FULL LENGTH | Iranian Biomedical Journal 29 (1 & 2): 9-19 January & March 2025

Combination Therapy of Oncolytic Newcastle Virus and Lenalidomide Enhanced Cytotoxicity in Prostate Cancer Cells

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OPEN ACCESS

Article type: Research Article
Received: September 29, 2024
Revised: December 10, 2024
Accepted: December 10, 2024
Published online: December 18, 2024

How to cite:
Jafari M, Abdoli S, Asgari M, Moghaddam Pour M, Shokrgozar MA, Sharifzadeh Z. Combination Therapy of Oncolytic Newcastle Virus and Lenalidomide Enhanced Cytotoxicity in Prostate Cancer Cells. *Iran. Biomed. J.* 2025; 29(1&2): 9-19.

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ABSTRACT

Background: Despite existing treatments, advanced solid tumors, such as PCa, require the development of novel anticancer therapies. OVs present a potential treatment option for solid tumors. NDV is one of the most promising OVs that can replicate within and destroys human cancer cells. This study aimed to evaluate the cytotoxic and apoptotic effects of the NDV strain on human PCa cells in vitro. Additionally, We examined a novel treatment for PCa by combining Len with NDV.

Methods: NDV strains La Sota, B1, and I2 were tested for cytotoxicity against several cell lines. A safety assessment was conducted in primary cells using PBMCs. Also, apoptosis induction was measured using annexin V/7AAD staining. Finally, the cytotoxic effects of NDV alone and in combination with Len, were assessed using MTT.

Results: The NDV showed cytotoxic effects on tumor cell lines and induced apoptosis in infected prostate cells compared to control cells. The NDV La Sota strain exhibited significant oncolytic capacity, reducing the viability of LNCaP and DU145 cells to less than 40% at specific concentrations, while showing no cytotoxic effects on primary PBMCs. Also, NDV induced apoptosis in the prostate cell line by 60%. Furthermore, Len enhanced the cytotoxicity of PCa cells when combined with NDV.

Conclusion: Our study confirms the efficacy of oncolytic NDV treatment for PCa, particularly utilizing the La Sota strain. When combined with Len, NDV indicates an enhanced effectiveness in destroying tumor cells. These findings suggest a prospective treatment approach that needs more preclinical and clinical studies to improve outcomes in PCa treatment.

DOI: 10.61882/ibj.4367

Keywords: Lenalidomide, Prostate cancer, Oncolytic Newcastle virus

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List of Abbreviations:
DMEM: Dulbecco's Modified Eagles Medium; **FBS:** fatal bovine serum; **FITC:** flow cytometry-based fluorescein isothiocyanate; **HA:** Hemagglutination; **HAU:** Hemagglutination unit; **HRPC:** hormone-resistant prostate cancer; **Len:** Lenalidomide; **MTT:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; **MYXV:** Myxoma virus; **NDV:** Newcastle disease virus; **OV:** oncolytic virus; **PBMC:** peripheral blood mononuclear cell; **PBS:** phosphate buffer saline; **PCa:** prostate cancer; **PSA:** prostate-specific antigen; **RBC:** red blood cell; **RPMI:** Roswell Park Memorial Institute 1640 Medium; **$TCID_{50}$:** tissue culture infectious dose 50

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INTRODUCTION

Prostate cancer is the leading cause of cancer-related death among men worldwide[1]. Therapies for PCa include radiation, chemotherapy, hormone therapy, and surgery. However, these treatments are often associated with serious adverse effects[2]. Therefore, it is imperative to develop novel therapeutic options that are both well-tolerated and effective. Immunotherapy has emerged as a promising new approach in the fight against various types of cancers[3,4]. One innovative area of immunotherapy in cancer treatment involves OVs. While these viruses can target, replicate within, and destroy various cancerous cells—whether they occur naturally or have been genetically modified—they cannot kill normal cells[5-8]. OVs have the potential to alter the paradigm in the solid tumor microenvironment and transform cold tumors into hot ones through a variety of mechanisms[9]. They also offer a significant opportunity for patients with PCa, particularly when combined with other therapies[10,11].

NDV is a member of the *Paramyxoviridae* family and causes serious illness in poultry and wild birds worldwide. Its safety for humans makes NDV a promising viro-therapeutic agent for treating human malignancies[12]. PSA-retargeted NDV effectively eliminated prostasphere tumor models, demonstrating its potential for OCa treatment[13]. Many solid tumors, including PC-3, epidermoid carcinoma, colon cancer, large cell lung carcinoma, breast carcinoma, and other xenografts, have shown significant regression when treated intratumorally with strain 73-T[14]. Oncolytic NDV induces the expression of immunogenic cell death in PCa cells[15]. Although OVs are efficacious treatments, their efficacy remains limited when administered as monotherapy. Hence, it is necessary to combine OV therapy with other cancer treatment methods[16]. Combining NDV and vanadyl sulfate enhances the innate immune response, accelerating tumor clearance[17].

A novel class of drugs known as immunomodulatory agents (IMiDs®), such as Len (REVLIMIDTM®), has several impacts on the human immune system and the tumor microenvironment[18,19]. The FDA has approved Len, an oral immunomodulator, for treating myelodysplastic syndromes and multiple myeloma. Patients with metastatic PCa have demonstrated therapeutic effectiveness and clinical immunoregulatory effects when treated with both Len and metronomic cyclophosphamide[20,21]. At present, the safety and efficacy of Len have not been fully established. However, Len may be beneficial for certain patients with castration-resistant PCa. The stability of the disease over time is maintained by administrating cyclooxygenase-2 inhibitors, along with thalidomide or Len, which are immunomodulatory drugs[22,23]. Preclinical and clinical studies suggest an immunomodulatory role for Len in HRPC. HRPC occurs when PCa becomes unresponsive to hormone treatment, which highlights the urgent need for more efficient therapies. Preclinical and clinical data support the role of the immunomodulatory drug Len in HRPC[11]. Treatment of metastatic PCa with Len and thalidomide has yielded varying results. In patients with metastatic PCa, Len has been indicated to increase cholesterol production and cell cycle progression[24].

In this study, we aimed to investigate the sensitivity of PCa cells to NDV and optimize its dosage, ensuring that NDV strains can be safely used as an anticancer treatment. We also demonstrated the cytotoxicity and apoptotic effects of La Sota strain infection on PCa cell lines compared to primary PBMCs. Our second objective was to explore whether combining the La Sota strain with Len could enhance its cytotoxicity in LNCaP cells.

MATERIALS AND METHODS

Cell lines and reagents

All cell lines used in this study were purchased from the National Cell Bank of Pasteur Institute of Iran (Tehran). Vero (kidney epithelial cells), LNCaP (prostate-specific membrane antigen-positive human PCa), DU145 (prostate-specific membrane antigen-negative human PCa), HeLa (human epidermoid cervical carcinoma cells), HEK-T293 (human embryonic kidney 293 cells), and human dermal fibroblast were cultured in high-glucose DMEM (Biosera, France) supplemented with 10% FBS (Biosera), penicillin (100 IU/mL), and streptomycin (100 µg/mL). Jurkat cell line (T lymphoblast) was cultured in RPMI 1640 medium (Biosera), 10% FBS, and antibiotic. All cell lines were cultured in 95% humidity and 5% CO_2 at 37 °C. Human PBMCs were selected as the control. Len (LENASOB™ 10 mg capsule), was purchased from Sobhan Darou Co. (Iran), dissolved in 10 mM DMSO and stored at -80 °C until use.

PBMC isolation

To assess the safety of NDV for non-transformed cells, PBMCs were selected as control normal cells. PBMCs were isolated from the blood of conditionally healthy donors using sedimentation in a Ficoll (Biosera) density gradient. The cells were concentrated by centrifugation at 290 ×g for 5 minutes. PBMC cells were cultured in RPMI medium containing 10% FBS and 1% antibiotic at 37 °C and 5% CO_2.

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NDV strains

In this study, we used three NDV strains: La Sota, B1 Hitchner, and I_2, donated by the Razi Vaccine and Serum Research Institute (Tehran, Iran). The viral strains were propagated in the 10-day-old specific pathogen-free embryonated chicken eggs. Each egg was given 10^9 EID_{50} (50% egg infectious dose) per 0.1 mL of inoculum. The eggs were incubated at 55% humidity and 37 ℃ for five days before being incubated at 4 ℃ overnight. Twenty-four hours post-inoculation, dead embryos were removed. After three days, all living embryos were inspected, and their chorioallantoic fluids were collected and tested for Haemagglutination assay (HA with 10% chicken RBCs). Then, the allantoic fluid was centrifuged at 3000 ×g for 10 minutes to remove debris. The viruses were then kept in aliquots at a temperature of -80 ℃.

HA assay

In a round-bottomed 96-well plate, 50 µl of PBS was added to each well, and 50 µl of the test sample was added to the first well. Two-fold dilutions of sample suspension was made across the plate, and then 50 µl of 1% chicken RBCs was added to test and PBS-only wells (RBC controls). The plates were incubated at room temperature for about 30 minutes until the positive antigen control wells agglutinated and the RBC control wells participated. By titling the plate and looking for tear-shaped streaming of the RBCs, the HA was determined.

Titration of NDV on Vero cell line

The Vero cell line was used to evaluate the cytotoxicity of the virus titer in mammalian cells. To figure out the median $TCID_{50}$, NDV titration was performed triplicate on Vero cells (3×10^4 cells/cm²) in 96-well plates. The next day, the cell monolayer was washed with PBS, and 10-fold viral dilutions were added to each well. The dilutions were prepared using DMEM maintenance medium without FBS. After incubating at 37 ℃ for 1 h to allow the virus to adsorb to the cell, and shaking every 15 min, the plates were replaced with a medium with 2% FBS. Each day, the cell monolayer was inspected under an inverted microscope to detect the presence of cytotoxic effect. The final titration was carried out on day seven. The infectious titers of the virus on the Vero cell line were calculated according to the Cerberus method in the Ashmarin's modification and presented as $TCID_{50}$/ml (1 HAU = 10^4-10^5 $TCID_{50}$)[25].

Cytotoxicity assay

The MTT test was used to assess the cytotoxicity of human tumor cell lines 72 hours after the administration of the viral strains. The triplicate overnight-cultured tumor monolayer cells (3×10^4 cells) in a 96-well plate was washed with PBS and treated with viruses (titer 2, 4, and 8 HAU per 10,000 cells). The dilutions were prepared using DMEM maintenance medium without FBS. The plates were incubated at 37 ℃ for 1 h to allow for virus adsorption while shaking every 15 min. The medium was replaced with fresh medium containing 2% FBS. Control tumor cells were incubated in the same condition without the virus. MTT solution was prepared in PBS at 5 mg/ml concentration. The cells were then treated with MTT solution in 5% CO_2 at 37 ℃ for 4 hours and lysed in DMSO (150 µl/well) in a dark place at room temperature for one hour. A wavelength of 570 nm was used to measure the optical density. Comparison of the infected cell survival to 100% viable uninfected control cells indicates cell viability. To examine the safety of the virus for PBMC, cells were concentrated by centrifugation at 1500 ×g for 5 minutes, then the supernatant was removed, and the cells were resuspended in new media. The cells were subsequently seeded on 96-well plates at a density of 1×10^4 cells per well with complete RPMI media and finally incubated with 5% CO_2 at 37 ℃ overnight. Similar to previous steps, the MTT assay was performed on these cells to evaluate their viability.

Evaluation of apoptosis in NDV-infected prostate tumor cells

To demonstrate if NDV administration induced apoptosis in LNCaP and DU145 cells, FITC-conjugated annexin V and 7AAD (Biolegend, CA, USA) was employed following the manufacturer's procedure. In summary, LNCaP and DU145 cells were seeded at 1×10^5 cells/ml in each six-well cell culture plate. After incubation for 24 hours, the DU145 and LNCaP cells were infected with La Sota at a titer of 4 and 8 HAU. Uninfected cells were used as control. After 72 hours of infection, the harvested cells were washed with PBS and resuspended in an annexin V binding buffer. FITC-conjugated annexin V and 7AAD were added to cell suspensions. After 15 minutes of incubation in the dark, the cells were analyzed using flow cytometry (Partec PAS-III). All data were analyzed by FlowJo software v. 7.0.

Cytotoxic evaluation of Lenalidomide and NDV combination

Four different groups were used to assess the impact of NDV and Len combination on tumor cells (Table 1). To do it, MTT assay was performed. Subsequently, 100 µL of 3×10^4 cells were added to each well of 96-well microplates. PBS was then added to the control cells, and the cells were treated with 8 HAU of NDV and 1 µM of Len. Afterwards, cell absorption was measured using a spectrophotometer at 570 nm wavelengths at 48 h and 72 hours after treatments.

Table 1. Experimental groups

No.	Groups	Treatment
1	NDV	8 HAU of NDV
2	Len	1 µM of Lenalidomide
3	Len&NDV	1 µM of Lenalidomide and 8 HAU of NDV
4	Len + NDV	Calculated total cytotoxicity for 1 µM of Lenalidomide and 8 HAU of NDV

Statistical analysis

The analysis of the data was performed using GraphPad Prism software v.8.0. The statistical analysis of results was examined using repeated measures of two-way ANOVA followed by Tukey's multiple comparisons test where appropriate. The viability of the tumor cell line was evaluated by comparing the average relative percentage of living cells post-treatment with the virus to the control cells that were not treated.

RESULTS

NDV strain preparation

The chorioallantoic fluid of the embryonated chicken eggs was collected after incubation (Fig. 1). The HA assay was carried out after collecting and clarifying chorioallantoic fluids to confirm the propagation of NDV. The HA assay and Vero cell titration demonstrated the effective titer of the NDV strains (Table 2).

Profile of susceptibility to NDV infection within different cell lines

To compare the viability of different NDV strains on different cells, we conducted cytotoxicity assays at three titers of NDV strains on different cell lines (Table 3). The cell lines were selected to represent a wide range of tissue types and demonstrate the cytotoxicity rates resulting from infection with NDV strains. Titers of 2, 4, and 8 HAU were selected for each strain, and cytotoxicity assays on the cell lines were then assessed by MTT after 72 h. The results showed that the cytotoxicity of NDV on LNCaP, DU145, and HeLa cell lines was greater than HEK and human dermal fibroblast cell lines; therefore, we suggest that tumor

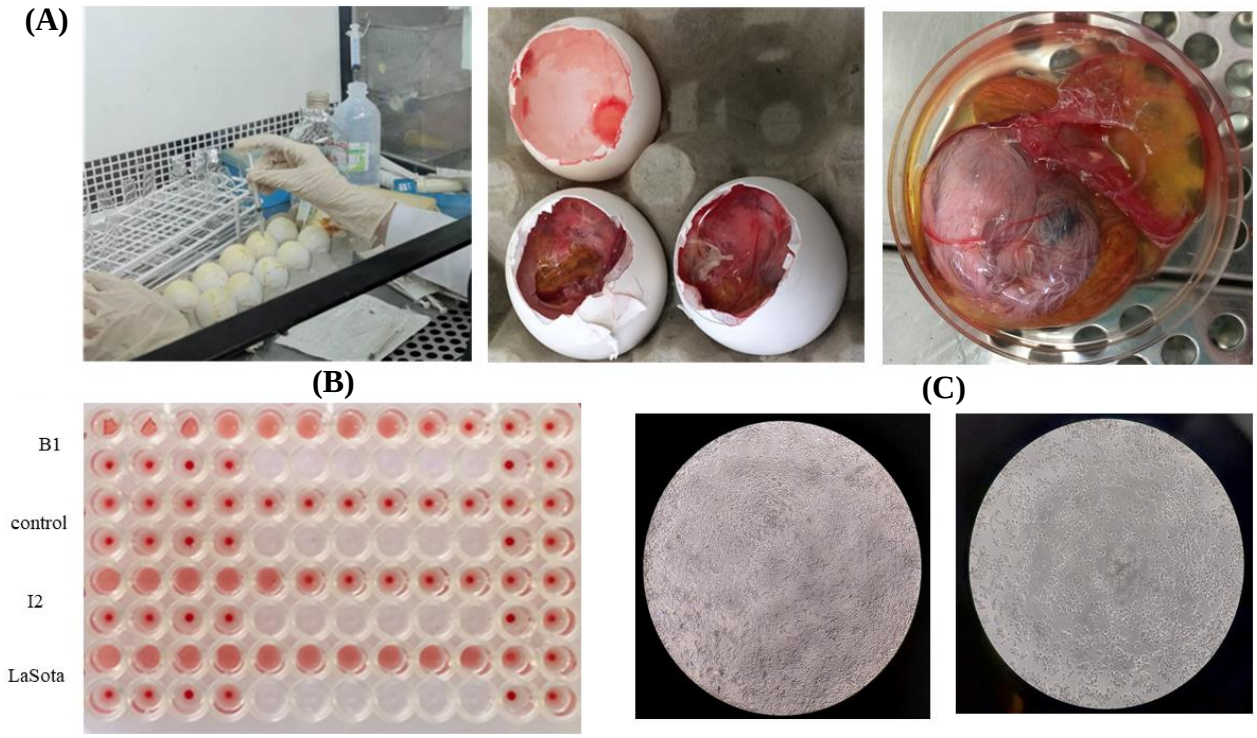

Fig. 1. Virus production and titration. (A) Each egg was inoculated with 0.2 ml of the virus by using a needle into the chorioallantoic cavity. (B) HA test: negative control (without virus); RBCs has a distinct pellet at the bottom of wells. Positive control (allantoic fluid + NDV): RBCs has a diffuse lattice structure. (C) The cytotoxic impact of La Sota on Vero cells; left and right pictures show control Vero cells and La Sota-treated Vero cells, respectively.

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Table 2. Titers of NDV strains

Strain	HA assay	logTCID$_{50}$/ml
La Sota	1024	6.2
B1	256	5.2
I2	512	6.5

cells are more susceptible to NDV infection than primary cells, as expected. PBMC showed the highest viability at 72 h; thus, the viral strains were safe for human cells (Fig. 2). Our data revealed that the studied strains had a significant cytotoxicity on different cell lines compared to primary cells. All three strains showed a significant difference in capability to kill various cancer cells. The cytotoxic properties of La Sota were investigated in lower titers on PCa cell lines. We evaluated different dilutions of the La Sota NDV on LNCaP and DU145 cells within the 2 to 256 HAU/10,000 cells. Our findings demonstrated a decreased NDV cytotoxicity with increasing dilutions of HAU. At infection with doses below 8 HAU per 10,000 cells, the NVD-infected PCa cells showed a significantly higher cytotoxicity than PBMC cells. There was also a significantly higher proportion of cytotoxicity in the LNcaP cells infected with La Sota at an infection with doses of 8, 4, and 2 HAU per 10,000 cells compared to the DU145 cells infected with La Sota at a dose of 2 HAU per 10,000 cells. Given their noticeable difference from the control cells, we chose the lowest titer for each cell line, at which the viability of the LNCaP and DU145 cells decreased to less than 40% after treatment (Fig. 3).

Apoptosis of NDV-infected PCa cells

Cell apoptosis was measured using Annexin V-FITC/7AAD apoptosis detection kit (Biolegend). The results demonstrated the apoptosis induction in PCa and PBMC cells after 72 h post-NDV infection. The number of tumor cells significantly decreased compared to that of PBMCs. The NDV-treated LNCaP and DU145 cell lines showed a 2.5-fold increase in percentage of apoptotic cells compared to PBMCs. The induction of cell death confirmed that the NDV virus can kill cancer cells in about 60% of PCa lines. It also showed that the La Sota strain can cause apoptosis in the tumor cell line. About 90% of PBMC cells remained viable after La Sota infection, indicating that NDV could not induce apoptosis similar to PCa cell lines. Collectively, our data revealed that NDV is safe for primary cells (Fig. 4).

Cytotoxic effect of La Sota alone and its combination with Len on LNCaP cell line

Using the MTT assay, we evaluated the impact of the Len and NDV combination on the viability of LNCaP cells. The results showed that La Sota dramatically affected the cytotoxicity of LNCaP cells at 8 HAU titers. We combined Len with La Sota 8 HAU and tested on LNCaP cells. Cell cytotoxicity increased in the combination group as compared to NDV aloneIndeed, the observed increase in cytotoxicity at 72 h after NDV treatment with Len suggests that this combination can be considered as a viable option for a therapeutic approach (Fig. 5).

DISCUSSION

PCa is the second most common cause of cancer-related death among men[8,26]. Current treatment methods for PCa primarily focuses on alleviating symptoms and offer slightly lower survival rates. However, the emergence of innovative approaches, such as oncolytic virotherapy, has created a unique strategy for treating PCa[27].

Comparative cytotoxicity tests of La Sota, B1, and I2 strains of NDV revealed that the La Sota strain had the highest efficacy in decreasing cell viability. This finding is supported by a recent study, indicating that the enhanced effectiveness of the La Sota strain is due to its stronger affinity for tumor cells[28]. Previous research has shown that wild-type NDV strains selectively target and kill tumor cells without affecting healthy PBMCs. Furthermore, the La Sota strain is considered a safe anticancer agent that warrants further investigation of NDV susceptibility[29,30]. It has been disclosed that NDV strains have the potential to act as highly effective

Table 3. Cell culture and growth conditions

Cell name	Tissue	Growth medium (+ 10% FBS)	Culture condition
LNCaP	Prostate adenocarcinoma cells	DMEM	Adherent, 37 °C, 5% CO_2
DU145	Prostate cancer cell	DMEM	Adherent, 37 °C, 5% CO_2
HeLa	Epidermoid cervical carcinoma	DMEM	Adherent, 37 °C, 5% CO_2
HEK-T293	Human embryonic kidney	DMEM	Adherent, 37 °C, 5% CO_2
HDF	Human dermal fibroblast	DMEM	Adherent, 37 °C, 5% CO_2
Jurkat	T lymphoblast	RPMI	Suspension, 37 °C, 5% CO_2
PBMC	PBMCs	RPMI	Suspension, 37 °C, 5% CO_2

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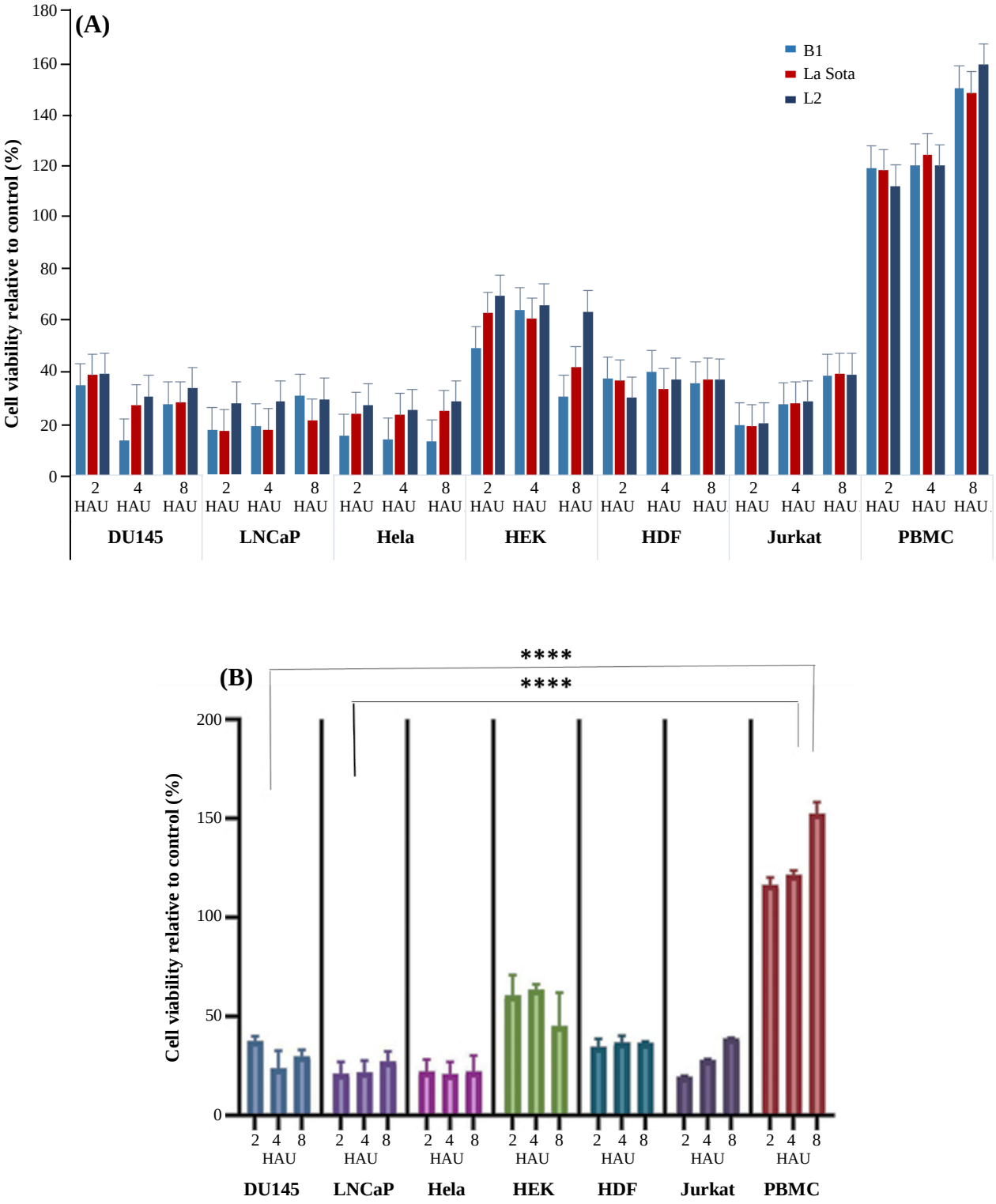

Fig. 2. In vitro oncolytic effects of NDV strains on human tumor cell lines and PBMC cells. (A) Cytotoxicity of NDV 72 hours after infection. The viability percentage was calculated by comparing treated with uninfected cells (not shown); untreated cells were considered 100% viable. (B) The cytotoxicity of La Sota on different cells. Data were analyzed from experiments in triplicate. ****significant difference with the control group ($p < 0.0001$).

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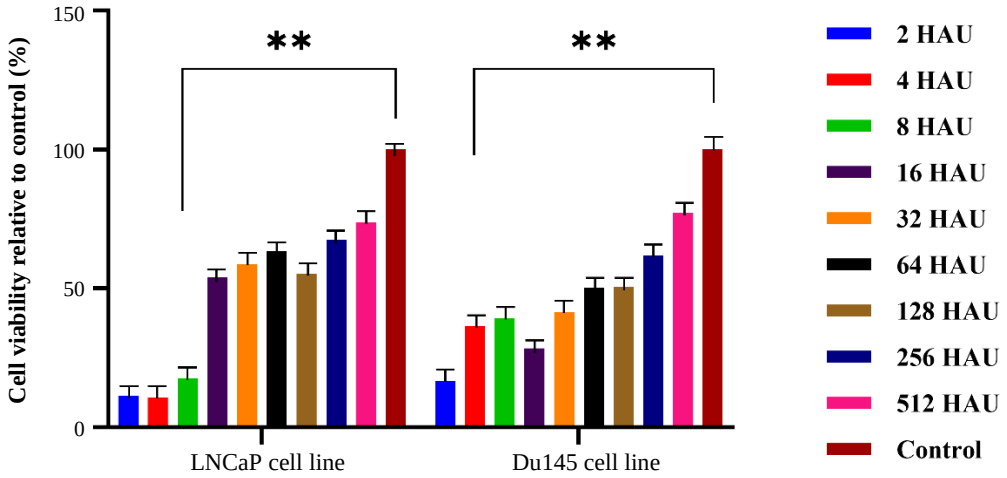

Fig. 3. The viability of prostate cell lines after NDV infection.**Significant difference to the control group ($p < 0.01$). ns: not significant.

oncolytic agents for PCa[31]. Some researchers have suggested that NDV retargeted with PSA could be an effective oncolytic drug against PCa[13]. The NDV La Sota strain demonstrated cytotoxic effects, particularly in the LNCaP and DU145 cell lines, reducing cell viability to less than 40% at specific dilution of the virus[32]. The oncolytic NDV enhances the production of immunogenic cell death markers in PCa cells by targeting STAT3[15]. NDV activates intrinsic and extrinsic apoptotic pathways specific to cell type and virus, killing cancer cells of ecto-, endo-, and mesodermal origin[33]. The primary mechanisms through which NDV infection induces cell death in tumor cells involve both endogenous and exogenous pathways[34,35]. Our results showed that PCa cells treated with NDV exhibit a 2.5-fold increase in apoptosis rate compared to PBMCs. Kalantari et al. determined the induction of apoptosis with NDV in breast cancer cells compared to normal cells[36] and indicated that numerous caspase-dependent apoptosis mechanisms, independent of interferon signaling, contribute to tumor cell cytotoxicity[33]. Findings have shown that the La Sota strain exhibits remarkable antitumor activity in breast cancer cell lines through cytolysis and apoptosis via the intrinsic pathway[17].

In this study, we evaluated the cytotoxic effects of Len alone and in the combination with NDV on the PCa cell line, LNCaP. We observed that Len increased cell cytotoxicity in the combination group compared to NDV alone. Keizmen et al. examined the effectiveness and safety of Len in men with nonmetastatic, relapsed PCa and exhibited that Len reduces PSA levels. They also reported that the toxicity of Len was barely noticeable[37]. Sundaresan et al. have suggested that Len accelerates the cell cycle progression and creates a hyperproliferative environment within tumors by enhancing cholesterol biosynthesis[24]. According to their research, Len facilitated cell cycle progression and promoted tumor hypergrowth. These findings necessitate further investigation to determine how these mechanisms could improve the treatment of metastatic PCa. According to the study by Blesa et al., antitumoral activity of Len and its low toxicity could make it an effective therapy for HRPC[11]. Treatment of patients with castration-resistant PCa using Len-based regimens has been associated with a modest response rate and tolerable toxicity[23,34,38,39]. Len is linked to sustained disease stability and reduction in PSA levels, with its toxicity remaining within acceptable limits[37]. In this study, we showed that the combination therapy of NDV with Len enhanced cytotoxicity in PCa cells, suggesting it as a potential therapeutic approach for treating PCa.

Len predominantly induces apoptosis by activating caspases, such as caspase-9 and caspase-3. The activation of caspase-8 suggests a potential interaction with the extrinsic pathway; however, these effects appear to be secondary to the primary mechanism[40,41]. Immunomodulatory drugs have also been shown to enhance apoptosis in chronic lymphocytic leukemia. By suppressing ERK1/2 and Akt, Len promotes cell death and impacts the phosphatidylinositol pathway in CLL cells[40]. Combining Bortezomib with Len reduces sensitivity, as caspase-8 cleaves CRBN[42]. Using Len alone or in conjunction with docetaxel considerably increases PC-3 PCa cell death[19]. Notably, increased rates of early apoptosis were observed in the MYXV, MYXV + Bortezomib, and MYXV + Len groups. patients with newly diagnosed and refractory multiple myeloma, the addition of MYXV resulted in a statistically significant increase in early apoptotic

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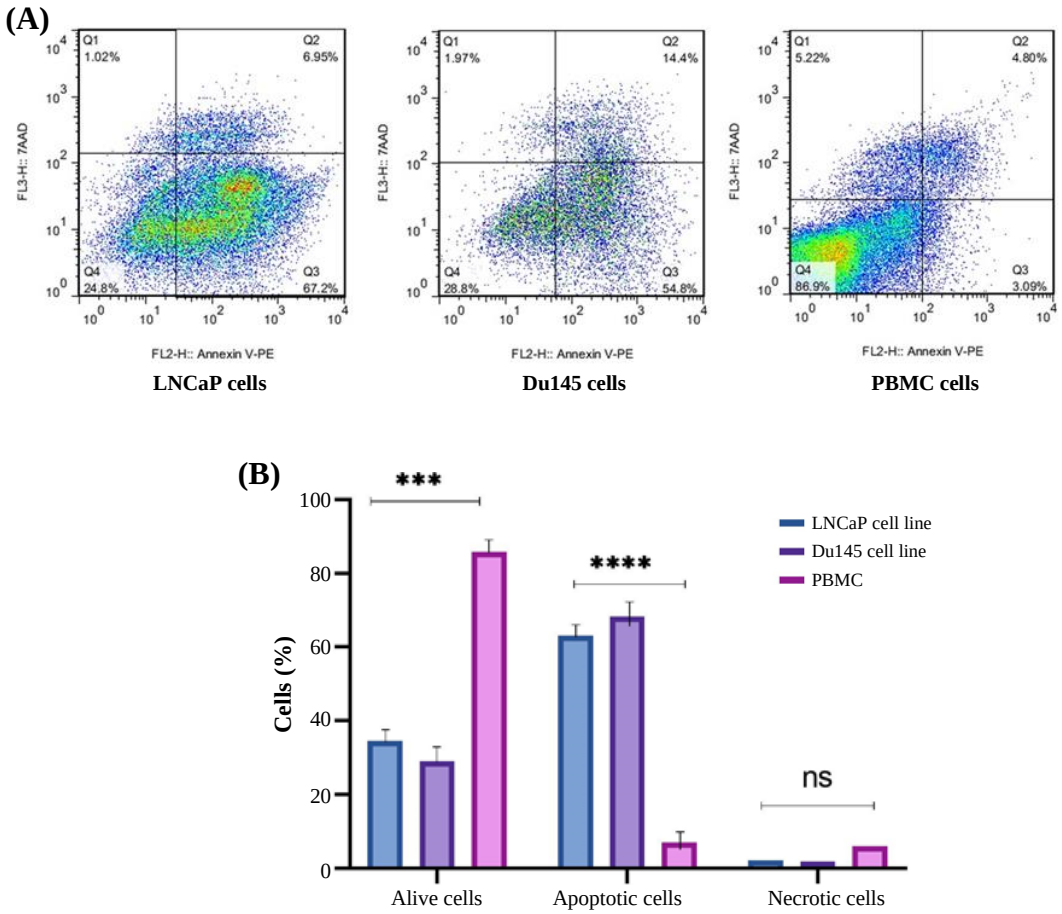

Fig. 4. Flow cytometry analysis of apoptotic induction in PCa and PBMC cells infected with NDV. (A) LNCaP, DU145, and PBMC cells infected with NDV at a dose of 8 HAU per 10,000 cells. Cells in the early apoptotic stage were stained only with annexin V, and those in the late apoptotic stage were stained with both annexin V and 7AAD. (Q1: necrosis, Q2: late apoptosis, Q3: early apoptosis, and Q4: live cells). (B) The percentage of living cells and apoptotic cells. A significant difference between PCa cell lines and PBMCs is indicated by $^{****}p < 0.0001$ or $^{***}p < 0.001$. ns: not significant.

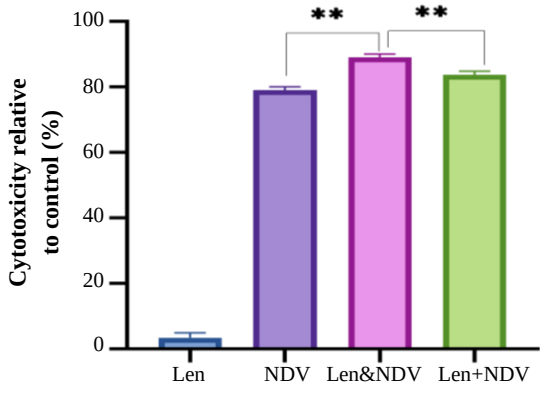

Fig. 5. Cytotoxicity of NDV alone and in combination with Len (Len & NDV), as well as total cytotoxicity of Len and NDV (Len and NDV). The combination of OV and Len demonstrated significant differences in cytotoxicity compared to NDV and Len + NDV groups. **significant difference compared to the control group ($p < 0.01$).

cells[16]. We implemented combination immunotherapy to address the challenges associated with oncolytic virotherapy in solid tumors. The combination of NDV and Len is likely to be more effective in LNCaP cells rather than NDV monotherapy. Studies have shown that Len can substantially improve the anti-tumor effects of other drugs in LNCaP cells, suggesting that it would be more effective when combined with NDV[39,43]. Clinical investigations, based on encouraging preclinical findings, yielded positive results, indicating a potential successful transition from laboratory experiments to practical application in patient care[29,44]. Due to their intrinsic characteristics as oncolytic agents, the lack of observable adverse effects in humans makes NDVs a promising candidate for future cancer therapies. Thus, we propose that NDVs can be employed as a viable option for cancer treatment. However, further clinical investigations are essential to confirm these findings and

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establish the appropriate different dilution of the virus and timing of NDV administration. Our work demonstrates that NDVs exhibit cytotoxic effects on cancer cells, promoting their promise as a therapeutic option for PCa and other solid tumors.

CONCLUSION

This research supports the use of oncolytic virotherapy in cancer treatment and highlights the promising role of NDV and Len in treating PCa. To actualize these findings, animal research and clinical trials are necessary. Our work demonstrates that NDVs exhibit cytotoxicity toward cancer cells, reinforcing existing knowledge and highlighting their potential as a therapeutic option for PCa and other solid tumors.

DECLARATIONS

Acknowledgments

We would like to acknowledge the personnel in the Department of Immunology, Pasteur Institute of Iran for their assistance and support of this research project. No artificial intelligence services were used for the preparation of this manuscript.

Ethical approval

All experiments have been conducted in accordance with the guidelines of the Ethics Committee of the Pasteur Institute of Iran (PII), Tehran (ethical code: IR.PII.REC.1398.009).

Consent to participate

Not applicable.

Consent for publication

All authors reviewed the results and approved the final version of the manuscript.

Authors' contributions

MJ: wrote the manuscript and developed the theory; SA: developed the theory and edited the manuscript; MA: wrote and edited the manuscript; MM: reviewed and edited the manuscript; MAS: reviewed the manuscript; ZS: reviewed and edited the manuscript.

Data availability

The data supporting the findings of this study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Funding

This project was funded by the Pasteur Institute of Iran (PII; Ph.D. thesis grant to MJ; Grant no. BD-9575).

Supplementary information

The online version does not contain supplementary material.

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