Combined Usage of Navitoclax and A-1210477 to Treat Acute Lymphoblastic Leukemia

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Abstract

Mcl-1 protein has been a key targeted therapeutic protein in treating acute lymphoblastic leukemia because of its resistance to other previously known drugs. However, a newly synthesized chemical A-1210477 has proven to possess enough affinity to bind to the Mcl-1 protein and disrupt its normal function. As a result, scientists started to test the potency of A-1210477 combined with other drugs to treat various cancers. This study will examine the effect of A-1210477 and Bcl-2 inhibitor navitoclax combined to treat acute lymphoblastic leukemia Bal-KHc cell line both in vitro by MTT and Annexin V/PI Facs as well as in vivo by measuring the tumor volume in xenograft.

Keywords: Navitoclax, A-1210477, Mcl-1 protein, Bcl-2 protein, Acute Lymphoblastic Leukemia

I. Introduction

Acute lymphoblastic leukemia (ALL) is a type of cancer in which the bone marrow produces too many lymphocytes, a type of white blood cell, which are also called leukemia cells.¹ These leukemia cells cannot fight infection very well, and as the number of leukemia cells increases in the blood and bone marrow, there is less room for other healthy cells like healthy white blood cells, red blood cells, and platelets [1]. This might lead to infection, anemia, and easy bleeding.¹ Furthermore, if acute lymphoblastic leukemia is not treated early, it can also quickly spread to the central nervous system, spleen, lymph nodes, liver, testicles, and other organs [1]. In the United States, the overall incidence of acute lymphoblastic leukemia occurrences is 1.5 per 100,000 population [1]. Therefore, finding the most efficient treatment and the most effective cure for acute lymphoblastic leukemia is imperative.

Although not being used clinically, two possible ways to treat acute lymphoblastic leukemia include navitoclax and A-1210477. Navitoclax, also known as ABT-263, is an orally taken bioavailable drug that mimics pro-death B-cell lymphoma-2 (Bcl-2) Homology 3 (BH3) domain-only proteins [2]. As a result, it has a high affinity toward prosurvival Bcl-2 family proteins such as Bcl-Xl, Bcl-2, and Bcl-W [3]. These Bcl-2 anti-apoptotic protein prevents the cell from entering apoptosis by isolating caspases, which is a death-driving cysteine protease, or by preventing the release of mitochondrial apoptogenic factors such as cytochrome c and AIF, apoptosis-inducing factor, into the cytoplasm [4]. Navitoclax binds to these anti-apoptotic proteins to prevent them from performing their original jobs, thus inhibit the cells from continuous replication of cancer cells and to induce cell apoptosis effectively [3,5]. However, clinical trials have observed that patients using navitoclax alone demonstrated limited anti-cancer activity, suggesting that proteins like Mcl-1 are inhibiting navitoclax from achieving its maximum efficacy [3,5,6]. Just like other Bcl-2 family proteins, Mcl-1 is an anti-apoptotic protein that regulates cancer cell survival [3]. It is also a known resistance factor for small-molecule Bcl-2 family inhibitors like navitoclax (ABT-263), rendering Mcl-1 protein a promising therapeutic target [3].

A-1210477 also serves as an alternative treatment for acute lymphoblastic leukemia. A-1210477 is a kind of indole-2-carboxylic acid that selectively binds to Mcl-1 protein with sufficient affinity to disrupt Mcl-1-BIM complexes in living cells [7]. Though the exact mechanism by which BIM protein protects Mcl-1 protein is still undiscovered, it is generally believed that BIM protein released from Bcl-2 stabilizes Mcl-1 protein, ultimately resulting in the survival of Mcl-1 protein [8]. In addition, the redistribution of Bim protein to Mcl-1 protein may displace ubiquitin ligases such as Mule, β-TRCP, and FBW7, resulting in increased Mcl-1 protein stability as well as Mcl-1 protein amount in a cell [8]. A-1210477 binds to Mcl-1 protein in order to destabilize the protein, regulate cancer cells, and send them to apoptosis eventually instead of letting them grow and replicate constantly [7].

Some of the ways a cell can die is through apoptosis, necrosis, necroptosis, and pyroptosis. Apoptosis is a form of cell death that prevents immune activation [9]. The cell activates caspases, a kind of proteins that are usually dormant [9]. These caspases then would dismantle the cell from within, and the apoptotic cell divides into little packages that can be consumed by other cells [9]. This

stops the contents of the dying cell from spilling out and allows the components to be recycled [9]. Necrosis occurs when a cell dies due to lack of a blood supply, or due to a toxin [10]. The cells' contents can leak out and damage neighboring cells and may also trigger inflammation [10]. Necroptosis is similar in appearance to necrosis because in both mechanisms, the dying cell's contents can leak out [10]. However, it is also very similar in apoptosis as necroptosis is also a programmed cell death process triggered by specific proteins inside the dying cell [10].

Pyroptosis is a form of cell death that occurs in some cells infected with certain viruses or bacteria [10]. A cell dying by pryoptosis releases cytokines, a kind of molecules that alert neighboring cells to the infection [10]. This would then trigger inflammation, which is a protective response that restricts the spread of the viruses and bacteria [10].

Recently, research has shown that combined usage of navitoclax and A-1210477 works significantly better than when they are used individually. The research examined cell lines that depend heavily on both Mcl-1 protein and Bcl-2 family proteins to avoid entering apoptosis. In all the cell lines examined and tested, the percent of cancer cell viability immediately decreased drastically when the cell was treated with combined navitoclax and A-1210477 [3].

This research paper intends to investigate the effect of navitoclax (ABT-263) and A-1210477 combination usage in the treatment of acute lymphoblastic leukemia and their role with the two anti-apoptotic protein, Bcl-2 protein and Mcl-1 protein. I predict that the combination of increasing amounts of Navitoclax and increasing amounts A-1210477 for various durations will treat acute lymphoblastic leukemia Bal-KHc cell line but that treatment with either individually does not kill the cells because Navitoclax binds Bcl-2 while A-1210477 binds Mcl-1 protein.

A. Materials and Methods

This research paper will perform three experiments: measuring apoptosis in vitro with MTT, with ANNEXINV/PI FACS, and killing in vivo with tumor size of drug treated Bal-KHc

B. MTT

MTT colorimetric assay is being used to measure the in vitro cell viability. Cells were to be incubated with different amounts of PBS/DMSO, Navitoclax, A-1210477, and Navitoclax and A-1210477 combination for various durations (0 hours, 2 hours, 4 hours, and 6 hours) followed by incubation with a 10 μ L MTT for 4 hours at 37°C. Lysis buffer is then added and mixed with the solution. The absorbance is measure at 570nm [11,12]. Assay 1 will contain 0.4% PBS/DMSO. Assay 2 will contain 1%

A-1210477. Assay 3 will contain 0.66% A-1210477. Assay 4 will contain 0.33% A-1210477. Assay 5 will contain 1% navitoclax. Assay 6 will contain 0.66% navitoclax. Assay 7 will contain 0.33% navitoclax. Assay 8 will contain 1% of A-1210477 and navitoclax combined. Assay 9 will contain 0.66% of A-1210477 and navitoclax combined. Assay 10 will contain 0.33% of A-1210477 and navitoclax combined. Assay 11 will contain 0.4% Taxol. This MTT assay will be repeated three times. The positive control is Taxol, and the negative control is PBS/DMSO.

C. Annexin V/Pi Facs

Cell apoptosis rate is measured with the Annexin V-FITC Apoptosis Detection Kit regarding the manufacturer's instructions. In general, the Bal-KHc cell lines are harvested and washed twice with cold PBS. Cells are then resuspended in the binding buffer and co-incubated with 5 μ L of the Annexin V-FITC and 5 μ L of PI for 15 min at room temperature in the dark. The cells were analyzed by a BD FACSAria TM III flow cytometer within 1 hour, and the data should be analyzed by Treestar Flowjo software [13,14]. This experiment will be repeated three times. The positive control is Taxol, and the negative control is PBS/ DMSO.

D. Caliper measurement of tumor size in xenografts

Each group will contain 7 mice that has acute lymphoblastic leukemia Bal-KHc cell line. There will be five groups in total treated individually with 2 μ L DMSO, 2 μ L taxol, 2 μ L navitoclax, 2 μ L A-1210477, and the combination of 1 μ L navitoclax and 1 μ L A-1210477. Tumor size will be measured every day for 14 days after the mice are being treated with different reagents. The tumor volume will be calculated as V=(W^2*L)/2 where V stands for volume, W stands for tumor width, and L stands for tumor length [15]. Each experiment will be repeated three times. The positive control is Taxol, and the negative control is PBS/DMSO.

E. Controls

In all three experiments, the positive control is the taxol, and the negative control is the PBS/DMSO

F. Statistical Analysis

All the results are presented in the way of the mean \pm SD (vertical error bars) from triplicate experiments. All experiments will be repeated three times. P < 0.05 indicated a statistical difference and P < 0.01 indicated a significant statistical difference [13].

II. Possible Results

Table 1 contains all the combined possible results for all

three experiments performed.

Table 1. Combined 1 ossible Results Table								
	1	2	3	4	5	6	7	8
Combined Navitoclax and A-1210477 increases MTT killing more than individually?	+	+	+	-	+	-	-	-
Combined Navitoclax and A-1210477 increases Annexin V/PI FACS+ more than individually?	+	+	-	+	-	+	-	-
Combined Navitoclax and A-1210477 decreases xenograft size more than individually?	+	-	+	+	-	-	+	-
Supporting hypothesis?	yes	partially	partially	partially	partially	partially	partially	no

 Table 1: Combined Possible Results Table

Note. "+" means "yes" to the question and exhibiting similar effect with Taxol positive control

"-" means "no" to the question and exhibiting similar effect with PBS/DMSO negative control

III. Discussion

The first possible result demonstrates significant cell apoptosis and tumor regression when using combined dosage of A-1210477 and navitoclax. This supports fully my hypothesis as all three experiments prove that using both A-1210477 and navitoclax is more effective than when using them individually. The next step for this result should be testing this combination clinically with actual patients who have acute lymphoblastic leukemia.

The second possible result demonstrates significant cell apoptosis only in vitro by using MTT assay and Annexin V/PI FACS, but not in vivo with the measurement of tumor growth in xenograft model. This only partly supports my hypothesis since combined usage of A-1210477 and navitoclax does not cure tumor growth in the xenograft model better than when used individually as predicted in the hypothesis. The next experiment for this result should aim to find out the reason why combined A-1210477 and navitoclax does not significantly trigger cell apoptosis as they did in the in vitro experiments.

The third possible result demonstrates more cell killing in MTT assay and xenograft model but not in Annexin V/PI FACS. This only partly supports my hypothesis because although combined A-1210477 and navitoclax has proven to be more effective in MTT assay and xenograft model, it does not do so in Annexin V/PI FACS. This is extremely weird since both MTT assay and Annexin V/PI FACS measure cell apoptosis. The next experiment for this result should be to redo the Annexin V/PI FACS experiment to see if it produces the same result. If it does, then we should investigate on the reason why MTT assay and V/PI FACS

The fourth possible result demonstrates more cancer cell apoptosis in Annexin V/PI FACS and xenograft model but not in MTT assay. This partly supports my hypothesis because in MTT assay combined A-1210477 and navitoclax does not kill cell better when they are used individually. Just like the third result, different results are shown for MTT assay and V/PI FACS. The next step for this should be to redo the MTT assay experiment, and if it provides the same result, we should then investigate the reason why these two experiments provide different result. One of the possible explanations for this phenomenon is because the MTT assay and Annexin V/PI FACS actually measure different things. MTT assay measure the in vitro cell viability while the Annexin V/PI FACS measure cell apoptosis. Therefore, if different results are shown for MTT assay and V/PI FACS, the cancer cells might have died some other ways other than cell apoptosis as mentioned in the introduction section.

The fifth possible result demonstrates significant apoptosis only MTT assay and not in Annexin V/PI FACS or the xenograft model. This partly supports my hypothesis since only MTT assay provides result that supports my hypothesis of combined A-1210477 and navitoclax works better. The next step for this result should be to redo all three experiments. If still the same results are being produced, then we should give up on this experiment because the in vivo xenograft model does not support my hypothesis.

The sixth possible result demonstrates significant cancer cell death only in Annexin V/PI FACS but not in the MTT assay or in the xenograft model. This partly supports my hypothesis because only Annexin V/PI FACS showed that

combined A-1210477 and navitoclax kill cancer cell better than when used individually. Just like the fifth possible result, the next step for this result should be to redo all three of the experiments. If the same results are showed, then we should consider giving up on this research as two of the three results have demonstrated undesirable results opposing to the hypothesis.

The seventh possible result demonstrates more cell apoptosis only in xenograft model but not in in vitro MTT assay or Annexin V/PI FACS. This only partly supports my hypothesis as only one out of the three experiments have shown result that supports my hypothesis. However, it is nearly impossible how the combined usage of A-1210477 and navitoclax work in the xenograft but not in the in vitro cell culture. It is highly possible that the xenograft models have been cured by some other factors other than the combined A-1210477 and navitoclax. As a result, the next experiment for this result should be to redo the xenograft model.

The eighth possible result demonstrate no significant cell apoptosis in combined A-1210477 and navitoclax compared to when used individually. This completely defies my hypothesis as all three experiments have shown results that are contradictory to my hypothesis saying combined A-1210477 and navitoclax will be more effective in cancer cell killing. The next step for this result should be to give up on this investigation of combined A-1210477 and navitoclax and adjust the direction of this research.

IV. Conclusion

In conclusion, this research intends to investigate the overall effect of navitoclax (ABT-263) and A-1210477 being used together to treat acute lymphoblastic leukemia. If this combination has been tested in the laboratory and has been proved to be effective, then this drug could be tested clinically. Perhaps one day, this combination of navitoclax and A-1210477 will be used in real-world therapy in acute lymphoblastic leukemia. Furthermore, since A-1210477 and navitoclax could treat acute lymphoblastic leukemia, it is very likely that this combination could treat other cancer that also has Mcl-1 protein and Bcl-2 protein which regulate the cell's apoptosis. A-1210477 could also combined with other drugs other than navitoclax and treat other kinds of cancer that also has Mcl-1 anti-apoptotic protein as one of their key regulators for cell apoptosis.

V. References

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