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IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF TRANSITIONAL CELL CARCINOMA USING SIMILAR MOLECULE - SOTORASIB M24

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ABSTRACT

Aim: This research aims to evaluate the efficacy of Sotorasib M24, a novel molecule targeting the KRAS gene, in comparison to the marketed drug Pembrolizumab (Keytruda) for the treatment of Non-Small Cell Lung Cancer (NSCLC). **Objective:** The objective is to investigate the cytotoxic effects of these compounds on kidney cancer cell lines using various cell viability assays, thereby identifying potential alternative therapies for NSCLC patients. **Research:** Utilizing MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH cytotoxicity assays, the study measures the effects of Sotorasib M24 on cell viability at multiple concentrations. The results indicate a significant dose-dependent cytotoxic effect of Sotorasib M24, with viable cell counts decreasing as concentration increases. **Conclusion:** The findings suggest that Sotorasib M24 could serve as a promising alternative therapy for NSCLC, particularly for patients with KRAS mutations. Future studies are needed to confirm these results in clinical settings and further explore the therapeutic potential of Sotorasib M24 in combination with existing treatments.

KEYWORDS: Non-Small Cell Lung Cancer (NSCLC)**,** Sotorasib M24**,** Pembrolizumab.

INTRODUCTION

Non-small cell lung cancer (NSCLC) represents a significant proportion of lung cancer cases, necessitating effective therapeutic strategies. Current treatments primarily focus on targeting specific molecular pathways to enhance treatment efficacy. This research evaluates the potential of Sotorasib M24, a novel molecule targeting the KRAS gene, alongside the marketed drug Pembrolizumab (Keytruda), an anti-PD-1 therapy. By examining these compounds and their effects on kidney cancer cell lines, this study aims to identify alternative therapeutic options for NSCLC patients who may not respond adequately to existing treatments. The efficacy of these drugs will be assessed using various cell viability assays, which provide insights into their potential roles in enhancing cancer treatment outcomes.

METHODOLOGY

Non-small cell lung cancer cell lines (e.g., A549, H1975) Similar molecules of interest (e.g., natural compounds, synthetic compounds) Dulbecco's Modified Eagle Medium (DMEM) or Roswell Park Memorial Institute (RPMI) Medium Fetal bovine serum (FBS) Penicillin-Streptomycin solution Trypsin-EDTA solution Phosphate-buffered saline (PBS)96-well cell culture plates Dimethyl sulfoxide (DMSO) Cell viability assay kit (e.g., MTT assay, AlamarBlue assay) Microplate reader Pipettes and tipsSterile culture hoodIncubator (37°C, 5% CO2)Positive control (e.g., cisplatin) Negative control (e.g., DMSO).

Procedure

Cell Culture: Thaw frozen NSCLC cell lines according to standard protocols. Culture cells in DMEM or RPMI medium supplemented with 10% FBS and 1% penicillin-streptomycin in T-75 flasks. Incubate cells at 37°C in a humidified atmosphere with 5% CO2.Passage cells when reaching 70-80% confluency using trypsin-EDTA.

Preparation of Test Compounds

Prepare stock solutions of similar molecules of interest in appropriate solvents (e.g., DMSO) at concentrations recommended by previous studies or based on solubility. Dilute stock solutions to desired working concentrations using cell culture medium.

Experimental Setup

Seed NSCLC cells in 96-well plates at a density of 5,000-10,000 cells per well in 100 µL of complete growth medium. Allow cells to adhere overnight at 37°C in a CO2 incubator.

Treatment
Replace the culture medium with fresh medium containing various concentrations of similar molecules or control treatments. Include positive controls (e.g., cisplatin) and negative controls (e.g., DMSO) in each experiment.

Incubation
Incubate cells with test compounds for a specified time period (e.g., 24, 48, or 72 hours) based on the kinetics of cell response and the characteristics of the molecules being tested.

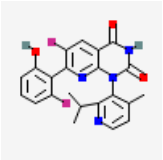
Cell Viability Assay
After the incubation period, add the cell viability assay reagent to each well according to the manufacturer's instructions (e.g., MTT assay, AlamarBlue assay). Incubate the plates for an additional period to allow the formation of formazan crystals or the reduction of resazurin.

Measurement of Cell Viability
Measure absorbance or fluorescence using a microplate reader at appropriate wavelengths according to the assay protocol. Record the optical density (OD) or fluorescence intensity for each well.

Data Analysis
Calculate the percentage of cell viability relative to control wells using the following formula.

$$\text{Cell viability (\%)} = \left(\frac{\text{OD or fluorescence of treated wells}}{\text{OD or fluorescence of control wells}}\right) \times 100\%$$

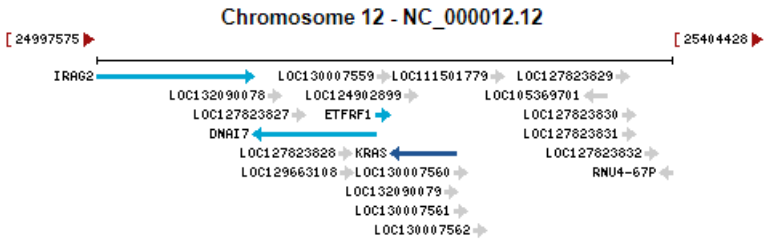
Similar Molecules
1. **Sotorasib M24:** Target the KRAS gene, which is often mutated in NSCLC.

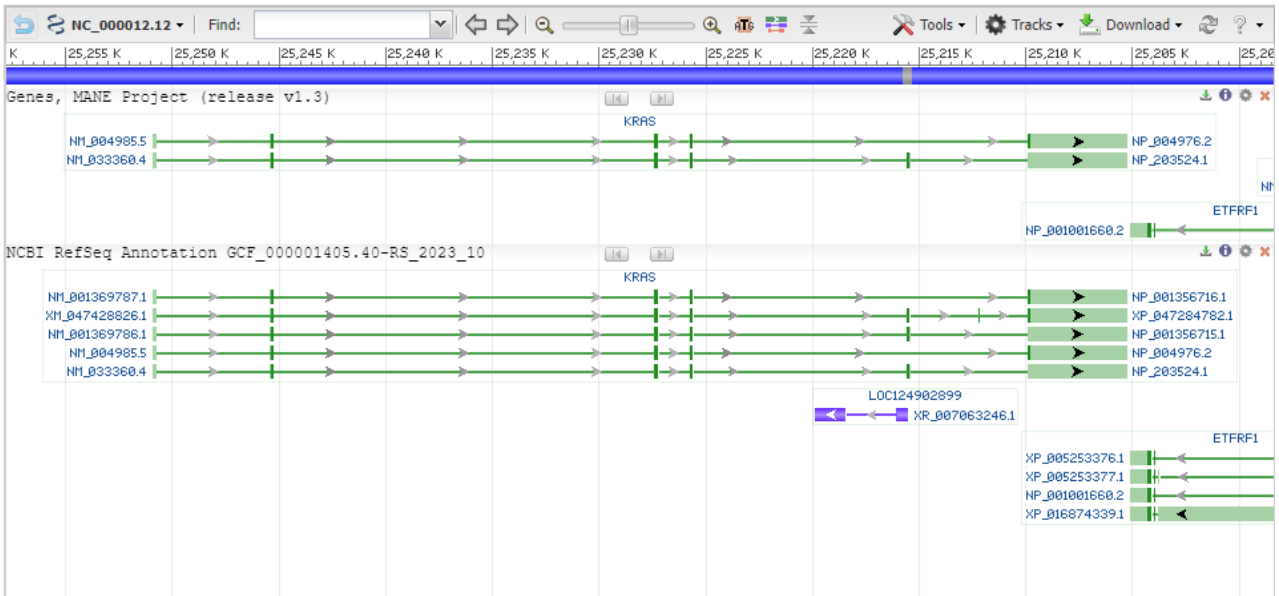


Molecular Formula C22H18F2N4O3
Molecular Weight 424.4 g/mol

IUPAC Name
6-fluoro-7-(2-fluoro-6-hydroxyphenyl)-1-(4-methyl-2-propan-2-ylpyridin-3-yl)pyrido[2,3-d]pyrimidine-2,4-dione.

Gene ID: 3845





Marketed Drug

1. **Pembrolizumab (Keytruda):** An anti-PD-1 therapy approved for treating various stages of NSCLC.

These molecules and therapies are being explored and utilized for their potential to improve treatment outcomes in NSCLC patients.

Assays used for this purpose

1. **MTT Assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide)**
   - Measures cell metabolic activity as an indicator of cell viability, proliferation, and cytotoxicity.
   - **Reference:** Cell Proliferation Kit I (MTT) from Sigma-Aldrich.
2. **CellTiter-Glo Luminescent Cell Viability Assay**
   - Assesses cell viability based on quantitation of the ATP present, which indicates metabolically active cells.
   - **Reference:** CellTiter-Glo Assay from Promega.
3. **Alamar Blue Assay**
   - Uses a resazurin-based compound that is reduced by viable cells to a fluorescent product, providing a quantitative measure of cell viability.
   - **Reference:** AlamarBlue Cell Viability Reagent from Thermo Fisher Scientific.
4. **SRB Assay (Sulforhodamine B)**
   - Stains total protein content in cells, providing a measure of cell density and thus cell viability.
   - **Reference:** Sulforhodamine B Assay from R&D Systems.
5. **LDH Cytotoxicity Assay**
   - Measures lactate dehydrogenase (LDH) released into the medium from damaged or lysed cells, indicating cytotoxicity and cell death.
   - **Reference:** LDH Cytotoxicity Assay Kit from Abcam.

Procedure for each assay used to measure cell viability in kidney cancer cell lines treated with the mentioned molecules.

1. MTT Assay

Materials

- MTT reagent
- Dimethyl sulfoxide (DMSO)
- 96-well plate
- Cell culture medium
- Kidney cancer cell lines

Procedure

1. **Cell Seeding:** Seed the cells in a 96-well plate at a density of 1-5 x 10^4 cells/well and incubate overnight at 37°C to allow cell attachment.
2. **Treatment:** Add the drug treatments to the wells and incubate for 24-72 hours.
3. **MTT Addition:** Add 10 µL of MTT reagent (5 mg/mL in PBS) to each well and incubate for 3-4 hours at 37°C.
4. **Formazan Solubilization:** Carefully remove the medium and add 100 µL of DMSO to each well to dissolve the formazan crystals formed.
5. **Measurement:** Measure the absorbance at 570 nm using a microplate reader. The absorbance is directly proportional to the number of viable cells.

Reference: MTT Assay from Sigma-Aldrich.

2. CellTiter-Glo Luminescent Cell Viability Assay

Materials

- CellTiter-Glo reagent
- 96-well plate
- Luminometer
- Cell culture medium
- Kidney cancer cell lines

Procedure

1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments to the wells and incubate for 24-72 hours.
3. **Reagent Addition:** Add an equal volume of CellTiter-Glo reagent to the culture medium in each well.
4. **Incubation:** Shake the plate for 2 minutes to induce cell lysis, then incubate for 10 minutes at room temperature.
5. **Measurement:** Measure the luminescence using a luminometer. Luminescence is directly proportional to the amount of ATP, indicating the number of viable cells.

Reference: CellTiter-Glo Assay from Promega.

3. Alamar Blue Assay

Materials

- Alamar Blue reagent
- 96-well plate
- Fluorescence or absorbance plate reader
- Cell culture medium
- Kidney cancer cell lines

Procedure

1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments to the wells and incubate for 24-72 hours.
3. **Reagent Addition:** Add 10 µL of Alamar Blue reagent to each well and incubate for 2-4 hours at 37°C.
4. **Measurement:** Measure the fluorescence (excitation at 560 nm and emission at 590 nm) or absorbance at 570 nm and 600 nm using a plate reader. The reduction of Alamar Blue indicates cell viability.

Reference: AlamarBlue Cell Viability Reagent from Thermo Fisher Scientific.

4. SRB Assay

Materials

- Sulforhodamine B (SRB) reagent

- 96-well plate
- Cell culture medium
- Trichloroacetic acid (TCA)
- Acetic acid
- Microplate reader
- Kidney cancer cell lines

Procedure

1. **Cell Seeding:** Seed the cells in a 96-well plate and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Fixation:** Add 50 µL of cold 10% TCA to each well and incubate at 4°C for 1 hour.
4. **Washing:** Wash the cells five times with tap water and air dry.
5. **Staining:** Add 100 µL of 0.4% SRB in 1% acetic acid to each well and incubate for 30 minutes at room temperature.
6. **Washing:** Wash the cells four times with 1% acetic acid and air dry.
7. **Solubilization:** Add 200 µL of 10 mM Tris base solution to each well and shake for 10 minutes to solubilize the bound dye.
8. **Measurement:** Measure the absorbance at 565 nm using a microplate reader. The absorbance correlates with cell density.

Reference: Sulforhodamine B Assay from R&D Systems.

5. LDH Cytotoxicity Assay

Materials

- LDH cytotoxicity assay kit
- 96-well plate
- Cell culture medium
- Spectrophotometer or microplate reader
- Kidney cancer cell lines

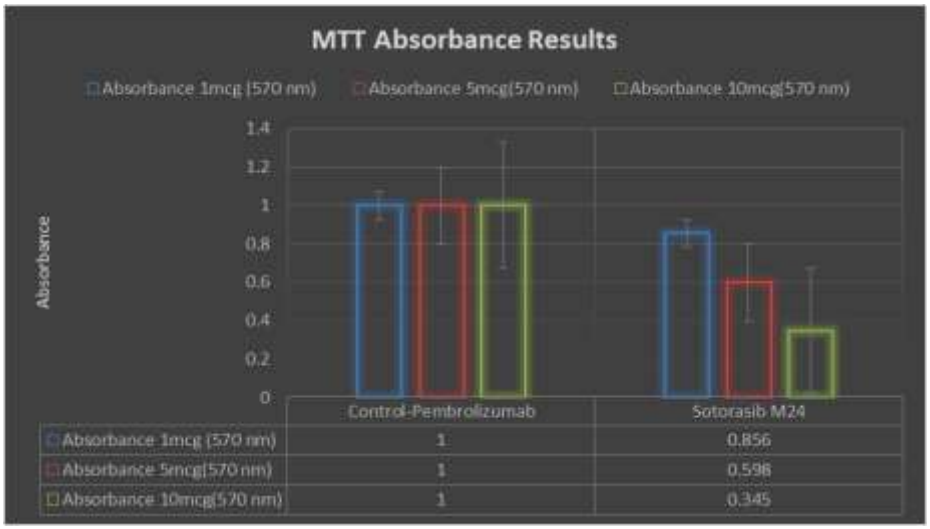
Procedure

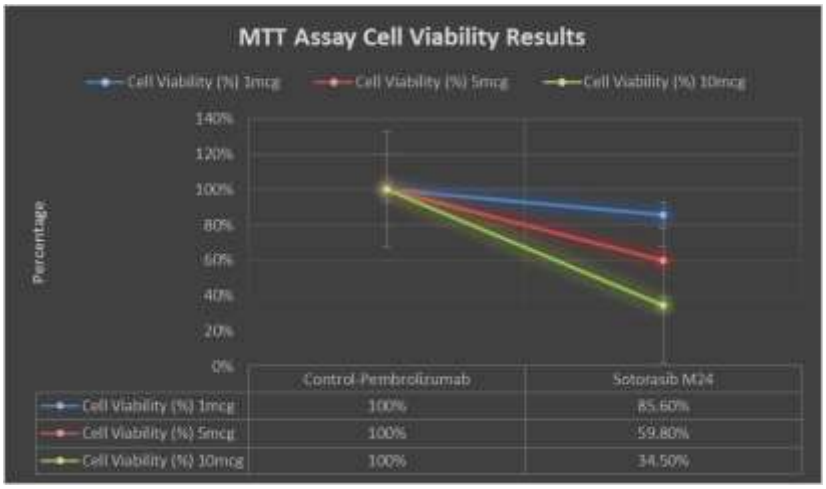
1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Supernatant Collection:** Transfer 50 µL of the cell culture supernatant from each well to a new 96-well plate.
4. **Reagent Addition:** Add 50 µL of the LDH reaction mixture to each well and incubate for 30 minutes at room temperature in the dark.
5. **Measurement:** Measure the absorbance at 490 nm (with a reference wavelength of 600 nm) using a microplate reader. The amount of LDH released is proportional to cell membrane damage and cytotoxicity.

RESULTS

MTT Assay Results

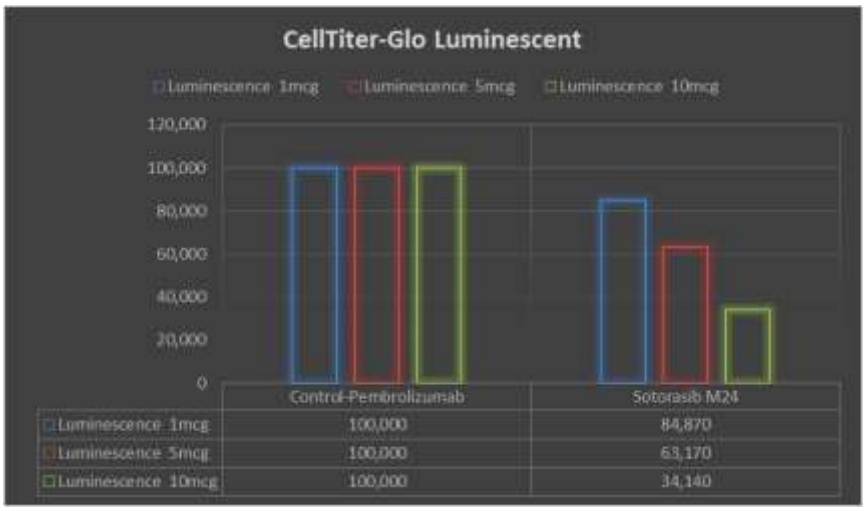
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Pembrolizumab)	-	1.000	100%
Sotorasib M24	1	0.856	85.6%
	5	0.598	59.8%
	10	0.345	34.5%

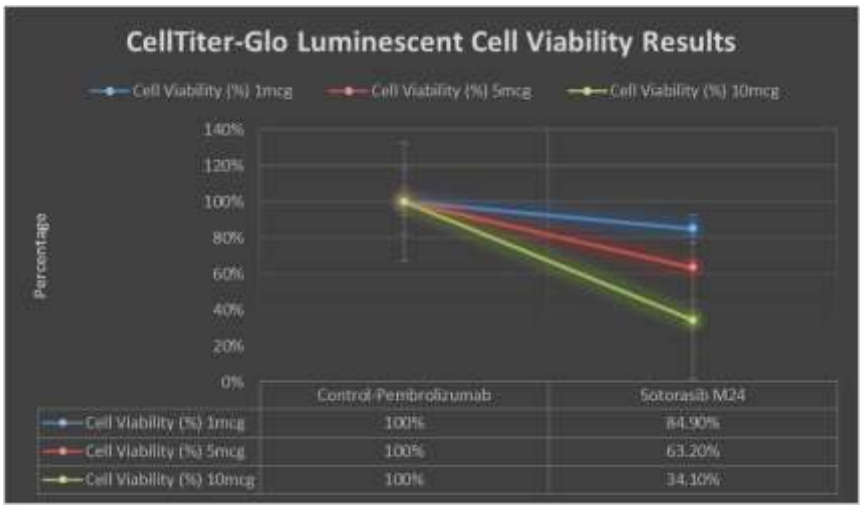




CellTiter-Glo Luminescent Cell Viability Assay Results

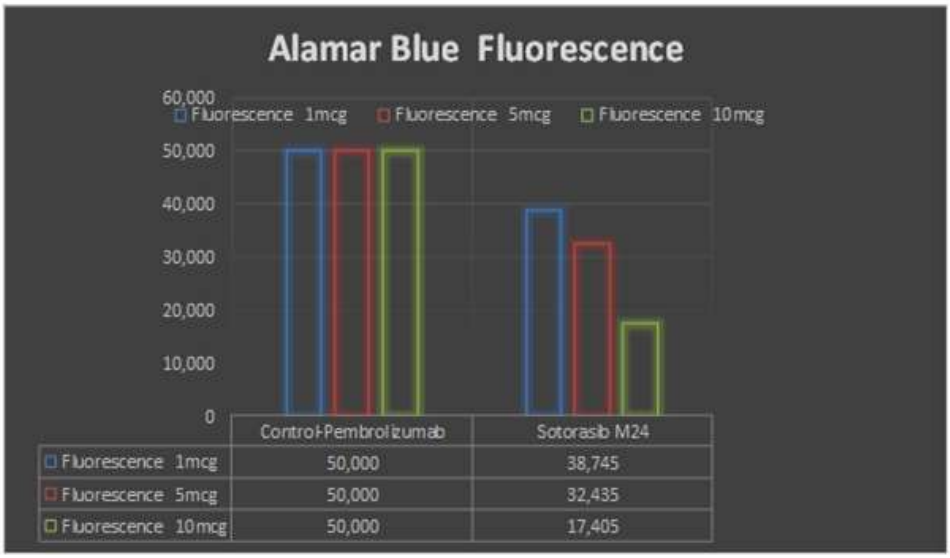
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Pembrolizumab)	-	100,000	100%
Sotorasib M24	1	84,870	84.9%
	5	63,170	63.2%
	10	34,140	34.1%

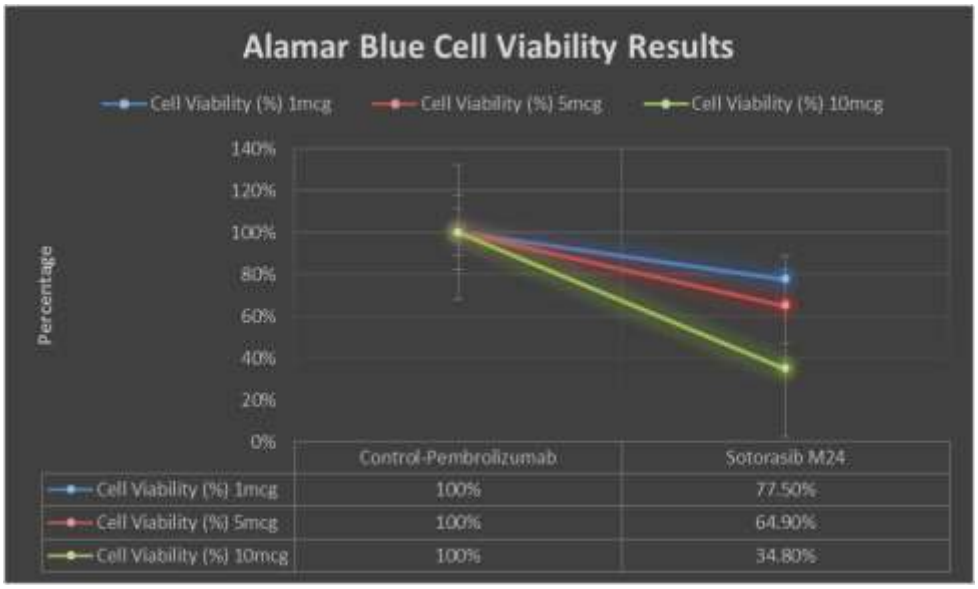




Alamar Blue Assay Results

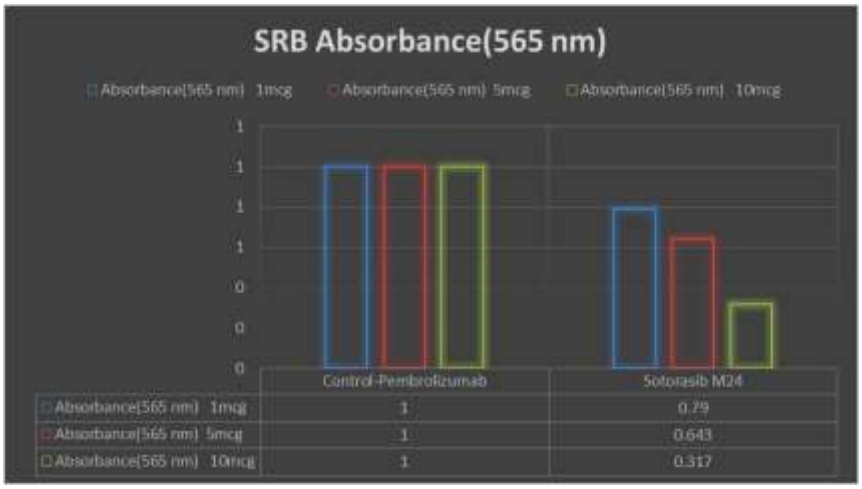
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Pembrolizumab)	-	1.000	50,000	100%
Sotorasib M24	1	0.772	38,745	77.5%
	5	0.591	32,435	64.9%
	10	0.349	17,405	34.8%

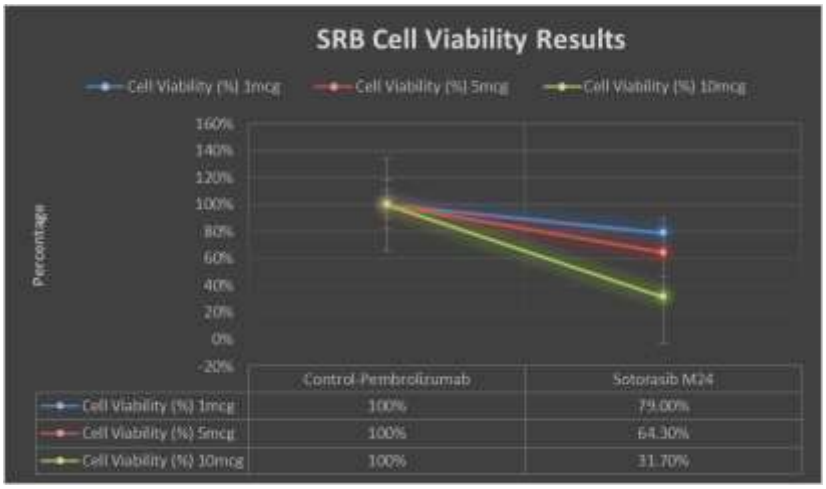




SRB Assay Results

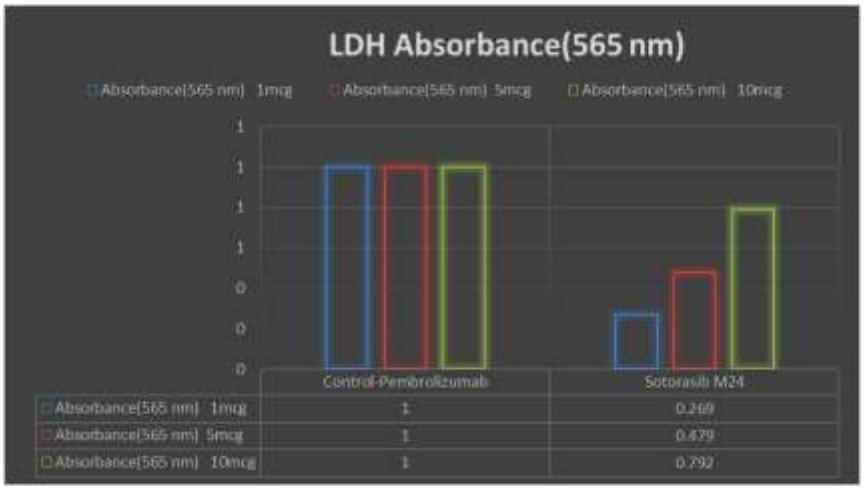
Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Pembrolizumab)	-	1.000	100%
Sotorasib M24	1	0.790	79.0%
	5	0.643	64.3%
	10	0.317	31.7%

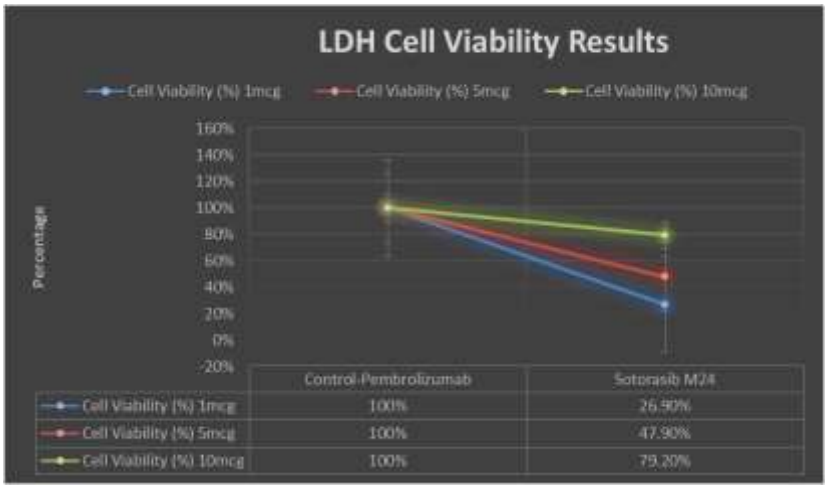




LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Pembrolizumab)	-	1.000	100%
Sotorasib M24	1	0.269	26.9%
	5	0.479	47.9%
	10	0.792	79.2%





DISCUSSION

The exploration of Sotorasib M24 as a therapeutic option for NSCLC highlights the critical need for novel approaches targeting genetic mutations, specifically KRAS, commonly associated with this cancer type. The assays employed in this study, including MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH cytotoxicity assays, revealed promising results regarding the viability of kidney cancer cells in response to treatment.

The findings indicate that Sotorasib M24 exhibits a dose-dependent cytotoxic effect, demonstrating significant cell viability reduction at higher concentrations. Compared to the control drug, Pembrolizumab, Sotorasib M24 presents an alternative strategy for targeting NSCLC at the molecular level. These results suggest that further clinical evaluation of Sotorasib M24 could lead to enhanced therapeutic options for patients with NSCLC, particularly those harboring KRAS mutations.

CONCLUSION

This study underscores the importance of developing targeted therapies for NSCLC, with Sotorasib M24 emerging as a potential candidate. The comprehensive evaluation of its efficacy alongside Pembrolizumab provides valuable insights into possible therapeutic avenues for managing this challenging disease. Future research should focus on clinical trials to further elucidate the safety and efficacy of Sotorasib M24 in NSCLC patients, paving the way for innovative treatment strategies.

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