis of this cancer can be either local, most often involving the spleen, adrenal glands and transverse colon, or distant.\(^\text{11,12}\)

**TREATMENT OF ADVANCED PANCREATIC CANCER**

**Conventional chemotherapies**

For over a decade, gemcitabine-based therapy has been considered a first-line treatment for locally advanced and metastatic pancreatic cancer. Preference for gemcitabine over 5-fluorouracil (5-FU) was established in the mid-1990s, when a phase III trial comparing gemcitabine monotherapy to 5-FU demonstrated clinical benefit in gemcitabine-treated patients with advanced pancreatic cancer.\(^\text{13}\) The value of radiotherapy in the management of locally advanced pancreatic cancer remains unclear.\(^\text{14}\)

Numerous attempts have been made to improve the efficacy of standard gemcitabine monotherapy in patients with advanced pancreatic cancer, but little success has been achieved. Several phase III trials were undertaken with gemcitabine in combination with a range of chemotherapy agents. However, the combinations of gemcitabine with 5-FU,\(^\text{8,13}\) as well as irinotecan, oxaliplatin, pemetrexed, exatecan and cisplatin,\(^\text{6-10}\) all failed to show superiority over gemcitabine monotherapy.

Capecitabine is an orally administered fluoropyrimidine that is metabolized in both liver and tumor cells into 5-FU, resulting in high intratumoral 5-FU concentrations. Phase III trial results on capcitabine and gemcitabine combination appeared to be contradictory. In a recent phase III trial, the combination significantly improved objective response rate and progression-free survival (PFS), but did not show superiority in overall survival (OS) in patients with advanced pancreatic cancer.\(^\text{11}\)

In addition, folfirinox (5-FU/leucovorin, irinotecan, and oxaliplatin) treatment of metastatic pancreatic cancer patients in a randomized phase II trial of folfirinox versus gemcitabine, indicated that response rate was more than 30%. Furthermore, the median OS was 11.1 mo in the folfirinox group as compared with 6.8 mo in the gemcitabine group, indicating that folfirinox is an option for the treatment of patients with metastatic pancreatic cancer.\(^\text{12}\)

**Targeted therapies**

Targeted therapies have also been investigated for advanced pancreatic cancer. The matrix metalloproteinase inhibitors (MMPIs) marimastat and talomastat (BAY 12-9566) inhibit enzymes that play a key role in extracellular matrix (ECM) degradation, and angiogenesis. In phase III trials, neither marimastat monotherapy nor marimastat with gemcitabine, improved OS compared with gemcitabine monotherapy.\(^\text{13}\)

The farnesyl transferase enzyme Kras regulator tipifarnib in combination with gemcitabine did not improve OS compared with gemcitabine monotherapy in a phase III trial.\(^\text{14}\)

Erlotinib is a small-molecule tyrosine kinase inhibitor (TKI) of the human epidermal growth factor receptor (EGFR). A multicenter, randomized, double-blind, placebo-controlled phase III clinical trial of erlotinib in combination with gemcitabine, in patients with locally advanced or metastatic pancreatic adenocarcinoma met its primary endpoint, with the combination regimen being the first gemcitabine combination to demonstrate a statistically significant survival advantage over gemcitabine monotherapy and the regimen was consequently approved for metastatic disease.\(^\text{15}\)

Cetuximab, an anti-EGFR monoclonal antibody, blocks the extracellular EGFR domain, preventing ligand-dependent or independent activation and downstream signaling. An open-label, randomized phase III trial, unfortunately, recently failed to demonstrate a clinically significant advantage of the addition of cetuximab to gemcitabine for response and OS.\(^\text{16}\)

Bevacizumab is a recombinant, humanized IgG1 monoclonal antibody that selectively binds to vascular endothelial growth factor (VEGF), inhibiting its interaction with VEGF receptor-1 and -2, on the surface of endothelial cells. Despite recently reported negative results, a number of phase II and III studies are underway in advanced pancreatic cancer that include bevacizumab and cetuximab in combination with other agents.\(^\text{17}\)

Finally, a wide range of molecular-targeted agents that interact with crucial pathways for cell survival in pancreatic cancer are currently being explored. These include agents that target polyADP-ribose polymerase, histone deacetylase (HDAC), Src/Abl kinases, and mammalian target of rapamycin.\(^\text{18}\)

Given the positive data observed in phase III trials, gemcitabine, erlotinib and capcitabine are likely to form the base for future treatment strategies for advanced pancreatic cancer.

**Histone acetyltransferases and HDACs**

The organization of chromatin is crucial for the regulation of gene expression. Accumulating evidence suggests that the acetylation and deacetylation of histones play significant roles in transcriptional regulation of eukaryotic cells. Histone acetylation and deacetylation are catalysed by specific enzyme families, Histone acetyltransferases (HATs) and HDACs, respectively.\(^\text{19,20}\) There are at least four groups of proteins with intrinsic HAT activity, while 18 HDACs belonging to four distinct classes have been described in mammalian cells. Eleven of the HDACs are zinc dependent, classified on the basis of homology to yeast HDACs: Class I includes HDACs 1, 2, 3 and 8; Class II A includes HDACs 4, 5, 7 and 9; Class II B, HDACs 6 and 10; and Class IV, HDAC11. Class III HDACs, sirtuins 1-7, have an absolute requirement for NAD⁺, are not zinc dependent, and generally not inhibiting.
ated by compounds that inhibit zinc-dependent deacetylases\(^{29}\). Apart from deacetylating histones, HDACs have also been reported to interact with non-histone proteins, involved in numerous important cell pathways including control of gene expression, regulation of cell proliferation, differentiation, migration and death. The balance between acetylation and deacetylation is an important factor in regulating gene expression and disruption of HAT or HDAC activity is possibly associated with cancer development\(^{22,23}\).

**HDAC inhibitors**

HDAC inhibitors (HDACIs) are divided into different classes based on their chemical properties, including hydroxamic acids, such as suberoylanilide hydroxamic acid (SAHA), trichostatin A (TSA), LBH589 (panobinostat) and PXD101 (belinostat); short chain fatty acids, such as sodium butyrate (NaBu), 4-phenylbutyrate (4-PB) and valproic acid; cyclic tetrapeptides, such as trapoxin, apicidin and depsipeptide—also known as FK228 or romidepsin; benzamides, such as MS-275, CI-994 and MGCD0103; and a variety of other chemical compounds and synthetic inhibitors. HDACIs have three common structural characteristics: a Zn-binding moiety, an opposite capping group, and a straight chain alkyl, vinyl or aryl linker connecting the two\(^{24,25}\).

HDAC inhibition is able to promote a variety of different anticancer mechanisms including apoptosis, cell differentiation and cell cycle inhibition. HDACIs are a new class of antineoplastic agents currently being evaluated in clinical trials. While these agents have been studied extensively in the laboratory, only recently has their mechanism of action begun to be elucidated. Several HDACIs are now in various stages of development, including clinical trials as monotherapy and in combination with other anticancer drugs and radiation\(^{26,27}\).

In the present review, the available so far data regarding the different classes of HDACIs used in clinical trials including patients with pancreatic cancer are presented in Table 1.

**Hydroxamic acids**

SAHA (N-hydroxy-N’-phenyl-octanamide, vorinostat) is a synthetic hydroxamic acid, which is structurally related to the natural product, TSA (7-[4-(dimethylamino)phenyl]-N-hydroxyl-4,6-dimethyl-7-oxo-(2E,4E,6R)-2,4-heptadienamide), produced by selected strains of *Streptomyces platensis*, *Streptomyces hygroscopicus* Y-50 or *Streptomyces inyoensis*. Hydroxamic acids have a high affinity to biometals, including Fe\(^{3+}\), Ni\(^{2+}\) and Zn\(^{2+}\). The synthesis of SAHA and its potency to induce differentiation of murine erythroleukemia (MEL) cells was first reported in 1996. SAHA and TSA comprise a hydroxamic-acid-based metal-binding domain that coordinates the catalytic Zn\(^{2+}\) in the HDAC active site, a 5 (TSA) or 6 (SAHA)-membered carbon-based linker that mimics the Ca functional group of lysine, and a hydrophobic motif that interacts with the periphery of the HDAC binding pocket\(^{28}\).

SAHA: SAHA (vorinostat) is now undergoing several clinical trials. One current phase I trial is studying the side effects and best dose of SAHA given together with flavopiridol in treating patients with advanced solid tumors. Another trial is studying SAHA in patients with metastatic or unresectable solid tumors or lymphoma and liver dysfunction. Combination with doxorubicin is also under survey for solid tumors, as well as that with bortezomib, vinorelbine, gemcitabine and other agents like paclitaxel and carboplatin, fluorouracil/leucovorin and oxaliplatin. A phase I / II trial is studying the highest tolerable dose of SAHA that can be given in combination with radiotherapy to patients with locally advanced pancreatic cancer, as well the efficacy of combined therapy. Another phase I clinical trial is now examining the safety, pharmacokinetics, pharmacodynamics and efficacy of iv proteasome inhibitor NPI-0052 in combination with oral SAHA in patients with non-small cell lung cancer, pancreatic cancer, melanoma or lymphoma. A phase I / II study of SAHA in combination with radiotherapy and infusional 5-FU in patients with locally advanced adenocarcinoma of the pancreas is under way. Finally, SAHA in combination with capecitabine plus radiotherapy is also being evaluated in patients with non-metastatic pancreatic cancer. The above studies are recruiting for participants and possible patients suffering from pancreatic cancer remain to determine the efficiency of these therapies\(^{29}\).

**Cyclic peptides**

FK228 (FR901228, depsipeptide, romidepsin): Depsipeptide (18,4S,7Z,10S,16E,21R)-7-ethyldiene-4,21-bis (1-methylletheyl)-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo[8.7.6]tricos-16-ene-3,6,9,19,22-penton e, is a bicyclic peptide isolated from *Chromobacterium violaceum* and has demonstrated potent *in vitro* cytotoxic activity against human tumor cell lines and *in vivo* efficacy against human tumor xenografts. Upon entering cells, FK228 is reduced to an active compound, capable of preferentially interacting with the zinc in the active site of the HDAC class I enzymes, however, it is still generally classified as a broad-spectrum inhibitor as it does inhibit class II enzymes. It was approved by the United States Food and Drug Administration (FDA) for the treatment of cutaneous T-cell lymphoma (CTCL)\(^{30}\).

Among other current clinical trials for patients with advanced solid tumors, FK228 is being studied in combination with gemcitabine in patients with pancreatic cancer. This phase I / II dose escalation trial is designed to determine the maximum tolerated dose for the combination, as well as evaluate toxicities and objective disease responses. Furthermore, another phase II trial is studying the effectiveness of FK228 in patients who have locally advanced or metastatic neuroendocrine tumors; among them pancreatic islet tumors\(^{30}\).

**Short-chain fatty acids**

Valproic acid: Valproic acid (VPA) is now an established antiepileptic drug, by affecting the function of the neu-
The patient with pancreatic cancer experienced grade 3 hypophosphatemia as DLT and was finally excluded from study after cycle 2 due to PD; at 100 mg/m² one patient with advanced pancreatic cancer, developed grade 4 hyperbilirubinemia associated with febrile neutropenia on day 3. The patient developed thrombocytopenia on day 8 of treatment and anemia, necessitating platelet and blood transfusions. This was followed by an episode of atrial fibrillation on day 13 and then acute renal failure. The patient finally died on d18. PC: Pancreatic cancer; PD: Progressive disease; PR: Partial response; RPTD: Recommended dose for phase II; SD: Stable disease.

Table 1  Clinical studies on histone deacetylase inhibitors for treatment of patients with pancreatic cancer

| HDACI | Patients total number | Patients with PC number | Time schedule | Dosage schedule (HDACI) | MTD (HDACI) | Responses (for patients with pancreatic cancer treated with HDACI) | Main toxicities | Ref. |
|-------|-----------------------|-------------------------|---------------|-------------------------|-------------|-------------------------------------------------------------------|----------------|-----|
| VPA + epirubicin phase I | 48 | 1 | q21 d | 15-160 mg/kg per day iv/po d1, d2 | 140 mg/kg per day | - | - | Neutropenia, thrombocytopenia, hypocalcaemia, fatigue, QTc prolongation | [33] |
| 4-PB phase I | 21 | 1 | q28 d | 60-360 mg/kg iv 2 × daily d1-d5, d8-d12 | 300 mg/kg per day | - | 1 | Fatigue, headache, nausea, vomiting, confusion | [36] |
| MS-275 + 13-cis retinoic acid phase I | Not defined | 1 | q28 d | 4-5 mg/m² po, once weekly | 5 mg/m² | - | 1 | Hypotension, hypocalcaemia, anemia, fatigue | [39] |
| CI-994 phase II | 17 | 17 | Continues | 8 mg/m² per day po | - | - | 2 | N/D | Thrombocytopenia, fatigue, anemia, nausea, vomiting, bruising, hematuria | [42] |
| CI-994 phase I | 53 | 1 | a: acute q21 d b: chronic q10 wk | a: 10-15 mg/m² per day po d1-d14 b: 5-12.5 mg/m² per day po d1-d56 | a: 15 mg/m² b: 8 mg/m² | - | 1 | Thrombocytopenia, hypocalcaemia, fatigue, vomiting, confusion, paresthesias, alopecia | [43] |
| CI-994 + capcetita-bine phase I | 54 | 4 sch. c | a: q21 d b: q42 d c: q21 d | a: 4-10 mg/m² per day po d1-d14 b: 6 mg/m² per day po d1-d35 c: 4-8 mg/m² per day po d1-d14 | a: 10 mg/m² b: (abandoned) c: 8 mg/m² | - | 4 | Thrombocytopenia, anemia, diarrhea, nausea, vomiting, fatigue | [44] |
| CI-994 + carboplatin paclitaxel phase I | 30 | 2 | q21 d | 4-6 mg/m² per day po d1-d14 or d1-d7 | 6 mg/m² (d1-d7) | - | 2 | Thrombocytopenia, neutropenia, fatigue, alopecia, anemia | [45] |
| CI-994 + gemcita-bine vs gemcita-bine alone phase II | 174 | 174 | q28 d (86 + 88) | 6 mg/m² per day po d1-d21 | - | 8/68 | 60/68 | Thrombocytopenia, neutropenia, anemia, nausea, vomiting, alopecia, diarrhea | [46] |
| MGCD-0103 phase I | 38 | 2 | q21 d | 12.5-56 mg/m² per day po d1, 3, 5, 8, 10, RPTD: 45 mg/m² | 56 mg/m² | - | 2 | Fatigue, anemia, nausea, vomiting, diarrhea, abdominal pain, dehydration | [48] |
| LAQ824 phase I | 39 | 3 | q21 d | 6-100 mg/m² per day iv d1-d3 | 72 mg/m² RPTD: < 72 mg/m² | - | 3 | Thrombocytopenia, fatigue, anemia, nausea, vomiting, diarrhea, nonspecific ST-segment, hyperbilirubinemia, transaminitis | [50] |

The patient with pancreatic cancer experienced grade 3 hypophosphatemia as DLT and was finally excluded from study after cycle 2 due to PD; 1At 100 mg/m² one patient with advanced pancreatic cancer, developed grade 4 hyperbilirubinemia associated with febrile neutropenia on day 3. The patient developed thrombocytopenia on day 8 of treatment and anemia, necessitating platelet and blood transfusions. This was followed by an episode of atrial fibrillation on day 13 and then acute renal failure. The patient finally died on d18. PC: Pancreatic cancer; PD: Progressive disease; PR: Partial response; RPTD: Recommended dose for phase II; SD: Stable disease.
rotransmitter GABA. The finding that VPA was an effective inhibitor of HDACs arose from the observations that VPA was able to relieve transcriptional repression of a peroxisomal proliferation and activation of a glucocorticoid receptor (GR)-PPARα hybrid receptor, suggesting that it acts on a common mechanism in gene regulation, such as histone deacetylation, rather than on individual transcription factors or receptors. Consistent with this finding, it was shown that VPA causes hyperacetylation of the N-terminal tails of histones H3 and H4 in vitro and in vivo, and was found to inhibit HDAC enzymatic activity at a concentration of 0.5 mmol/L. VPA has shown potent antitumor effects in a variety of in vitro and in vivo systems, by modulating multiple pathways including cell cycle arrest, apoptosis, angiogenesis, metastasis, differentiation and senescence. Most preclinical and clinical data on the anticancer effects of VPA have been generated for malignant hematological diseases.

A phase I clinical trial investigated the safety, toxicity and maximum-tolerated dose of VPA and the topoisomerase II inhibitor epirubicin in solid tumors. Forty-eight patients with different malignancies were enrolled; one with pancreatic cancer. The patient suffering from pancreatic cancer experienced a partial response at 100 mg/kg VPA and 100 mg/m² on day 3 of the cycle, while no dose-limiting toxicities occurred. All patients with a partial response had at least a twofold increase in histone acetylation.

Phase I clinical trials are currently testing VPA in combination with other agents such as erlotinib, 5-FU, cyclophosphamide, bevacizumab, and azacytidine, as well as epirubicin to determine safety, tolerability and effectiveness in treating patients with advanced solid tumors. In addition, another phase II trial is studying VPA combined with the hypomethylating factor hydralazine. A phase I clinical trial is undertaking recruitment to determine maximum tolerated doses of VPA in combination with sunitinib, sorafenib, dasatinib, erlotinib, lapatinib, or lenalidomide for the treatment of patients with advanced solid tumors, as well as to estimate the safety and treatment response.

4-PB: 4-PB is a short-chain fatty acid known to inhibit reversibly class I and II HDACs. It is considered as an HDAC inhibitor of the first generation, as the HDAC inhibitory effect is not specific. Working concentrations are rather high, in the millimolar range, and the effects are pleiotropic. 4-PB is known to exert multiple effects in the cell, including the modulation of protein isoprenylation, which importantly regulates the ras proto-oncoprotein, and activation of the nuclear steroid PPARG. 4-PB exerts a potent antitumor effect in vitro and has been shown to cause growth inhibition and differentiation in various human cancer cell lines.

A phase I dose escalating trial to evaluate twice daily in 4-PB infusion has been undertaken. 4-PB was administered for five consecutive days for a total of 20 doses over two consecutive weeks from 60 to 360 mg/kg per day. Twenty-one patients with different malignancies, including one with pancreatic carcinoma, participated in the trial. Dose limiting toxicities were fatigue and headache, while no significant myelosuppression was seen. Three patients with brain malignancies remained stable for an average of 6 mo.

A current phase I clinical trial is investigating oral phenylbutyrate three times daily in patients with refractory solid tumors. Combination with azacitidine is also being studied to determine effectiveness and maximum dose in patients with advanced or metastatic solid tumors.

NaBu: NaBu has multiple effects on cultured mammalian cells that include inhibition of proliferation, induction of differentiation and induction or repression of gene expression. Sodium butyrate inhibits most HDACs except class II HDAC and class II HDAC6 and-10. Promoters of butyrate-responsive genes have butyrate response elements, and the action of butyrate is often mediated through Sp1/Sp3 binding sites.

In a recruiting phase I clinical trial, the butyrate prodrug tributyrin is being studied in patients with various advanced solid tumors.

Benzamides

MS-275: This synthetic benzamide derivative (3-pyridylmethyl-N-[(2-aminophenyl)carbamoyl]benzyl)carbamate) has been shown to inhibit HDACs, and has antitumor activity in many preclinical models. The first clinical trial with this agent in 2005 included patients with advanced solid tumors or lymphoma. At high concentrations of MS-275, there is a marked induction of reactive oxygen species, mitochondrial damage, caspase activation and apoptosis. Treatment of sensitive tumor cell lines with MS-275 induces gelsolin, a maturation marker, and produces a change in the cell cycle distribution with a decrease in S phase and an accumulation of cells in G1. The in vivo therapeutic efficacy of MS-275 has been shown in a variety of human tumor xenograft models.

A phase I study determined the maximum tolerated dose, the dose limiting toxicity and the pharmacokinetic or pharmacodynamic profile of MS-275 in combination with 13-cis-retinoic acid. Patients with advanced solid tumors were treated with MS-275 orally, once weekly, and 13-cis-retinoic acid orally, 1 mg/kg twice daily, for 3 wk every 4 wk. One patient suffering from pancreatic cancer remained on treatment for 6 mo and a patient with renal cell carcinoma showed a partial response in the lungs. Side effects included hyponatremia, neutropenia, anemia and fatigue.

Another phase I study evaluated the toxicity and pharmacokinetic profiles of MS-275 in patients with refractory solid tumors and lymphomas, including one patient suffering from metastatic pancreatic cancer, on three different schedules. The patient with metastatic pancreatic cancer developed grade 3 hypophosphatemia, thus meeting criteria for dose limiting toxicity, and was
finally removed from study due to disease progression after cycle 2. Objective responses were only observed in patients receiving the every-other-week dosing schedule, but the numbers of patients enrolled were too small to determine whether this dosing regimen was truly more efficacious.

CI-994: CI-994 or N-acetylindolinaline [4-(acylamino)-N-(2-amino-phenyl) benzamide] is a novel oral compound with a wide spectrum of antitumor activity in preclinical models. The mechanism of action may involve inhibition of histone deacetylation and cell cycle arrest. CI-994 is currently undergoing clinical trials. Although several changes in cellular metabolism induced by the drug have been characterized, the primary molecular mechanism of its antitumor activity remains unknown.

A phase II trial of CI-994 in patients with advanced pancreatic cancer evaluated the antitumor activity and safety of CI-994. CI-994 was administered orally at 8 mg/m^2 per day. Seventeen patients were enrolled, including 15 with metastatic disease. Among patients evaluable for response, stable disease for 8 wk occurred in two patients (12%). Overall, median time to progressive disease was 6 wk and median survival was 10 wk, with one patient alive at 41 wk. Grade 3 thrombocytopenia occurred in eight patients but grade 4 in none. Most common non-hematological toxicities were generally grade 1 or 2 and were identical with both treatments. Consequently, in this study, CI-994 in combination with gemcitabine did not appear to offer any benefit compared to gemcitabine as a single agent for the treatment of pancreatic cancer.

A randomized, double-blind, placebo-controlled, multicenter study compared whether CI-994 plus gemcitabine improved OS, duration of response, time to treatment failure, and quality of life compared to gemcitabine alone. Patients had diagnosis of advanced or metastatic adenocarcinoma of the exocrine pancreas and were not considered surgical candidates. A total of 174 patients received CI-994 6 mg/m^2 per d orally on days 1-21 plus gemcitabine 1000 mg/m^2 on days 1, 8 and 15 or placebo plus gemcitabine 1000 mg/m^2 on days 1, 8 and 15 of each 28-d cycle. There was no observed difference in survival time between the two cases. The estimated median survival was 194 and 214 d and objective response rates based on investigator assessments were 12% and 14%, respectively. In addition, pain responses did not differ significantly. Treatment with CI-994 was associated with more cases of grade 3/4 thrombocytopenia, anemia and leukopenia than with placebo, while non-hematological toxicities such as nausea, vomiting, anorexia and diarrhea were identical with both treatments. Consequently, in this study, CI-994 in combination with gemcitabine did not appear to offer any benefit compared to gemcitabine as a single agent for the treatment of pancreatic cancer.

MGCD0103: MGCD0103 is an isotype-specific amino-phenylenbenzamide that inhibits HDAC classes I and IV, with almost no class II effect. MGCD0103 is well tolerated and exhibits favorable pharmacokinetic and pharmacodynamic profiles, demonstrating target inhibition and clinical responses. It induces cell death and autophagy, synergizes with proteasomal inhibitors and affects non-histone targets, such as microtubules.

In a phase I study MGCD0103 was given three times weekly orally to patients with advanced solid tumors to determine safety, tolerability and pharmacokinetics. Thirty-eight patients were enrolled and completed a total of 99 cycles of MGCD0103, including two with pancreatic cancer. No objective tumor responses were observed. Five patients with previously progressive colorectal, renal cell and lung cancers had stable disease for four or more cycles. Furthermore, MGCD0103 exerted dose-dependent HDAC inhibitory activity and was able to induce histone acetylation in peripheral leukocytes.

Oral MGCD0103 three times weekly is currently being studied in combination with gemcitabine, in patients with advanced solid tumors. In this phase I/II study, patients with locally advanced or metastatic pancreatic cancer were treated into five treatment cohorts, including two suffering from pancreatic cancer. Five patients achieved a partial response (non-small cell lung, colon, unknown primary origin) and two patients achieved a complete response (esophageal and bladder cancer). It was observed that patients whose histone H3 acetylation in peripheral blood lymphocytes was at least 1.5-fold greater after treatment had an objective clinical response or stable disease.

A randomized, double-blind, placebo-controlled, multicenter study investigated the toxicity profile, maximum tolerated dose and pharmacokinetics of CI-994 in combination with capecitabine. Fifty-four patients were treated according to three different dosing schemes in combination with capecitabine. Fifty patients were treated with CI-994 daily for treatment duration of 15 with metastatic disease. Among patients evaluable for response, stable disease for 8 wk occurred in two patients, most of them suffering from colorectal, pancreatic cancer, received CI-994 daily for treatment duration of 5 wk. Objective responses were only observed in patients receiving the every-other-week dosing schedule, but the numbers of patients enrolled were too small to determine whether this dosing regimen was truly more efficacious.

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cancer can participate. Maximum tolerated dose of MGCD0103 and objective response of patients remain to be determined\[^{28,29}\].

**Other HDACIs**

A phase I study of LAQ824 has determined the safety, maximum tolerated dose, and pharmacokinetic-pharmacodynamic profile in patients with advanced solid tumors. Thirty-nine patients were recruited and were eligible for assessment of toxicity, including three with pancreatic cancer. At 100 mg/m\(^2\), one patient who had advanced pancreatic cancer, developed grade 4 hyperbilirubinemia associated with febrile neutropenia on day 3. The patient finally died after an episode of atrial fibrillation and acute renal failure 18 d after the first infusion. All patients treated with LAQ824 at 12 mg/m\(^2\) or above showed increase in histone acetylation. No objective responses were documented. One patient with hepatocellular carcinoma and two with fibrosarcoma and papillary carcinoma of the thyroid showed disease stabilization\[^{24}\].

A phase I study is investigating safety, pharmacokinetic and antitumor activity, and pharmacokinetics of PXD101 (belinostat) alone and in combination with 5-FU in patients with advanced solid tumors or lymphoma\[^{51}\].

Another recruiting phase I trial is studying the safety, tolerability and pharmacokinetics of orally administered PXD101 in combination with carboplatin and/or paclitaxel in patients with advanced solid tumors or lymphoma\[^{54}\].

A recruiting phase I study is evaluating the pharmacokinetics and safety of oral LBH589 (panobinostat) in patients with advanced solid tumors and varying degrees of renal function. Another phase II trial will determine tumor response, toxicity and tolerability of LBH589 in patients with gastrointestinal neuroendocrine tumors. A phase I A, dose-escalating study of iv LBH589 in adult patients with advanced solid tumors is ongoing. Another phase I study will evaluate the safety and tolerability of the combination of LBH589 and paclitaxel/carboplatin in patients with metastatic or locally advanced solid tumors. Finally, a phase I dose escalation trial of LBH589 and gemcitabine in patients with solid malignancies has been temporarily suspended\[^{25,28}\].

Other HDACIs, such as CHR-3996, CRA-024781, SB939 and R306465, are under phase I trials for the treatment of patients with advanced solid tumors\[^{28}\].

**CONCLUSION**

The experimental results of HDACIs led to their use in clinical trials, especially in pretreated and multiply relapsed patients at an advanced cancer stage. Additionally, HDACIs constitute a promising treatment for cancer due to their low toxicity. The first HDACIs tested in clinical trials have shown encouraging antitumor effects, at doses well tolerated by patients. Vorinostat (SAHA) was the first HDACI to be approved by the FDA for clinical use in patients with hematological malignancy (CTCL). HDACIs alone and in combination with a variety of cytotoxic or other targeted anticancer agents are now being tested. To date, at least 10 different HDACIs, including SAHA, VPA, NaBu, MS-275, CI-994, FK228, PXD101, LAQ824 and others, are in phase II or III clinical trials for the treatment of hematological and solid tumors.

Despite efforts in recent decades, conventional treatments such as surgery, radiation and chemotherapy, have slightly affected the course of pancreatic cancer. The development of effective systemic treatments, capable of reversing the biology of this aggressive disease, remains a critical requirement. Unfortunately, clinical data for HDACIs on patients with pancreatic cancer are inadequate (Table 1). So far, only a few studies have included patients suffering from this type of neoplasm. Additionally, the number of pancreatic cancer patients that entered HDACIs phase II/III trials, among others with advanced solid tumors, is very limited. Although HDACIs are recognized as some of the most promising agents, more studies recruiting candidates with pancreatic cancer remain to determine the efficacy of these therapies.

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