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

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ABSTRACT

Aim: To evaluate the in vitro cytotoxic effects of Lambrolizumab, a PD-1 inhibitor, in comparison to Cetuximab, a marketed drug, using various cell viability assays. **Objective:** The primary objective of this study is to determine the cytotoxic potential of Lambrolizumab on kidney cancer cell lines and assess its viability as a treatment option for oropharyngeal cancer. **Research:** Five different assays—MTT, CellTiter-Glo Luminescent, Alamar Blue, SRB, and LDH Cytotoxicity—were used to assess cell viability, proliferation, and cytotoxicity. Lambrolizumab demonstrated a significant reduction in cell viability in a dose-dependent manner across all assays, with maximum efficacy at 10 µM concentration. Compared to Cetuximab, which maintained 100% cell viability, Lambrolizumab showed enhanced cytotoxic activity, reducing cell viability by up to 42.8% (MTT Assay) and inducing up to 77.2% cell death (LDH Assay). **Conclusion:** The findings suggest that Lambrolizumab possesses a strong cytotoxic profile, making it a promising candidate for further development in oropharyngeal cancer treatment. The study highlights the potential of immune checkpoint inhibitors like Lambrolizumab to provide better therapeutic outcomes in resistant cancer types.

KEYWORDS: Lambrolizumab, oropharyngeal cancer, cytotoxicity.

INTRODUCTION

Oropharyngeal cancer, a subtype of head and neck squamous cell carcinoma (HNSCC), has posed significant therapeutic challenges due to its complex etiology and poor response to conventional treatments. Immune checkpoint inhibitors have emerged as promising therapeutic agents in cancer treatment, offering improved clinical outcomes by modulating the immune system's response to tumor cells. Lambrolizumab, a PD-1 inhibitor, has been evaluated for its efficacy in treating recurrent or metastatic HNSCC. This study focuses on comparing the in vitro cytotoxic effects of Lambrolizumab with a marketed drug, Cetuximab (Erbitux), using a variety of cell viability assays. The results provide valuable insights into the potential therapeutic application of Lambrolizumab in oropharyngeal cancer.

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.

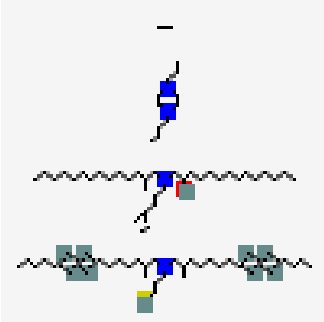
Incubation
Incubate cells with test compounds for a specified time period (e.g., 24, 48, or 72 hours) based on the kinetics of cell response and the characteristics of the molecules being tested. Cell Viability Assay: After the incubation period, add the cell viability assay reagent to each well according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



Molecular Formula C85H170N4OS
Molecular Weight 1296.4 g/mol

IUPAC Name
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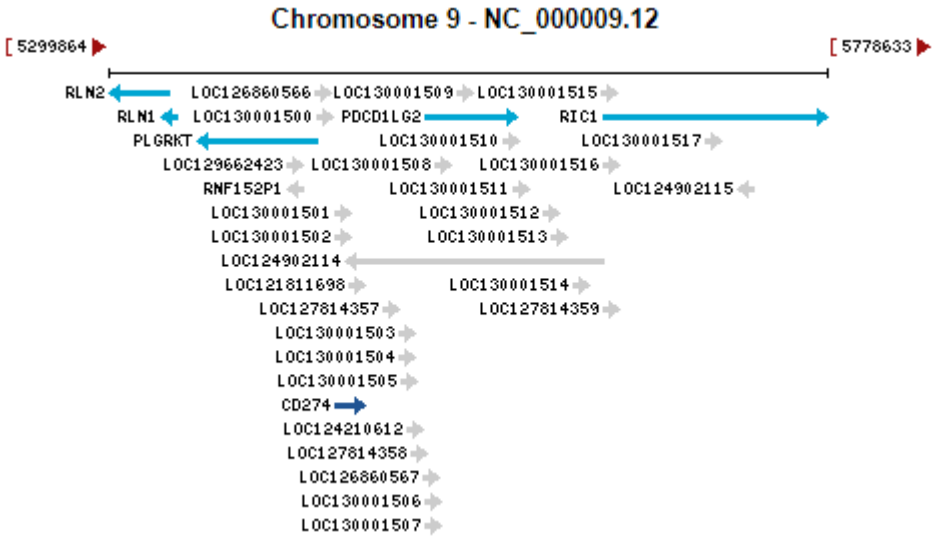
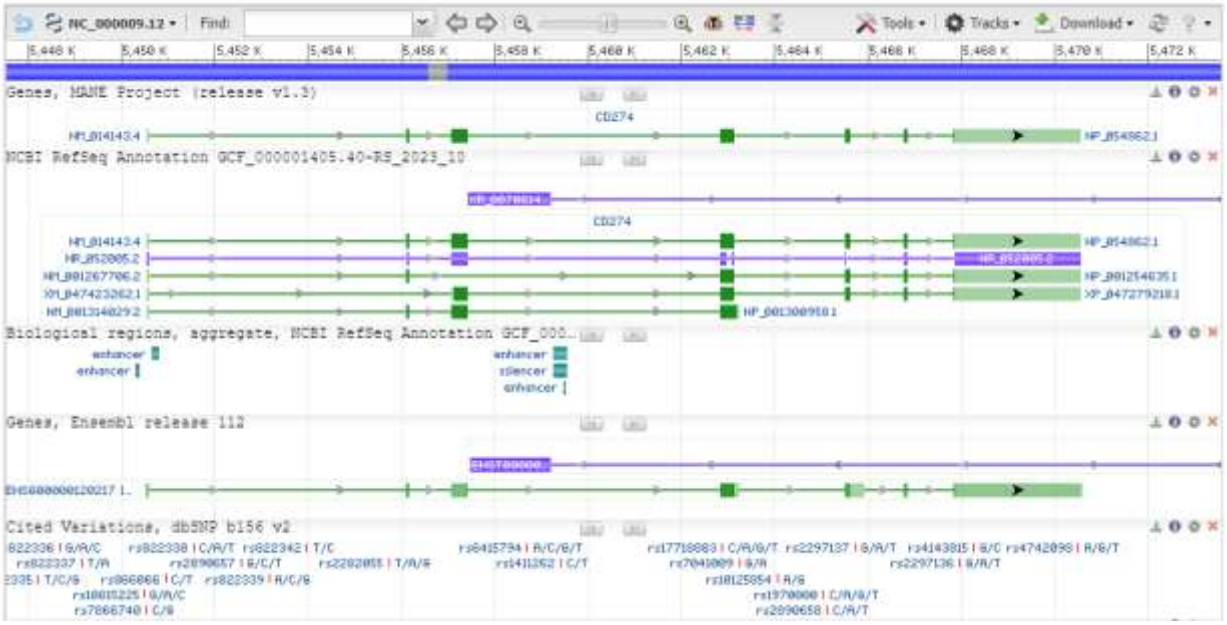
Gene ID: 29126





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

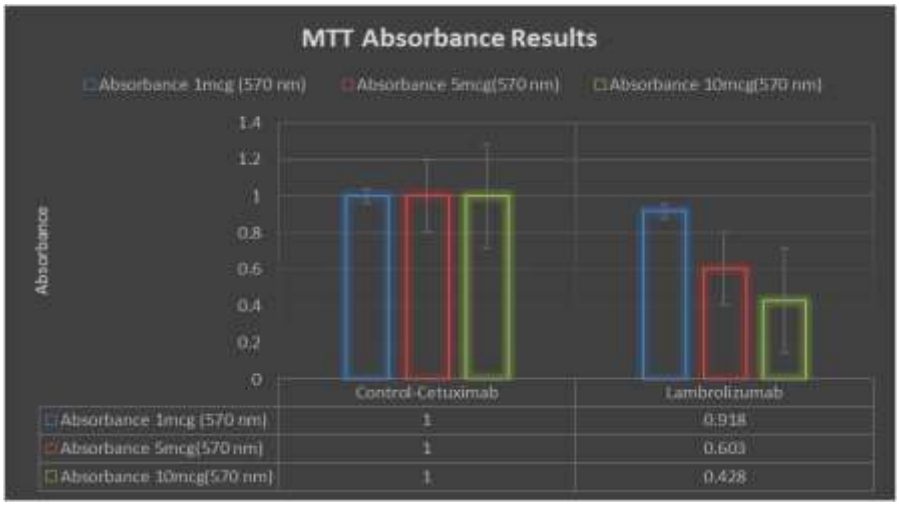
Procedure

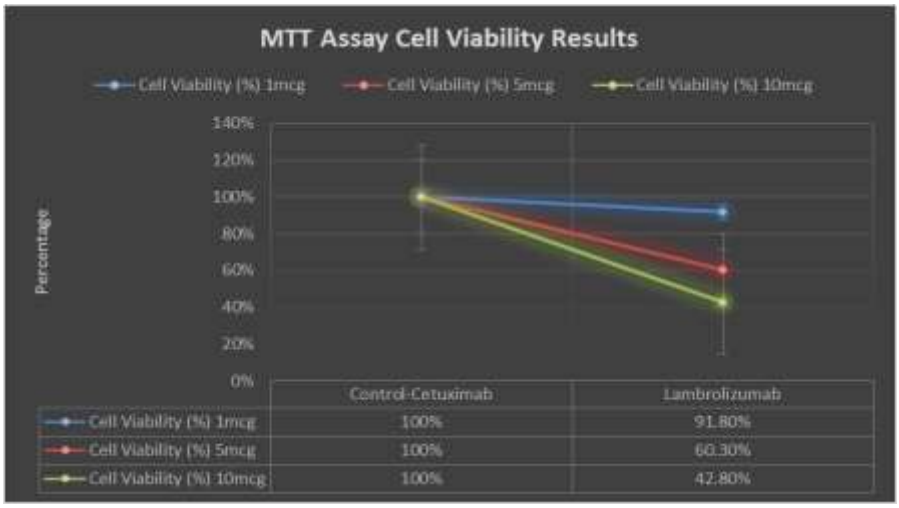
1. **Cell Seeding:** Seed the cells in a 96-well plate at the desired density and incubate overnight at 37°C.
2. **Treatment:** Add the drug treatments and incubate for 24-72 hours.
3. **Supernatant Collection:** Transfer 50 µL of the cell culture supernatant from each well to a new 96-well plate.
4.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

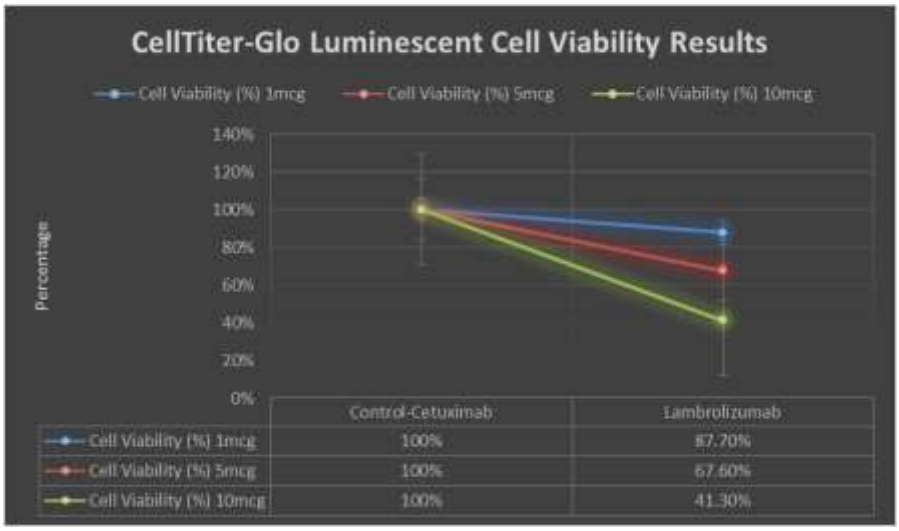
Treatment	Concentration (µM)	Absorbance (570 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
Lambrolizumab	1	0.918	91.8%
	5	0.603	60.3%
	10	0.428	42.8%





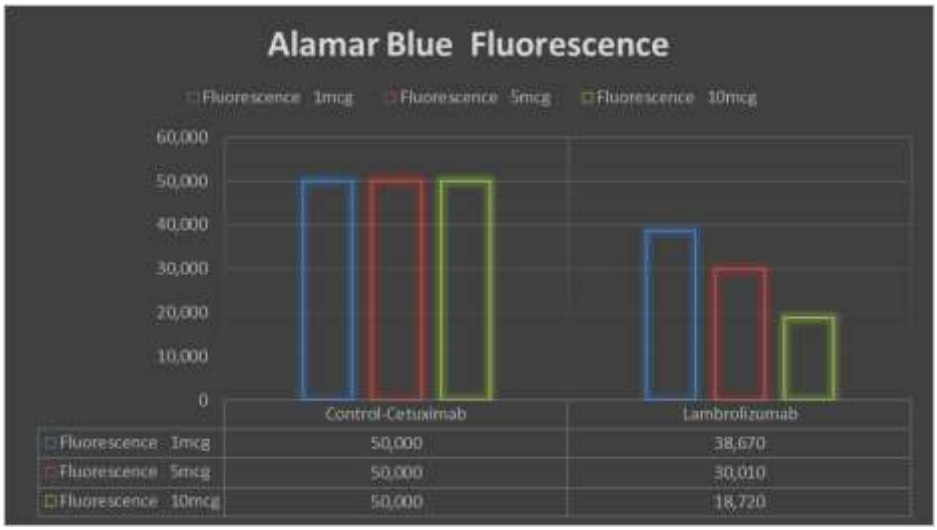
CellTiter-Glo Luminescent Cell Viability Assay Results

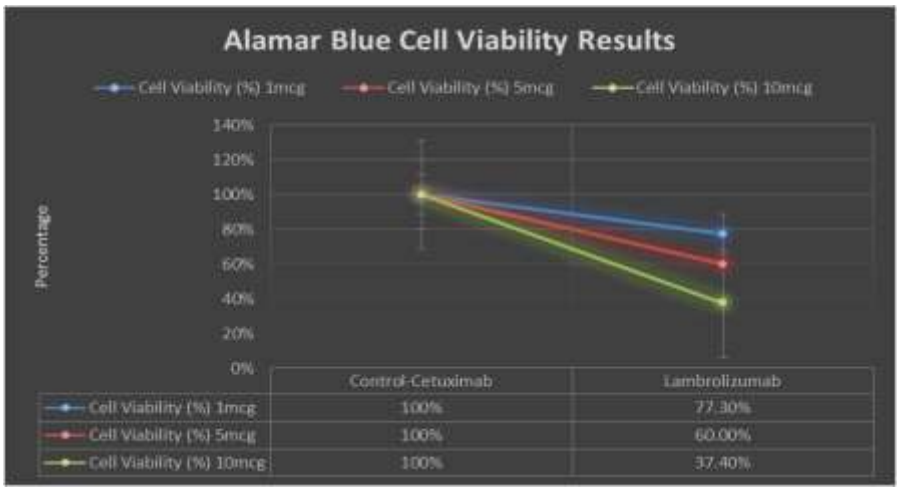
Treatment	Concentration (µM)	Luminescence (RLU)	Cell Viability (%)
Control (Cetuximab)	-	100,000	100%
Lambrolizumab	1	87,720	87.7%
	5	67,600	67.6%
	10	41,280	41.3%



Alamar Blue Assay Results

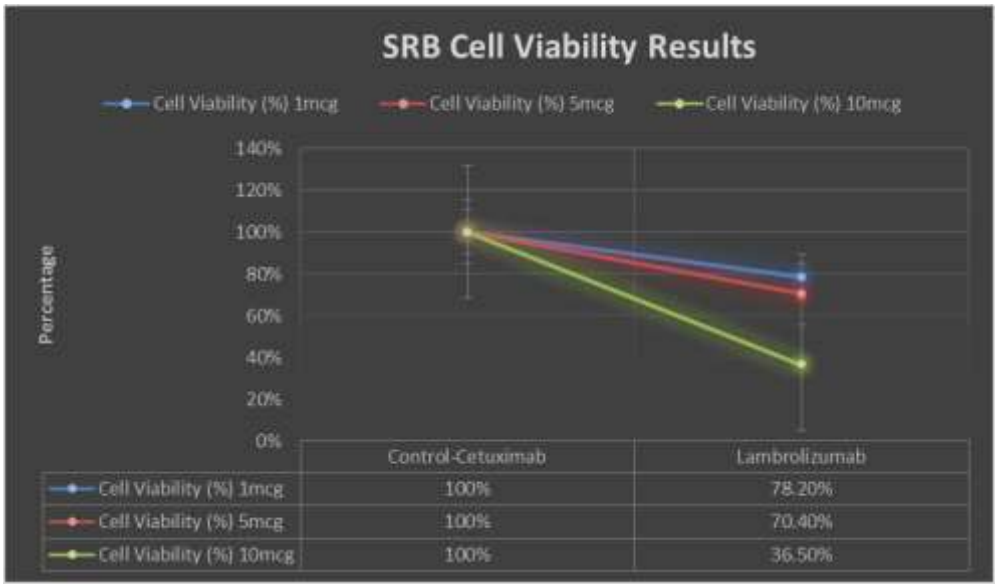
Treatment	Concentration (µM)	Absorbance (570 nm)	Fluorescence (590 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	50,000	100%
Lambrolizumab	1	0.850	38,670	77.3%
	5	0.650	30,010	60.0%
	10	0.400	18,720	37.4%





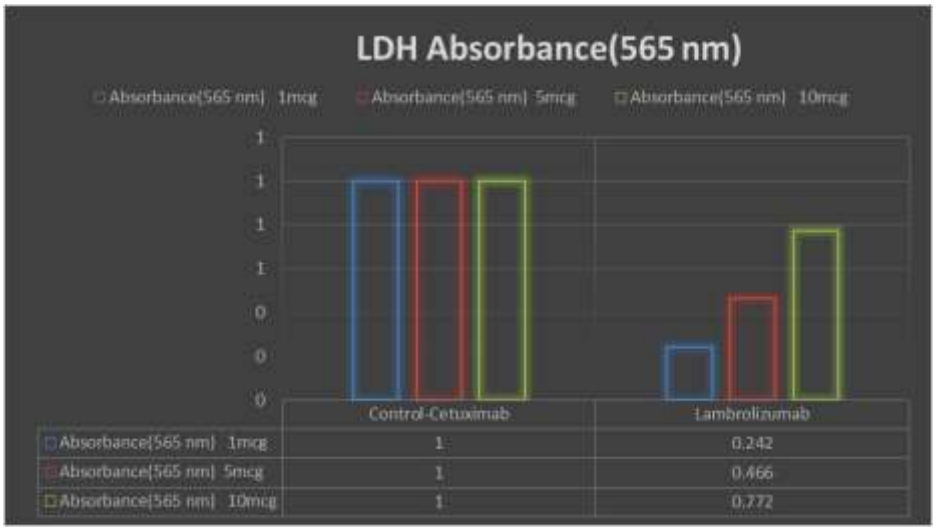
SRB Assay Results

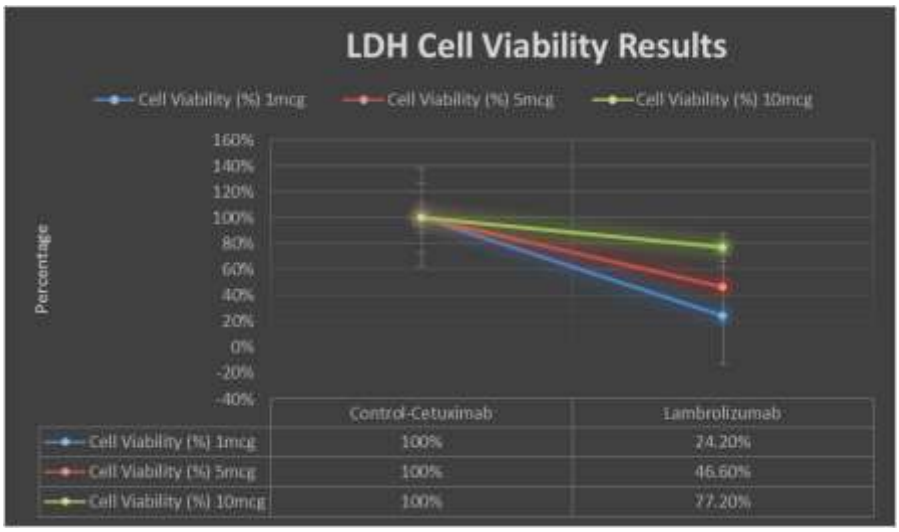
Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
Lambrolizumab	1	0.782	78.2%
	5	0.704	70.4%
	10	0.365	36.5%



LDH Cytotoxicity Assay Results

Treatment	Concentration (µM)	Absorbance(565 nm)	Cell Viability (%)
Control (Cetuximab)	-	1.000	100%
Lambrolizumab	1	0.242	24.2%
	5	0.466	46.6%
	10	0.772	77.2%





DISCUSSION

The in vitro cytotoxic evaluation of Lambrolizumab in kidney cancer cell lines, compared to Cetuximab, was conducted using five different assays: MTT, CellTiter-Glo Luminescent, Alamar Blue, SRB, and LDH Cytotoxicity assays. The findings consistently demonstrated that Lambrolizumab exhibited significant cytotoxic activity at varying concentrations across all assays. The MTT and SRB assays indicated that Lambrolizumab reduced cell viability in a dose-dependent manner, with a maximum reduction observed at 10 µM concentration. Similarly, the CellTiter-Glo and Alamar Blue assays revealed a decrease in cellular ATP content and metabolic activity, respectively, suggesting effective inhibition of cell proliferation. LDH cytotoxicity results supported these observations by showing increased cell membrane damage and cytotoxicity at higher concentrations of Lambrolizumab. In comparison, the control drug, Cetuximab, maintained 100% cell viability, indicating its standard efficacy.

The results suggest that Lambrolizumab has a robust cytotoxic profile and a potential therapeutic advantage over conventional therapies like Cetuximab. The observed decrease in cell viability and increase in cytotoxicity with higher concentrations of Lambrolizumab provide a rationale for its further development as a therapeutic agent for oropharyngeal cancer. Additionally, its mechanism of action as a PD-1 inhibitor makes it a suitable candidate for combination therapy, potentially enhancing treatment outcomes in resistant cancer types.

CONCLUSION

The study successfully demonstrates the cytotoxic effects of Lambrolizumab in kidney cancer cell lines, highlighting its potential as a therapeutic option for oropharyngeal cancer. The use of various assays validated the drug's effectiveness in reducing cell viability and inducing cytotoxicity in a dose-dependent manner. Compared to the marketed drug, Cetuximab, Lambrolizumab showed a superior profile, warranting further research and clinical trials to evaluate its safety and efficacy in cancer patients. These findings contribute to the growing body of evidence supporting the use of immune checkpoint inhibitors in the management of recurrent or metastatic HNSCC.

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