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Reactivation of Mutant p53 in NSCLC: Cytotoxic Potential of PRIMA-1(Met) and Etoposide Combination

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ABSTRACT

Lung cancer, particularly non-small cell lung cancer (NSCLC), remains a leading cause of cancer-related mortality. Mutations in the tumor suppressor protein p53 contribute to tumor progression and chemoresistance, making p53 reactivation a promising therapeutic strategy. PRIMA-1(Met), a small molecule designed to restore mutant p53 function, has shown potential in various cancers. This study investigates the cytotoxic effects of PRIMA-1(Met), alone and in combination with etoposide, on NSCLC cell lines. Human NSCLC cell lines A549 (p53-wild type) and NCI-H1975 (p53-mutant) were treated with PRIMA-1(Met) and/or etoposide. Cell viability was assessed using the WST-1 assay at 24, 48, and 72 hours. Statistical analyses were performed using one-way ANOVA followed by Tukey's test. PRIMA-1(Met) exhibited a dose- and time-dependent cytotoxic effect, with NCI-H1975 cells showing greater sensitivity compared to A549 cells ($p<0.001$). Etoposide treatment alone induced cytotoxicity, but its combination with PRIMA-1(Met) significantly enhanced cell death in both cell lines, particularly in p53-mutant NCI-H1975 cells ($p<0.001$). These findings suggest that PRIMA-1(Met) is more effective in p53-mutant NSCLC cells and that its combination with etoposide enhances its therapeutic potential. This study supports further investigation into PRIMA-1(Met) as a targeted therapy for p53-mutant NSCLC, particularly in combination with standard chemotherapy.

1. Introduction

Cancer remains one of the leading causes of mortality worldwide, with an increasing number of cases reported each year. While advances in chemotherapy have improved survival rates, limitations such as drug resistance and severe side effects continue to drive the search for novel therapeutic approaches. As a result, combination therapies and targeted treatments have gained significant attention, particularly those aimed at modulating key molecular pathways involved in tumor progression. Among these, strategies targeting the apoptosis pathway have emerged as promising alternatives to conventional chemotherapy.

The tumor suppressor protein p53 plays a crucial role in maintaining genomic integrity by regulating the cell cycle and apoptosis. Often referred to as the "guardian of the genome", p53 prevents tumor formation by halting the proliferation of damaged or mutated cells [1]. However, p53 mutations occur in nearly 50% of human cancers, leading to loss of its tumor-suppressive function and contributing to cancer progression and drug resistance [2]. Mutant p53 is not only inactive but can also acquire oncogenic properties, making it an attractive target for cancer therapy. As a result, pharmacological reactivation of mutant p53 has been proposed as a novel therapeutic strategy [3].

Over the past two decades, multiple small molecules have been identified to restore wild-type p53 function, with PRIMA-1 (p53 reactivation and induction of massive apoptosis) and its methylated derivative PRIMA-1(Met) (also called APR246) being among the most widely studied [4,5]. PRIMA-1(Met) has demonstrated significant potential in preclinical studies by converting mutant p53 into an active conformation, thereby restoring its tumor-suppressive functions in various cancers [6].

Studies suggest that mutant p53 levels can be further manipulated using chemotherapeutic agents to enhance tumor cell sensitivity to PRIMA-1(Met). This has led to the exploration of combination therapies, where PRIMA-1(Met) is used alongside conventional chemotherapy drugs to maximize anticancer effects [7]. In this study, we aimed to evaluate the anticancer effects of PRIMA-1(Met) in combination with the widely used chemotherapeutic agent etoposide in non-small cell lung cancer (NSCLC) cell lines. Although PRIMA-1(Met) has been extensively studied in various cancers, its effects in NSCLC, particularly in combination with etoposide, remain largely unexplored.

To address this, we have examined the cytotoxic effects of PRIMA-1(Met) and etoposide individually, as well as in combination, on two NSCLC cell lines: NCI-H1975 (p53-mutant) and A549 (p53-wild type). Our objective was to

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determine whether PRIMA-1(Met) exhibits enhanced efficacy in p53-mutant cells and whether its combination with etoposide provides a synergistic effect. Understanding the interplay between PRIMA-1(Met) and etoposide could provide valuable insights into the development of more effective therapeutic strategies for NSCLC, particularly in patients harboring p53 mutations.

2. Material and Method

2.1. Cell Culture and Reagents

Cell Culture and ReagentsPRIMA-1(Met) was purchased from Santa Cruz Biotechnology (USA) and dissolved in ultrapure water (Sigma, USA) before use. Etoposide was prepared in DMEM supplemented with 1% FBS.

2.2. Cell Viability Assay

Cell viability was assessed using the WST-1 colorimetric assay. The WST-1 assay is a colorimetric method used to assess cell viability and proliferation. It is based on the cleavage of the tetrazolium salt WST-1 by cellular mitochondrial dehydrogenases in viable cells, producing a soluble formazan dye. The amount of the produced formazan is directly proportional to the number of metabolically active cells and can be measured by absorbance at 440–480 nm.

NCI-H1975 and A549 cells were seeded in 96-well microtiter plates at a density of 5×10^3 cells/well in 200 µL of culture medium and incubated overnight to allow cell attachment. After 24, 48, and 72 hours of exposure to PRIMA-1(Met) and/or etoposide, 10 µL of WST-1 reagent (Roche Diagnostics GmbH, Mannheim, Germany; Catalog#: 11.644.807.001) was added to each well. The plates were incubated for a further period of 4 hours at 37°C, and absorbance was measured at 450 nm using an ELISA microplate reader (Thermo Labsystem Multiscan Spectrum, Thermo Labsystem, Chantilly, VA, USA).

Cell viability was calculated using the following formula [8]: % Cytotoxicity = (1-(OD450 treated/OD450 control) x 100).

All experiments were performed in quadruplicate and repeated four independent times. Morphological changes in the cells were observed using an Olympus IX71 inverted microscope.

2.3. Statistical Analysis

All data were expressed as mean ± standard error of the mean (SEM). The Kolmogorov-Smirnov test was used to assess data normality. Differences between the control and experimental groups were analyzed using one-way ANOVA, followed by Tukey's multiple comparison test using Instat 3.0 software. Statistical significance was set at: $p<0.05$ (statistically significant, *), $p<0.01$ (highly significant, **), $p<0.001$ (extremely significant, ***). All graphs were generated using Microsoft Excel software.

3. Results

3.1 Effect of PRIMA-1(Met) on Cell Viability in A549 Cells

The cytotoxic effects of PRIMA-1(Met) on A549 (p53-wild type) cells were evaluated after 24, 48, and 72 hours of treatment. Statistical analyses revealed that after 24 hours, the highest cytotoxic effects were observed at 10 and 20 µM doses compared to the control group ($p<0.001$). After 48 hours, the highest cytotoxic effects were detected at 10, 40, and 50 µM doses ($p<0.001$). After 72 hours, the most significant reduction in cell viability was observed at 40 and 50 µM doses ($p<0.001$). These results indicate a time- and dose-dependent cytotoxic effect of PRIMA-1(Met) in A549 cells (Fig. 1).

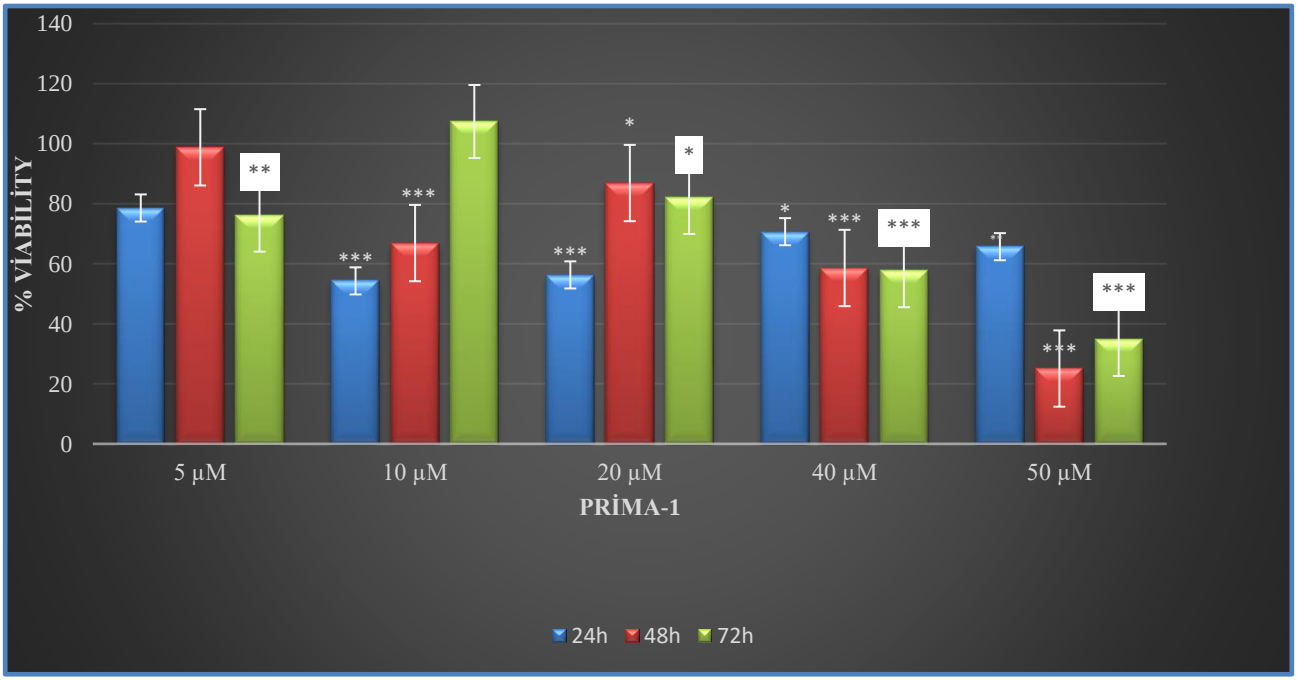

Fig. 1 Effect of PRIMA-1(Met) on cell viability in A549 cells (*, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

3.2. Effect of Etoposide on Cell Viability in A549 Cells

To assess the cytotoxicity of etoposide in A549 cells, cell viability was analyzed at 24, 48, and 72 hours post-treatment. After 24 hours, the highest cytotoxic effects were observed at 20 and 40 µM doses ($p<0.001$). However, after 48 hours, no significant difference was detected between the control group and any of the treatment doses. By the end of 72 hours, a dose-dependent increase in cytotoxicity was observed, with the highest effect seen at 20 µM ($p<0.01$) and at 40 and 50 µM ($p<0.001$). (Fig. 2)

3.3. Cytotoxicity of PRIMA-1(Met) and Etoposide Combination in A549 Cells

Following the determination of effective PRIMA-1(Met) and etoposide doses, their combination was analyzed for synergistic cytotoxic effects in A549 cells. At the end of 24 hours, all combination treatments demonstrated significantly enhanced cytotoxicity compared to the control ($p<0.001$), suggesting a potential additive or synergistic interaction PRIMA-1(Met) and etoposide in p53-wild type NSCLC cells (Fig. 3).

3.4. Effect of PRIMA-1(Met) on Cell Viability in NCI-H1975 Cells

The cytotoxic effects of PRIMA-1(Met) were also evaluated in NCI-H1975 (p53-mutant) cells after 24, 48, and 72 hours of treatment. PRIMA-1(Met) exhibited a dose- and time-dependent cytotoxic effect in these cells (Fig. 4). The lowest tested dose (5 µM) had no significant effect at 24 and 48 hours ($p > 0.05$), but after 72 hours, it caused a significant decrease in cell viability compared to the control ($p<0.001$). The 10 µM

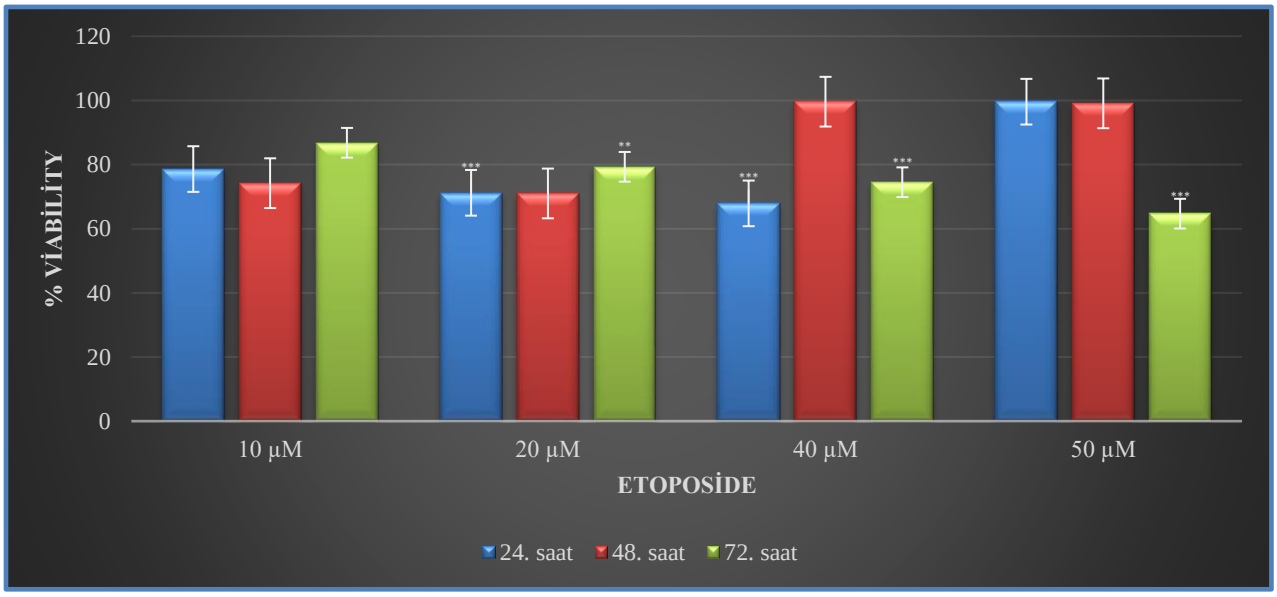

Fig. 2 Cytotoxic effects of Etoposide on A549 cells (**, $p<0.01$; ***, $p<0.001$).

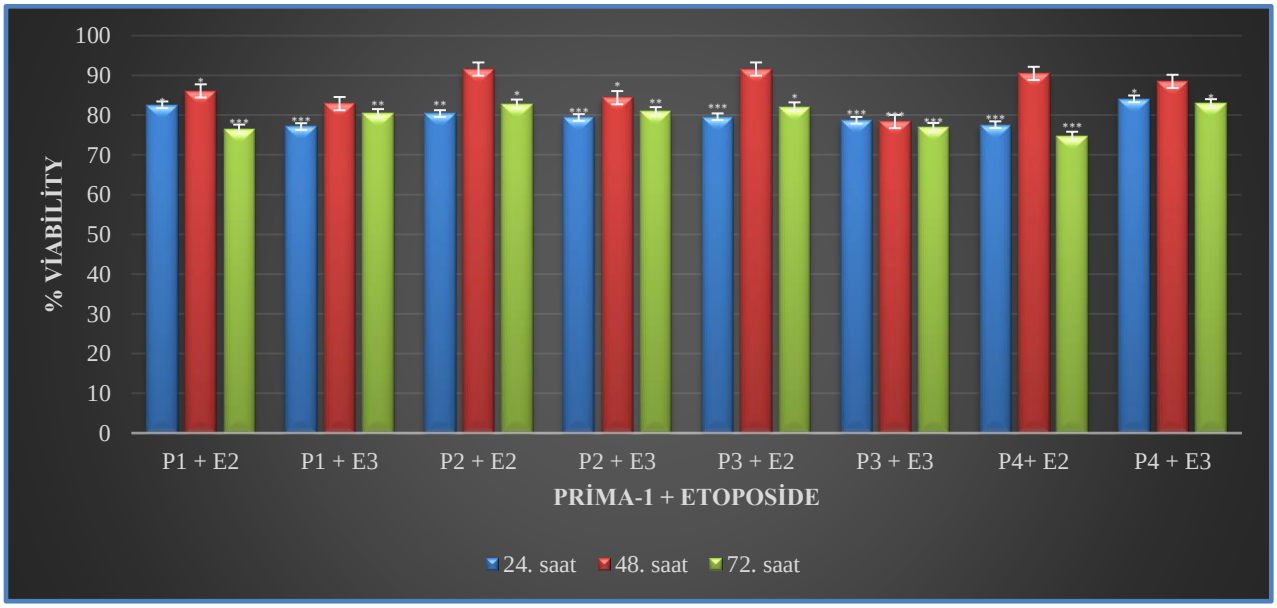

Fig. 3 PRIMA-1(Met) and Etoposide Combination-Induced Cytotoxicity in A549 Cells (P: Prima-1; E: Etoposide; P1: 5 µM, P2: 10 µM, P3: 20 µM, P4: 40 µM; E2: 20 µM, E3: 40 µM; *, $p<0.05$; **, $p<0.01$; ***, $p<0.001$).

dose showed moderate cytotoxicity at 24 hours ($p<0.01$) and a stronger effect at 48 and 72 hours ($p<0.001$). Higher doses of PRIMA-1(Met) (20, 40, and 50 µM) resulted in a dose-dependent decrease in cell viability across all time points ($p<0.001$). These findings indicate that PRIMA-1(Met) is highly effective in p53-mutant NSCLC cells, particularly with prolonged exposure.

3.5. Effect of Etoposide on Cell Viability in NCI-H1975 Cells

The cytotoxic effects of etoposide on NCI-H1975 cells were examined at 24, 48, and 72 hours. A dose- and time-dependent decrease in cell viability was observed, with the highest cytotoxic effects detected at 20 and 40 µM doses ($p<0.001$). These findings indicate that etoposide alone effectively reduces cell viability in p53-mutant NSCLC cells (Fig. 5).

3.6. Cytotoxicity of PRIMA-1(Met) and Etoposide Combination in NCI-H1975 Cells

In order to investigate the potential synergistic effects of PRIMA-1(Met) and etoposide, their combination was evaluated in NCI-H1975 cells (Fig. 6). The results demonstrated a strong cytotoxic effect in a time-dependent manner, with significant reductions in cell viability observed at all tested combinations ($p<0.001$). Notably, the combination treatment was more effective in NCI-H1975 cells compared to A549 cells, suggesting that PRIMA-1(Met) is particularly potent in p53-mutant cancer cells when used alongside etoposide, possibly through an additive or synergistic mechanism.

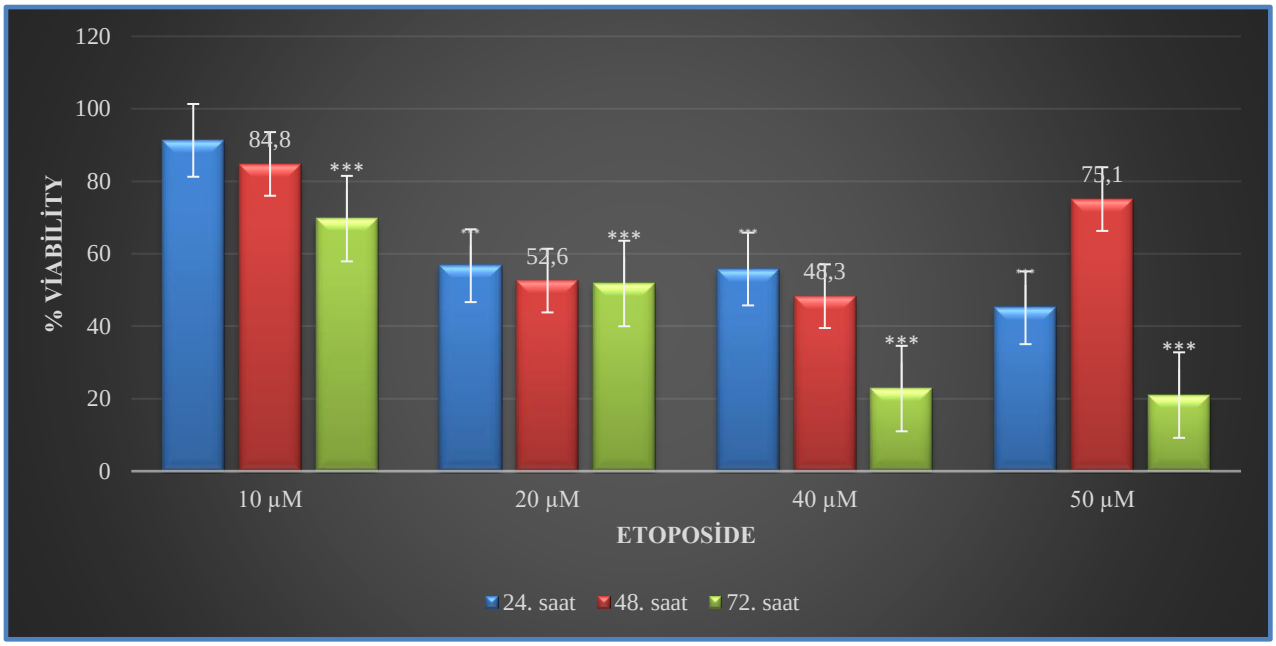

Fig. 5 Cytotoxic effects of Etoposide on NCI-H1975 cells (*, $p<0.05$; ***, $p<0.001$).

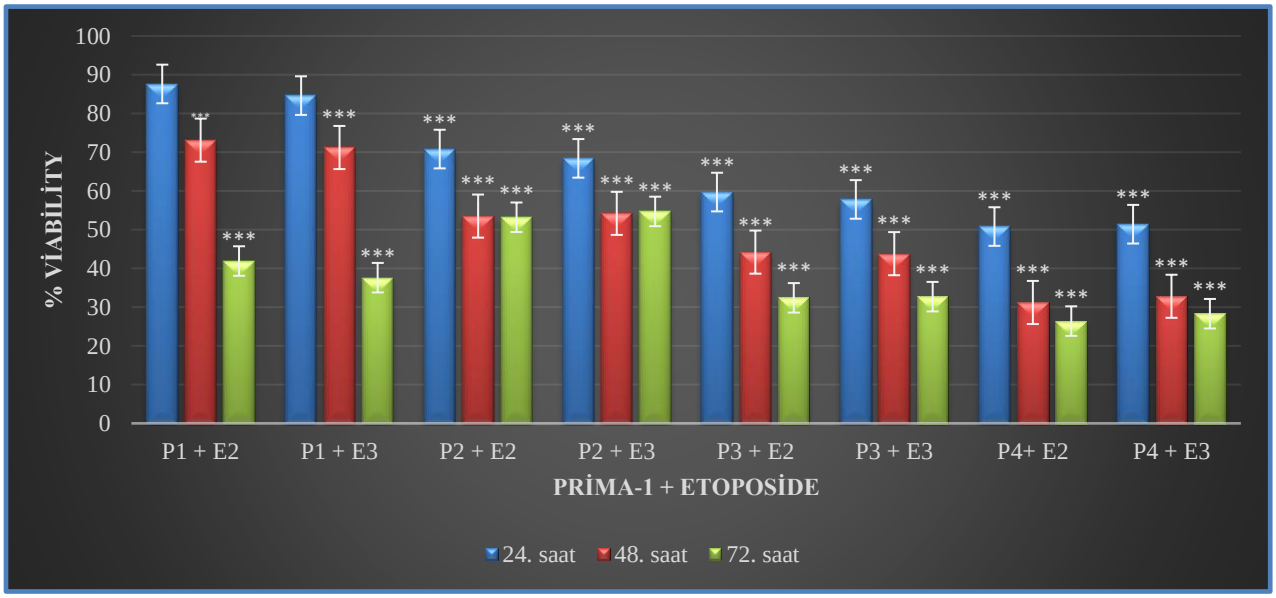

Fig. 6 PRIMA-1(Met) and Etoposide Combination-Induced Cytotoxicity in NCI-H1975 Cells (P: Prima-1; E: Etoposide; P1: 5 µM, P2: 10 µM, P3: 20 µM, P4: 40 µM; E2: 20 µM, E3: 40 µM; ***, $p<0.001$).

4. Discussion

The tumor suppressor p53 plays a fundamental role in maintaining cellular homeostasis by triggering apoptosis and cell cycle arrest in response to genetic damage. However, p53 mutations are highly prevalent in cancer, often resulting in loss of its protective functions and contributing to tumorigenesis [1]. Given its pivotal role, reactivating mutant p53 has emerged as a promising therapeutic strategy for treating cancers resistant to conventional therapies. Among the small molecules designed to restore p53 function, PRIMA-1(Met) has demonstrated significant potential in preclinical studies by inducing apoptosis in p53-mutant cancer cells [4;9-14].

Our findings confirm that PRIMA-1(Met) exhibits significant cytotoxic effects on NSCLC cells in a dose- and time-dependent manner. Notably, p53-mutant NCI-H1975 cells displayed greater sensitivity to PRIMA-1(Met) compared to p53-wild type A549 cells, reinforcing the hypothesis that PRIMA-1(Met) is more effective in tumors harboring p53 mutations. These results line up with previous reports demonstrating that PRIMA-1(Met) preferentially induces apoptosis in mutant p53-expressing cancer cells [15].

Given that p53-mutant tumors are generally more resistant to chemotherapy, strategies to enhance their sensitivity are critical. Our study explored the potential of combining PRIMA-1(Met) with etoposide, a DNA-damaging agent commonly used in NSCLC treatment. Our results revealed that etoposide significantly enhanced PRIMA-1(Met)-induced cytotoxicity, particularly in p53-mutant NCI-H1975 cells. This finding is consistent with previous studies in small-cell lung cancer (SCLC) models, where PRIMA-1(Met) in combination with etoposide exhibited strong synergistic effects [16].

The observed synergy between PRIMA-1(Met) and etoposide can be attributed to their complementary mechanisms of action. Etoposide induces DNA damage, leading to the accumulation of mutant p53, which in turn enhances PRIMA-1(Met)-mediated apoptosis. Additionally, PRIMA-1(Met) has been shown to modulate cellular redox balance, further sensitizing tumor cells to chemotherapeutic agents [5;11]. These findings suggest that the combination of PRIMA-1(Met) with standard chemotherapy regimens could be a viable strategy for treating p53-mutant NSCLC. Although our data showed significantly increased cytotoxicity in combination treatments, further studies utilizing drug interaction models such as Chou-Talalay or Bliss independence analysis are required to definitively characterize the interaction as additive or synergistic.

Recent studies have increasingly supported the use of PRIMA-1(Met) in combination with DNA-damaging agents to enhance therapeutic efficacy in TP53-mutant cancers. For instance, in TP53-mutant myelodysplastic syndromes (MDS) and acute myeloid leukemia (AML), the combination of PRIMA-1(Met) with azacitidine has demonstrated significant synergistic effects, leading to higher complete remission rates and improved overall survival compared to azacitidine alone [17]. Furthermore, a Phase I study combining PRIMA-1(Met) with azacitidine and venetoclax in TP53-mutant AML patients showed promising preliminary efficacy, suggesting that this triplet regimen may overcome resistance observed with standard therapies [18]. In solid tumors, PRIMA-1(Met) has been shown to restore p53 function and enhance the efficacy of DNA-damaging agents. For example, in breast cancer cell lines harboring TP53 mutations, PRIMA-1(Met) treatment led to increased DNA repair activity and reduced genomic instability when combined with cisplatin [19]. Similarly, in esophageal cancer models, PRIMA-1(Met) induced apoptosis and enhanced chemosensitivity to 5-fluorouracil, particularly in TP53-mutant contexts [20]. These findings align with our current study and highlight the broad applicability of PRIMA-1(Met) in combination therapies targeting mutant p53-driven tumors.

Despite these promising findings, some limitations may yet be considered. First, our study was conducted in vitro, and the efficacy of PRIMA-1(Met) in combination with etoposide should be further evaluated in in vivo models to confirm its clinical potential. Second, protein-level validation of mutant p53 reactivation via western blot or immunohistochemistry would strengthen our mechanistic insights. Lastly, while our results highlight the enhanced cytotoxicity of PRIMA-1(Met) in p53-mutant NSCLC, further studies are needed to explore its effects on additional NSCLC subtypes and in patient-derived tumor models.

5. Conclusion

Our study provides new insights into the anticancer effects of PRIMA-1(Met) in NSCLC, demonstrating that p53-mutant cells are more sensitive to its effects than p53-wild type cells. Furthermore, our findings suggest that combining PRIMA-1(Met) with etoposide significantly enhances its cytotoxic effects, supporting the rationale for exploring this combination in p53-mutant NSCLC therapy. These results contribute to the growing body of evidence supporting the clinical development of PRIMA-1(Met) and highlight the need for further in vivo validation to assess its full therapeutic potential.

Declaration

Author Contribution: Conceive– N. I.; Design– N. I.; Supervision- N. I.; Experimental Performance, Data Collection and Processing– F.T.K., N. I.; Analysis and Interpretation– F.T.K., N. I.; Literature Review– F.T.K., N. I.; Writer– F.T.K., N. I.; Critical Review– N. I.

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