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

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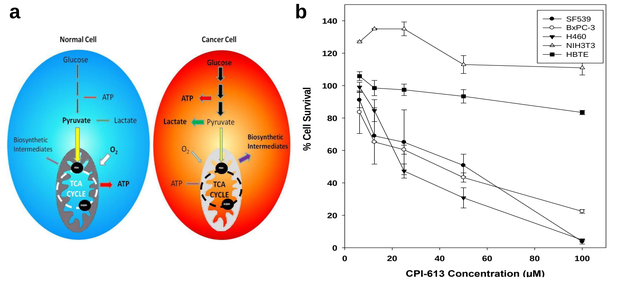
Abstract

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 H460 (human non-small cell lung), but not on non-transformed NIH 3T3 (murine fibroblast) and HBTEC (normal human bronchial/ tracheal epithelial) cells. Specifically, gene microarray data demonstrates that CPI-613 affects signal transduction pathways and 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; pyruvate metabolism; and the citric acid (TCA) cycle. CPI-613 regulates the ATM (Ataxia Telangiectasia Mutated Protein) signaling pathway, to activate p53 signaling leading to downregulation of cyclins and CDK2. Activation of p53 signaling pathway also plays a role in inducing apoptosis in cells. The gene expression of cyclin E, A, and B and CDK2 was notably downregulated in cancer cells treated with CPI-613 compared to normal cells, thereby halting the cell-cycle at multiple points in cancer cells. Further study of this phenomenon may elucidate additional mechanisms of action of CPI-613 treatment.

CPI-613 is selectively toxic to cancer cells



c

Cell Line	IC_{50} Value of CPI-613 (μM)
H460	48 ± 5
BxPC-3	51 ± 7
SF539	57 ± 9
HBTC	N/D
NIH 3T3	N/D

Figure 1. CPI-613 is selectively toxic to H460, BxPC-3, and SF539 cancer cells but not to NIH 3T3 (normal) cells.
A Illustrates the difference between cancer cells and normal cells in the conversion of glucose to energy. Nearly all tumor cells utilize aerobic glycolysis, a phenomenon known as the Warburg effect. CPI-613, developed by Cornerstone Pharmaceuticals Inc., disrupts biochemical alterations in the conversion of glucose to energy.
B The dose-response curves for cell kill by CPI-613 in BxPC-3 human pancreatic cancer cells, H460 non-small cell lung carcinoma cells, and SF539 gliosarcoma cells, as well as non-transformed, normal NIH 3T3 mouse fibroblast cells and HBTE normal human bronchial/tracheal epithelial cells grown in their appropriate complete media (either in RPMI-1640, DMEM, or bronchial life media containing 10% Fetal Bovine Serum (FBS)). Cells were treated with 0-100 μM of CPI-613 in their appropriate media containing 0.5% FBS. The cell survival was measured using CellTiter Glo® Luminescent Cell Viability Assay (Promega).
C The IC_{50} value for this cell lines was determined using mean relative cell growth as a function of the concentration of CPI-613 with the aid of SigmaPlot, V11 software.

Cell death pathways induced by CPI-613 in BxPC-3 human pancreatic cancer cells

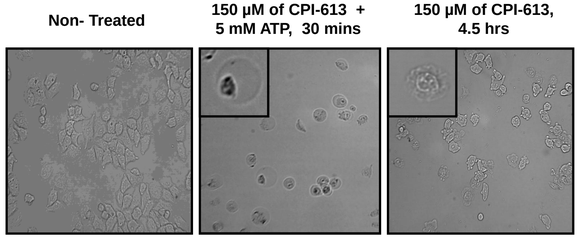


Figure 2. CPI-613 induces both apoptotic and non-apoptotic cell death in BxPC-3 human pancreatic cancer cells. BxPC-3 cells were treated with either 150 μM of CPI-613 or a combination of 150 μM CPI-613 and 5 mM ATP (equaling five-times (x5) the normal cell ATP concentration). The cell death pathway and the time taken to first see the effect was followed under the microscope. Figures are at x40 magnification.

Profiling genes regulated by CPI-613 using the Illumina HumanHT-12 v4 Expression Array

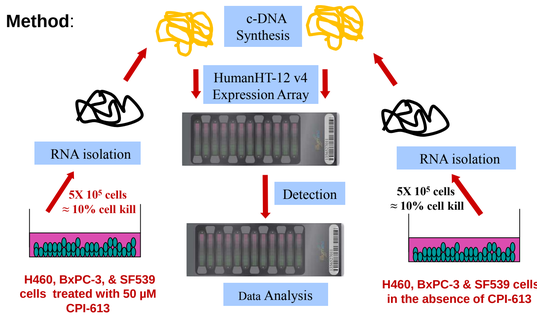


Figure 3. Schematic of the method used for profiling of genes regulated by CPI-613 using the HumanHT-12 v4 Expression BeadChip Kit array (Illumina, Inc.). 50 μM CPI-613 was added for 16 hrs in H460 and SF539 cells and 6 hrs for BXPC-3 cells. 100 μM CPI-613 was added for 6 hrs to NIH 3T3 cells. Control cells in the absence of CPI-613 were also maintained at the specific time points. The time points selected were based on a time course experiment (data not shown), which measured the time taken to see the first cell kill effect (where 10-15% cells were killed). Microarray expression profiling was performed on these samples using the HumanHT-12 v4 Expression BeadChip Kit. The data was analyzed and normalized by using GeneSpring GX, version 9 (Agilent Technologies). Genes with a associated p-value of < 0.05 was considered statistically significant and termed differentially expressed genes (DEG). and the 1.5-fold was used as the cut-off.

CPI-613 regulates cell-cycle genes preventing cell-cycle progression in cancer cells

a

Cell Cycle Regulator	H460 Cells	BxPC-3 Cells	SF539 Cells	NIH 3T3 Cells
Cyclin D1	5.9 (↓)	N/D	1.9 (↑)	N/D
Cyclin D2	2.0 (↑)	N/D	N/D	N/D
Cyclin D3	2.3 (↓)	2.6 (↓)	1.8 (↓)	N/D
Cyclin E1	N/D	2.5 (↓)	N/D	N/D
Cyclin E2	2.3 (↓)	3.7 (↓)	1.8 (↓)	N/D
Cyclin F	3.8 (↓)	2.9 (↓)	3.8 (↓)	N/D
Cyclin A2	2.6 (↓)	1.8 (↓)	2.4 (↓)	N/D
Cyclin B1	2.8 (↓)	2.4 (↓)	2.4 (↓)	N/D
Cyclin B2	1.7 (↓)	N/D	1.9 (↓)	N/D
Cyclin G2	2.4 (↑)	N/D	N/D	N/D
Cyclin H	1.8 (↑)	1.5 (↑)	1.7 (↑)	N/D
Cyclin L1	N/D	N/D	N/D	1.5 (↑)
p27	1.6 (↓)	N/D	N/D	N/D
p21	N/D	N/D	2.2 (↑)	N/D
p15	N/D	N/D	2.2 (↑)	N/D
p19	1.8 (↓)	N/D	N/D	N/D
p57	N/D	N/D	1.8 (↓)	N/D
CDK2	2.1 (↓)	2 .0(↓)	2.1 (↓)	N/D

Only genes with fold changes by ≥1 .5 units are shown;
"N/D" represents not detected or fold changes <1.5-fold .

b

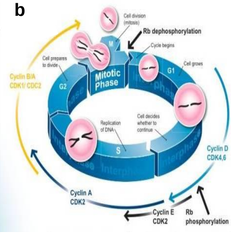


Figure 4. Illumina HumanHT-12 v4 Expression BeadChip array data for cell-cycle associated genes regulated by CPI-613 in H460, BxPC-3, SF539 and NIH 3T3 cells. Comparison of the gene expression profile reveled that the expression of cyclin E, A and B, p27, p19, and CDK2 are specifically noted to be down-regulated in cancer cells treated with CPI-613, thereby halting the cell cycle progression at multiple points. CPI-613 had no effect on these genes in NIH-3T3 cells.

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

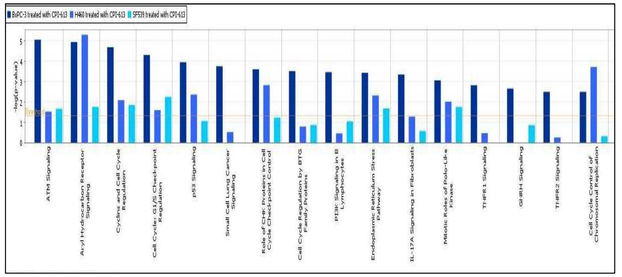


Figure 5. Identification of signaling pathways regulated by CPI-613 in cancer cells using Ingenuity® Pathway Analysis Software (IPA). The differentially expressed genes (DEG) by CPI-613 were mapped to signaling pathways and tested by the Fishers Exact Test p-value using IPA software. The pathways were represented as a histogram of pathway vs –log(p-value).

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

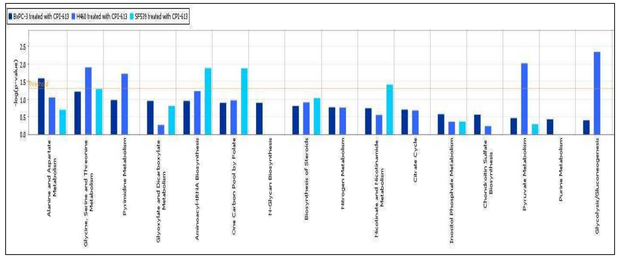


Figure 6. Identification of metabolic pathways regulated by CPI-613 in cancer cells using Ingenuity® Pathway Analysis Software (IPA). The differentially expressed genes (DEG) by CPI-613 were mapped to metabolic pathways and tested by the Fishers Exact Test p-value using IPA software. The pathways were represented as a histogram of pathway vs. –log(p-value).

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 cells: BXPC3 human pancreatic cancer; H460 non-small cell lung carcinoma; and SF539 gliosarcoma cell lines. This effect was not seen in the normal, non-transformed NIH 3T3 mouse fibroblast cells and HBTE normal human bronchial/ tracheal epithelial cells *in vitro.***
- **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 BeadChip Kit revealed that the expression of cyclin E, A and B was downregulated in cancer cells. These cyclins regulate the conversion of cells from the G1 to S, S to G2, and G2 to M phases of the cell-cycle, respectively. Further, the expression of CDK2, which is necessary for progression of cells from G1 to S phase and S to G2 phase was downregulated in these cells, thereby halting the cell-cycle progression at multiple points. The expression of these genes was not regulated by treatment with CPI-613 in NIH 3T3 non-transformed cells vs. non-treated control NIH 3T3 non-transformed cells.**
- **Analysis of signaling pathways revealed that CPI-613 regulates the ATM signaling pathway to activate p53 signaling that leads to the downregulation of cyclins and CDK2, further confirming the cell-cycle arrest effects seen from the HumanHT-12 v4 Expression BeadChip Kit. p53 signaling also plays a role in inducing apoptosis in cells.**
- **Further, the main metabolic pathways regulated by CPI-613 includes: amino acid metabolism; pyrimidine metabolism; pyruvate metabolism; and the citric acid (TCA) cycle.**