Regulation of Pancreatic, Gliosarcoma, and Non-Small Cell Lung Cancer via CPI-613, a Novel Selective Anticancer Therapeutic Agent

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AACR Annual Meeting 2012 Abstract # 3807

Abstract

The novel anticancer agent, CPI-613, is primarily known to affect cancer cell energy metabolism. Here we demonstrate that CPI-613 also shows cancer cell-cycle specific effects on human cancer cell lines BxPC-3 (human pancreatic), SF539 (human gliosarcoma), and H20 (human non-small cell lung) but on non-transformed NIH 3T3 (mouse fibroblasts) and BBE (normal human bronchial epithelial) cells. Specifically, gene regulation studies were performed by comparing differences in gene expression between treated and control cells using the Ingenuity® software. The results showed that CPI-613 altered expression of genes related to cell-cycle progression as well as energetic metabolic processes and pathways.

The main metabolic pathways regulated by CPI-613 include amino acid metabolism; pyrimidine metabolism; and the citric acid (TCA) cycle. CPI-613 regulates the ATP (adenosine triphosphate) relevant pathways to activate cell signaling leading to downregulation of cdk2 and cdk4. Activation of p32 signaling pathway also plays a role in inducing apoptosis in cells. Additionally, Cdk 3 and 4 were downregulated in cancer treated with CPI-613 compared to normal cells, thereby helping the apoptosis multiple genes in in further. This study may suggest multiple mechanisms of action of CPI-613 treatment.

CPI-613 is selectively toxic to cancer cells

Metabolic pathways regulated by CPI-613 in BxPC-3, H460, and SF539 cancer cells

Figure 5. Identification of metabolic pathways regulated by CPI-613 in cancer cells using Ingenuity® software. CPI-613 impairs glycolysis, pentose phosphate pathway, and tryptophan degradation; this may indicate that CPI-613 induces cancer cell death through metabolically mediated mechanisms.

Figure 4. Schematic representation of the effects of CPI on cancer cell cycle progression. CPI-613 induces cell cycle arrest at G1/S phase in gliosarcoma, pancreatic cancer, and non-small cell lung cancer cell lines.

Figure 3. Profiling of genes regulated by CPI-613 using the Illumina HumanHT-12 v4 Expression Array. The results indicated that CPI-613 regulates cell-cycle genes preventing cell-cycle progression in cancer cells.

Figure 2. CPI-613 induces both apoptotic and non-apoptotic cell death in BxPC-3 human pancreatic cancer cells. BxPC-3 cells were treated with 12.5µM CPI-613 for 24 hours. A) Western blot analysis of active caspase-3, a marker of apoptosis, and B) Trypan blue staining, a non-apoptotic marker of cell death.

Figure 1. CPI-613 is selectively toxic to NIH3T3, BxPC-3, and human gliosarcoma cells but not to BBE cells. The data show that CPI-613 selectively affects cancer cell lines in a concentration of glucose to energy. Nearly all human cancer cell lines were killed by CPI-613 at 50µM. However, normal NIH3T3 fibroblasts and BBE cells were not affected.

This work was supported by the National Institutes of Health (NIH) under grant numbers 1R01CA177770-01A1, 1R01CA185572-02, and 1R01CA185572-02S1, and the Cornerstone Pharmaceuticals Inc. (CPI) of Cranbury, NJ. CPI-613 is currently in clinical trials.

Metabolic pathways regulated by CPI-613 in BxPC-3, H460, and SF539 cancer cells

Signal pathways regulated by CPI-613 in BxPC-3, H460, and SF539 cancer cells

Summary

CPI-613, developed by Cornerstone Pharmaceuticals, is a novel compound currently in Phase I/II clinical trials that has been identified to selectively disrupt biochemical alterations in the conversion of glucose to energy (cancer cell metabolism). In this study, we determined some of the downstream effects of this process and elucidated the possible mechanisms by which CPI-613 causes its selective anticancer activity.

CPI-613 selectively kills all tested cancer cell lines: BxPC3 human pancreatic cancer; H460 human non-small cell lung carcinoma; and SF539 gliosarcoma cell lines. This effect was not seen in the normal, non-transformed NIH 3T3 mouse fibroblasts and HBE normal human bronchial epithelial cells.

CPI-613 can induce both apoptotic and necrotic cell death in BxPC-3 cells. The cell death mechanism appears based on the levels of ATP present, with higher levels of ATP triggering apoptosis.

Profiling of genes regulated by CPI-613 using the HumanHT-12 v4 Expression Array revealed that expression of cdk2 and cdk4 were downregulated in cancer cells. These results confirm the reduction of cell activity from the G1 to S phase on human and non-human cancer cell lines. This is consistent with studies which have shown that the downregulation of cell cycle inhibitors such as p27 and p21 is necessary for progression of cells from G1 to S phase and S to G2 phase was downregulated in these cells, tumour cell killing due to cell cycle progression is likely play a role in these events.

The expression of these genes was not regulated by treatment with CPI-613 in NIH 3T3 non-transformed cells. Analysis of signaling pathways showed that CPI-613 regulates the ATM signaling pathway to activate p53 signaling that leads to the downregulation of cdc2 and cdk2, further confirming the cell-cycle arrest effects seen from the HumanHT-12 v4 Expression Array. CPI-613 may represent a novel treatment option for patients suffering from cancer.