



Comprehensive Assessment of Time- and Dose-Dependent Oncolytic Activity, Apoptotic Induction, and Metabolic Reprogramming by Newcastle Disease Virus (NDV) LaSota in p53-Deficient H1299 Non-Small Cell Lung Cancer Cells

Omar Hussien Harran^{1*}

¹ Department of Agricultural Biotechnology, College of Biotechnology, University of Al-Qadisiyah, Al-Diwaniyah, Iraq

Corresponding Author: Omar Hussien Harran, PhD, Assistant Professor, Department of Agricultural Biotechnology, College of Biotechnology, University of Al-Qadisiyah, Al-Diwaniyah, Iraq. Tel: +9647859314564, E-mail: o.h.harran@qu.edu.iq

Received August 25, 2026; Accepted September 17, 2026; Online Published September 30, 2026

Abstract

Introduction: Newcastle disease virus (NDV) is a candidate oncolytic virus. This study assessed the preferential *in vitro* effects of the attenuated LaSota NDV strain in p53-deficient H1299 non-small cell lung cancer (NSCLC) cells compared with BEAS-2B bronchial epithelial cells.

Materials and Methods: H1299 and BEAS-2B cells were infected with NDV LaSota at multiplicities of infection (MOIs) of 1, 5, and 10 and evaluated at 24, 48, and 72 h post-infection. MTT reduction, cytopathic effect (CPE) microscopy, Annexin V-FITC/PI flow cytometry, glucose and lactate measurements, intracellular ATP assays, and hemagglutination (HA) assays were performed. Each condition was assessed in three independent experiments. Publicly available LaSota-NDV RNA-seq data from KKKU-055 and KKKU-100 cells (NCBI BioProject PRJNA1114906) were used for cross-cancer molecular validation.

Results: NDV induced a time- and MOI-dependent reduction in MTT-based metabolic activity that was more pronounced in H1299 than in BEAS-2B cells. H1299 cells also exhibited greater CPE severity, higher Annexin V-positive fractions, and more pronounced reductions in glucose consumption, lactate production, and intracellular ATP. At 48 h post-infection, HA titers increased with MOI, reflecting hemagglutinating activity; however, HA titers are not equivalent to infectious virus titers. In the external dataset, LaSota NDV was associated with 368 differentially expressed genes (DEGs) in KKKU-055 cells (321 upregulated and 47 downregulated) and 1,807 DEGs in KKKU-100 cells (892 upregulated and 915 downregulated). In total, 131 genes were commonly altered, including six shared hub genes (DDX58, IFIH1, IRF7, ISG15, GBP1, and DHX58), consistent with involvement of apoptosis, interferon, TNF- α /NF- κ B, and IL-6/JAK/STAT3 pathways.

Conclusions: H1299 cells exhibited a preferential *in vitro* response to LaSota NDV across multiple cellular and metabolic parameters. However, direct evidence of infectious viral replication and its relationship to cytotoxicity remains to be established. Transcriptomic data support a distinction between cell-killing programs in H1299 cells and the comparatively preserved responses in BEAS-2B cells. Further studies are required to confirm infectious virus production and *in vivo* relevance.

Keywords: Newcastle Disease Virus, Oncolytic Virotherapy, Non-small Cell Lung Cancer, Apoptosis, Public Transcriptomics, RNA-seq

Citation: Harran OH. Comprehensive Assessment of Time- and Dose-Dependent Oncolytic Activity, Apoptotic Induction, and Metabolic Reprogramming by Newcastle Disease Virus (NDV) LaSota in p53-Deficient H1299 Non-Small Cell Lung Cancer Cells & Quot. J Appl Biotechnol Rep. 2026;13(3):2116-2127. doi:10.30491/jabr.2026.599070.2053

Introduction

Lung cancer remains the leading cause of cancer-related mortality worldwide, and non-small cell lung cancer (NSCLC) accounts for the majority of cases.^{1,2} Although molecularly targeted therapies and immunotherapies have transformed the management of selected NSCLC subgroups, treatment resistance, tumor heterogeneity, and treatment-related toxicity continue to limit durable disease control.^{2,3} These limitations support continued investigation of therapeutic strategies that can selectively damage malignant cells while engaging antitumor immunity.

Oncolytic virotherapy employs replication-competent viruses to preferentially infect and lyse tumor cells and can also convert an immunologically cold tumor microenvironment

into a more inflammatory state.⁴⁻⁷ Newcastle disease virus (NDV), an avian paramyxovirus, has received sustained attention as an oncolytic platform because of its tumor-selective replication, broad experimental antitumor activity, and favorable safety record in humans.⁸⁻¹⁰ NDV-mediated tumor killing involves direct viral cytotoxicity as well as intrinsic and extrinsic apoptotic pathways and immunomodulatory mechanisms.^{11,12} The attenuated LaSota strain is a well-characterized vaccine strain that provides a practical experimental platform for evaluating NDV–host cell interactions.^{13,14}

Recent studies continue to expand the evidence base for NDV-based cancer therapy. In lung cancer models, NDV-

based vaccination and virotherapy have been shown to suppress tumor growth and promote antitumor immune responses.¹⁵ NDV can also interact with cancer-cell metabolism, including glycolytic and mitochondrial pathways.¹⁶ Contemporary mechanistic studies have further identified host factors and cell-death programs that influence NDV susceptibility and oncolysis.^{17,18}

The H1299 human NSCLC cell line lacks functional p53 expression and is therefore a useful model for investigating oncolytic responses in a genetically defined, treatment-relevant context.¹⁹ This is important because metabolic reprogramming, including increased dependence on aerobic glycolysis, is a recognized feature of malignant cells.²⁰ For oncolytic viruses, productive viral propagation and the extent of tumor-cell killing are also closely linked, although viral replication is not the only determinant of cytotoxicity.^{21,22} NDV has consequently remained of translational interest as both a direct oncolytic agent and a potential component of combination cancer therapy.²³

In this respect, the present study assessed the *in vitro* performance of the NDV LaSota vaccine strain in the H1299 lung cancer line and used BEAS-2B bronchial epithelial cells for comparison. The study evaluated multiple parameters, including MTT-based metabolic activity, cytopathic morphological changes, Annexin V/PI cell-death profiles, cellular metabolic indicators, and hemagglutinating activity. To provide molecular context without pooling data from biologically distinct cell types, previously published RNA-seq data from two human cholangiocarcinoma cell lines infected with LaSota NDV were used as an independent cross-cancer dataset. This approach was intended to characterize the time- and dose-dependent oncolytic effects of LaSota NDV in a p53-deficient NSCLC model while acknowledging that direct infectious-virus titration remains necessary to define the relationship between viral replication and cellular damage.

Materials and Methods

Cell Lines and Culture Conditions

H1299 human non-small cell lung cancer cells and BEAS-2B human bronchial epithelial cells were used. Cell-line identifiers are provided for reference: NCI-H1299 corresponds to ATCC CRL-5803 (19), and BEAS-2B corresponds to ATCC CRL-9609;²⁴ a manufactured progeny of the BEAS-2B lineage is available as ATCC CRL-3588.^{24,25} These accession numbers are provided solely for cell-line identification and do not indicate the original supplier, as supplier records were not available in the archived data for this revision. H1299 cells were maintained in RPMI-1640 medium (Gibco, USA), and BEAS-2B cells were maintained in DMEM/F12 (Gibco, USA). Both media were supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 µg/ml) (Sigma,

USA). Cells were incubated at 37 °C with 5% CO₂. Subculturing was performed using 0.25% trypsin-EDTA when cultures reached 80–90% confluence.

Newcastle Disease Virus LaSota Vaccine Strain

The attenuated LaSota vaccine strain of Newcastle disease virus (NDV) was used as the oncolytic agent. NDV was propagated in 9–11-day-old specific-pathogen-free embryonated chicken eggs. Allantoic fluid was collected after 48–72 h, centrifuged at 3000 rpm for 10 min, aliquoted, and stored at –80 °C. Virus-associated hemagglutinating activity was determined using the hemagglutination (HA) assay and expressed as hemagglutination units per milliliter (HAU/ml).¹⁴

Experimental Design and Viral Infection

H1299 and BEAS-2B cells were seeded in 96-well plates for the MTT-based metabolic-activity assay and in 6-well plates for apoptosis and metabolic analyses. At approximately 70–80% confluence, cells were assigned to an untreated mock-control group or to NDV-treated groups at the experimentally recorded MOIs of 1, 5, or 10. Virus adsorption was performed for 1 h at 37 °C with gentle agitation. The inoculum was then removed, cells were washed with phosphate-buffered saline (PBS), complete medium was added, and samples were evaluated at 24, 48, and 72 h post-infection. Post-infection HA measurements were used to quantify hemagglutinating activity and were not treated as a substitute for infectious-virus titration.

MTT-Based Metabolic Activity Assay

MTT reduction was used as an index of cellular reducing/metabolic activity rather than as a direct cell-count viability assay. At each time point, 20 µl of MTT solution (5 mg/ml) (Sigma, USA) was added to each well and incubated for 3–4 h at 37 °C. Formazan crystals were dissolved in dimethyl sulfoxide (Sigma, USA), and absorbance was measured at 570 nm using a microplate reader. The normalized MTT signal was calculated as (A₅₇₀ of NDV-treated cells / A₅₇₀ of the corresponding mock-treated control) × 100 and expressed as percent of the matched mock control.²⁶

Morphological Assessment of Cytopathic Effects

NDV-induced morphological changes were examined by inverted phase-contrast microscopy at 24, 48, and 72 h post-infection. Cell rounding, shrinkage, detachment, membrane blebbing, syncytium formation, and loss of monolayer integrity were recorded. Cytopathic effect (CPE) severity was summarized using the semi-quantitative scoring approach applied in the original experimental dataset.¹¹

Annexin V-FITC/PI Flow-Cytometry Analysis

Cell-death analysis was performed using Annexin V-FITC in conjunction with propidium iodide (PI) dual staining at 24,

48, and 72 h post-infection. Cells were collected, rinsed with cold PBS, and stained according to the manufacturer's recommendations.²⁷ The quadrants were designated as follows: Q4, Annexin V⁻/PI⁻ viable cells; Q3, Annexin V⁺/PI⁻ early apoptotic cells; Q2, Annexin V⁺/PI⁺ double-positive cells compatible with late apoptosis and/or secondary necrosis; and Q1, Annexin V⁻/PI⁺ primary necrotic cells. The Q2 quadrant cannot be defined exclusively as late apoptosis because it does not differentiate between late apoptosis and secondary necrosis. The sum of Q2 and Q3 was reported quantitatively as the Annexin V-positive fraction (Q2+Q3), not as total apoptotic cells.

Measurement of Cellular Metabolic Activity

Culture supernatants were collected at 24, 48, and 72 h and used for glucose and lactate measurements with commercial kit (Promega, USA) according to the manufacturer's recommendations. In all comparative graphs, glucose consumption and lactate production are expressed as percentages relative to the corresponding mock control, which was set at 100%; lower percentages indicate lower metabolic activity than the control.

ATP Measurement

Cellular ATP was quantified using the ATP Detection Assay Kit-Luminescence (Item No. 700410; Cayman Chemical, USA) according to the manufacturer's protocol. Following NDV infection, cells were washed with pre-chilled PBS and lysed with ice-cold 1× ATP Detection Sample Buffer. Ten microliters of each lysate were mixed with 100 µl of ATP Detection Assay Buffer containing the D-luciferin/luciferase reaction mixture. After 15–20 min at room temperature in the dark, luminescence was measured using a microplate reader with luminescence-detection capability. ATP concentrations were derived from the assay standard curve and expressed relative to the corresponding mock-infected control, which was set at 100%.

Hemagglutinating Activity Assay

Supernatants obtained at 24, 48, and 72 h after infection were serially diluted in PBS and combined with 1% chicken red blood cells. Hemagglutination was determined visually, and HA endpoints were expressed in log₂ HA units. HA titers measure hemagglutinating ability but not virus concentration, infectious agents, or successful viral replication. Thus, TCID₅₀ or PFU could not be calculated from HA data.¹⁴

External Public Transcriptomic Evidence

For external inter-cancer molecular validation, publicly available data from Thanyaphoo et al.²⁴ were used. The original study investigated lentogenic NDV LaSota in human cholangiocarcinoma cell lines KKKU-055 (JCRB

1551) and KKKU-100 (JCRB 1568). RNA-sequencing data were analyzed 24 h after LaSota NDV exposure, while cell counting and trypan-blue exclusion data were acquired 72 h post-infection using 16 HAU per 10,000 cells. The authors deposited raw FASTQ data in the NCBI Sequence Read Archive under BioProject PRJNA1114906 and provided processed differential expression, pathway enrichment, and hub-gene tables in PLOS ONE Supporting Information S1–S4. In the present study, these published results were organized into Supplementary Dataset S1 and used only to assess whether cellular events observed in H1299 cells are also evident in independently conducted human cancer models treated with the same LaSota strain.

Biosafety and Ethical Considerations

Experiments involving live NDV and embryonated chicken eggs were conducted under the institution's approved laboratory procedures and biosafety requirements. The study used established cell lines and did not involve recruitment of human participants or collection of identifiable human specimens.

Statistical Analysis

Data are presented as mean ± standard deviation (SD) of three independent experiments (n = 3). GraphPad Prism was used for analysis. Two-way ANOVA with Tukey's post hoc test was used to assess MTT-based metabolic activity and Annexin V-positive fractions within each cell line, with infection time and MOI as factors. One-way ANOVA was used for H1299 CPE at fixed MOI and for HA titers at 48 h; two-way ANOVA was used for BEAS-2B CPE and metabolic endpoints. A *p*-value < 0.05 was considered statistically significant. Exact *p* values or statistical symbols are presented in the relevant figure legends. Public transcriptomic data were summarized descriptively and were not pooled with the primary H1299/BEAS-2B data for inferential testing.

Results

Time- and MOI-Dependent MTT Reduction in H1299 Cells

Treatment with NDV led to a greater reduction in the normalized MTT signal in H1299 cells than in BEAS-2B cells. In H1299 cells, MTT activity remained moderately reduced at 24 h, with some MOIs showing reductions to 75–80% of the mock control. At 48 h, the normalized MTT activity decreased to approximately 55% at MOI 5 and 40% at MOI 10. The most pronounced reduction occurred at 72 h and MOI 10, reaching approximately 30% of mock activity. In contrast, BEAS-2B cells-maintained MTT activity above 85% even at the highest MOI. Two-way ANOVA showed significant effects of both MOI and time (*p* < 0.0001). These changes in MTT signal should be interpreted as alterations in

overall cellular reducing/metabolic activity rather than as direct measurements of cell number. Because the original figure and reader export file were unavailable, missing mean values and error bars for some conditions, particularly at MOI 1, could not be reconstructed; the reported values therefore represent the descriptive data retained in the archived manuscript.

Cytopathic Effects in H1299 Cells

CPE was assessed in H1299 cells at MOIs 1, 5, and 10 at 24, 48, and 72 h post-infection. Morphological injury increased progressively with infection duration and viral dose. At MOI

1, CPE scores increased from 0.4 at 24 h to 0.8 at 48 h and 1.3 at 72 h. At MOI 5, scores were 1.2, 2.0, and 2.6 at 24, 48, and 72 h, respectively. At MOI 10, scores increased from 0.8 at 24 h to 1.8 at 48 h and 2.9 at 72 h. Early changes consisted mainly of cell rounding. By 48 h, shrinkage, membrane blebbing, loss of adhesion, and detachment became more evident. At 72 h, the highest MOI produced extensive monolayer disruption, syncytium formation, and widespread cell death. The MOI-specific H1299 profiles are shown in Figures 1–3. One-way ANOVA across time for each fixed MOI confirmed significant progression of CPE severity ($p < 0.05$).

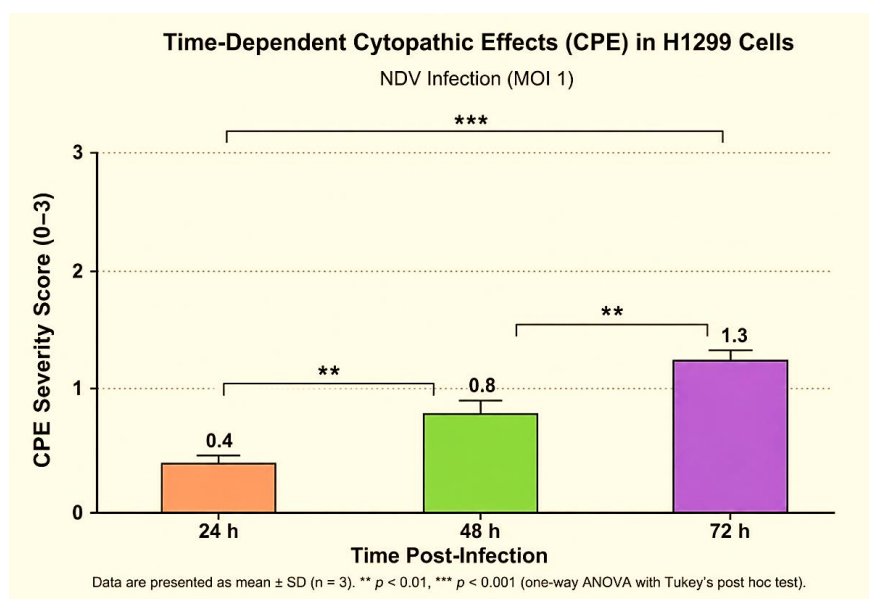


Figure 1. Semi-Quantitative Assessment of NDV-Induced Cytopathic Effects in H1299 Cells at MOI 1. CPE severity increased from mild cell rounding at 24 h to greater shrinkage and detachment at 48 h and advanced morphological damage at 72 h. Data are presented as mean ± SD (n = 3).

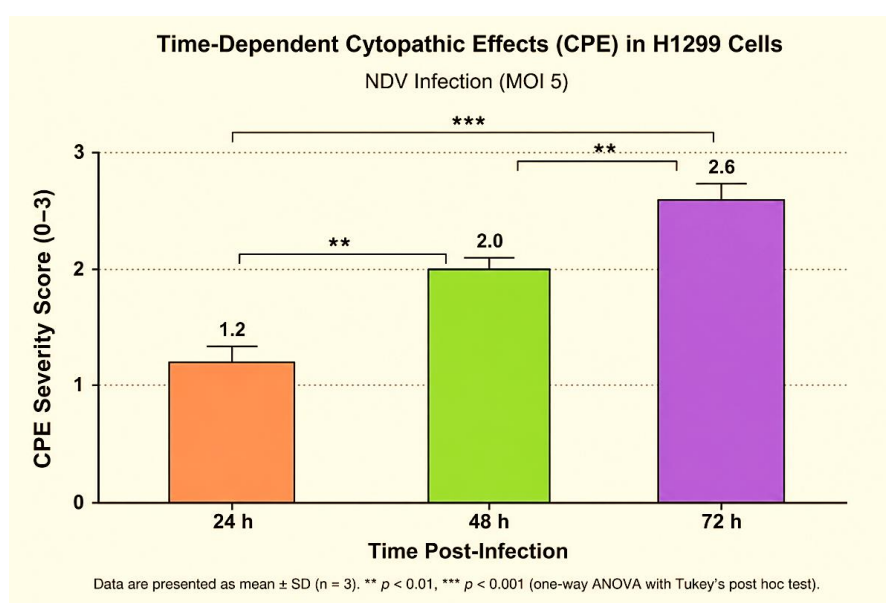


Figure 2. Semi-Quantitative Assessment of NDV-Induced Cytopathic Effects in H1299 Cells at MOI 5 Across 24, 48, and 72 h Post-Infection. Data are presented as mean ± SD (n = 3).

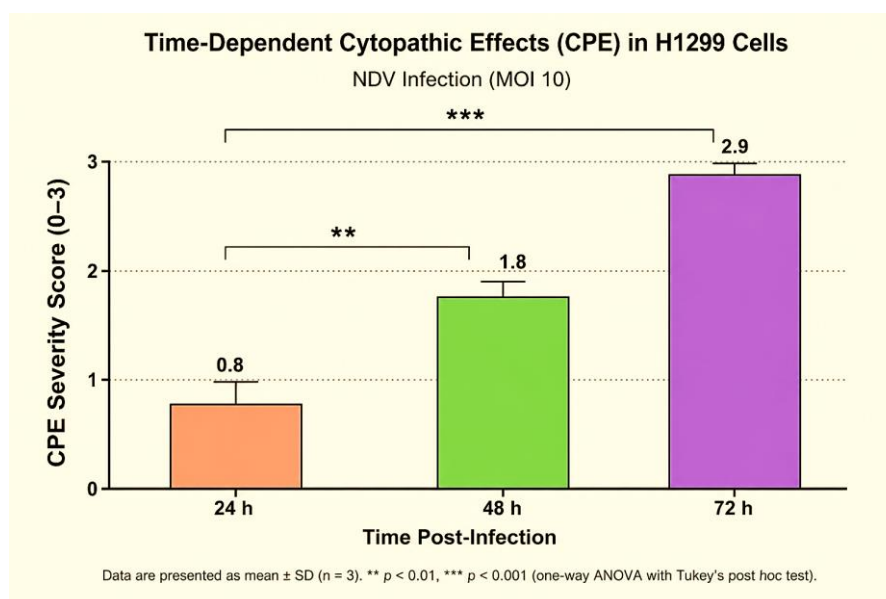


Figure 3. Semi-Quantitative Assessment of NDV-Induced Cytopathic Effects in H1299 Cells at MOI 10 Across 24, 48, and 72 h Post-Infection. Data are presented as mean ± SD (n = 3).

Cytopathic Effects in BEAS-2B Cells

BEAS-2B cells displayed substantially less morphological injury than H1299 cells across the tested time points and MOIs. CPE scores in BEAS-2B cells remained low, ranging from 0.10 to 0.62 across all conditions. At 24 h, scores were 0.10, 0.18, and 0.30 for MOIs 1, 5, and 10, respectively. At 48 h, the corresponding scores were 0.22, 0.28, and 0.46, and at 72 h they were 0.30, 0.40, and 0.62. By comparison, H1299 cells reached a CPE score of 2.9 at MOI 10 and 72 h. Only minor time- and dose-associated changes were observed in BEAS-2B cells, and monolayer integrity was largely preserved. Figure 4 presents the BEAS-2B data together with the corresponding H1299 CPE panels to facilitate direct comparison across cell types, time points, and MOIs. Two-way ANOVA with time and MOI as factors confirmed significantly lower CPE severity in BEAS-2B cells than in H1299 cells.

NDV Suppresses Cellular Metabolic Activity in H1299 Cells

NDV infection was accompanied by progressive suppression of metabolic endpoints in H1299 cells. At MOI 10, glucose consumption in H1299 cells declined to 75%, 62%, and 50% of mock levels at 24, 48, and 72 h, respectively. Lactate production declined to 78%, 60%, and 55%, and intracellular ATP declined to 70%, 58%, and 50% over the same time points. In BEAS-2B cells at MOI 10, glucose consumption remained at 95%, 93%, and 88% of mock levels; lactate production at 95%, 93%, and 90%; and ATP at 96%, 93%, and 91%.

At MOI 5, H1299 cells showed glucose consumption of 80%, 65%, and 55% of mock levels at 24, 48, and 72 h;

lactate production of 85%, 65%, and 60%; and ATP of 78%, 63%, and 52%. The corresponding BEAS-2B values remained above 90% for glucose, 90% for lactate, and 92% for ATP across all time points.

At MOI 1, H1299 cells exhibited a milder but progressive reduction, with glucose consumption of 90%, 80%, and 70% of mock levels at 24, 48, and 72 h; lactate production of 88%, 75%, and 65%; and ATP of 88%, 72%, and 60%. BEAS-2B cells at MOI 1 maintained glucose consumption at 97%, 95%, and 93%; lactate production at 97%, 94%, and 92%; and ATP at 98%, 96%, and 94%. Overall, metabolic suppression in H1299 cells was time- and MOI-dependent, with the largest reductions observed at MOI 10 and 72 h. BEAS-2B cells showed much smaller changes, generally below 15%. Figures 5–7 present H1299 (Panels A–C) and BEAS-2B (Panels D–F) in corresponding side-by-side panels for direct comparison at each fixed MOI. Two-way ANOVA evaluated time and cell type for each metabolic endpoint, followed by Tukey-adjusted comparisons where applicable.

Time- and MOI-Dependent Annexin V/PI Changes

Flow cytometry using Annexin V-FITC/PI showed that NDV infection in H1299 cells resulted in a progressive shift from viable cells to populations positive for Annexin V. At MOI 10, the Annexin V-positive fraction (Q2+Q3) was 37.1% at 24 h, increasing to 48.7% at 48 h and 62.5% at 72 h. In contrast, BEAS-2B cells at the same MOI and time points showed markedly lower Annexin V-positive fractions of 8.7%, 9.2%, and 9.9%, respectively. At lower MOIs, Annexin V-positive fractions were lower but increased with time; however, the archived record provided quantitative

Side-by-side comparison of NDV-induced cytopathic effects

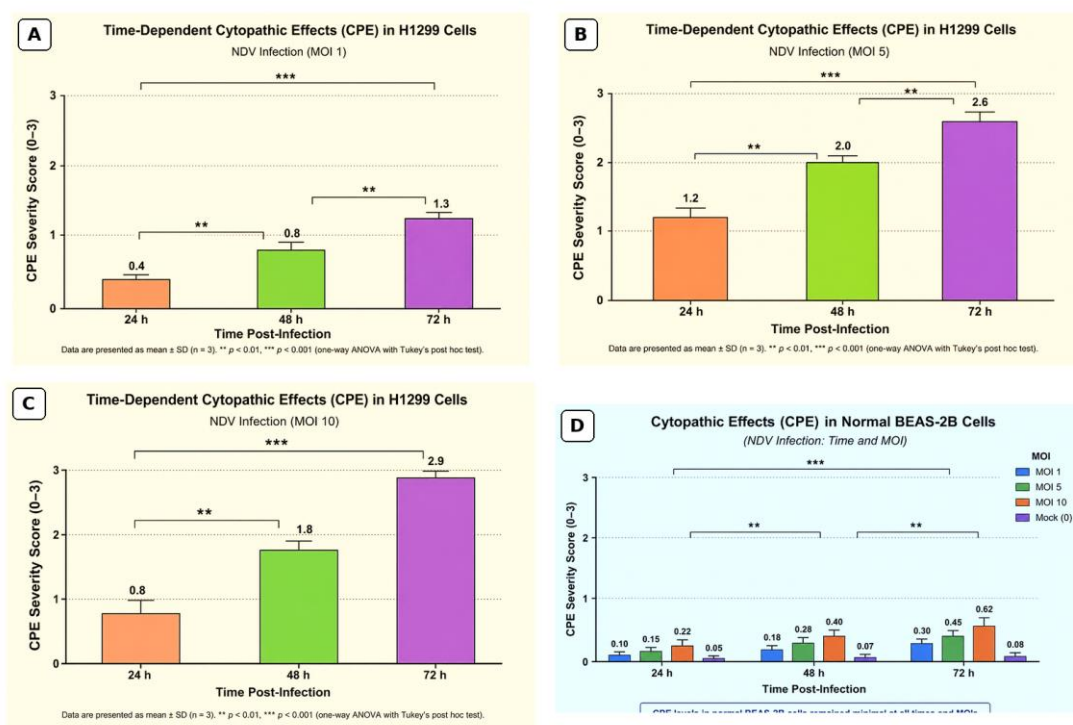


Figure 4. Side-by-side comparison of NDV-induced cytopathic effects (CPE) in H1299 and BEAS-2B cells. Panels A–C show the time-dependent H1299 CPE profiles at MOIs 1, 5, and 10, respectively, whereas Panel D shows BEAS-2B CPE across the corresponding times and MOIs. H1299 cells displayed substantially greater morphological injury under the tested conditions, while BEAS-2B CPE remained comparatively low. H1299 fixed-MOI panels were analyzed by one-way ANOVA across time; the BEAS-2B panel used two-way ANOVA with time and MOI as factors, followed by Tukey-adjusted comparisons.

NDV-Associated Metabolic Changes at MOI 10

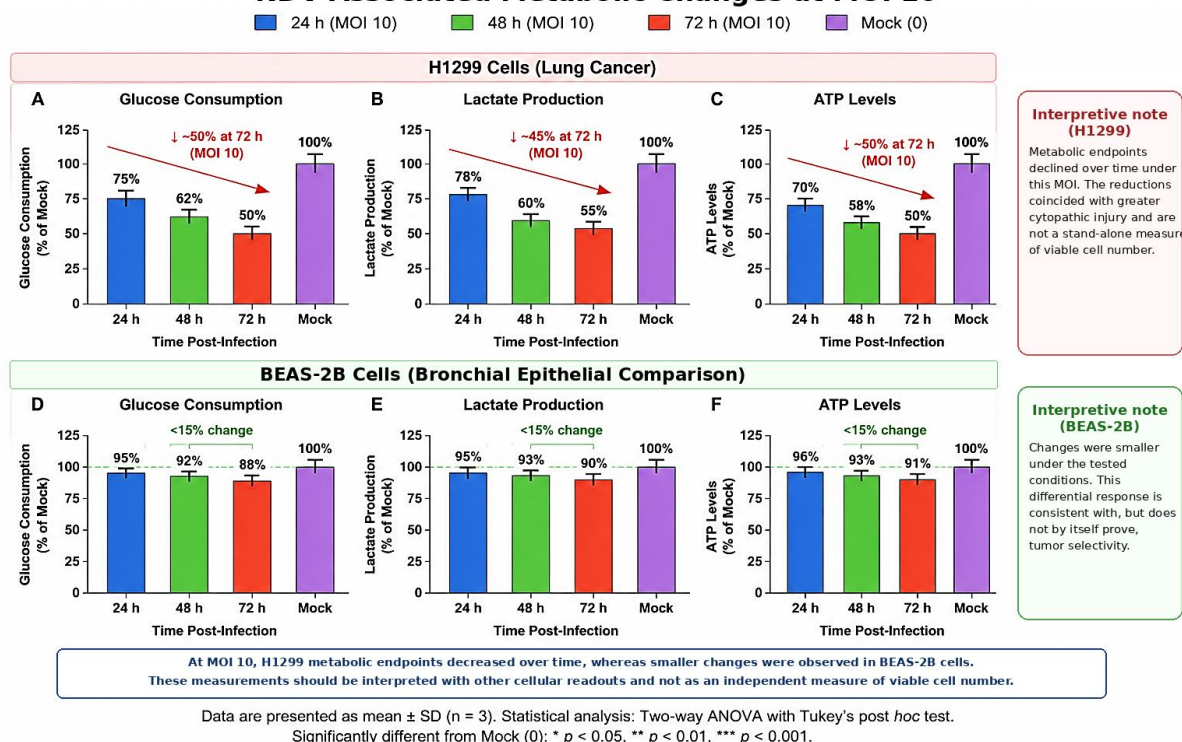


Figure 5. NDV-associated metabolic changes at MOI 10 presented in corresponding panels for direct cell-type comparison. Panels A–C show H1299 glucose consumption, lactate production, and intracellular ATP, respectively; Panels D–F show the matched BEAS-2B endpoints at 24, 48, and 72 h. Data are mean $\pm$ SD (n = 3). Two-way ANOVA evaluated time and cell type for each endpoint, followed by Tukey-adjusted comparisons as applicable.

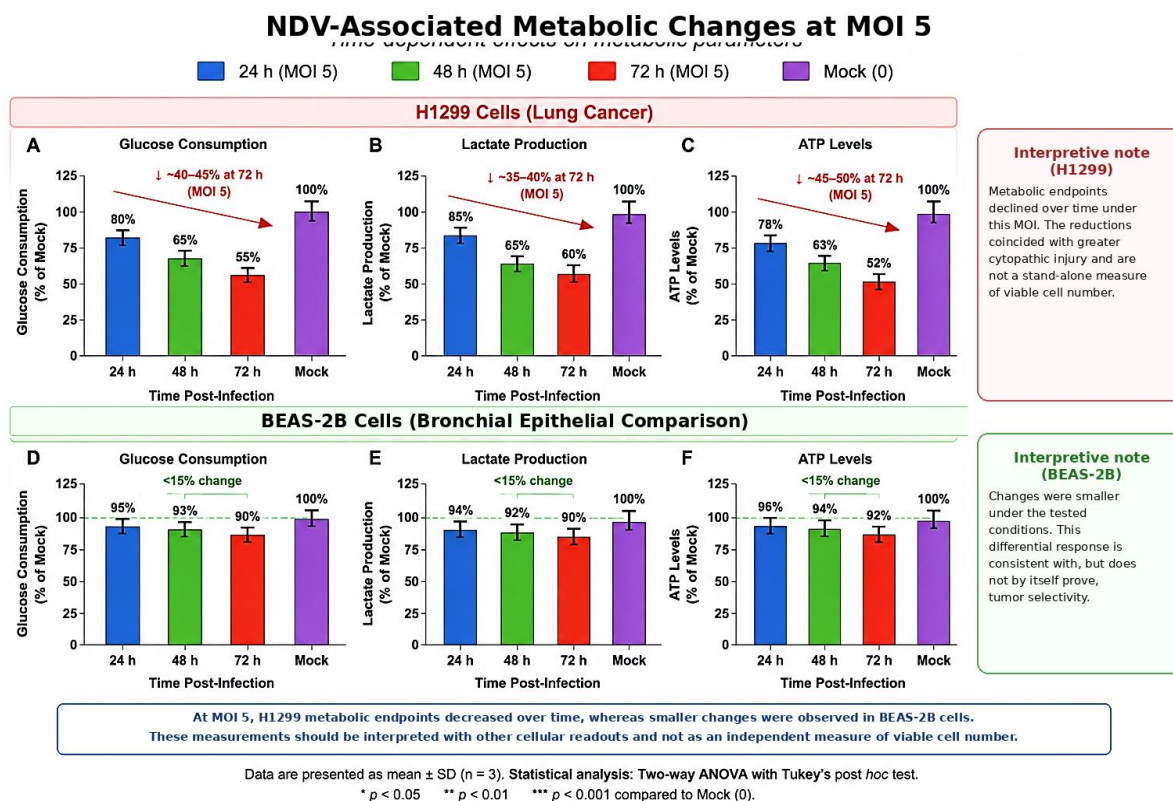


Figure 6. NDV-associated Metabolic Changes at MOI 5 Presented in Corresponding Panels for Direct Cell-type Comparison. Panels A–C show H1299 glucose consumption, lactate production, and intracellular ATP, respectively; Panels D–F show the matched BEAS-2B endpoints at 24, 48, and 72 h. Data are mean $\pm$ SD (n = 3). Two-way ANOVA evaluated time and cell type for each endpoint, followed by Tukey-adjusted comparisons as applicable.

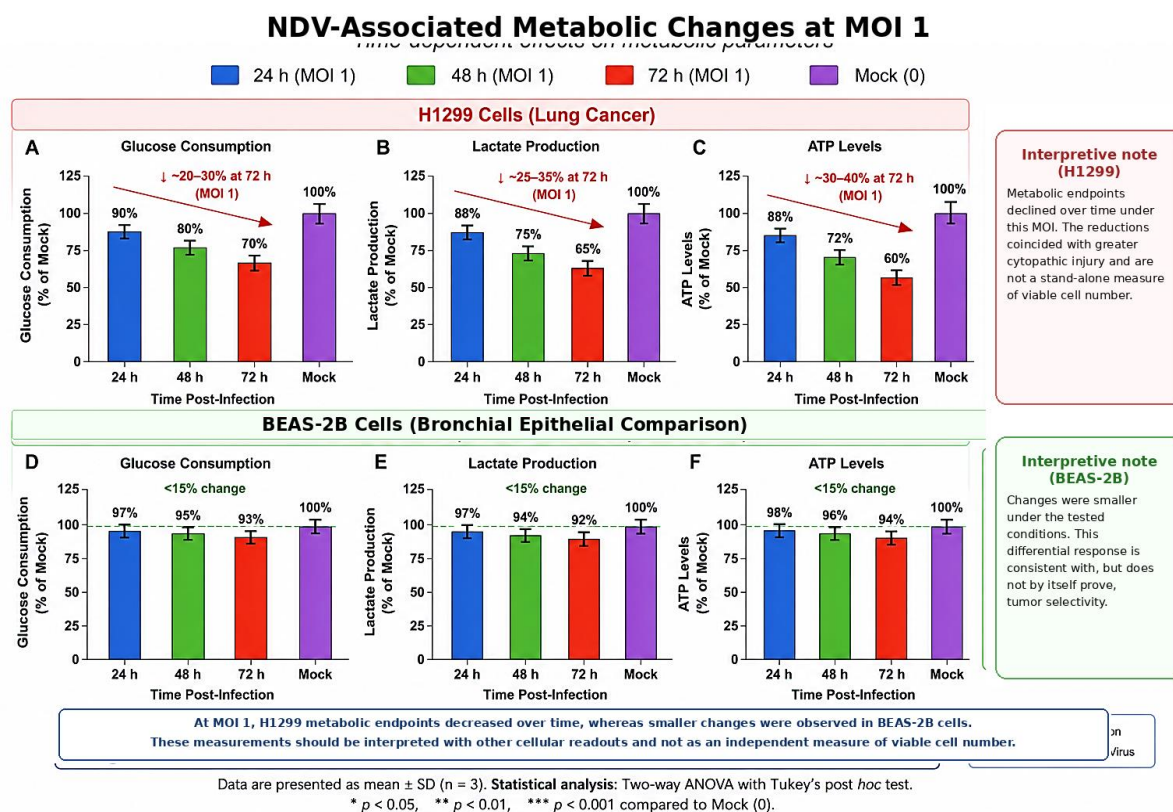


Figure 7. NDV-associated Metabolic Changes at MOI 1 Presented in Corresponding Panels for Direct Cell-type Comparison. Panels A–C show H1299 glucose consumption, lactate production, and intracellular ATP, respectively; Panels D–F show the matched BEAS-2B endpoints at 24, 48, and 72 h. Data are mean $\pm$ SD (n = 3). Two-way ANOVA evaluated time and cell type for each endpoint, followed by Tukey-adjusted comparisons as applicable.

values only for MOI 10. Q3 indicates early apoptotic Annexin V+/PI- cells, whereas Q2 refers to Annexin V+/PI+ cells that may include both late-apoptotic and secondary-necrotic cells; therefore, Q2 cannot be strictly interpreted as late apoptosis. Q4 represents viable cells, and Q1 indicates primary necrotic cells. Representative gating results are presented in Figure 8, and quantitative Annexin V-positive fractions are shown in Figure 9. Two-way ANOVA with time and MOI as factors confirmed significant increases in Annexin V positivity in H1299 cells and significantly lower values in BEAS-2B cells.

MOI-Dependent HA Activity at 48 h

HA titers in the culture supernatants of H1299 cells at 48 h post-infection increased with MOI. The mean HA titers were 6.2, 8.4, and 9.8 log₂ units for MOIs 1, 5, and 10,

respectively. One-way ANOVA with Tukey's test indicated significant differences between MOI groups, as shown in Figure 10. These data demonstrate a clear MOI-dependent increase in hemagglutinating activity; however, HA values are not considered reliable for estimating the concentration of infectious virus. The correlation between TCID₅₀ and cytotoxicity could not be analyzed because post-infection TCID₅₀ data were not available in the archived dataset.

Public LaSota NDV Transcriptomes: Conserved Cell-Death and Antiviral Programs

The external public dataset provided molecular data from independent cell comparisons using two human cholangiocarcinoma cell lines infected with LaSota.²⁴ At 24 h post-infection, a total of 368 differentially expressed genes (DEGs) were reported in KKKU-055 cells, comprising 321 upregulated

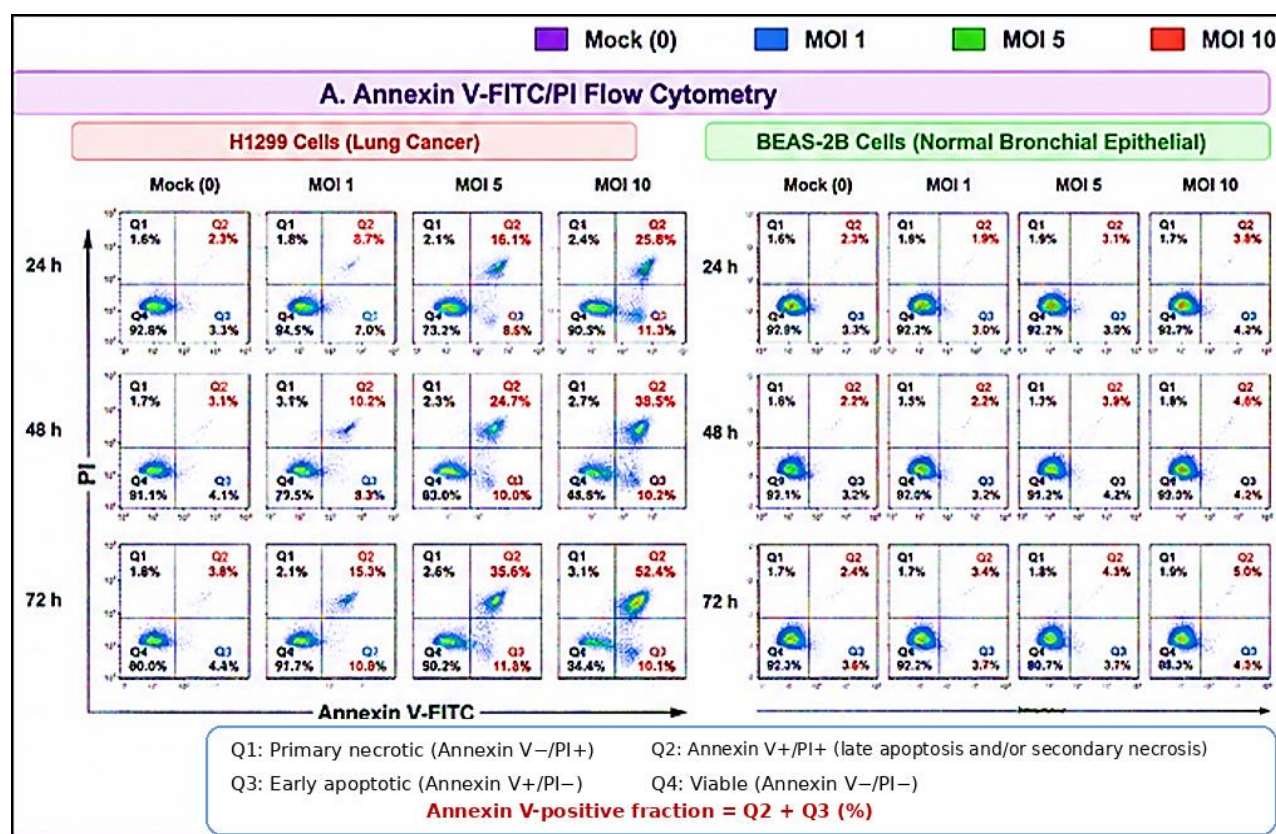


Figure 8. Representative Annexin V-FITC/PI Flow Cytometry Plots for H1299 and BEAS-2B Cells Across the Tested Times and MOIs. Q4 (Annexin V-/PI-) represents viable cells, Q3 (Annexin V+/PI-) early apoptotic cells, Q2 (Annexin V+/PI+) a double-positive population compatible with late apoptosis and/or secondary necrosis, and Q1 (Annexin V-/PI+) primary necrotic cells. Q2 was not interpreted exclusively as late apoptosis. The descriptive Annexin V-positive fraction is Q2+Q3.

and 47 downregulated genes. In KKKU-100 cells, 1,807 DEGs were identified, including 892 upregulated and 915 downregulated genes (Table 1). At 72 h, KKKU-055 cells reached total-cell levels of 23.31% and 68.46% of corresponding mock controls, with live-cell fractions of 16.77% and 60.75%, respectively. These data indicate significant variation in LaSota NDV effects across human

cancer cell lines, although the magnitude of the H1299 response remains unconfirmed.

In total, 131 genes were commonly affected across both models. Network analysis identified DDX58, IFIH1, IRF7, ISG15, GBP1, and DHX58 as six hub genes whose expression was upregulated by LaSota NDV. Hallmark enrichment showed that apoptosis, interferon-alpha response, interferon-

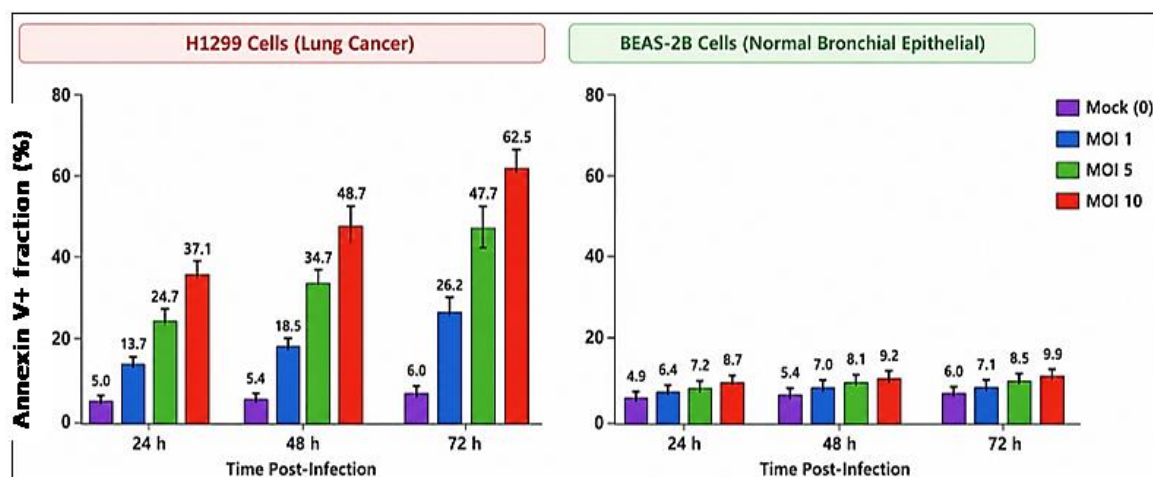


Figure 9. Quantitative Annexin V-positive Fractions (Q2+Q3) in H1299 and BEAS-2B Cells Following NDV Infection. The plotted quantity is reported as Annexin V positivity rather than definitive total apoptosis because Q2 (Annexin V+/PI+) may contain both late-apoptotic and secondary-necrotic cells. The H1299 fraction increased with time and MOI, whereas BEAS-2B values remained comparatively low. Data are mean $\pm$ SD ($n = 3$); two-way ANOVA used time and MOI as factors within each cell line, followed by Tukey-adjusted comparisons as applicable.

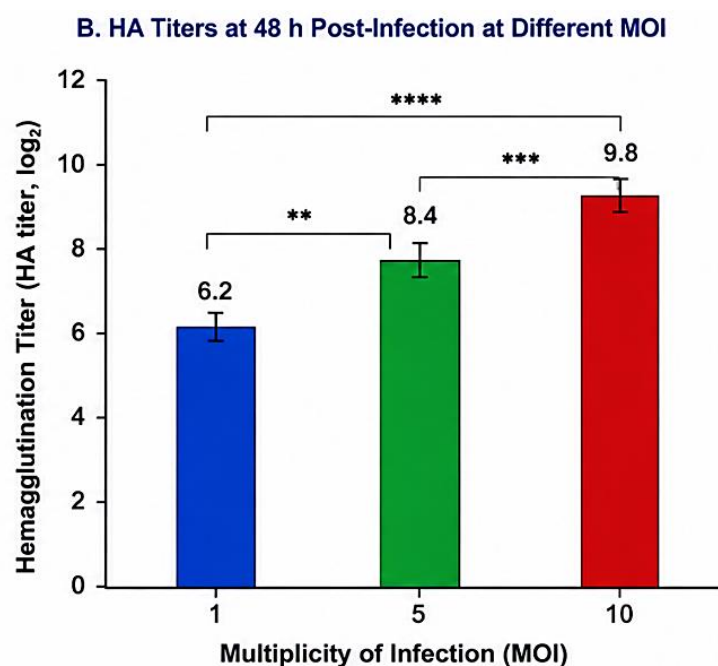


Figure 10. Hemagglutination (HA) Titers in H1299 Culture Supernatants at 48 h Post-Infection Across MOIs 1, 5, and 10. Mean HA titers were 6.2, 8.4, and 9.8 log₂ units, respectively. Data are mean $\pm$ SD ($n = 3$); ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ (one-way ANOVA with Tukey's post hoc test). HA titers represent hemagglutinating activity and are not equivalent to infectious-virus TCID₅₀ or plaque titers.

Table 1. Public LaSota NDV Transcriptomic Dataset Used for Cross-Cancer Molecular Validation. DEGs, differentially expressed genes; data from Thanyaphoo et al.²⁴

Public model	RNA-seq time point	Total DEGs	Up DEGs	Down DEGs	72-h total cells (% of mock)	72-h live cells (% of mock)
KKU-055	24 h	368	321	47	23.31	16.77
KKU-100	24 h	1,807	892	915	68.46	60.75

gamma response, TNF-alpha signaling via NF- κ B, IL-6/JAK/STAT3 signaling, inflammatory response, and hypoxia-related programs were activated in both cell lines.²⁴ Thus, the observed cross-model agreement is consistent with the

cell-death phenotype observed in H1299 cells, although it should be regarded as indirect corroboration rather than as direct transcriptomic evidence from H1299 cells themselves (Table 1).

Discussion

The present study demonstrates that the attenuated NDV LaSota vaccine strain exerts time- and MOI-dependent effects in p53-deficient H1299 NSCLC cells, with substantially smaller effects in BEAS-2B bronchial epithelial cells. The concurrent reduction in MTT-based metabolic activity, increased CPE severity, higher Annexin V-positive fractions, suppressed glucose consumption, lactate production and intracellular ATP, and increased HA activity in H1299 cultures provide internally consistent evidence of a preferential *in vitro* response in the malignant cell line. These findings are relevant to ongoing efforts to engineer NDV-based platforms through transgene expression and rational viral modification.²⁸⁻³⁰

The differential response between H1299 and BEAS-2B cells supports, but does not prove, tumor selectivity. Previous work has shown that NDV can preferentially kill malignant cells with altered apoptotic and antiviral responses.³¹ In the present experiments, increasing cell rounding, shrinkage, detachment, syncytium formation, and monolayer loss at higher MOI and longer infection times are consistent with progressively stronger viral cytopathic activity. MTT reduction provides additional evidence of decreased cellular reducing/metabolic activity; however, it should not be equated with direct viable-cell counting, particularly because ATP, glucose consumption, and lactate production were concurrently reduced. Because the study was performed in established monolayer cultures, the magnitude of the observed differential effect should not be extrapolated directly to intact lung tissue or *in vivo* therapeutic safety.

Flow-cytometric findings indicate that programmed cell-death processes are an important component of the H1299 response. Previous studies with LaSota and other NDV strains have documented apoptosis induction in cancer cells,³² and work in lung cancer systems has shown that mitochondrial quality-control pathways can modulate the balance between NDV propagation and intrinsic apoptosis.³³ In the present analysis, Annexin V+/PI- cells were interpreted as early apoptotic, whereas Annexin V+/PI+ cells were reported separately because this double-positive population can include both late-apoptotic and secondary-necrotic cells. The increase in the combined Annexin V-positive fraction (Q2+Q3) therefore indicates greater Annexin V positivity but is not treated as proof that every Q2 cell represents late apoptosis.

The metabolic findings add another dimension to the cellular response. Decreases in glucose consumption, lactate production, ATP, and MTT reduction were greatest under conditions that also produced the strongest cytopathic and Annexin V/PI changes. NDV infection is known to interact with cellular energy metabolism and mitochondrial pathways.³⁴ Nevertheless, reductions in metabolite consumption or

production and MTT signal in a strongly injured culture can partly reflect loss of metabolically active cell mass. Accordingly, the present data support an association between NDV infection and metabolic disruption but do not establish a specific causal metabolic pathway. Future work using normalization to independently measured viable cell number and pathway-specific metabolic-flux assays would help distinguish direct metabolic reprogramming from secondary effects of cell injury or loss.

The HA results showed that in H1299 culture supernatants, hemagglutinating activity increased with MOI at 48 h. The HA method can only show that virus-related hemagglutination occurred and does not differentiate between infectious and non-infectious virus particles; therefore, it cannot be used to infer TCID50 or plaque titers. Consequently, no conclusion can be drawn regarding infectious virus output or its relationship to cytotoxicity from HA alone. Further studies based on direct infectious-virus titration of supernatants at different MOIs and time points will be needed to determine whether infectious viral output correlates with the cytotoxicity observed in cells. Another aspect of NDV that could contribute to its therapeutic potential is the possibility of triggering immune-mediated cell death and antitumor immunity,³⁵ which cannot be assessed *in vitro*.

The external transcriptomic evidence introduces an independent molecular layer over the cellular findings while illustrating that LaSota NDV responses are not uniform across cancer types. The two publicly available CCA models varied considerably in the magnitude of gene-expression changes and in cell survival at 72 h but shared antiviral and apoptosis-related pathways involving DDX58/IFIH1/IRF7, interferon responses, apoptosis, TNF-alpha/NF-κB, and IL-6/JAK/STAT3 signaling.^{35,36} The agreement between apoptosis signaling and the H1299 Annexin V/PI and CPE results increases biological credibility for a conserved death signal associated with LaSota. However, the public data do not confirm the magnitude of the H1299 phenotype, nor do they validate the metabolic endpoints or infectious virus production, because the external models use different cancer genetics, doses, and molecular readouts.

Several limitations were identified. The study used two previously validated cell lines, three independent experiments, and semi-quantitative CPE assessment, without three-dimensional cultures, additional NSCLC genotypes, animal models, or independent direct viable-cell-count assays. Annexin V/PI staining cannot differentiate late apoptosis from secondary necrosis in the double-positive population. Analyses of caspases, interferon pathways, mitochondrial function, and viral gene expression were not included. In addition, infectious virus titers were not determined, and HA was not used as a surrogate for infectious titer; therefore, the relationship between input MOI and productive infection remains undefined. Moreover, certain manufacturer- and

instrument-specific metadata were lost in the archival data available for revision, making them impossible to reconstruct without speculation. Another limitation is that the external transcriptomic evidence was derived from cholangiocarcinoma rather than NSCLC and was curated from published studies rather than generated from matched H1299 RNA-seq. Future work should include RNA sequencing or targeted validation using H1299 and BEAS-2B cells under precisely the same conditions as those outlined in these assays.

Conclusion

The attenuated NDV LaSota strain induced preferential *in vitro* effects in p53-deficient H1299 NSCLC cells compared with BEAS-2B bronchial epithelial cells. Increasing MOI and infection duration resulted in decreased MTT-based metabolic activity, progressive cytopathic changes, increased Annexin V positivity, and reduced glucose consumption, lactate production, and intracellular ATP. HA titers increased with MOI at 48 h, indicating enhanced hemagglutinating activity; however, HA does not measure infectious virus, and no infectious viral replication was demonstrated. Overall, these observations indicate that LaSota NDV causes cellular damage in H1299 cells that is less pronounced in BEAS-2B cells. Further studies are required, including direct infectious-virus titration, alternative viability assays, additional NSCLC models, three-dimensional cultures, and *in vivo* experiments, to confirm the therapeutic potential and safety of this approach.

Authors' Contributions

The conceptualization was done by MG and HEG. The formal analysis and interpretation were done by MG and HEG. The resource and writing-original draft preparation were carried out by MG, HEG and SMY. The writing review and editing were performed by MKh. The supervision was done by SMY. The whole manuscript was read and approved by all the authors.

Ethical Approval

This study used established human cell lines and did not involve the recruitment of human participants, identifiable human-derived specimens, or the collection of personal data; therefore, informed consent was not applicable. Experiments involving live NDV and embryonated chicken eggs were conducted in accordance with institutional laboratory and biosafety procedures and applicable local requirements. As no human participants were involved, no human-participant ethics approval number is applicable to the work described in this manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts of interest.

Declaration of Generative AI and AI-assisted Technologies

Generative artificial intelligence (ChatGPT, OpenAI) was used to assist with language editing, manuscript organization, and reference formatting during revision. In addition, AI-assisted tools were used to arrange and aesthetically enhance the presentation of figures, graphs, diagrams, and other visual elements. These tools did not generate or alter the underlying data, results, or scientific content. The author critically reviewed and verified all scientific content, figures, and images and accepts full responsibility for the final manuscript.

Data Availability

Publicly available data analyzed in this study were obtained from Thanyaphoo et al.²⁴ Raw RNA-seq FASTQ files are available from the NCBI Sequence Read Archive under BioProject PRJNA1114906. Processed data, including differential expression, pathway enrichment, and hub-gene tables, are provided in the PLOS ONE Supporting Information S1–S4 (DOI: 10.1371/journal.pone.0322307). The publicly obtained data used in this research are compiled in Supplementary Dataset S1 (Supplementary_Dataset_S1_NDV_LaSota_Public_Evidence.xlsx).

Acknowledgment

The author gratefully acknowledges the University of Al-Qadisiyah, Al-Diwaniyah, Iraq, for its support of this study.

References

- Bray F, Laversanne M, Sung H, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin.* 2024;74(3):229-63. doi:10.3322/caac.21834
- Herbst RS, Morgensztern D, Boshoff C. The biology and management of non-small cell lung cancer. *Nature.* 2018;553:446-54. doi:10.1038/nature25183
- Tan WL, Jain A, Takano A, Newell EW, Iyer NG, Lim WT, et al. Novel therapeutic targets on the horizon for lung cancer. *Lancet Oncol.* 2016;17(8):e347-e362. doi:10.1016/S1470-2045(16)30123-1
- Russell SJ, Peng KW, Bell JC. Oncolytic virotherapy. *Nat Biotechnol.* 2012;30(7):658-70. doi:10.1038/nbt.2287
- Ribas A, Dummer R, Puzanov I, VanderWalde A, Andtbacka RHI, Michielin O, et al. Oncolytic virotherapy promotes intratumoral T cell infiltration and improves anti-PD-1 immunotherapy. *Cell.* 2017;170(6):1109-19. doi:10.1016/j.cell.2017.08.027
- Fukuhara H, Ino Y, Todo T. Oncolytic virus therapy: a new era of cancer treatment at dawn. *Cancer Sci.* 2016;107(10):1373-9. doi:10.1111/cas.13027
- Pathak U, Pal RB, Malik N. The viral knock: ameliorating cancer treatment with oncolytic Newcastle disease virus. *Life (Basel).* 2023;13(8):1626. doi:10.3390/life13081626
- Zamarin D, Palese P. Oncolytic Newcastle disease virus for cancer therapy: old challenges and new directions. *Future Microbiol.* 2012;7(3):347-67. doi:10.2217/fmb.12.4
- Mehta D, Gujar S, Kumar S. Interplay between viral oncolysis and host immunity in Newcastle disease virus-

- based cancer therapy. *Semin Immunol.* 2026;82:102032. doi:10.1016/j.smim.2026.102032
10. Kooti W, Gouvarchin Ghaleh Esmaeili H, Farzanehpour M, Dorostkar R, Jalali Kondori B, Bolandian M. Oncolytic Newcastle disease virus effects on immune response: a new issue in cancer treatment. *Klin Onkol.* 2023;36(2):124-9. doi:10.48095/ccko2023124
11. Elankumaran S, Rockemann D, Samal SK. Newcastle disease virus exerts oncolysis by both intrinsic and extrinsic caspase-dependent pathways of cell death. *J Virol.* 2006;80(15):7522-34. doi:10.1128/JVI.00241-06
12. Zaher KA, Alrahimi JS, Basingab FS, Aldahlawi AM. Newcastle disease virus virotherapy: unveiling oncolytic efficacy and immunomodulation. *Biomedicines.* 2024;12(7):1497. doi:10.3390/biomedicines12071497
13. Schirmacher V. Oncolytic Newcastle disease virus as a prospective anti-cancer therapy: a biological agent with potential to break therapy resistance. *Expert Opin Biol Ther.* 2015;15(12):1757-71. doi:10.1517/14712598.2015.1088000
14. Alexander DJ. Newcastle disease and other avian paramyxoviruses. *Rev Sci Tech.* 2000;19(2):443-62. doi:10.20506/rst.19.2.1231
15. Wang H, Nan F, Zeng Z, Zhang X, Ke D, Zhang S, et al. Tumor cell vaccine combined with Newcastle disease virus promote immunotherapy of lung cancer. *J Med Virol.* 2023;95(2):e28554. doi:10.1002/jmv.28554
16. Al-Ziaydi AG, Al-Shammari AM, Hamzah MI, Kadhim HS, Jabir MS. Newcastle disease virus suppress glycolysis pathway and induce breast cancer cells death. *Virusdisease.* 2020;31(3):341-8. doi:10.1007/s13337-020-00612-z
17. Zhang Y, Feng S, Yi G, Jin S, Zhu Y, Liu X, et al. Genome-wide screening reveals the oncolytic mechanism of Newcastle disease virus in a human colonic carcinoma cell line. *Viruses.* 2025;17(8):1043. doi:10.3390/v17081043
18. Qu Y, He S, Shen L, Liao Y, Qiu X, Tan L, et al. Oncolytic Newcastle disease virus promotes tumor cell death via the anoikis effector Bit1 translocation. *Virol Sin.* 2025;40(5):842-52. doi:10.1016/j.virs.2025.10.004
19. American Type Culture Collection. NCI-H1299 (ATCC CRL-5803) [Internet]. Manassas (VA): ATCC; [cited 2026 Aug 21]. Available from: <https://www.atcc.org/products/crl-5803>
20. Vander Heiden MG, Cantley LC, Thompson CB. Understanding the Warburg effect: the metabolic requirements of cell proliferation. *Science.* 2009;324(5930):1029-33. doi:10.1126/science.1160809
21. Russell SJ, Peng KW. Viruses as anticancer drugs. *Trends Pharmacol Sci.* 2007;28(7):326-33. doi:10.1016/j.tips.2007.05.005
22. Chu Z, Gao X, Liu H, Ma J, Wang C, Lu K, et al. Newcastle disease virus selectively infects dividing cells and promotes viral proliferation. *Vet Res.* 2019;50:27. doi:10.1186/s13567-019-0644-0
23. National Cancer Institute. Newcastle Disease Virus (PDQ®)-Health Professional Version [Internet]. Bethesda (MD): National Cancer Institute; [cited 2026 Aug 21]. Available from: <https://www.cancer.gov/about-cancer/treatment/cam/hp/ndv-pdq>
24. Thanyaphoo S, Sae-Lee C, Thaopech W, Amornrit W, Junking M, Yenchitsomanus P-T, Pongvarin N. Transcriptome insights into Newcastle disease virus-mediated eradication of cholangiocarcinoma cells. *PLoS One.* 2025;20(5):e0322307.
25. American Type Culture Collection. BEAS-2B (ATCC CRL-3588; manufactured progeny of CRL-9609) [Internet]. Manassas (VA): ATCC; [cited 2026 Sep 12]. Available from: <https://www.atcc.org/products/crl-3588>
26. Khorrami S, Dogani M, Mahani SE, Moghaddam MM, Taheri RA. Neuroprotective activity of green synthesized silver nanoparticles against methamphetamine-induced cell death in human neuroblastoma SH-SY5Y cells. *Sci Rep.* 2023;13(1):11867. doi:10.1038/s41598-023-37917-0
27. Rahmani D, Taheri RA, Moghaddam MM. Targeted delivery of curcumin and CM11 peptide against hepatocellular carcinoma cells based on binding affinity of PreS1-coated chitosan nanoparticles to SB3 protein. *Amino Acids.* 2025;57(1):12. doi:10.1007/s00726-024-03438-x
28. Wu Q, Jin Y, Li S, Guo X, Sun W, Liu J, et al. Oncolytic Newcastle disease virus carrying the IL24 gene exerts antitumor effects by inhibiting tumor growth and vascular sprouting. *Int Immunopharmacol.* 2024;136:112305. doi:10.1016/j.intimp.2024.112305
29. Liu T, Liu Z, Yu D, Jiang S, Yin C, Zhang Y, et al. Development and preclinical evaluation of ovNDV-28: a chimeric Newcastle disease virus expressing human IL-2 for cancer therapy. *Cancer Gene Ther.* 2026;33(1):85-101. doi:10.1038/s41417-025-00981-x
30. Sun J, Wang J, Xiao M, Chen L, Guan Y. Research progress on recombinant NDV in cancer therapy. *Front Immunol.* 2025;16:1735440. doi:10.3389/fimmu.2025.1735440
31. Mansour M, Palese P, Zamarin D. Oncolytic specificity of Newcastle disease virus is mediated by selectivity for apoptosis-resistant cells. *J Virol.* 2011;85(12):6015-23. doi:10.1128/JVI.01537-10
32. Kalantari A, Farashi Bonab S, Keyvanfar H, Mortazavi P. Evaluation of apoptosis induction by Newcastle disease virus LaSota strain in human breast carcinoma cells. *Arch Razi Inst.* 2020;75(3):367-76. doi:10.22092/ARI.2019.125824.1322
33. Meng G, Xia M, Wang D, Chen A, Wang Y, Wang H, et al. Mitophagy promotes replication of oncolytic Newcastle disease virus by blocking intrinsic apoptosis in lung cancer cells. *Oncotarget.* 2014;5(15):6365-74. doi:10.18632/oncotarget.2219
34. Gong Y, Tang N, Liu P, Sun Y, Lu S, Liu W, et al. Newcastle disease virus degrades SIRT3 via PINK1-PRKN-dependent mitophagy to reprogram energy metabolism in infected cells. *Autophagy.* 2022;18(7):1503-21. doi:10.1080/15548627.2021.1990515
35. Gujar S, Pol JG, Kroemer G. Heating it up: oncolytic viruses make tumors 'hot' and suitable for checkpoint blockade immunotherapies. *Oncoimmunology.* 2018;7(5):e1442169. doi:10.1080/2162402X.2018.1442169
36. Sinkovics JG, Horvath JC. Newcastle disease virus in cancer therapy. *Cancer Detect Prev.* 2000;24(2):127-35. doi:10.1371/journal.pone.0322307