Chinese-German J Clin Oncol
DOI 10.1007/s10330-014-1286-3
April 2014, Vol. 13, No. 4, P185–P188

Progress in clinical trial of histone deacetylase (HDAC) inhibitors for non-small cell lung cancers*

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Received: 5 January 2014 / Revised: 1 March 2014 / Accepted: 18 March 2014

Abstract Histone deacetylase (HDAC) inhibitors, which represent a structurally diverse group of molecules, have emerged as a novel therapeutic class of molecules with significant anticancer potential. Vorinostat and romidepsin, known as the first generation of HDAC inhibitors, were approved in the United States for the treatment of T-cell lymphomas. Preliminary activity of HDAC inhibitors has also been observed in non-small cell lung cancer (NSCLC) in combination with the existing treatment regimens, of which is the focus of the current review.

Key words histone deacetylase (HDAC) inhibitor; non-small cell lung cancer (NSCLC); treatment; progress

Histone deacetylase (HDAC) inhibitors, a new class of anti-tumor drugs functioning in epigenetic regulation, have been developed rapidly over the past decade. HDAC inhibitors were proven to be effective not only in hematological malignancies as single agents, but also in solid tumors combined with other treatments. This paper will briefly review the clinical research progress of HDAC inhibitors in treating non-small cell lung cancers (NSCLC).

HDAC and HDAC inhibitors

Chromatin histone acetylation and deacetylation are key steps in epigenetic regulation. These two reversible processes, jointly regulated by histone acetyltransferase (HAT) and HDAC, are in dynamic equilibrium under normal physiological conditions. HDACs are a large family of enzymes, which can be divided into four major class with 18 subtypes. It has been found that HDACs play crucial roles in tumor genesis and development, such as tumor suppressor gene silencing, cell differentiation, angiogenesis, cell migration, cell cycle abnormalities, signal transduction, and cell adhesion. Specifically, cancer metastasis, recurrence, and drug resistance are closely associated to such processes as epithelial-mesenchymal transition, cancer stem cells differentiation, and immune escape with reversible signaling pathways. By changing the acetylation status of chromatin histone, HDAC inhibitors may epigenetically regulate gene transcription and thus disrupt these aberrant processes and finally induce suppression of tumor growth. Meanwhile, HDAC inhibitors also have the potential of synergistic actions with other drugs or therapies (such as chemotherapy, radiotherapy and immunotherapy) with different anti-tumor mechanisms in clinical applications.

Currently, there are mainly three categories of HDAC inhibitors in the market or valuable for in-depth clinical studies. (1) Hydroxamic acids: Vorinostat (SAHA) developed by Merck was approved by American FDA in late 2006 for the treatment of cutaneous T-cell lymphoma (CTCL). A series of other hydroxamic acids, all unselective HDAC inhibitors, are also in various stages of clinical research, including Panobinostat, Belinostat (PXD101), Resminostat (4SC-201), LAQ824, and IFT2357. (2) Cyclic tetrapeptides: HDAC inhibitor Romidepsin (FK228), a macrolide peptide developed by Celgene Corporation, was approved by the U.S. FDA for the treatment of CTCL in 2009 and for relapsed/refractory peripheral T-cell lymphoma (PTCL) in 2011. (3) Benzamides: Clinical trials in different phases are currently examining benzamides for several different indications. These benzamides include Entinostat (MS-275) from Syndax Corporation and Chidamide developed by Chipscreen in China, both of which are class I selective HDAC inhibitors.

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* Supported by a grant of New Key Drug Formulation Grand in the 12th Five-Year Plan of the National Science and Technology Project of China (No. 2012zx09303-012).

Single-agent efficacy of HDAC inhibitors in different tumors

Apart from other potential mechanisms, HDAC inhibitors suppress cell cycle of the tumor cells and induce apoptosis, and therefore can be used in clinical treatment alone or in combination with other drugs. There have been more than 20 HDAC inhibitor drugs at various stages of clinical research over the past decade. Monotherapy showed remarkable results in treating hematological malignancies. Vorinostat (SAHA) and Romidepsin (FK228) were approved as monotherapy for the treatment of CTCL by FDA in 2006 and 2009, respectively, and Romidepsin was also approved as monotherapy for the treatment of PTCL in 2011. Chipscreen in China completed phase II clinical trials of Chidamide, and submitted a new drug application to the China Food and Drug Administration (CFDA) in early 2013. Additionally, other HDAC inhibitors, including Entinostat (MS-275), Panobinostat (LBH589), Belinostat (PXD101), and Resminostat (4SC-201), are in phase II clinical studies for various hematological cancers, such as PTCL, CTCL, multiple myeloma, diffuse large B-cell lymphoma, and myelodysplastic syndrome.

For patients with solid tumors, although better tolerated, the monotherapy of HDAC inhibitors showed no significant effects and the objective response rates were often less than 10%. In some single-agent clinical trials, not even one patient responded. For example, in a phase II clinical trial of Vorinostat as monotherapy treating recurrent pleomorphic interstitial cell tumors, only two out of 52 patients had shown partial response (PR) for only a week [1]. Still, in other clinical trials investigating Vorinostat as monotherapy for non-small cell lung cancer [2], breast cancer [3], prostate cancer [4], head and neck cancer [5] and thyroid cancer [6], no patients had gained any observable benefits. Similarly, Romidepsin monotherapy showed only minimal efficacy in lung cancer [7], prostate cancer [8], colorectal cancer [9], renal cell carcinoma [10], and neuroendocrine tumors [11]. Other drugs such as Panobinostat [12] did not demonstrate satisfactory results in treating solid tumors, either. However, the results of many preclinical studies and some exploratory clinical studies indicated that the inhibitory mechanisms of HDAC inhibitors were complementary to the anti-tumor effects of other treatment, especially for tumor cells that are insensitive to chemotherapy, stem cell-like, and of no genetic mutations or obvious cell proliferation. Therefore, HDAC inhibitors might have significant synergistic effects in combination with various chemotherapeutic drugs and targeted therapeutic drugs. Up to date, great progresses have been made in the treatment of NSCLC using combination therapies with HDAC inhibitors.

HDAC inhibitors in combination with other drugs in the treatment of NSCLC

Vorinostat (SAHA)

In a phase I clinical trial of Vorinostat combined with paclitaxel and carboplatin for solid tumors [13], 19 patients with NSCLC were enrolled, and 10 (52.6%) of them showed partial response.

In a randomized, double-blind phase II clinical trial [14] to further investigate effects of the three-drug combination as first-line therapy for patients with stages IIIB and IV NSCLC, the response rates of the Vorinostat-treatment group (Vorinostat + paclitaxel + carboplatin) and the control group (placebo + paclitaxel + carboplatin) were 34% and 12.5% ($P = 0.02$), respectively. The Vorinostat-treatment group also showed a trend of longer progression-free survival (PFS) (6.0 months vs 4.1 months, $P = 0.48$) and overall survival (OS) (13.0 months vs 9.7 months, $P = 0.17$). However, the addition of Vorinostat also led to the aggravation of toxicity in the experimental group, in which the incidence of grade 4 thrombocytopenia was significantly higher than that of the control group (18% vs 3%, $P < 0.05$), and nausea, vomiting, fatigue, dehydration and hyponatremia were also more common.

However, the positive results from the exploratory phase II clinical trials were not confirmed in the phase III clinical trial. The latter trial enrolled 253 patients with stage IIIb or IV NSCLC without biomarker screening or systemic therapy till interim analysis. The results showed that 28 cases (22.2%) in the experimental group (Vorinostat + paclitaxel + carboplatin, $n = 126$) achieved response, while 36 cases (28.3%) in the control group (placebo + paclitaxel + carboplatin, $n = 127$). The median OS and PFS of the experimental group were both shorter than those of the control group, which were 11 months vs 14 months and 4.3 months vs 5.5 months, respectively. These differences between the two groups, however, were insignificant, and the study was discontinued because the preset primary endpoint was not reached in the interim analysis.

In addition, the phase II clinical trials to investigate Vorinostat and bortezomib regimens as the third-line NSCLC therapy were also terminated due to disappointing results [15]. Nevertheless, Vorinostat combined with cisplatin and gemcitabine in the treatment of NSCLC has shown initial effects in another phase I clinical trial. Among the 19 cases of evaluable patients, there were 9 cases (47%) of PR, 8 cases of stable disease (SD) and 2 cases of progressive disease (PD) [16].

Entinostat (MS-275)

Currently, many prospective clinical studies [17–19] suggested that epithelial cadherin (E-cadherin, E-cad), a type of cell adhesive molecule, had a close relationship with

lung cancer invasion and metastasis, cell differentiation and disease prognosis, and that E-cad expression was an important favorable prognostic factor for the survival of NSCLC patients.

In a randomized, double blind, placebo-controlled phase II clinical trial [20] of Entinostat for NSCLC, the researchers conducted a subgroup analysis on the responses of patients with varying degrees of E-cad expression. This trial enrolled a total of 132 patients with advanced/progressive NSCLC who had previously received one or two chemotherapy regimens but not with EGFR inhibitors. The patients were randomly assigned by 1:1 to one group treated with Entinostat + erlotinib (EE group) and another group with erlotinib + placebo (EP group). In the population with high expression of E-cad (grade 3+), the median OS of EE group was 9.4 months (n = 14), which was significantly higher than 5.4 months of the EP group (n = 12) (HR = 0.35, P = 0.03). Meanwhile, EE group also had relatively longer PFS compared with EP group, and PFSs of the two groups were 3.7 months vs 1.9 months (HR = 0.55, P = 0.19). However, in the population with low expression of E-cad (≤ 2+) and in all enrolled patients, the median OS and PFS were not statistically different between the EE and EP groups. This study suggested that HDAC inhibitors may have more pronounced efficacy in patients with high expression of E-cad. Since NSCLC patients with high E-cad expression account for 40% of the entire NSCLC population, this study might provide new ideas and strategies for the treatment of NSCLC.

Chidamide

Chidamide (Ai Pu Sha, CS055), belonging to benzamides and highly selective in class I HDAC1, 2, 3 subtypes, was independently designed and developed by Shenzhen Chipscreen Biosciences Ltd., China. The registrational clinical trial of Chidamide as monotherapy was completed with PTCL as the indication and shown definite therapeutic effects.

Combination therapy with PC for advanced NSCLC

A phase Ib clinical trial of Chidamide in combination with paclitaxel and carboplatin (PC) for the treatment of advanced NSCLC was conducted at the Cancer Hospital in Chinese Academy of Medical Sciences initiated in March 2011. An open-labelled, single-center, dose-escalation design was adopted in the trial. The dose of PC was fixed in the trial, while that of Chidamide was incremental. Each treatment cycle lasted three weeks with intravenous infusion of paclitaxel (175 mg/m^2) and carboplatin (AUC = 5 mg/mL/min) on the fifth day. Chidamide, starting at 20 mg, was administered twice a week without withdrawal intervals. The three patients in the 20 mg dose group did not present with protocol-defined dose-limiting toxicity (DLT), and two of the four patients in the 30 mg group showed DLT (the second cycle of chemotherapy was delayed due to low platelet counts). To further identify the maximum tolerated dose of Chidamide in combination with PC for the treatment of NSCLC, the combination of 25 mg Chidamide with the PC was further studied after the completion of the 30 mg dose group. Three patients receiving this combination regimen showed no DLT.

As for these combination therapies, the main adverse events were hematologic toxicity, including leukopenia/neutropenia, thrombocytopenia and low hemoglobin. Non-hematologic toxicity included gastrointestinal reactions, numbness of extremities, hair loss, pain, fatigue, and nosebleed. Hematologic toxicity was mainly grade 1 to 2. Patients with grade 4 toxicity included one case of leukopenia/neutropenia, one case of thrombocytopenia, and another case of neutropenia. Non-hematologic toxicity was mainly grade 1 without grade 3 to 4. These adverse events were mostly mild to moderate. After symptomatic treatment, toxic reaction was either reduced or ceased, suggesting that the regime of Chidamide combined with PC had controllable toxicity.

Four of the 10 patients who participated in the trial completed the predefined four cycles of Chidamide combined with PC therapy. In the second cycle, eight patients were evaluated with regard to the efficacy of the combination therapy. The three patients in the 20 mg group were in PR, SD and PD, the 2 patients in the 30 mg group were in SD and PD, and the 3 patients in the 25 mg group were in SD, PD and PD, respectively.

Among all patients enrolled, four patients had brain metastasis upon enrollment, and were subsequently treated with combination chemotherapy of PC and Chidamide. Two of them had their brain metastasis remitted, which suggested that the regime of Chidamide combined with PC might be potentially efficacious for brain metastasis.

Phase II clinical trials of the combination therapy with PC for advanced NSCLC

A multi-center phase II clinical trial of the combination of PC and Chidamide was launched for the treatment of advanced NSCLC in March 2013. The trial was aimed to explore the clinical benefits of this combination regime for NSCLC patients with negative EGFR mutation and thus lacking of effective drug treatment. Considering that E-cad is closely associated with the invasion and prognosis of NSCLC and that Chidamide could upregulate E-cad expression in lung cancer cells as demonstrated by preclinical studies, this trial also set up a correlation analysis between the level of E-cad expression and the efficacy. Specific designs of the trial will be presented in future relevant occasions.

Discussion

With encouraging efficacy and mild adverse reactions, molecular-targeted therapy has opened up new ways for

NSCLC treatment and brought new hopes to NSCLC patients in recent years. However, there are also many problems to be solved for molecular targeted therapy, such as recognizing suitable population with specific clinical features, managing drug resistance and combining with chemoradiotherapy. For the NSCLC patient population, those with positive EGFR and ALK mutations can be effectively treated with EFGR inhibitors and ALK inhibitors, respectively. However, there is still lacking of ideal chemotherapy or targeted therapy for patients with negative EGFR or ALK mutations and for patients resistant to EGFR and ALK targeted drugs. Therefore, it is both necessary and urgent to conduct clinical studies on HDAC inhibitors and other new targeted drugs with novel antitumor mechanisms for this group of patients.

Besides, a large number of prospective clinical studies have shown that E-cad is a favorable prognostic factor for the survival of NSCLC patients. Clinical trials of HDAC inhibitors also preliminarily confirmed that they could further promote the survival of the patients with high expression of E-cad. These studies will contribute to the development and improvement of personalized treatment of NSCLC.

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