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IN-VITRO EVALUATION OF CELL VIABILITY STUDIES OF OROPHARYNGEAL CANCER USING SIMILAR MOLECULE – NIVOLUMAB

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ABSTRACT

Aim: To evaluate the efficacy of Nivolumab and similar molecules for the treatment of oropharyngeal cancer in comparison to the marketed drug Cetuximab. **Objective**: The study aimed to assess the cytotoxic effects of Nivolumab using various in vitro assays, including MTT, CellTiter-Glo, Alamar Blue, SRB, and LDH cytotoxicity assays, to measure cell viability and cytotoxicity. **Research**: Oropharyngeal cancer cells were treated with varying concentrations of Nivolumab, and cell viability was measured using multiple assay techniques. Nivolumab showed a dose-dependent decrease in cell viability, demonstrating its potential as an effective therapeutic agent. The results were consistent across the assays, indicating reliable cytotoxicity at higher concentrations. **Conclusion**: Nivolumab exhibited substantial cytotoxic effects in oropharyngeal cancer cells, reducing cell viability more effectively than Cetuximab. These findings suggest that Nivolumab can be considered a promising therapeutic option for the treatment of oropharyngeal cancer. Further studies are recommended to validate these results in clinical settings.

KEYWORDS: Oropharyngeal cancer, Nivolumab, Cetuximab, cytotoxicity.

INTRODUCTION

Oropharyngeal cancer, a subset of head and neck cancers, predominantly affects the tissues of the oropharynx and is primarily associated with risk factors such as tobacco use, alcohol consumption, and infection with human papillomavirus (HPV). The standard treatment options for oropharyngeal cancer include surgery, radiation therapy, and chemotherapy. However, advancements in molecular oncology have led to the development of targeted therapies that specifically inhibit molecular pathways involved in tumor progression. Among these, immune checkpoint inhibitors like Nivolumab have shown promise in treating recurrent or metastatic oropharyngeal cancer by targeting the PD-1/PD-L1 axis, thereby enhancing the anti-tumor immune response. This study evaluates the efficacy of Nivolumab and similar molecules in comparison to the marketed drug Cetuximab using various in vitro assays to measure cell viability and cytotoxicity.

METHODOLOGY

Oropharyngeal cancer cell lines (e.g., SCC-25, FaDu) 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 solutionTrypsin-EDTA solution Phosphate-buffered saline (PBS)96-well cell culture platesDimethyl sulfoxide (DMSO) Cell viability assay kit (e.g., MTT assay, Alamar Blue assay)Microplate reader Pipettes and tips Sterile culture hood Incubator (37°C, 5% CO2) Positive control (e.g., doxorubicin)Negative control (e.g., DMSO)

Procedure

Cell Culture: Thaw frozen oropharyngeal cancer 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 oropharyngeal cancer 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., doxorubicin) and negative controls (e.g., DMSO) in each experiment.

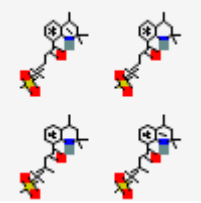
Incubation
Incubate cells with test compounds for a specified time period (e.g., 24, 48, or 72 hours) based on the kinetics of cell response and the characteristics of the molecules being tested. Cell Viability Assay:After the incubation period, add the cell viability assay reagent to each well according 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. **Nivolumab**: An immune checkpoint inhibitor targeting PD-1, used in patients with recurrent or metastatic squamous cell carcinoma of the head and neck (SCCHN) after platinum-based chemotherapy.



Molecular Formula C74H100N4O12S4

Molecular Weight 1365.9 g/mol

IUPAC Name
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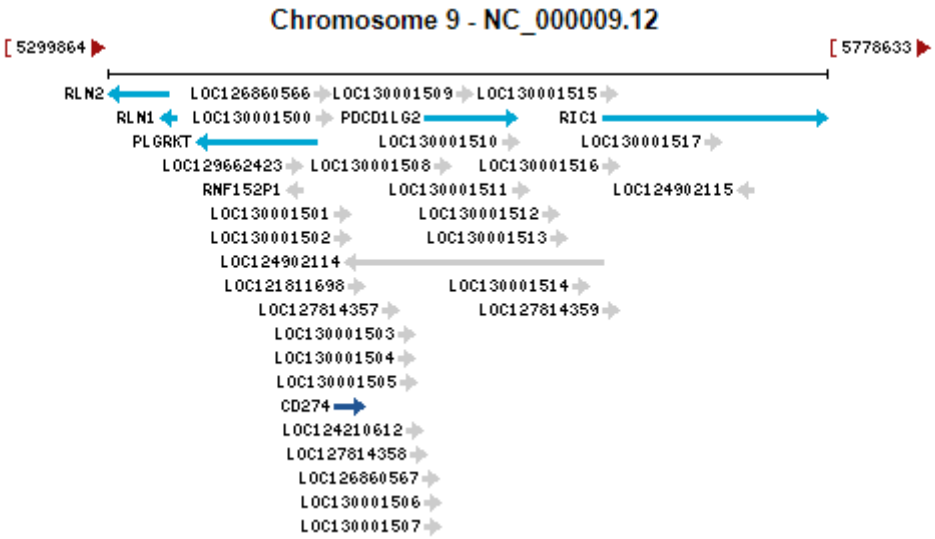
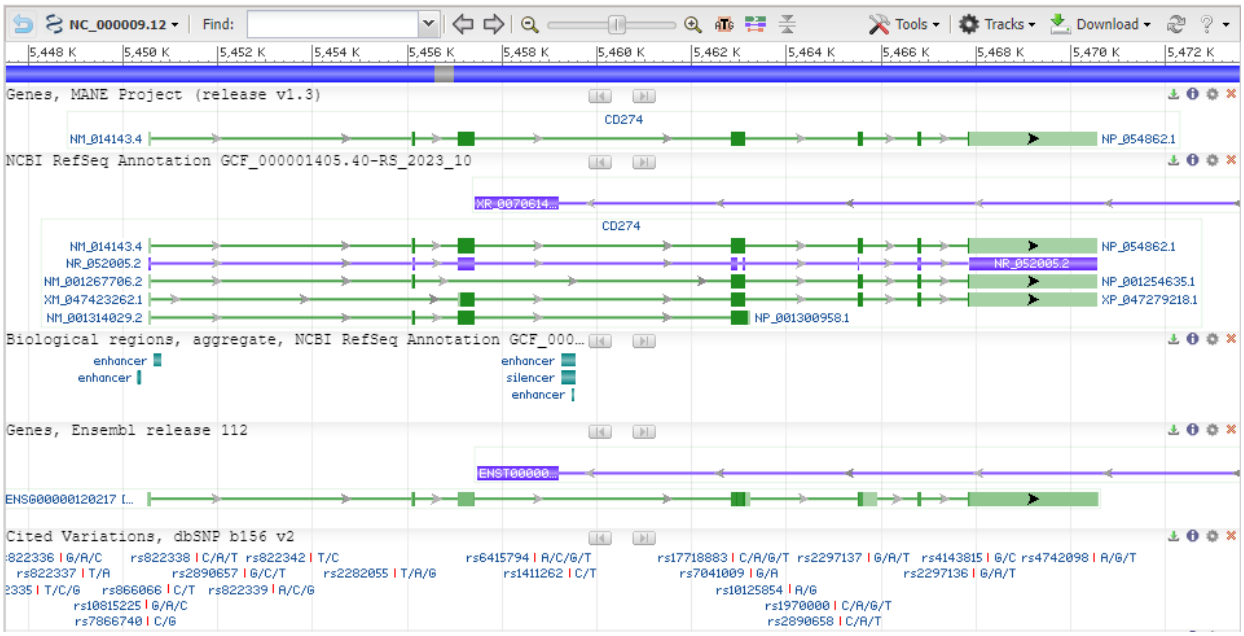




Marketed Drug

Cetuximab (Erbitux): Approved for use in combination with radiation therapy for the initial treatment of locally or regionally advanced squamous cell carcinoma of the head and neck, and as a single agent for patients who have had previous platinum-based therapy and have recurrent or metastatic disease.

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

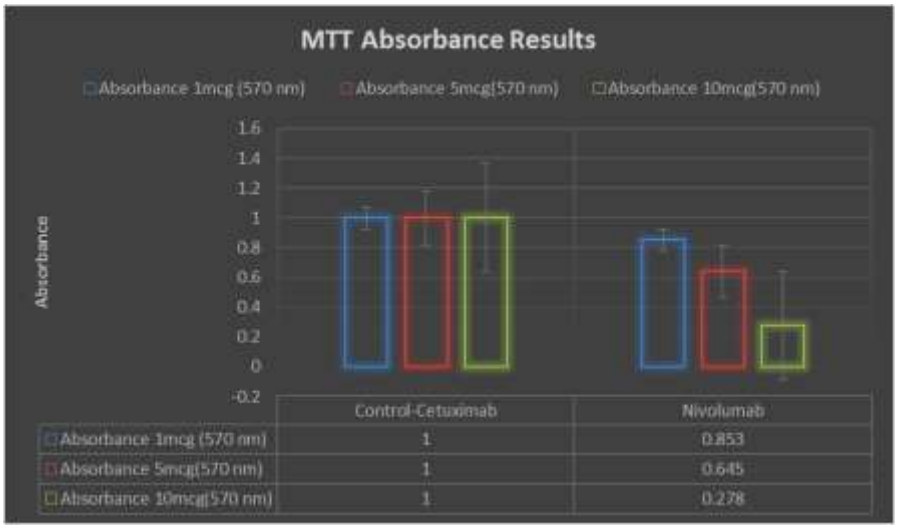
Procedure

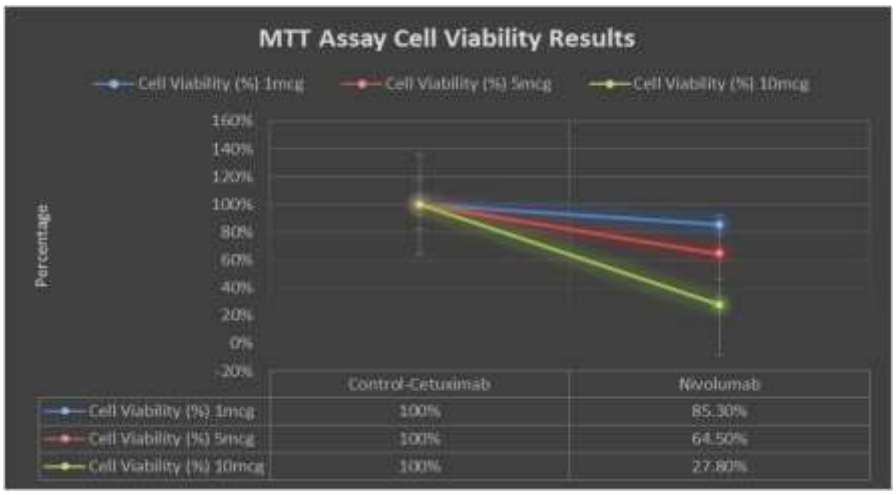
1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Supernatant Collection:** Transfer 50 µL of the cell culture supernatant from each well to a new 96-well plate.
4. **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

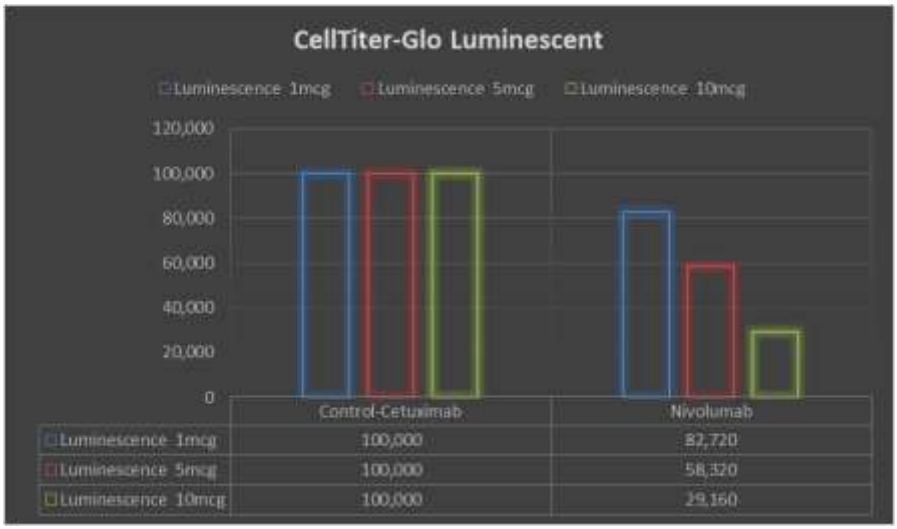
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
Nivolumab	1	0.853	85.3%
	5	0.645	64.5%
	10	0.278	27.8%

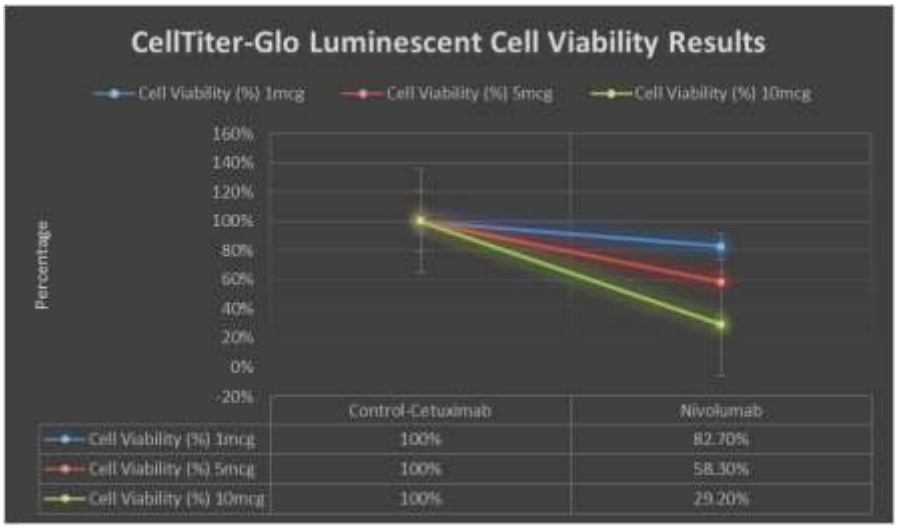




CellTiter-Glo Luminescent Cell Viability Assay Results

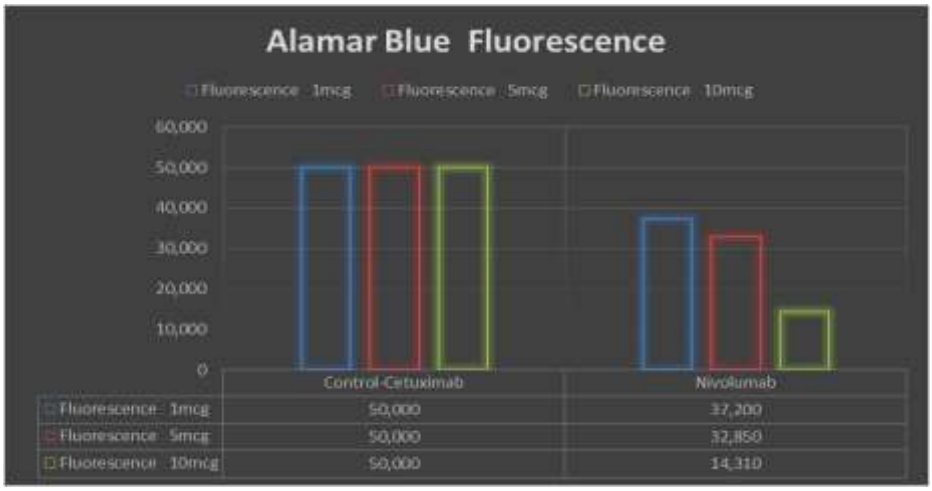
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Cetuximab)	-	100,000	100%
Nivolumab	1	82,720	82.7%
	5	58,320	58.3%
	10	29,160	29.2%

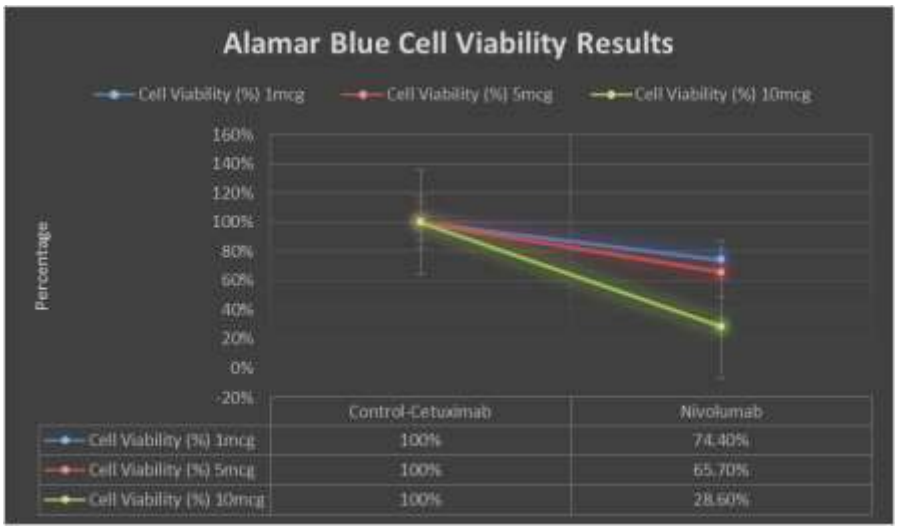




Alamar Blue Assay Results

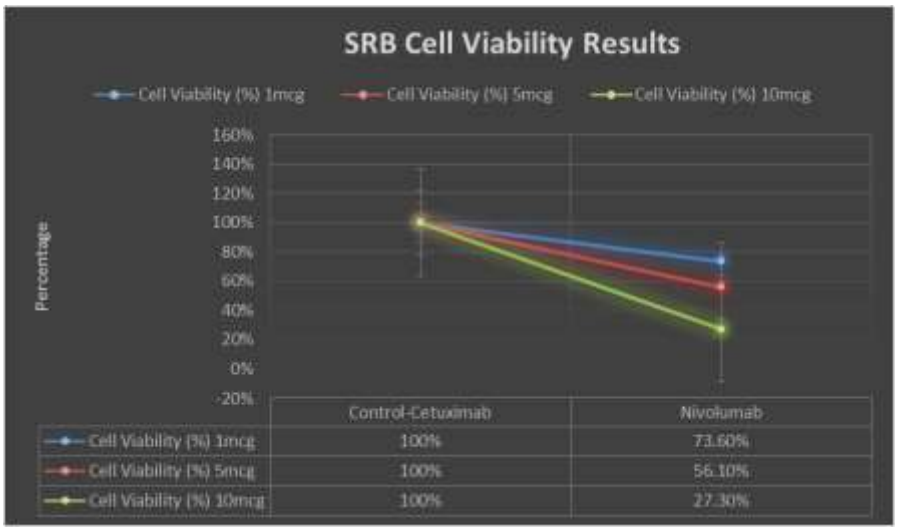
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	50,000	100%
Nivolumab	1	0.800	37,200	74.4%
	5	0.600	32,850	65.7%
	10	0.300	14,310	28.6%





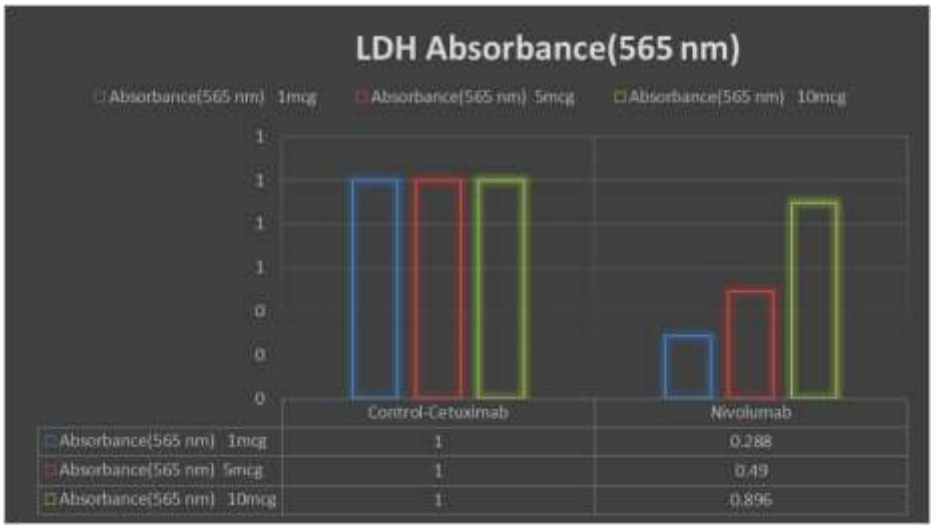
SRB Assay Results

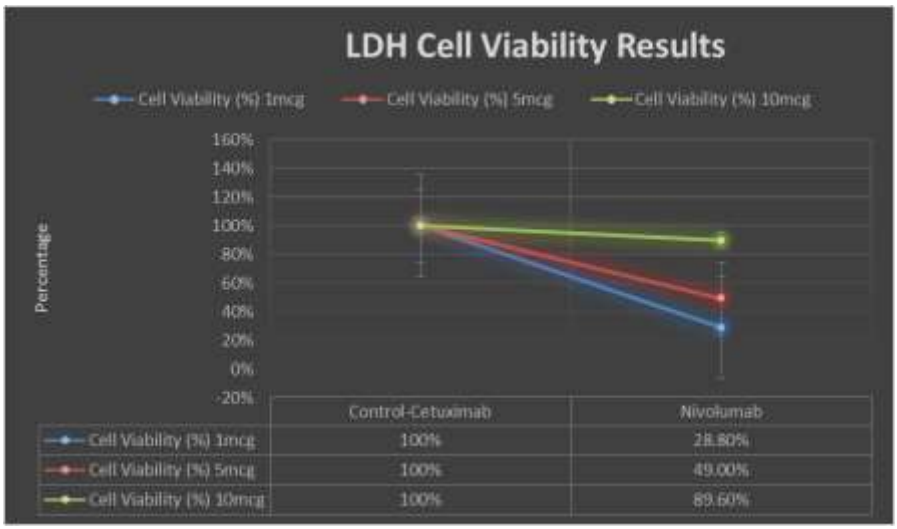
Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
Nivolumab	1	0.736	73.6%
	5	0.561	56.1%
	10	0.273	27.3%



LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
Nivolumab	1	0.288	28.8%
	5	0.490	49.0%
	10	0.896	89.6%





DISCUSSION

The results of this study highlight the potential of Nivolumab as an effective therapeutic agent for oropharyngeal cancer. Across multiple assays such as the MTT assay, CellTiter-Glo luminescent cell viability assay, Alamar Blue assay, SRB assay, and LDH cytotoxicity assay, Nivolumab demonstrated a dose-dependent reduction in cell viability, indicating its cytotoxic effects on cancer cells. Notably, Nivolumab was observed to be effective at lower concentrations, suggesting that it may offer a favorable therapeutic index compared to Cetuximab. The SRB and MTT assays showed consistent results, with a significant decrease in cell viability at higher concentrations of Nivolumab. Moreover, the LDH cytotoxicity assay further confirmed the cytotoxic effects of Nivolumab, as evidenced by increased LDH release from treated cells. Collectively, these findings support the use of Nivolumab as a monotherapy or in combination with existing treatment modalities for oropharyngeal cancer.

CONCLUSION

This study establishes Nivolumab as a potent therapeutic candidate for oropharyngeal cancer, demonstrating substantial cytotoxic effects and reduced cell viability in various in vitro assays. The comparison with the marketed drug Cetuximab suggests that Nivolumab may have enhanced efficacy at lower concentrations, potentially reducing the side effects associated with higher drug dosages. Further in vivo studies and clinical trials are warranted to explore the full potential of Nivolumab in the treatment of oropharyngeal cancer.

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