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Report on Investigational Drugs

A Novel Approach to Cancer Therapy using PX-478 as a HIF-1α Inhibitor

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Hypoxia-inducible factor-1α (HIF-1α) is a transcription factor produced by tumor cells under hypoxic conditions, and a key regulator of a number of genes important in cancer biology. Over-expression of HIF-1α in human tumors is associated with poor prognosis and poor therapeutic outcomes and HIF-1α has been suggested as a novel target for cancer therapy. This article provides a review of PX-478 as the first novel HIF-1α inhibitor in clinical stage for the treatment of solid tumors.

Hypoxia is a feature of solid tumors. As they grow, oxygen supply to them is limited. In order to survive and sustain growth under hypoxic conditions, tumor cells upregulate specific genes that promote angiogenesis and survival (Kung et al., 2000). HIF-1α is a major regulatory transcription factor required for adaptation to hypoxia. Under hypoxic conditions, HIF-1α is stabilized and translocates to nucleus and activates target genes in the form of heterodimer along with HIF-1β. More than 70 putative HIF-1 target genes have currently been identified and many of these are known to play important roles in tumor progression such as tumor survival, neovascularization and energy metabolism (Lee et al., 2004). Clinically, increased tumor HIF-1α levels are a marker of aggressive disease, and are associated with poor patient prognosis and treatment failure for a number of malignancies, including breast, lung, ovarian, cervical, esophageal, and oropharyngeal cancers (Aebersold et al., 2001). Given this background, it was suggested that HIF-1α could be a novel and effective target of cancer therapy and its inhibitors have the potential to target multiple cancer processes such as angiogenesis, cell proliferation, apoptosis, glucose metabolism and cell invasion (Belozerov and Van Meir, 2005). So far, various HIF-1α inhibitors, including known anticancer drugs acting on the HIF-1 pathway and novel HIF-1α inhibitors, are in preclinical and clinical trials (Onnis et al., 2009). For example, YC-1 is in preclinical stage and a Phase I clinical trial for topotecan as an HIF-1α inhibitor was completed. Our research team is also developing small molecule HIF-1α inhibitors, which modulate the expression and stability of HIF-1α and are now in a preclinical stage of drug development with support from the Ministry of Education, Science and Technology in Korea. Among these, PX-478 is one of the leading novel HIF-1α inhibitors developed by Oncothyreon Inc (Macpherson and Figg, 2004). Last year, a Phase I clinical trial of PX-478 was completed. PX-478 was tested against advanced solid tumors and lymphomas. Herein, we will discuss the development of PX-478, the first novel HIF-1α inhibitor as a form of anticancer chemotherapy.

PX-478 (*S*-2-amino-3-[4-*N*,*N*,-bis(2-chloroethyl)amino] phenyl propionic acid *N*-oxide dihydrochloride), which is derived from melphalan and which is orally bioavailable, was reported to suppress HIF-1α levels in human tumor xenografts and inhibit the expression of HIF-1 target genes including vascular endothelial growth factor (*VEGF*) and the glucose transporter-1 (GLUT-

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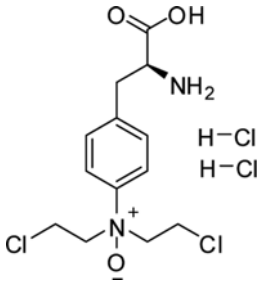

Fig. 1. Chemical structure of PX-478

1). PX-478 had marked antitumor activity against even large tumor xenografts, which correlated positively with HIF-1α levels (Welsh et al., 2004). Even though the upstream targets have not been fully elucidated, PX-478 inhibited HIF-1α at multiple levels that together or individually might contribute to its antitumor activity against HIF-1α-expressing tumors (Koh et al., 2008). It was reported that PX-478 inhibited HIF-1α protein levels and HIF-1 transactivating activity in a variety of cancer cell lines. The inhibition occurred in both normoxic and hypoxic conditions and did not require pVHL or p53. Three mechanisms were identified as contributing to the decrease in HIF-1α levels by PX-478: reduction in HIF-1α mRNA levels, and inhibition of HIF-1α translation play major roles; inhibition of HIF-1α deubiquitination, appears to play a minor role. HIF-1α was up-regulated in irradiated tumors and thus might serve as a promising target for radiosensitization. PX-478 reduced HIF-1α protein levels and signaling *in vitro* in a dose-dependent manner and provided direct radiosensitization of hypoxic cancer cells in clonogenic survival assays using C6 glioma, HN5 and UMSCCa10 squamous cells, and Panc-1 pancreatic adenocarcinoma cell lines. Of note, PX-478 yielded striking *in vivo* tumor sensitization to a single-dose irradiation, prevented postradiation HIF-1 signaling, and abrogated downstream stromal adaptation in C6 and HN5 reporter xenografts (Schwartz et al., 2009, 2010).

A Phase I trial was done at two sites in the US and the result was reported at the 2010 ASCO Annual Meeting (Tibes et al., 2010). The Phase I trial of PX-478 was an open-label, dose escalation trial in 41 patients with advanced cancer that was designed to examine safety, tolerability, pharmacokinetics, pharmacodynamics and antitumor activity. PX-478 was administered orally on days 1-5 of a 21 day cycle at doses ranging from 1 mg/m^2 to 88.2 mg/m^2. Adverse events occurring in more than 10 percent of patients were nausea, fatigue, diarrhea and vomiting. One patient experienced prolonged Grade 3 thrombocytopenia at the highest dose level. Thirteen of 37 evaluable patients (35%) had stable disease. Pharmacodynamic studies revealed dose-proportional inhibition of HIF-1α levels. Pharmacokinetic studies demonstrated low levels of PX-478 with evidence for conversion to melphalan and other metabolites. The result was promising in that clinical treatment with PX-478 was associated with a relatively high proportion of patients achieving stable disease and a dose-dependent inhibition of HIF-1α. Metabolite identification and related metabolism studies might be done to identify metabolic pathways and active entities from PX-478 besides inactive melphalan.

Conclusively, HIF-1α was proven to be a valid target of cancer therapy and modulates multiple tumorigenic processes. The information from preclinical and clinical trials of PX-478 will be useful for developing a novel approach to cancer therapy with HIF-1α inhibitors and give positive impacts to following developments and appilications of HIF inhibitors.

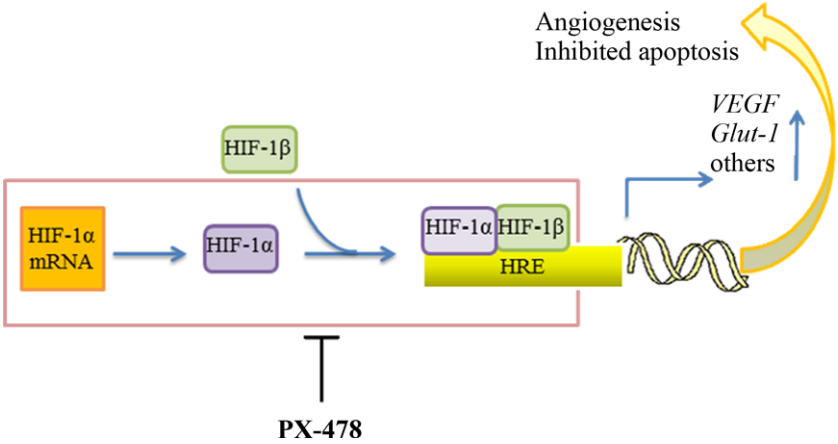

Fig. 2. Mode of action of PX-478. PX-478 inhibits HIF-1α at multiple levels that together or individually might contribute to its antitumor activity against HIF-1α-expressing tumors.

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Main Research Area

Cancer therapeutics: HIF-1, Runx-3, PRX, HDAC
Lead optimization: efficacy, PK, toxicity
Development of new methods of pharmacology