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Synergy of Bosutinib and Chk-1 Inhibitor (PF) in Chemotherapy

Akhil Kolluri
Virginia Commonwealth University

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Akhil Kolluri, Mentors: Dr. Steven Grant and Dr. Tri Nguyen
Virginia Commonwealth University

Introduction

Chronic myelogenous leukemia (CML) is a form of cancer. This disease affects the bone marrow cells that develop into the cells of the blood (Chen, 2014). CML is caused by a deleterious protein encoded by the chromosome called the Philadelphia (Ph) chromosome (Chen, 2014). Current treatments for CML include imatinib, the trade name for which is Gleevec, and bone marrow transplants (Chen, 2014).

CML can be studied in clinical research using various cell lines. BA/F3 is a model cell line that is commonly used to study CML (Warmuth et al., 2007). This is because BA/F3 cells work well in studying the signaling of kinases in the cell cycle, and how those kinases can be inhibited by drugs like imatinib (Warmuth et al., 2007). Cell lines that have been mutated are also very useful in researching and modeling cancer cells in the body. According to Watanabe et al. (2012), the T315I mutation is generally found in cancer cells from patients who have relapsed after a period of remission. The T315I mutation has been shown to cause resistance to tyrosine kinase inhibitors like imatinib, one of the most common treatments to CML (Watanabe et al., 2012).

Bosutinib is a newly tested kinase inhibitor that also functions in cell signaling pathways important in the cell cycle of CML cells (Boschelli et al., 2010). The chemical structure of bosutinib is shown in Figure 1. Bosutinib blocks the phosphorylation of key proteins like Bcr-Abl and CrkL in these cells that allow them to proliferate (Boschelli et al., 2010). It has been shown that bosutinib is effective in slowing the growth of CML cells (Boschelli et al., 2010).

Chk-1 is a protein that has a function in regulating the many checkpoints in the cell cycle (Zhang et al., 2009). PF-00477736 (PF) inhibits the Chk-1 protein, and has been shown to enhance the effect of chemotherapy drugs like docetaxel (Zhang et al., 2009).

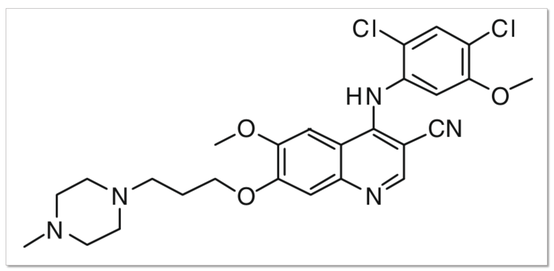

Figure 1. The chemical structure of bosutinib. From Boschelli et al. (2010).

Methods

The purpose of this research was to find out if bosutinib can be combined with a Chk-1 inhibitor (PF) to increase the efficacy of the treatment. Two cell lines from CML were used in this experiment: BAF3/T315I and Adult/T315I. Both of these cell lines are resistant to imatinib due to the T315I mutation. Cultures of these cells were incubated and maintained at 37° C. These cell lines were also xenotransplanted into mice, and then the xenograft tumors were extracted. Western blot analysis was performed on the extracted tumor cells to determine protein expression levels.

The cells were allowed to grow in RPMI medium with 10% fetal bovine serum. The cell cultures were cut every Monday, Wednesday, and Friday as cell density increased. The tumors and the lysed proteins were stored in -80 ° C.

The cultured cells were treated with both bosutinib at 0.3 – 0.4 μM and PF at 1 – 1.2 μM individually as well as simultaneously. The cell death was examined in each of these conditions over a 72-hour period.

Results

Individual treatment of BAF3/T315I and Adult/T315I cell cultures with bosutinib or with PF produced little cell death (less than 25%). However, the combined treatment of bosutinib and PF showed a remarkable increase in cell death (around 65 – 75%). These results are shown in Figure 2.

This chemotherapy combination was also tested on cells from three CML patients. In these cells as well, individual treatments of bosutinib and PF were ineffective, while the combined treatment increased cell death. Also, normal $CD34^{+}$ cells were not as effected by the treatment as cancerous cells, only showing around 20% lethality compared to around 50% for cancerous cells.

Results also showed that the simultaneous treatment of bosutinib and PF caused an increase in the expression of the pro-apoptotic protein Bim and a decrease in the expression of pro-survival protein Mcl-1.

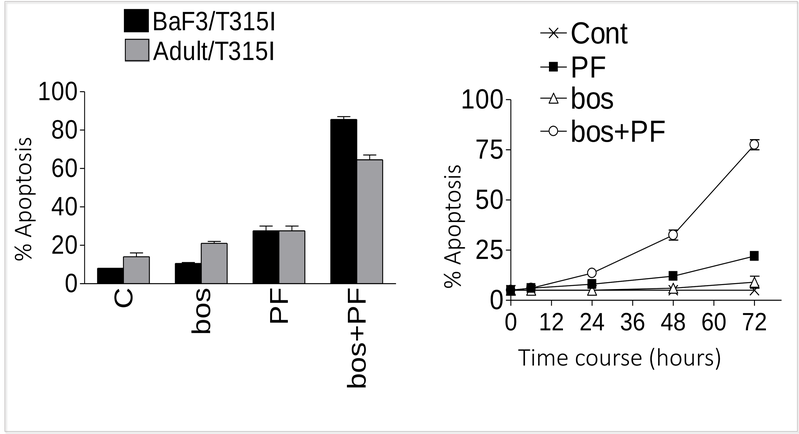

Figure 2. The results of individual and simultaneous treatment of bosutinib and PF, showing apoptosis percentage at the end (left) and throughout (right) the 72-hour period.

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References

Chen, Y. (2014). Chronic myelogenous leukemia (CML). In *A.D.A.M. Medical Encyclopedia*, in *MedlinePlus*.

Warmuth, M., Kim S., Gu X., Xia G., & Adrián F. (2007). Ba/F3 cells and their use in kinase drug discovery. *Current Opinion in Oncology, 19*, 55-60.

Watanabe, K., Minami, Y., Ozawa, Y., Miyamura, K., & Naoe, T. (2012). T315I mutation in Ph-positive acute lymphoblastic leukemia is associated with a highly aggressive disease phenotype: Three case reports. *Anticancer Research, 32*, 1779-84.

Boschelli, F., Arndt, K., & Gambacorti-Passerini, C. (2010). Bosutinib: A review of preclinical studies in chronic myelogenous leukaemia. *European Journal of Cancer, 46*, 1781-89.

Zhang, C., Yan, Z., Painter, C. L., Zhang, Q., Chen, E., Arango, M. E., . . . Christensen J. G. (2009). PF-00477736 mediates checkpoint kinase 1 signaling pathway and potentiates docetaxel-induced efficacy in xenografts. *Clinical Cancer Research, 15*(14), 4630-40.