

# Combined Action of Virus Injection and Local Tumor Irradiation on Tumor Growth in Mice

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## ABSTRACT

*The dynamics of SCCVII transplantable tumor growth in C3H/H mice was determined after local tumor irradiation and/or virus (NDV LaSota) i.p. injection. The virus applied alone significantly suppressed tumor growth, particularly until the 19<sup>th</sup> day after tumor transplantation. Local irradiation with 30 Gy resulted in tumor disappearance followed with its regrowth about 15 days later. However, if the virus was injected after the irradiation, there was no tumor growth until the end of the 31 day observation period. It should be noted that virus application prior to local irradiation did not have any additional influence on tumor growth. Thus, the pronounced efficacy of virus applied after tumor irradiation deserves attention. It is possible that the virus injected after irradiation induced a chain of cytokine production joining the action of tumor destruction induced by irradiation. This should be further studied in clarifying the approaches to combined tumor therapy with possible cell-free vaccine production.*

**Key words:** mouse tumors, tumor irradiation, NDV virus, combined antitumor therapy

## Introduction

The problem of tumor growth control is still an open question. Numerous approaches were attempted and some of them are still in clinical practice, as surgery, radiotherapy and chemotherapy. However, besides their antitumor action, a pronounced detrimental effects on normal tissue were observed. The larger the dose (or »radical« surgery), the better control of tumor was achieved, yet also the more pronounced destruction of normal structures. So, the combination of these classical approaches involving lowered doses is the basis of numerous protocols in treating tumorous patients.

Besides the above mentioned classical treatments several others were tested in experimental models and then in clinical approaches. One of them is the application of a particular virus as a single anticancer agent. Several oncolytic viruses have been identified that selectively attack cancer cells but spare normal cells. Some of these viruses, classified as natural tumor selective viruses (e.g., Newcastle disease virus, some strains of reoviruses, vesicular stomatitis virus and parvovirus), are used without any genetic manipulation in cancer treatment<sup>1-5</sup>. Others are genetically modified (e.g., herpes simplex virus type 1 and adenovirus) to increase selectivity and to

mediate oncolytic effects in animal models and in human clinical trials<sup>6-9</sup>.

Newcastle disease virus (NDV), an avian paramyxovirus, is classified as a virus with inherent oncolytic activity. NDV strains have been shown to directly destroy different cancer cells in vitro while being significantly less aggressive toward normal cells<sup>10,11</sup>. Preclinical data also suggested that NDV may enhance both T-cell specific antitumor immunity and tumor nonspecific immunity possibly either through induction of cytokines such as tumor necrosis factor (TNF), interferon, IL-1, IL-6 and TNF related apoptosis inducing ligand (TRAIL), or through the activation of tumoricidal macrophages<sup>12,13</sup>.

Even though there are many studies dealing with the oncolytic properties of NDV as a single anticancer agent, no studies have been carried out on the simultaneous use of NDV and classical approach in cancer treatment such as radiotherapy. So, this study is designed to evaluate a multimodal cancer therapy approach utilizing a naturally attenuated strain of NDV, LaSota strain, in combination with radiotherapy in an animal tumor model.

## Materials and Methods

### Animals

Male mice of C3H/H strain were used in the experiments. The animals were about 3 months old and weighed 20–23 g. They were provided standard diet (Mucedola, Italy) and tap water ad libitum. The light regime was natural. Animals were treated according to the Animal Welfare Regulations.

### Virus

An apathogenic strain of Newcastle disease virus, LaSota strain, (hereinafter LS) was used in the study. The virus was cultivated in the allantois fluid of fertilized ten-day old SPF (Specific Pathogen Free) chicken eggs. Allantois fluid was collected and EID<sub>50</sub> was determined. Virus titre was adjusted on 10<sup>9</sup> EID<sub>50</sub>/ml allantois fluid and subsequently allantois fluid was lyophilized. (Pestikal LaSota spf; Veterina d.o.o, Zagreb, Croatia).

### Tumor treatment

C3H/H mice were subcutaneously injected into the right thigh with  $5 \times 10^5$  SCCVII mouse carcinoma cells in 100  $\mu$ L RPMI using a tuberculin syringe and a 25-gauge needle. Eight days later, when the tumors were 5–7 mm in diameter, particular tumor treatments were performed. In the first experiment, tumors were irradiated with 6 Gy and LaSota virus ( $5 \times 10^8$  EID<sub>50</sub>/mouse) was applied 24 and 48 hours after the irradiation. In the second experiment, tumors were irradiated with 10, 20, and 30 Gy, respectively and LaSota virus ( $1 \times 10^9$  EID<sub>50</sub>/mouse) was injected intra peritoneally in all mice, either 24 hours prior to or 24 hours after local tumor irradiation. The control group received in the same way 100  $\mu$ L of saline. Tumor growth dynamics was followed by measuring three tumor diameters (a,b,c) with a caliper (Lange Skin fold Caliper, Cambridge Scientific Industry, USA) starting on day 10 after inoculation, and subsequently on every 2<sup>nd</sup> or 3<sup>rd</sup> day until day 31. Tumor volume was calculated by using the formula  $a \times b \times c \times 0.526$ .

### Irradiation

Mouse right thigh (with growing tumor) was irradiated by using linear accelerator Varian Clinac 1800 with a 20 MeV electron beam, dose rate 2 Gy/min at room temperature. The other leg was protected with lead blocks. Homogeneous tumor irradiation was obtained by using 1 cm bolus.

### Statistical analysis

The differences between effects of particular treatments were statistically evaluated by an unpaired Student t-test. This test was performed using a standard statistical package, STATISTICA for Windows.

## Results

C3H/H mice were injected with squamous cell carcinoma (SCCVII) cells into the right thigh.

Tumor irradiation with 6 Gy followed with two LS injections

Preliminary experiment included local tumor irradiation with the dose of 6 Gy followed by i.p. LS injections ( $5 \times 10^8$  EID<sub>50</sub>/mouse) 24 and 48 hours later. As presented in Figure 1, irradiation with 6 Gy significantly postponed tumor growth ( $p < 0.01$ ) during the observation period (27 days), and the addition of LS was even more effective. In comparison to irradiation only, the differences were significant ( $p < 0.01$ ) between days 12 and 21 after tumor transplantation. LS virus applied alone significantly influenced tumor growth, especially until day 19 after tumor transplantation.

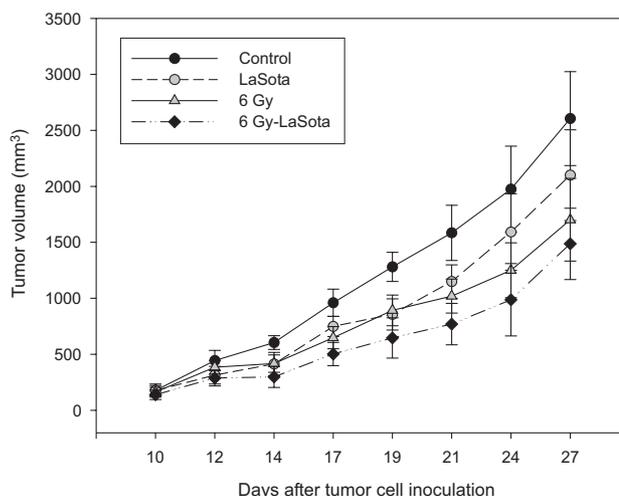


Fig. 1. The influence of local tumor irradiation and LaSota virus application on the growth of SCCVII mouse carcinoma transplanted in C3H/H mice. LaSota virus ( $5 \times 10^8$  EID<sub>50</sub>/mouse) was injected 24 and 48 hours after local tumor irradiation with the dose of 6 Gy. The irradiation was performed at day 8 after tumor transplantation.

In the following experiments, local irradiation dose was increased (10, 20 and 30 Gy respectively) and virus was injected as a single dose ( $1 \times 10^9$  EID<sub>50</sub>/mouse) either 24 hours prior to, or 24 hours after irradiation.

### Tumor irradiation with 10 Gy and/or LS injection

As presented in Figures 2a and 2b, tumor growth was slightly postponed following 10 Gy application ( $p < 0.01$ ) between days 22 and 31 after tumor transplantation, but not if LS was applied alone. LS injection prior to 10 Gy irradiation (Figure 2a) did not additionally influence tumor growth rate. However, in comparison with the irradiation only, the addition of LS after the irradiation caused a significant reduction in tumor growth ( $p < 0.05$ )

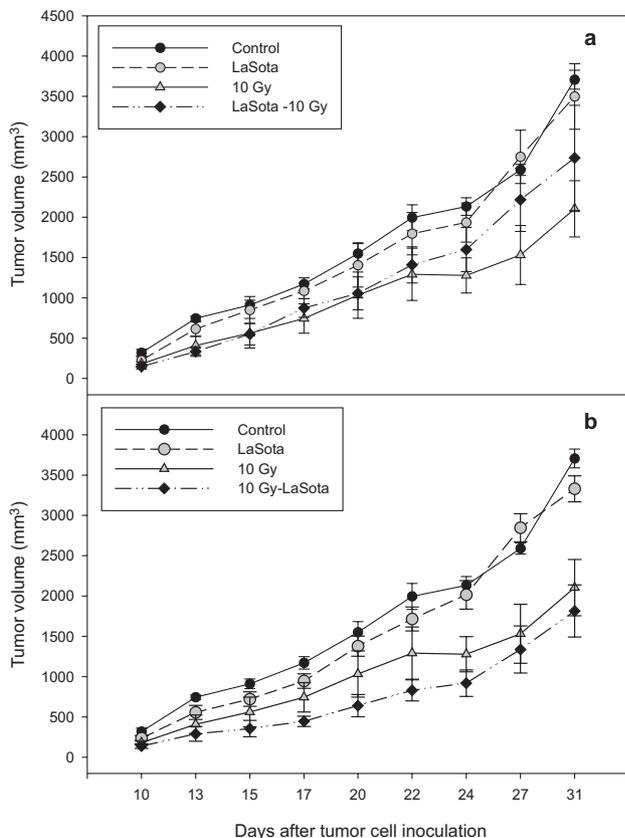


Fig. 2. The influence of local tumor irradiation and LaSota virus application on the growth of SCCVII mouse carcinoma transplanted in C3H/H mice. Tumors were irradiated with the dose of 10 Gy and LaSota virus ( $1 \times 10^9$  EID<sub>50</sub>/mouse) was injected either 24 hours prior to (a) or 24 hours after (b) the irradiation.

between days 15 and 24 after tumor transplantation (Figure 2b).

#### Tumor irradiation with 20 Gy and/or LS injection

Tumor irradiation with 20 Gy significantly ( $p < 0.0001$ ) suppressed its growth, as presented in Figures 3a and 3b. During the first week after the treatment there were no signs of tumor growth, which was followed by an increase in tumor growth rate, but less pronounced than in the controls. The application of LS either prior to (Figure 3a) or after the irradiation (Figure 3b) did not additionally affect tumor growth.

#### Tumor irradiation with 30 Gy and/or LS injection

As presented in Figures 4a and 4b, tumor growth was significantly suppressed ( $p < 0.0001$ ) during approximately two weeks after the treatment. Tumor re-growth was noticed later except in the mice treated with LS following tumor irradiation (Figure 4b). Only in this group

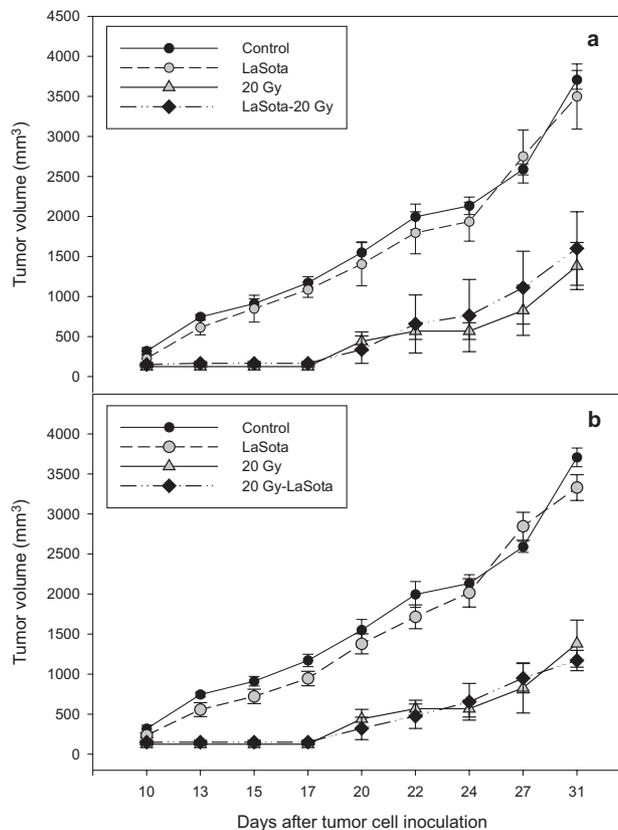


Fig. 3. The influence of local tumor irradiation and LaSota virus application on the growth of SCCVII mouse carcinoma transplanted in C3H/H mice. Tumors were irradiated with the dose of 20 Gy and LaSota virus ( $1 \times 10^9$  EID<sub>50</sub>/mouse) was injected either 24 hours prior to (a) or 24 hours after (b) the irradiation.

of animals was tumor growth completely controlled until the end of the observation period (day 31).

## Discussion

Numerous data point to beneficial effects of local tumor irradiation as more useful with the increase in the irradiation dose<sup>14</sup>. However, normal surrounding tissue is damaged too, limiting the dose of irradiation of the tumor<sup>14</sup>. So, combined approaches, sufficiently detrimental to tumor but sparing normal tissues, are the choice in a successful treatment<sup>15</sup>. Currently there is a satisfactory choice of protocols for a particular tumor, but also a permanent need to improve their efficiency. On the other hand, particular viruses were shown to destroy selectively tumor cells in vitro<sup>11</sup>. Further, the use of virus vaccines was beneficial in particular clinical approaches<sup>3,4,9</sup>.

Besides direct destruction of tumor cells, as was shown in the cultures, virus induced modification of host immune reaction against the tumor seems to be important. It is well known that the key steps in the generation of an immune response to tumor cells include the loading of tumor antigens onto antigen presenting cells, present-

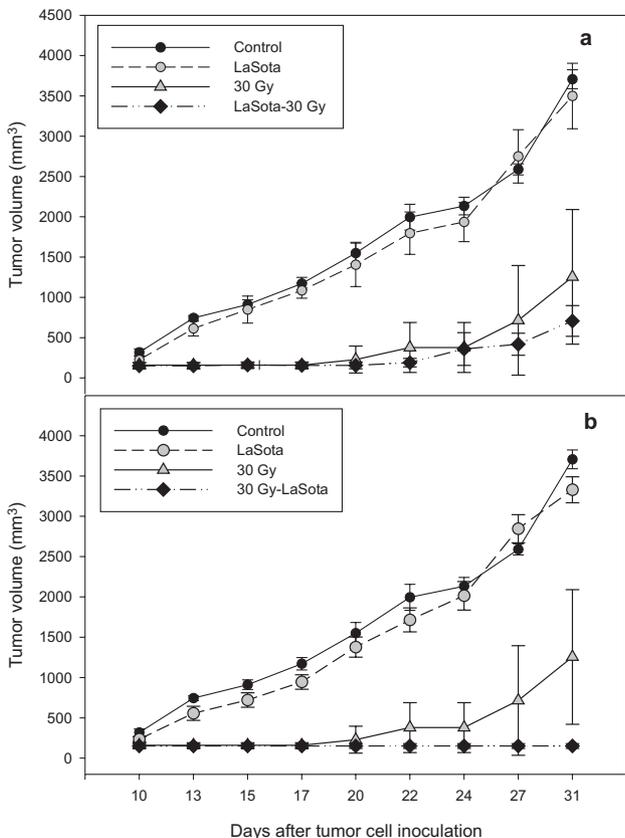


Fig. 4. The influence of local tumor irradiation and LaSota virus application on the growth of SCCVII mouse carcinoma transplanted in C3H/H mice. Tumors were irradiated with the dose of 30 Gy and LaSota virus ( $1 \times 10^9$  EID<sub>50</sub>/mouse) was injected either 24 hours prior to (a) or 24 hours after (b) the irradiation.

ing the antigen in an appropriate immune stimulatory environment, activating cytotoxic lymphocytes and blocking autoregulatory control mechanisms<sup>16,17</sup>.

Numerous immunologically active vaccines usually require costly and laborious ex vivo cellular cultures, whereas cell-free vaccines that can be directly adminis-

tered from an easily stored and transported vials are usually less immunologically active but more suitable for widespread clinical testing<sup>18</sup>.

The pronounced efficacy of viruses applied after tumor irradiation deserves particular attention. It is considered that virus stimulates TNF related apoptosis by inducing ligand – TRAIL<sup>12,19</sup>, which can lead to cell destruction. Further, virus induces the production of particular cytokines (IL-1, IL-6, TNF, INF  $\alpha$ , INF  $\beta$ )<sup>20,21</sup>, leading to NF kappa B expression<sup>22</sup>. However, the cells expressing kappa B were shown to be more resistant to irradiation, which might be the explanation for elimination of destructing effect of irradiation if virus had been injected previously<sup>23</sup>.

According to the data of Raju et al.<sup>24,25</sup>, the production of NF kappa B was increased in irradiated tissue (tumor) about 2 hours after irradiation. It was followed by a return to the basal level about 8 hours after irradiation<sup>24,25</sup> and, if the irradiation was repeated, there was no increase in NF kappa B dynamics.

However, the virus injected i.p. after tumor irradiation might induce cytokine production, joining the action of tumor destruction induced by previous irradiation. By increasing irradiation dose, tumor destruction is more pronounced and additional application of virus (penetrating better in the cells damaged by irradiation!?) is also more effective. The time schedule of LS injection following irradiation seems to be detrimental and should be further studied in this model, particularly by using fractionated irradiation. Is it possible that virus stimulates the attraction of antigen presenting cells in tumor that is being destroyed after irradiation? In the case of positive answer this could be one of new advances in cancer vaccine productions. These approaches require a more precise knowledge of the generation of cellular immune response to tumor antigens, together with the current ability to closely monitor cellular immune response<sup>26,27</sup>. This will likely provide powerful cell-free vaccine in the near future.

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## KOMBINIRANO DJELOVANJE VIRUSA I LOKALNOG ZRAČENJA NA RAST TUMORA U MIŠEVA

### SAŽETAK

Praćena je dinamika rasta karcinoma SCCVII presađenog u miševе soja C3H/H nakon lokalnog zračenja tumora i/ili intraperitonejske (i.p.) primjene virusa. Primjena samog virusa značajno je usporila rast tumora do 19. dana nakon ubrizgavanja tumorskih stanica. Lokalno zračenje tumora dozom od 30 Gy rezultira nestankom tumora do 15. dana, nakon čega je uslijedio njegov ponovni rast. Međutim, ako je virus primijenjen nakon zračenja tumorski rast nije zabilježen do 31. dana kada je eksperiment završen. Primijećeno je da virus primijenjen prije lokalnog zračenja tumora nema dodatni utjecaj na rast tumora. Ova jasno izražena učinkovitost primjene virusa nakon lokalnog zračenja tumora zaslužuje pozornost. Moguće je da virus primijenjen nakon zračenja inducira produkciju citokina koji pomažu destrukciju tumora induciranu zračenjem. Ovo bi trebalo nadalje razraditi u kombiniranim terapijskim pristupima.