Examining P53 Mutant Triple Negative Breast Cancer Cell Viability and Sphingosine Kinase 1 in Response to CHK1 Inhibitor and Doxorubicin

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ABSTRACT

Triple Negative Breast Cancer [TNBC] is breast cancer that lacks abnormal expression of the progesterone receptor, estrogen receptor, or human epidermal growth factor receptor 2. Without this abnormal expression, targeted hormone therapy cannot be used for TNBC. In addition, 44% of TNBCs have nonfunctional p53 genes. Since p53 responds to DNA damage by halting cell cycle progression and inducing apoptosis, TNBC without functional p53 is also more resilient to genotoxic chemotherapy agents. In this investigation, check kinase 1 [CHK1], which functions by blocking cell cycle progression to allow DNA repair, was inhibited in combination with genotoxic stress. It was hypothesized that genotoxic stress with the CHK1 inhibition would damage the genome of a cell and then prevent the cell from repairing such damage, resulting in cell death. Cell viability and sphingosine kinase 1 [SK1], an enzyme associated with cell proliferation, levels were observed to assess the effectiveness of the combination treatment as compared to a single treatment of each drug and no treatment. No statistically significant differences in cell viability were observed among the treatment groups. However, there was a trend in the data suggesting that CHK1 inhibition alone and CHK1 inhibition combined with genotoxic stress are equally effective at reducing cell viability compared to genotoxic stress alone or no treatment. If supported by more data, this would suggest the potential of using CHK1 inhibition alone as a TNBC cancer treatment. This would be beneficial as this could avoid the side effect of cardiotoxicity from using genotoxic agents.

1. INTRODUCTION

Sphingolipids, a class of bioactive lipids, have received extensive attention over the past few decades for their role in cancer cell signaling. Sphingolipids serve as structural molecules in cell membranes, but they are also crucial in regulating biological processes such as growth, apoptosis, and cell migration [10]. Sphingolipids are composed of multiple biomolecules (Fig. 1). The sphingolipids most connected to cell survival are ceramide, sphingosine, and sphingosine 1 phosphate [S1P]. Ceramide and sphingosine are largely involved in the pro-death processes apoptosis and cell cycle arrest, while S1P promotes cell survival and proliferation [4]. The contrasting effects of these different sphingolipids makes a cell's survival largely dependent on a sphingolipid balance (Fig. 1A) [4]. This balance must be considered in tandem with the fact that increases and decreases in concentrations of the over 40 enzymes involved in sphingolipid metabolism can affect a cell's sphingolipid levels (Fig. 1B) [4]. The lack of regulation of sphingolipids is characteristic of multiple cancers, including various lymphomas and sarcomas [5], where often the enzymes associated with the synthesis of ceramide are downregulated and the enzymes associated with S1P synthesis are upregulated [9]. In particular, an upregulation of SK1 levels, in many cancers can be seen as leading to tumor progression (Fig 1B) [9].

Improper levels of SK1 can be a result of a defect in the major tumor suppressor gene TP53, which produces the p53 protein [5]. TP53 is mutated in over 50% of human cancers, and SK1 has been found to be an enzyme regulated by the protein p53 [5]. In response to genotoxic stress, TP53 is activated resulting in the production of the p53 which has the downstream effect of reducing SK1 levels [5]. Genotoxic stress is an agent that cause DNA damage, such as radiotherapy and chemotherapy. Recently, the downregulation of SK1 has been shown to occur through caspase mediated proteolysis of SK1 in response to increases in p53 [2]. Likewise, another effect of p53 is an accumulation of cellular ceramide [6]. These two results taken together demonstrate the susceptibility of cells with nonfunctional p53 to properly regulate sphingolipids, and therefore regulate cellular growth and proliferation. SK1 levels are universally elevated in multiple types of cancer, and this is further supported by evidence that p53 knockdown in mice exhibit spontaneous tumor development, with thymic lymphoma being the most prevalent cancer type [5]. In contrast, a functional TP53 will induce the downregulation of

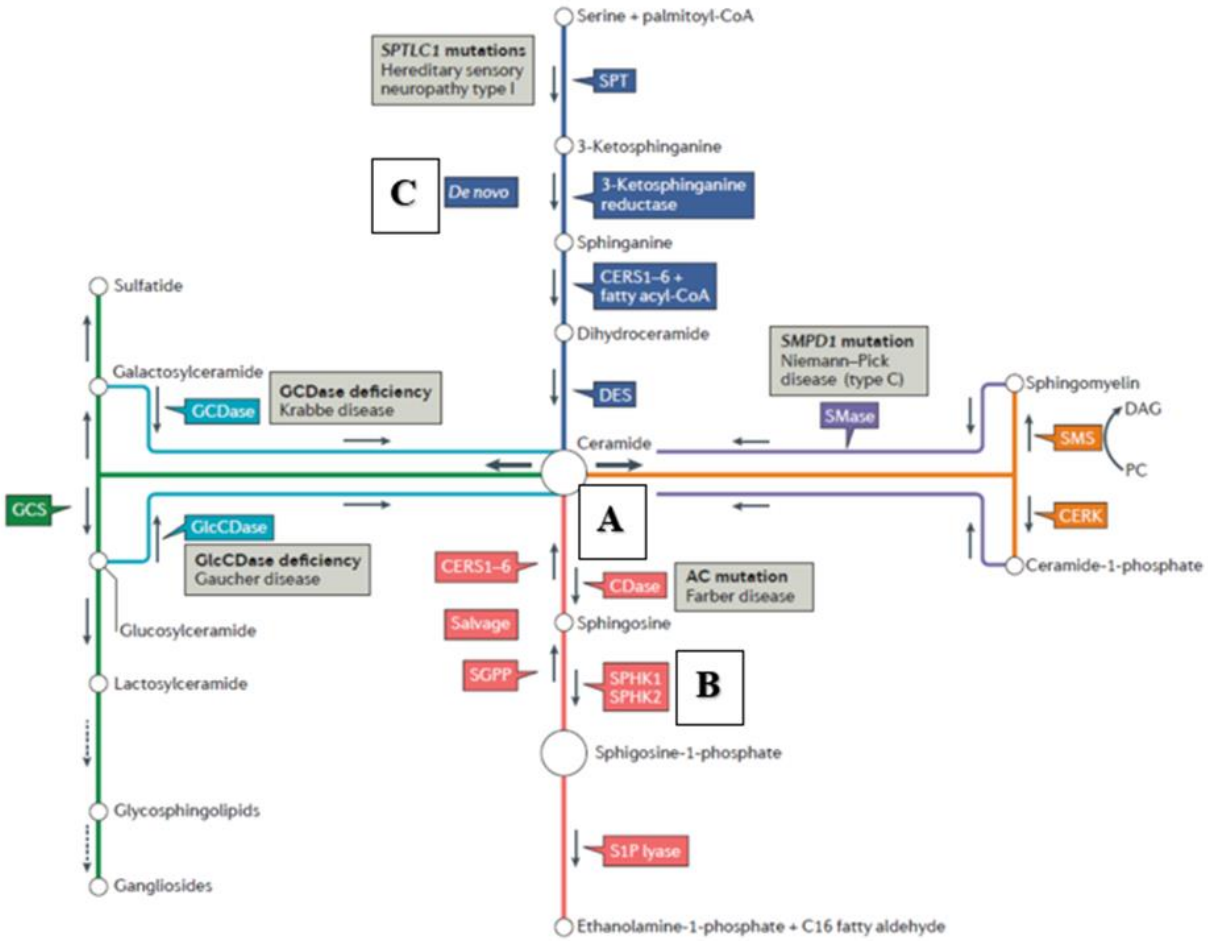

Figure 1. **Sphingolipid Pathways** (Retrieved from [10]).

(A) The wide array of sphingolipids is centered around ceramide. (B) Sphingosine 1 kinase [SK1], denoted as SPHK1 here, has a key role in converting pro-death ceramide into pro-life S1P. Since these sphingolipids are both the reactants of each other's synthesis, the regulation of their associated enzymes is essential for cell cycle regulation. (C) *De novo* synthesis of ceramide is represented by the top pathway, which allows the creation of new sphingolipids.

SK1 in response to DNA damage, a genotoxic stress. Studies have shown that cells with functional TP53 treated with the DNA damaging agent, actinomycin D, exhibited such SK1 downregulation, demonstrating that cancers with mutated TP53 can avoid apoptosis in response to DNA damage [13].

The necessity of p53 to induce an apoptotic response to genotoxic stress makes multiple chemotherapeutic therapies that utilize DNA damaging less effective. Doxorubicin is a widely used chemotherapeutic drug that is largely theorized to function via inducing DNA double strand breaks, which induces the TP53 response to genotoxic stress (Fig. 2A) [11]. Therefore, the lack of functional p53 in this pathway would prevent the apoptotic response to the DNA damage. This

lack of p53 functionality adds to the severity of triple negative breast cancer [TNBC], which lacks abnormal estrogen receptor, progesterone receptor, human epidermal growth factor receptor 2 expression [7]. TNBC, which is especially aggressive, lacks targeted therapies due to its non-amplified expression of the given receptors, and exhibits a TP53 mutation in 44% of cases [7]. These factors require the need for novel therapies for TNBC, as receptor targeting and DNA damaging are ineffective in treating TNBC [7]. Interestingly, a recent study demonstrated that increasingly concentrated doses of doxorubicin on the MDA-MB-231 cell line, a human TNBC cell line that harbors a TP53 mutation, resulted in increasing SK1 levels [2]. This result

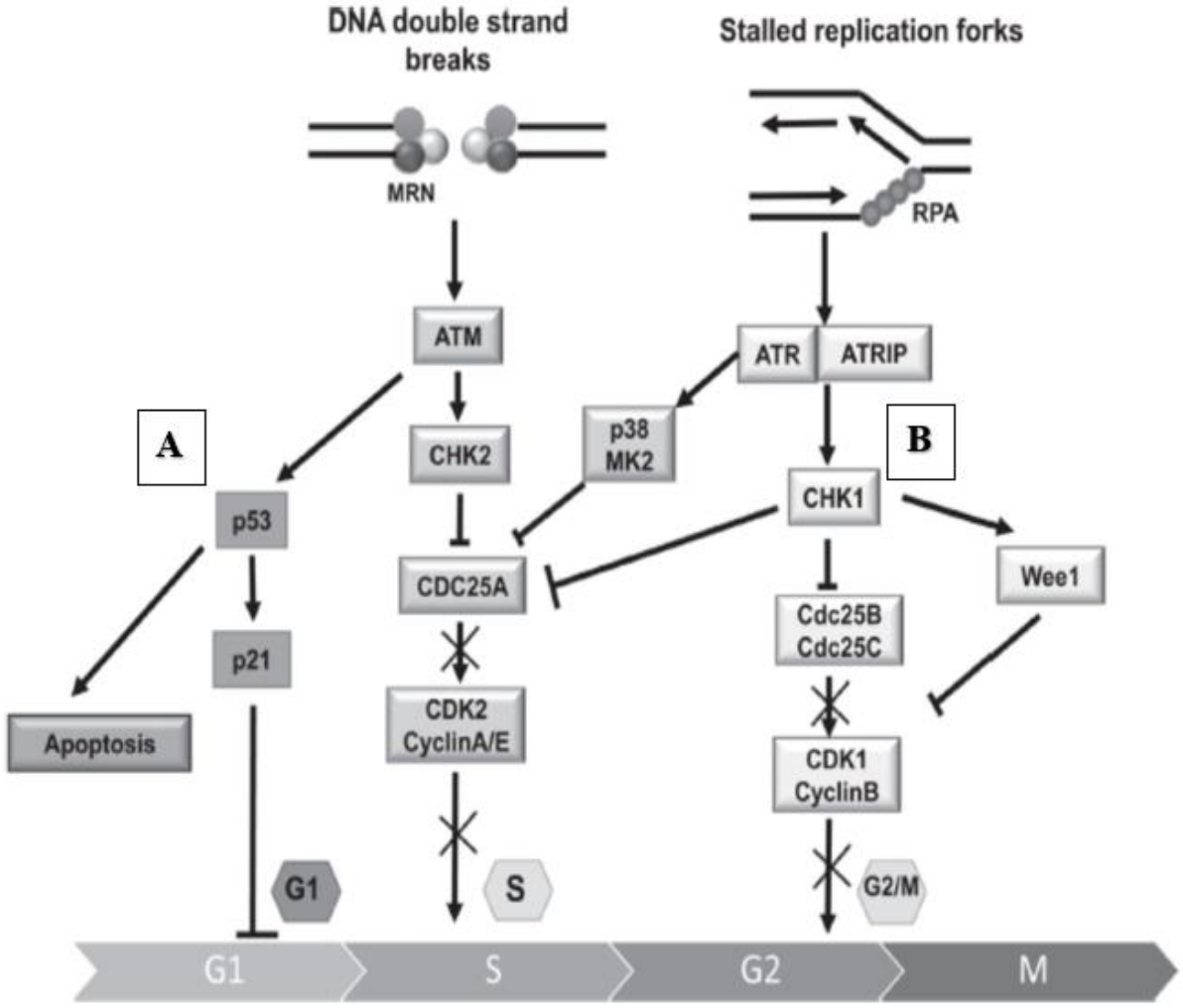

Figure 2. **Pathways of p53 and CHK1 in Inhibiting Cell Cycle Progression** [Retrieved from 12].

(A) In response to DNA double strand breaks, p53 is blocks progression through the G1 cell cycle checkpoint. (B) CHK1 is activated downstream of stalled DNA replication forks and causes the blocking of cell cycle progression at the S checkpoint or the late G2 checkpoint.

serves as further evidence of the inability of genotoxic agents to inhibit cell proliferation in TP53 mutant cancers. In addition, long-term doxorubicin use in cancer treatment can result in cardiotoxicity and skeletal muscle impairment [3].

A potential alternative to use of solely doxorubicin and other genotoxic agents are Check Kinase 1 [CHK1] inhibitors. Under normal conditions, the kinase ATR responds to replication stress and DNA damage and activates the downstream effector protein CHK1 [8]. CHK1 then functions by delaying cell cycle progression. This essentially gives the cell a time window to resolve DNA replication conflicts before progressing into mitosis [8]. CHK1 activation results in the inhibition of the cell cycle in either the middle of the S-phase or at the end of G2 phase just prior to mitosis (Fig. 2B) [12]. In contrast, p53 functions by blocking progression through the G1 phase checkpoint (Fig. 2A), and TP53 mutant cells have been shown to be reliant on CHK1 function to protect genome integrity [12]. This then leads to the hypothesis that inhibition of CHK1 function along with non-functional TP53 would sensitize a cell to DNA damage, as the cell will be unable to properly respond to and repair DNA damage (Fig. 3).

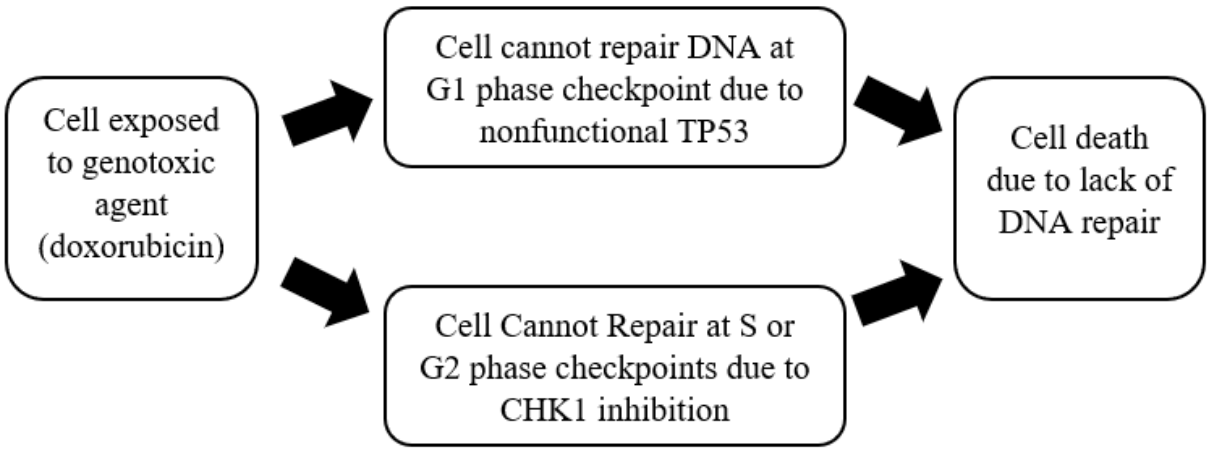

Figure 3. **Hypothesis Illustration**

This therapeutic method is not entirely new, as inhibitors of CHK1 have been developed for the potential use in cancer therapy [12]. A study utilizing human-in-mouse TP53 mutant TNBC models tested the idea that CHK1 inhibition would sensitize the tumors to the DNA damaging agent irinotecan, and the study was successful in demonstrating that only tumor

models that lacked TP53 exhibited enhanced apoptosis [7]. The purpose of my study was to build upon this finding by examining the efficacy of a combination therapy of the DNA damaging agent doxorubicin and CHK1 inhibition via the inhibitor AZD7762 on the human TNBC TP53 mutant MDA-MB-231 cell line. To carry out this study, I looked at a combination treatment group of AZD7762 and doxorubicin [AD], a group that contained just the vehicles of both AZD7762 and doxorubicin [VV], a group of AZD7762 and the doxorubicin vehicle [AV], and a group of the AZD7762 vehicle and doxorubicin [VD]. A vehicle denotes the solution a drug or inhibitor was delivered to a cell in, without the drug or inhibitor. A greater presence of cell death and lesser extent of cell life were viewed as greater effectiveness in a treatment. I also probed for SK1 levels to observe if changes in this pro-cell progression protein occurred concurrently with any changes in cell life and death. Recent research found that SK1 proteolysis occurs downstream of CHK1 inhibition [2], I observed SK1 levels as an indicator in measuring the effectiveness of a treatment to inhibit cell growth and cause cell death.

2. METHODS

2.1 Cell Culture and Drug Treatment

The TNBC cell line MDA-MB-231 was purchased from ATCC (Catalog number HTB – 26). Cells were grown in Dulbecco's modified Eagle's medium (DMEM) with 10% Fetal Bovine Serum (FBS) added as growth factor. Both of these products came from Life Technologies (Grand Island, NY). The cell line was split into a new flask every 2 to 3 days to prevent the accumulation of metabolic waste products and reduce the chance of contamination. For MTT and LDH assays, cells were plated on 6 well 35 mm plates. For Western Blot Analysis, cells were plated on 60 mm plates. Cells for these experiments were plated into 3 mL DMEM with 10% FBS and given about one to two days to reach near confluency of cells in the plate. About one hour prior to drug treatment, the medium was removed from the plates and FBS-free DMEM media was placed into the dishes. Then, CHK1/2 inhibitor AZD7762, purchased from Selleckchem (Houston, TX, USA), was added to the cells at a concentration of 0.3 μM. About an hour later, Doxorubicin, purchased from Amresco (Solon, OH, USA), was added at a concentration of 0.8 μM. The length of time that the cells remained in treated medium was dependent on the intended assay of the cells. In all experiments, the four treatments groups of

vehicle-vehicle [VV], vehicle-doxorubicin [VD], AZD7762-vehicle [AV], and AZD7762-doxorubicin [AD] were used.

2.2 LDH Assay for Cell Death (Fig. 5)

Originally, 75,000 cells per well were plated into 4 wells of a 6 well 35 mm plate (Fig 4). Cells part of a 24-h lactate dehydrogenase [LDH] assay would remain in the treated medium for 24 h, while cells part of a 48-h LDH assay would remain in the treated medium for 48 h. The LDH and thiazolyl blue tetrazolium bromide [MTT] assays would be done in conjunction with each, such that the same 6-well plate would first be used for an LDH assay then an MTT assay (Fig. 5). This was possible as an LDH assay only utilized a 50 μL aliquot of the treated medium the cells were suspended in. As such, 50 μL of the medium from each group was extracted and placed into separate wells on a 96-well plate. For each treatment group, this procedure was doubled to serve as an internal control for variation in the aliquots extracted in each treatment group, bringing the total of wells used in the 96-well plate to 8. Then, 50 μL of LDH substrate, purchased from BioVision, Inc. (Milpitas, CA, USA) was added to each of the 8 wells. LDH is a cytoplasmic enzyme that is released into cell culture medium after membrane disruption occurs due to cell death [1]. Therefore, a greater presence of cell death in a sample is indicated by a greater concentration of LDH in the medium [1], which allowed the activity of LDH to serve as a measure of cell death. The LDH substrate and medium mixture was then incubated at 37ºC in the dark for 30 minutes. The samples were then scanned for light absorbance values in a spectrophotometer at wavelengths of 490 nm and 680 nm. The presence of the product of the

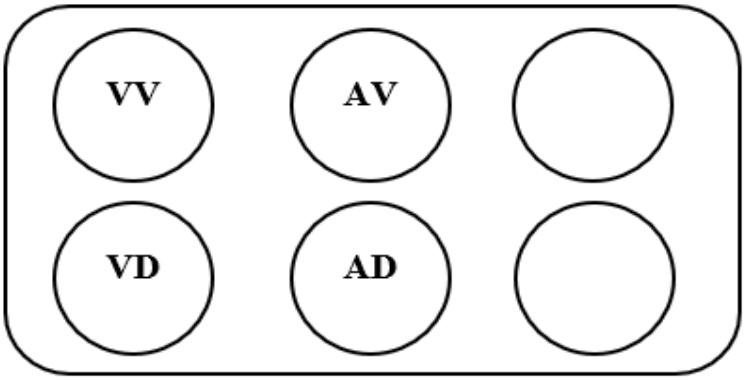

Figure 4. **Representation of Treatment Groups on 6 Well Plate** (VV = No AZD7762 or Doxorubicin, VD = Only Doxorubicin, AV = Only AZD7762, AD = AZD7762 and Doxorubicin)

LDH reaction, reduced tetrazolium salt, was responsible for the absorbance at 490 nm [1]. The plate was also read at 680 nm to serve as correction factor for any absorbance caused by the DMEM. To attain the final LDH value of a sample, the absorbance value for 680 nm was subtracted from 490 nm for each of the two wells for one sample. These two values were then averaged to provide the data point for the given sample.

2.3 MTT Assay for Cell Life (Fig. 5)

During the incubation period of the LDH assay, the MTT assay was prepared. The medium in the 6-well plate was removed from each of the samples. One mL of fresh medium and 1 mL of MTT purchased from Amresco (Solon, OH, USA), were added to the samples in the 6 well samples. MTT works as a substrate for mitochondrial enzymes present in living cells, therefore a greater conversion of MTT into its product of insoluble formazans will represent a greater presence of cells with functional mitochondria [1]. Viable, proliferating cells had a much higher capability of converting MTT than dead cells [1]. The 6-well plate was then incubated at 37ºC for 30 min in the dark. After this incubation period, the MTT and medium mixture was removed and replaced with 2 mL of dimethyl sulfoxide [DMSO] as a solvent to stop the MTT reaction. The 6-well plate was then gently rocked for 10 minutes. Two 200 µL aliquots from each treatment group were then placed into a 96-well plate. The 96-well plate was measured for absorbance in a spectrophotometer at 570 nm. As done with LDH assay, the average of the two absorbance values for a sample was used as the data point for the given sample.

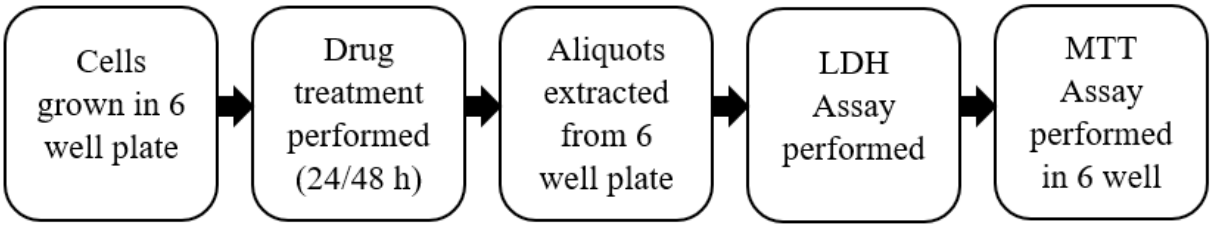

Figure 5. **Overview of LDH Assay then MTT Assay Procedure**

2.4 Western Blot Analysis for SK1 and Imaging (Fig. 6)

Cells were plated in 60 mm plates at 75,000 cells per plate. 24 h after the drug treatment and prior to the procedure for Western Blot Analysis, imaging was done on an EVOS Digital Inverted Microscope to provide visual observations on the viability of the treated cells. For the western blot analysis procedure (Fig. 6), the treated media was first removed. Cells were then lysed in RIPA buffer, purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), which contained 1 mM sodium orthovanadate, 2 mM PMSF, and multiple protease inhibitors. The cell lysate mixture was then sonicated to further breakdown any intact membranes. The cell lysates then underwent centrifugation at 14,500 g for 10 min at 4ºC. A BCA Protein assay kit from Thermo Scientific (Suwanee, GA, USA) was then used to find the protein concentration in each sample. This information was then used to create new samples of each of the treatment groups where the total protein concentration was 20 μg/ 20 μL. These samples were then placed onto an SDS/ PAGE (4% - 15% Tris/HCl) gel to separate the different proteins in the sample based on molecular weight. The separated proteins were then transferred onto a nitrocellulose membrane. To prevent the nonspecific binding of the antibodies that would later be added to identify the specific proteins, the membrane after the transfer was blocked with 5% nonfat milk in phosphate buffered saline PBS with 0.1 % Tween-20 [PBS-T] for 1 h. The primary antibody for SK1 was diluted 1:1000 while the primary antibody for β-Actin was diluted 1:10000. Measuring the β-Actin served as an internal control as the levels of β-Actin should remain consistent between all samples. If the levels of β-Actin were similar, then this meant that any differences in levels of SK1 were not a result of more total protein being in one sample as opposed to another sample. The membranes were then incubated at 4ºC in the primary antibodies overnight. The next day, the primary antibodies were removed, the membranes then washed 2 times with PBS-T, and then incubated at room temperature for 1 h in the secondary antibodies (diluted 1:1000 for SK1 and 1:5000 for β-Actin). The membranes were then washed 4 times in PBS-T, and then briefly soaked in Pierce ECL Western Blot Substrate (Pierce, Waltham, MA, USA). The membranes were then exposed to X-ray film. The burning of the X-ray films was due to the reaction of the secondary antibodies and the Pierce ECL Western Blot Substrate showed the presence of protein, as greater burning of the X-ray indicated more protein. The X-ray films were then scanned and formatted in ImageJ for presentation.

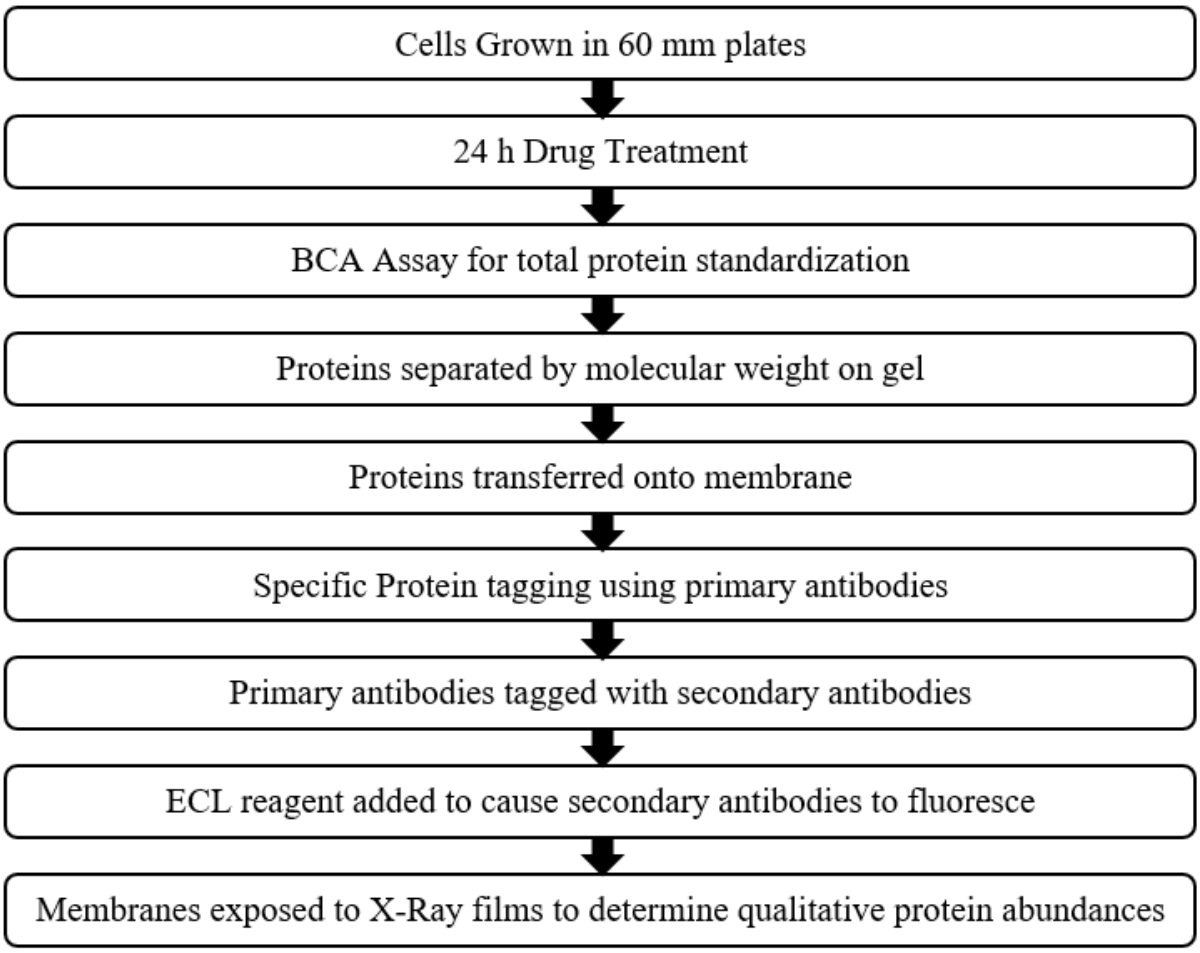

Figure 6. Steps for Western Blot Analysis

2.5 Statistical Analysis

Quantitative data was acquired from the LDH assays and MTT assays. Data was then entered into Microsoft Excel. Using the Data Analysis Tool Pack in Microsoft Excel, a One-Way ANOVA was performed for the 24 h LDH assay, 48 h LDH assay, 24 h MTT assay, and 48 h MTT assay. A p-value was then acquired for each assay, and an Alpha value of 0.05 was used to determine any significant differences among the four treatment groups. All graphs were made using Microsoft Excel.

3. RESULTS

3.1 LDH Absorbance is not Significantly Impacted among Treatment Groups

For the both 24 h and 48 h LDH assays, 3 trials were performed. The absorbance values for both the 24 h and 48 h (Table 1, 2) LDH assays were used in performing One-Way ANOVAs. The One-Way ANOVA for the 24 h LDH assays resulted in an insignificant difference in absorbance values among the treatment groups (p= 0.334). The One-Way ANOVA for the 48 h LDH assays resulted in an insignificant difference in absorbance values among the treatment groups (p=0.258). Despite a lack of a statistically significant differences among the

Table 1. 24 h LDH Values

Group	Count	Average	St Dev
VV	3	0.086	0.006
VD	3	0.104	0.016
AV	3	0.112	0.010
AD	3	0.120	0.039

Table 2. 48 h LDH Values

Groups	Count	Average	St Dev
VV	3	0.092	0.010
VD	3	0.151	0.065
AV	3	0.158	0.031
AD	3	0.144	0.036

(VV = No AZD7762 or Doxorubicin, VD = Only Doxorubicin,
AV = Only AZD7762, AD = AZD7762 and Doxorubicin)

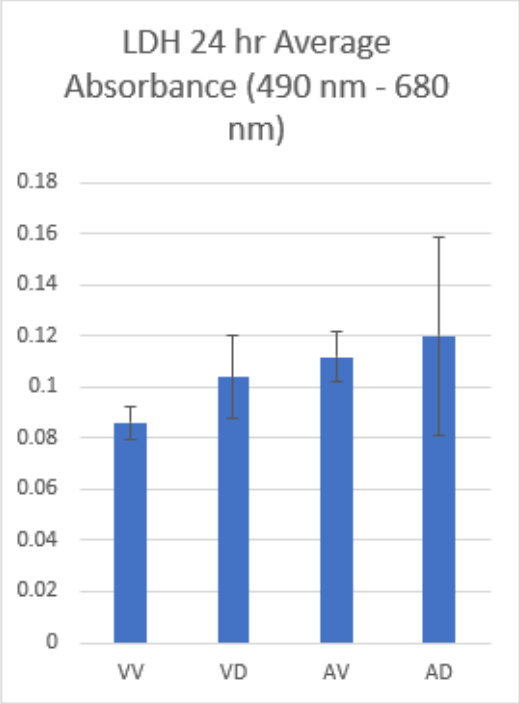

Figure 7. LDH 24 h Average Absorbances
(Error Bars: ±1 St. Dev.)

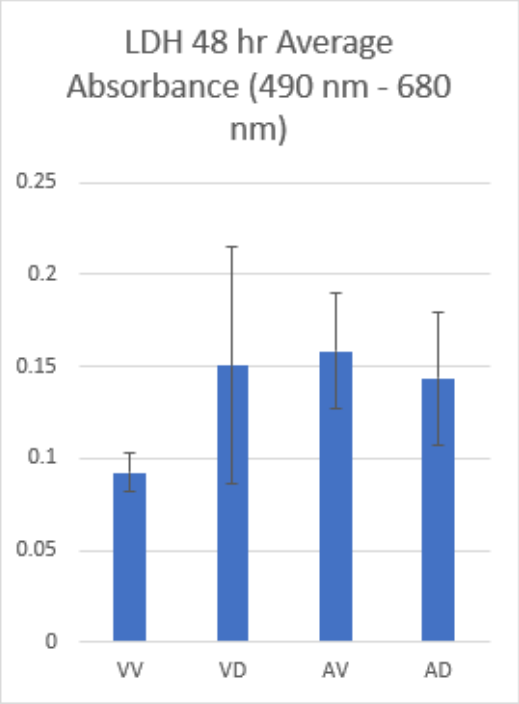

Figure 8. LDH 48 h Average Absorbances
(Error Bars: ±1 St. Dev.)

treatment groups, there was an observable trend in the absorbance averages. The VD, AV, and AD treatment groups all had greater absorbance values in both LDH assays as compared to the VV treatment group (Fig. 7, 8), however, each treatment group had high standard deviations relative to each group's average absorbance (Table 1, 2).

3.2 MTT Absorbance is not Significantly Impacted Across Treatment Groups

Each 6 well plates used for an LDH assay would also be used in an MTT assay. Therefore, there were 3 MTT 24 h assays since there were 3 LDH 24 h assays. Likewise, there

Table 3. 24 h MTT Values

Groups	Count	Average	St Dev
VV	3	0.225	0.093
VD	3	0.189	0.063
AV	3	0.144	0.038
AD	3	0.142	0.050

Table 4. 48 h MTT Values

Groups	Count	Average	St Dev
VV	3	0.275	0.124
VD	3	0.160	0.070
AV	3	0.132	0.038
AD	3	0.111	0.020

(VV = No AZD7762 or Doxorubicin, VD = Only Doxorubicin,
AV = Only AZD7762, AD = AZD7762 and Doxorubicin)

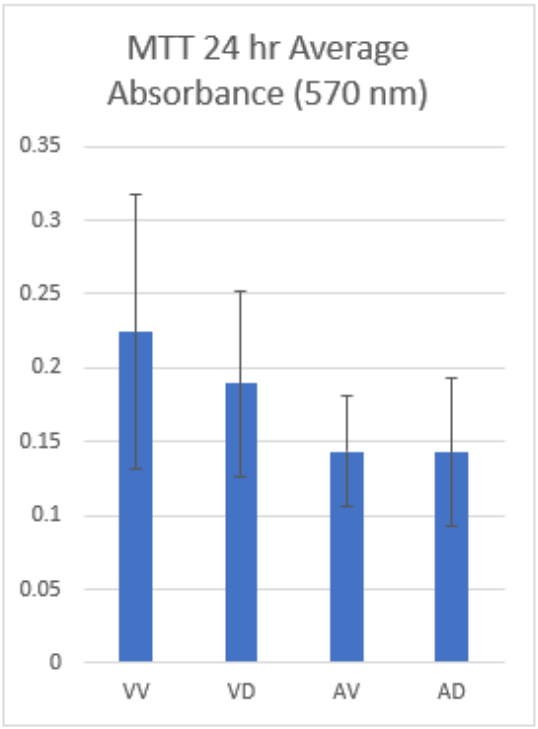

Figure 9. MTT 24 h Average Absorbances
(Error Bars: ±1 St. Dev.)

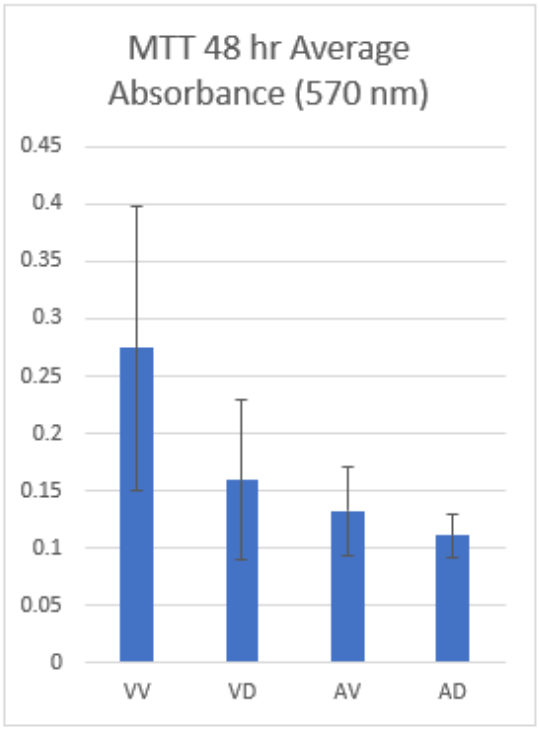

Figure 10. MTT 24 h Average Absorbances
(Error Bars: ±1 St. Dev.)

were 3 MTT 48 h assays since there were 3 LDH 24 assays. The One-Way ANOVA for the MTT 24 h assays resulted in an insignificant difference in absorbance values ($p=0.392$), and the One-Way ANOVA for the MTT 48 h assays resulted in an insignificant difference in absorbance values ($p=0.101$). This result is similar for the LDH assays, the standard deviations of each treatment group were high as compared to the means for each treatment group (Table 1, 2). However, the AZD7762 treatment groups (AV and AD) had lower absorbance averages as compared to both the untreated group (VV) and the only doxorubicin treated group (VD) (Fig. 9, 10).

3.3 CHK1 Inhibited Cells Exhibit Lower SK1 Abundance

β-Actin protein abundances were consistent across the treatment groups (Fig. 11), therefore equal total protein amounts were used for this western blot. Nonspecific binding of the SK1 antibody resulted in the reduced clarity in the SK1 protein image (Fig. 11). For the SK1 protein abundances among the treatments relative to the control group (VV), the VD group appeared to have a greater SK1 abundance, while both the AV and AD groups appeared to have slightly less SK1 abundance (Fig. 11). The differences in SK1 levels among the treatment groups is not drastic, since SK1 was present in each of the treatment groups. A total lack of SK1 by one treatment group would have represented a drastically lesser abundance of SK1.

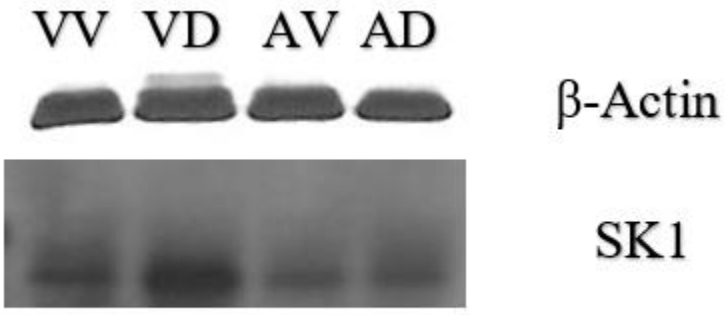

Figure 11. Western Blot Analysis Assay Images

3.4 EVOS Imaging Displays Decreased Cell Viability

Analysis of the presented images (Fig.12) was used as a qualitative analysis of the overall viability of the cells within each treatment group. Greater cell viability was marked as greater cell density and a greater proportion of healthy cells. Healthy, proliferating cells had a more defined cell membrane and thicker shape. In contrast, dying or dead cells had a very thin cell membrane and structure or appeared to be only a round particle. Using these criteria, the VV treatment group had the greatest cell viability due to its greater cell density and healthy cells (Fig. 12). In comparison, the VD, AV, AD treatment groups all exhibited less cell viability (Fig. 12). These 3 treatment groups all had extensive cell death, while the AV treatment group appeared to have the lowest cell density out of these three treatment groups (Fig. 12).

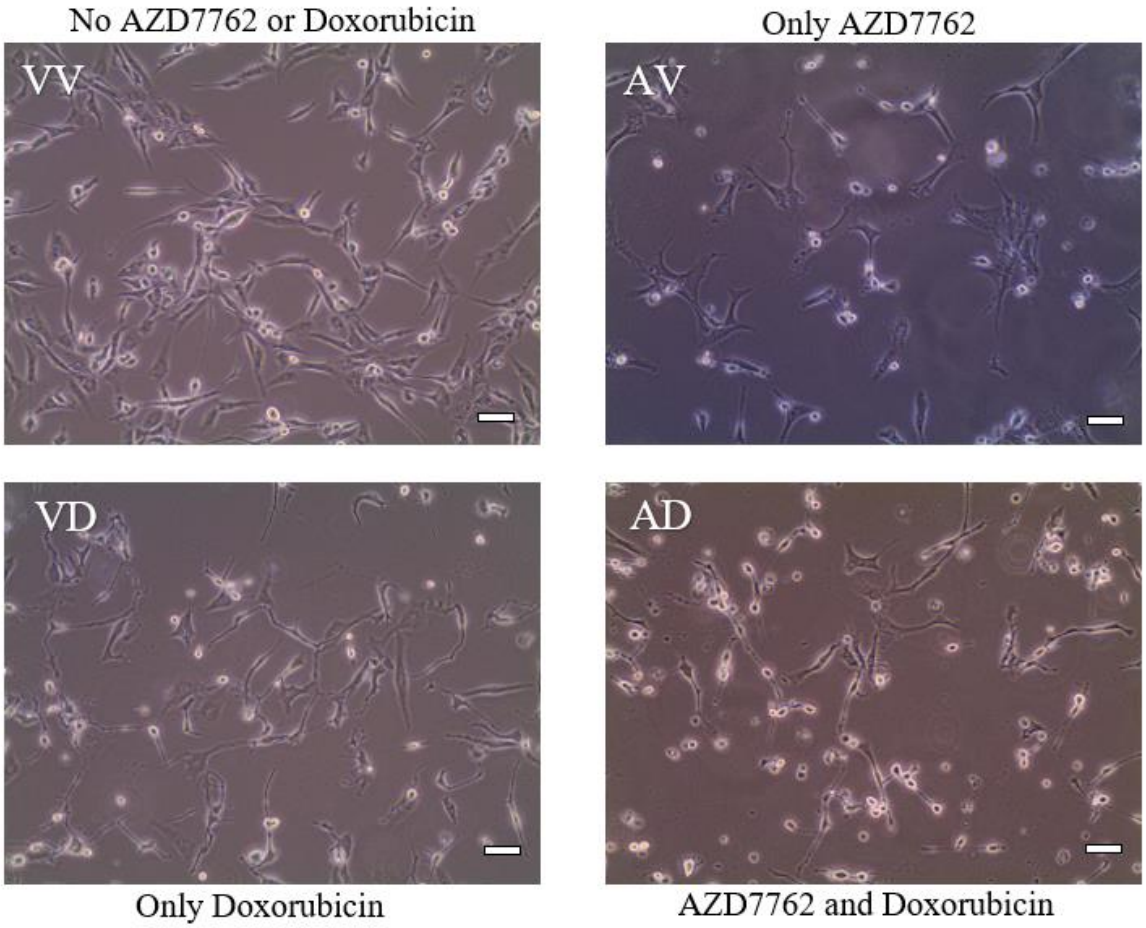

Figure 12. EVOS Imaging of Cells 24 h After Treatment
Scale Bar = 100 μm

4. DISCUSSION

The purpose of this study was to examine the effectiveness of a combination treatment of doxorubicin, a genotoxic chemotherapeutic drug, and AZD7762, a Check Kinase 1 [CHK1] inhibitor, on the cell viability of the TP53 mutated Triple Negative Breast Cancer [TNBC] cell line MDA-MB-231. This was accomplished through LDH cell death assays, MTT cell life assays, Western Blot Analysis of sphingosine 1 kinase [SK1], and EVOS microscope imaging of the cells. This project evolved based on the information that p53 deficient cells are unable use the G1 cell cycle checkpoint to repair DNA damage [11] and CHK1 inhibition prevents DNA repair from occurring during the S or late G2 phase cell cycle checkpoints [12]. This then led to the hypothesis that CHK1 inhibition in p53 deficient cells in combination with a DNA damaging agent would result in decreased cell viability as opposed to the DNA damaging agent alone, CHK1 inhibition alone, or no treatment. This was hypothesized to occur as it was expected that the lack of cell cycle checkpoints to repair the DNA damage would result in cell death and halted cell proliferation. This research also built upon a previous study where p53 deficient TNBC human-in-mouse tumor models showed the greatest reduction in tumor size in response to a similar combination treatment [7].

The MTT and LDH data did not support the hypothesis as no significant differences were observed. The small number of trials performed in conjunction with the high variation in absorbance values among the trials resulted in the high standard deviations for each treatment group (Table 1, Table 2, Table 3, & Table 4), so the data was not as robust as it could have been if more samples were analyzed. Based solely on the average absorbances in each assay for each group, the AZD7762 treated groups, both with and without doxorubicin, exhibited the lowest MTT absorbance values and greatest LDH absorbance values (Figs. 7, 8, 9, 10). Since greater absorbance values for an MTT assay indicate greater cellular proliferation and viability and greater absorbance values for an LDH assay indicate greater cellular death [1], the AZD7762 treated groups appear to have the least cellular proliferation and greatest cell death. Such examination suggests that the effect of the AZD7762 is generating this result. AZD7762, although not observed to be statistically more effective in reducing cell viability than in untreated cells, appears to be as effective as the combination treatment of AZD7762 and doxorubicin in reducing cellular viability.

The Western Blot Analysis somewhat validates this trend seen in the LDH and MTT data, as the both AZD7762 treated groups had slightly less SK1 abundance as compared to the untreated cells (Fig. 11). SK1 has the function of synthesizing S1P, which signals cellular proliferation [4], therefore the observed slight decreases in SK1 suggests that the AZD7762 treatment is slightly effective in reducing cellular proliferation. In addition, the doxorubicin only treated group had greater SK1 protein abundance than the control (Fig. 11). The upregulation of SK1 following doxorubicin treatment in p53 deficient cells had been demonstrated in a previous study [2]. This upregulation of SK1 as well as the pro cellular life impact of SK1 leads to the idea that doxorubicin alone is somewhat ineffective in reducing cellular proliferation. Based upon the EVOS imaging, doxorubicin alone does result in greater cellular death and slightly reduced cellular proliferation, as shown by cellular density, when compared to untreated cells (Fig. 12). This then conflicts the observation from the Western Blot Analysis that doxorubicin is ineffective in reducing cellular proliferation. Overall in this cell line, doxorubicin treatment alone serves to somewhat reduce cellular viability. However, as asserted by the even greater cell death and lesser cellular proliferation demonstrated in the EVOS imaging (Fig. 12), the AZD7762 treated groups demonstrate a greater reduction in cell viability. This qualitative result, taken together with the trend in MTT and LDH data and the qualitative reduction in SK1 protein abundance, provides evidence for the conclusion that AZD7762 is more effective in reducing cellular viability, regardless if combined with the genotoxic agent doxorubicin or on its own.

While it had previously been observed that the combination of a genotoxic agent and a CHK1 inhibitor has the greatest effectiveness as a TP53 mutated TNBC model [7], my data suggests that, based upon the TP53 and TNBC MDA-MB-231 cell line, that CHK1 inhibition is just as effective at reducing cell viability as compared to the combination treatment. Such a finding may be a result of CHK1 inhibition causing a loss of functionality of the G1, S, and late G2 cell cycle checkpoints in p53 deficient cells [7]. Such a loss of these checkpoints, regardless of the presence of an agent that damages the DNA, may be enough to induce a halting of the cell cycle's progression. Not using doxorubicin to serve as a genotoxic agent would be beneficial as extended use of doxorubicin results in the harmful side effect of cardiotoxicity [3]. Currently, there is a lack of targeted therapies for TNBC, as TNBC cannot be targeted for hormone therapy [7]. Therefore, the potential for the use of CHK1 inhibition as a therapy, without the cardiotoxic

effects of doxorubicin, may have its uses in the clinical environment. Yet, it must be acknowledged that this study did not have statistically significant results in the quantitative data from the MTT and LDH assays for cellular life and death, respectively. This, as well as the short time window allocated for this study, serve as major limitations to the strength of the given conclusion over the effectiveness of CHK1 inhibition alone by AZD7762 treatment. More research into the levels of cellular life and death, in addition to further Western Blot Analysis, would assist in demonstrating if CHK1 inhibition is as effective in reducing cellular viability as compared to CHK1 inhibition in combination with genotoxic stress.

5. REFERENCES

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