# ONCOLYTIC ACTIVITY OF ATTENUATED MEASLES VACCINE STRAIN AGAINST BREAST CANCER CELL LINE (MDA) AND REF NORMAL CELL LINE

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Received: 02/05/2024
Accepted: 10/05/2024

**Online ISSN: 2735-3540**

## ABSTRACT:

***Background and objective***: Breast cancer continues to be one of the primary causes of death for women globally. There is a pressing need to find novel therapeutic techniques despite advancements in cancer therapeutics. Here, we examined the anticancer potential of a new viral cause: an attenuated measles strain genetically modified to produce carcinoembryonic antigen (CEA) against breast cancer and derived from the Edmonston vaccination lineage.

***Subject and Methods:*** The VERO-hSLAM cells were use spread the MV. The virus's ability to destroy human Breast cell line (MDA) & REF normal cell line was tested using the crystal violet cytotoxicity assay. Study effected measles vaccine strain against breast cancer cell line (MDA) and REF normal cell line by used different multiplicities of infection (MOIs) from 0.1, 0.5, 1, 3, 5, 10, 15 and 20. for different exposure time (24, 48 and 72 hr.).

***Results:*** At 24, 48, and 72 hours after infection, the data demonstrated a strong cytopathic effect that included widespread syncytia development and enormous cell death.

The outcomes demonstrated that the live attenuated measles virus vaccination may be administered infect and kill breast cancer cells. At 24, 48, and 72 hours after infection, the infected cell line exhibited a significant cytopathic effect, with a noteworthy impact on MDA cells (IC50 values were 4.928 for 24 hours, 2.153 for 48 hours, and 0.2433 for 72 hours).

***Conclusion:*** MeV vaccination has strong oncolytic action against breast cancer cells, as demonstrated by the current study. Virotherapy active and apoptotic instruction results suggest the virus's therapy probable in the treatment of breast cancer.

**Keywords:** Cancer therapy, measles vaccine, tumor targeting, virotherapy.

## 1. INTRODUCTION:

A naturally occurring or genetically engineered virus known as an oncolytic virus has the ability to specifically replicate and destroy cancer cells while posing no damage to healthy cells [(1)]. Oncolytic virotherapy uses the virus as an active pharmacological agent, as opposed to gene therapy, which only uses it as a carrier for transgene delivery. Adenoviruses, hepatitis, WND, Yellow fever., and dengue fever viruses were among the wild-type or naturally attenuated viruses used in a number of clinical trials conducted between 1950 and 1980 to cure cancer [(2)]. However, because there was no known mechanism to lower virulence while still permitting viral replication in cancer cells at the time, these viruses were not considered

therapeutic agents. Giving oncolytic viruses immune-stimulatory or cancer-treatment genes might be beneficial. In terms of the oncolytic virus's mode of action, it disrupts the tumor cell's regular physiological function by seizing control of the protein factory after infection and preventing the tumor cells from producing enough protein to meet their needs [3]. The most common adenocarcinoma in the world, breast cancer is still regarded as one of the most serious and potentially fatal illnesses that affect women, even with the use of current treatment options like hormone-therapy, radiation, chemo-therapy, and surgery [4]. Breast cancer is incurable, hard to cure, and needs special, potent therapies. Measles virus (MV), VS virus, vaccines, HSV, and reo-virus are among the viruses that have been preclinical investigated as oncolytic viral therapy agents with extremely encouraging outcomes [5]

Oncolytic viruses infect cancer cells by binding to their receptors on the target cell's surface or by fusing two membranes together [6]. Because live attenuated MV vaccine strains can target a variety of human tumor types, they have garnered a lot of attention [7].

Developments in gene editing and viral treatment have produced a range of vectors belonging to multiple viral families that can replicate exclusively in tumor cells. Due to the way viruses are delivered, infected, and replicate, oncolytic virotherapy is a promising new treatment option for cancer patients, particularly those with incurable tumors [8].

The measles virus used in the Edmonston vaccine is very effective and selective against a variety of tumor types. This virus or its genetically altered strains cause tumor cells to release viral proteins that enable the cells to merge with nearby cells to create syncytia, which eventually die [9].

Adenovirus, (VACV), (HSV), (VSV), measles virus (MeV), and reo-virus are among the viruses that have been thoroughly investigated in breast cancer[s] study to evaluate the vireo activities. MeV is a member of the Paramyxoviridae family's order Mono-negavirales and genus Morbillivirus [10]. MV interacted by three distinct types of human cell receptors via (CD - 46), (SLAM) or (CD - 150), and the host cell receptor. Nectin4 was recently discovered to operate as a receptor for both latent and measles virus's vaccine strain [11]. Since SLAMm and C-D 46 typically overexpressed through tumors cell, MV has selectively targeted cancered cell by inhibiting their growth through oncogenic cell [12]. MV vaccination (Edmons Strain) have studied cure numerous cancers like glioblastoma, epithelial ovarian carcinoma, prostate cancer and hepatocellular carcinoma hematological cancer [13].

## AIM OF THE STUDY:

This study aimed to investigates:

1. Virus proliferated using VERO-H-SLAM cell.
2. Oncolytic activity of attenuated measles vaccine strain against breast cancer cell line (MDA).
3. Approve of MV vaccine strain don't affect on normal cell.

## 2. MATERIALS AND METHODS:

### *2.1. Cell - lines*

Three cell lines (VERO-hSLAM), (MDA), and (normal cell line (REF)), were providing via cell banks in biotechnology Research Center, Nahrin University.

VERO-hSLAM, MDA, and REF cells were as cultures in RPM-1640 culture contain 10 percent serum fetal calf.

### *2.2. MV*

Attenuate measles virus vaccinated (Institute India PVT. LTD., India), were acquired from the Division of Vaccination, Department of Public Health, Alnajaf Al Ashraf. The virus was grown in 5 milliliters of free serum RPMI-1640 media at 37 degrees Celsius using VERO hSLAM cells (4.5 x 106 cells / T75 flasks). cultured have removed and swapped with fresh, free serum culture after two hours. Following a 5-7-day incubation period at 37 °C, the cells were extracted at 80% to 90% syncytia. Finally, three cycles of freezing and thawing were used to gather the multiplying viruses. VEROhSLAM cells in 96-well plates were used in a 50% endpoint dilution test to determine the titer of viral stocks.

### *2.3. Cytotoxicity of the MV.*

The crystal violet test, which uses violet dye that is simply engaged via live cell and decreased by mitochondria dehydrogenase activity, was used to determine the viability of breast cancer (MDA) and normal cell (REF). 10,000 cells per well were cultivated on 96-well plates, and different multiplicities of infection (MOIs) of the measles vaccine were added (0.1, 0.5, 1, 3, 5, 10, 15, and 20). Cells in PBS were rinsed after 72 hours, and then 100 µg/ml solution was added for up to two hours. Spectrophotometric analysis was used to estimate the extinction values at 620 nm photometrically after the supernatant was discarded and the cells were allowed to dry [14].

The following formula was used to calculate the cytotoxicity percentage or rate of growth inhibition:

$$G.I = \frac{\mathrm{A} - B}{\mathrm{A}} \mathrm{X}\, 100$$

everywhere A and B represent the mean optical densities of the treated and control cells, respectively [15].

The inhibitory multiple of infections killing 50% infection cell remained determined using the software Graphpad Prism 8.4.3 (type 2018).

### 2.4. Statistical analysis

All three-count observation readings were presented as mean ± SD. Utilizing statistical significance evaluation, multiple comparisons of a single sample t test were carried out to show organizational differences (GraphPad Prism version 8.4.3 for Windows by GraphPad Software, San Diego, CA.), To evaluate the cytotoxicity and apoptosis of MeV on MDA and REF cell lines in vitro, statistical significance was defined as $p < 0.05$.

### Ethical Consideration:

this study was approved by College of Health and Medical Technology/ Kufa (7\37\3294 in 29\10\2023).

## 4. RESULTS:

### 4.1. Cytotoxic effect MeV on MDA.

The inhibitory dose 50 (IC50) was calculated by inoculating the measles virus vaccine at various MOIs (0.1, 0.5, 1, 3, 5, 10, 15, 20) on breast and normal cell lines. Crystal violet dye was added after 24, 48, and 72 hours of incubation. Growth inhibition (GI) and IC50 were calculated by comparing the proportion of viable cells following MV infection of controlled cell that hadn't been exposing via viruses. The resulted exposed that MV vaccination of cytotoxicity affected on the breast malignant cells line (MDA) but not on normal cells, with IC50 values of (4.928 for 24 hr., 2.15 for 48 hr., and 0.243), inhibition effect of virus is increasing via growing virus MOI from MOI 5 and decreased more than MOI 5. Figure (1).

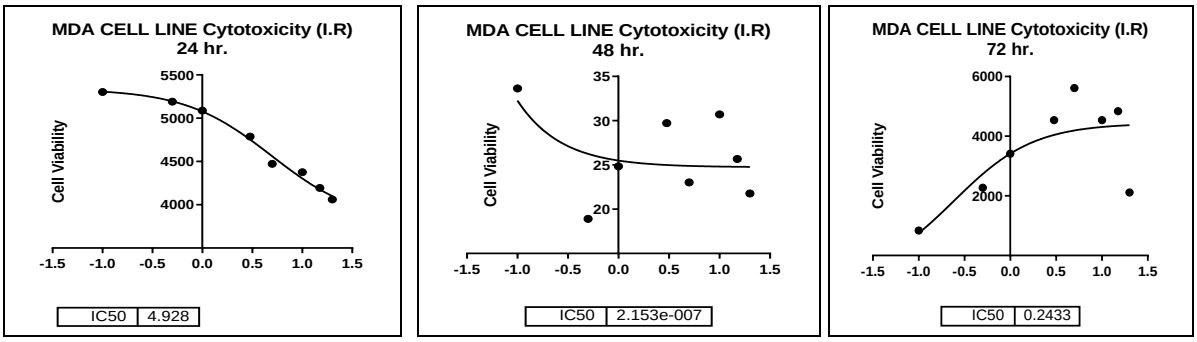


**Figure 1:** The MeV vaccine's in vitro anti-cancer activity was tested on breast cancer (MDA) cell lines, and its IC50 was determined after 24, 48, and 72 hours.

The production of multinucleated giant cells, or syncytia, as a result of cell-cell fusion, was the typical CPE of MeV. The live attenuated measles virus replicated and killed tumor cells with excellent efficiency. After being exposed for 24, 48 and 72 hours, cancer cells had several morphological changes, including rounding shrinkage, aggregation, and hollow areas filled with debris from lysed and dead cells. In contrast, uninfected cells did not exhibit any morphological changes Figure (2).

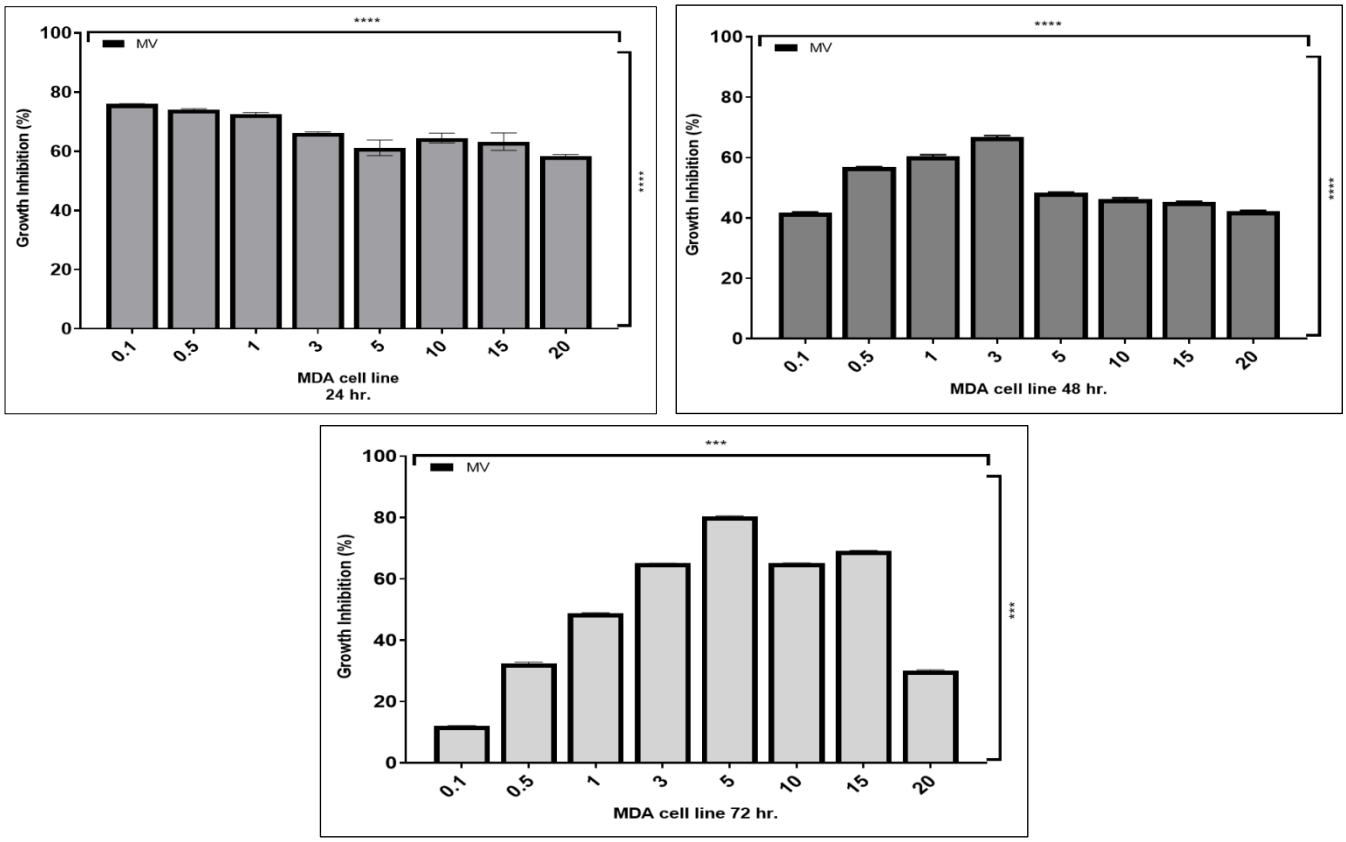


**Figure 2:** The MeV vaccine's in vitro anti-cancer activity was tested for different exposure time 24, 48 and 72 hours on MDA cell line.

### 4.2. Cytotoxicity effect of measles attenuated vaccine on REF normal cell line

Cytotoxic effect of measles attenuated vaccine do not effect on REF normal cell line in different exposure time, this results shown in Figure (3).

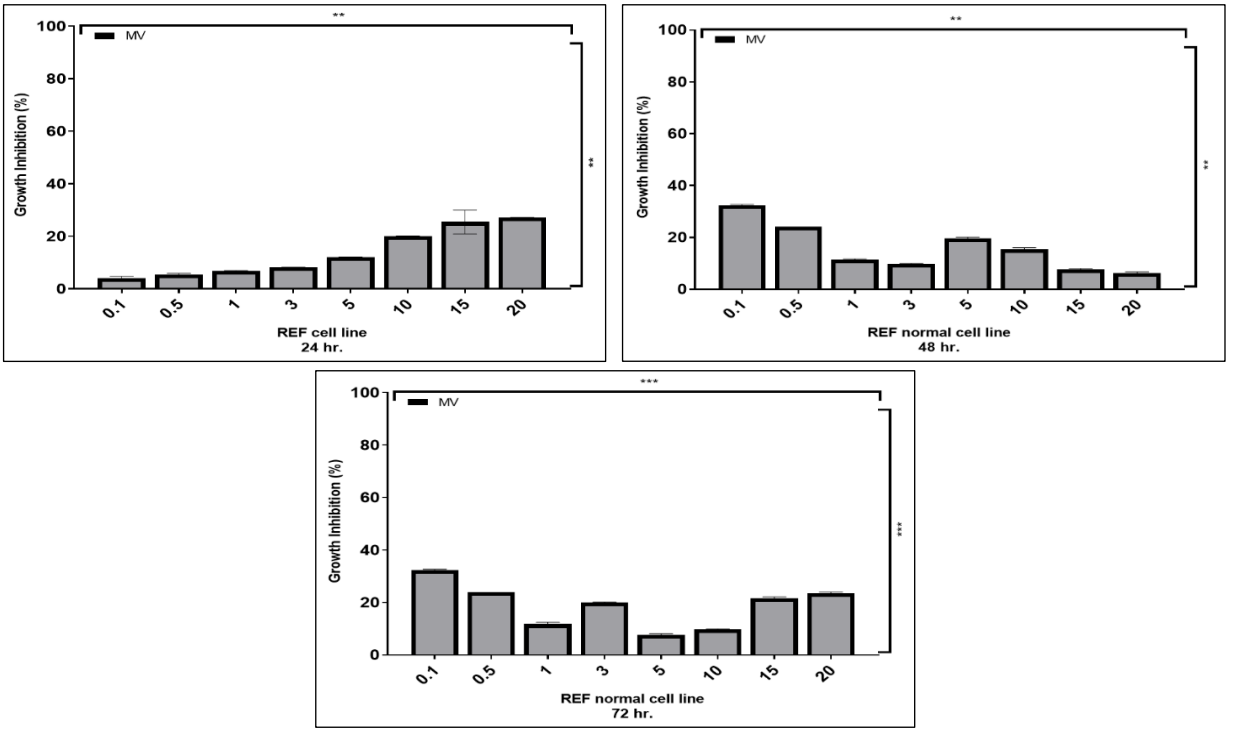


**Figure 3:** MeV vaccination's in vitro effects on normal cell lines (REF) lasting 24, 48, and 72 hours.

## 5. DISCUSSION:

The purpose of this study was to explore the effect of virotherapy with attenuated measles virus vaccine on breast cancer cells.

To determine the inhibitory dose 50 (IC50), several MOI (0.1, 0.5, 1, 3, 5, 10, 15, 20) of measles virus vaccination were inoculated MDA. The crystal violet test was carried out 24, 48 and 72 hours after incubation. Growth inhibition (GI) and IC50 are percentages of viable cells after MV effected compare with un-affected cells that's not exposure to virus. The resulted exposed that numerous breast cancer cell lines are cytotoxically affected by the MeV vaccine [16].

The outcome shown that the MV vaccine effectively infects and kills breast cancer cells. It also significantly induces a cytopathic effect in the infected cell lines 24–48–72 hours after infection, with a particularly strong effect on MDA cells.

The measles virus (MV) binds to the (CD-46) using hemagglutinin (H) protein. Cell membrane and the fusion (F) protein cooperate to allow the virus to enter the vulnerable cell more easily [17].

Syncytia, or multinucleated giant cells, are formed when newly generated glycoproteins accumulate in tissue of the infection cells and merge with near cells. After a few days of infection, syncytia are typically lysed in the following steps. As a result, the virus can propagate from one cell to another without requiring the full generation of viral particles [10]. Syncytial infection cells are unable to proliferate and thus don't contribute to development of additional malignant cell. Moreover, if several syncytia remained eliminated, they capacity release free virus that could infect other cells. The current study's cytotoxicity test results for the MeV vaccine on infected MDA cell lines demonstrated dose-dependent inhibition rates, with the maximum inhibition rate occurring at multiplicity of infection (MOI = 20) and the lowest inhibition rate occurring at (MOI = 0.1). Prior research has demonstrated that tiny amounts virus that have infection cells cannot be used to infect

further cells, potentially resulting in an imbalance in virotherapy. In vivo [13].

The cytotoxic effect of MeV and apoptosis indicate that, when compared to control cells, MeV therapy significantly reduced cancer cell viability and increased apoptosis. MeV may multiply itself to cause malignant cell death. This viral replication featured gave constant contribution doses that remains until it is terminated by an immunity responses or a lacked susceptible cell [18]. Particularly, this might be related to MeV's entrance mechanism. Due to MeV's lytic nature, malignant cells that it replicates in are killed and lysed.

A normal growth, because of the metabolic of normal cell is low Compared to cancer cells, the mechanism of cytotoxicity of MeV is dependent on mechanisms that involve, genotoxic effects that lead to cell cycle arrest at G2/M phase, and (ROS) Reactive Oxygen Species, JNK signaling and mitochondria dependent apoptosis ,that produce radicals inside the cell. The therapeutic index is low for the metastatic REF cells line [19].

## 6. Conclusion:

MeV vaccination shows strong oncolytic action against breast cancer cells, according to the study. These findings of apoptosis induction and oncolytic activity support the virus's therapeutic utility in the management of breast cancer and point to encouraging clinical results. The results shown MeV vaccine don't effect on normal cell line.

## 7. Potential Conflicts of Interest :

Regarding these studies, the authors have no conflicts of interest.

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## دراسة الفعالية الفايروسية للقاح فايروس الحصبة ضد خط خلايا سرطان الثدي (MDA) والخط الطبيعي REF

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**الخلفية والهدف:** يظل سرطان الثدي أحد الأسباب الرئيسية لوفاة الإناث في جميع أنحاء العالم على الرغم من التقدم في علاجات السرطان، وهناك حاجة ملحة لتطوير أساليب علاجية جديدة. في هذه الدراسة، قمنا بدراسة الفايروس كعلاج جديد للورم، وهو سلالة مخففة من فيروس الحصبة المستمدة من سلالة لقاح إدمونستون المعدلة وراثيا لإنتاج المستضد السرطاني المضغي (CEA) ضد سرطان الثدي.

**الموضوع والطرق:** تم تكاثر الفيروس في خلايا VERO-hSLAM. حيث تم استخدام صبغة البنفسج البلوري في اختبار السمية الخلوية واختبار قدرة الفيروس على قتل خطوط خلايا الثدي البشرية (MDA) وخط الخلايا الطبيعية REF. حيث تم استخدام تعددات مختلفة من العدوى (MOIs) من 0.1، 0.5، 1، 3، 5، 10، 15 و 20. لأوقات التعرض المختلفة (24، 48 و 72 ساعة.).

**النتائج:** أظهرت النتائج وجود تأثير كبير للاعتلال الخلوي يتكون من تكوين syncetia واسع النطاق وموت الخلايا على نطاق واسع من خلال اوقات تعريض مختلفة 24 و 48 و 72 ساعة. من الاصابة.

أظهرت النتائج أن خلايا سرطان الثدي يتم إصابتها وتدميرها بشكل فعال بواسطة لقاح فيروس الحصبة الحي الموهن، وتسبب في تأثير اعتلال خلوي كبير في خط الخلايا المصابة بعد 24، 48 و 72 ساعة. من العدوى مع تأثير ملحوظ على خلايا MDA من خلال حساب IC50 لتأثر الفايروس وكان 4.928 لمدة 24 ساعة، 2.153 لمدة 48 ساعة. و0.2433 لمدة 72 ساعة).

**الاستنتاج:** أثبتت الدراسة الحالية أن لقاح MeV له نشاط قوي ضد خلايا سرطان الثدي من خلال تحفيز الموت المبرمج للخلايا وعدم تأثير الفايروس المضعف على الخلايا الطبيعية.