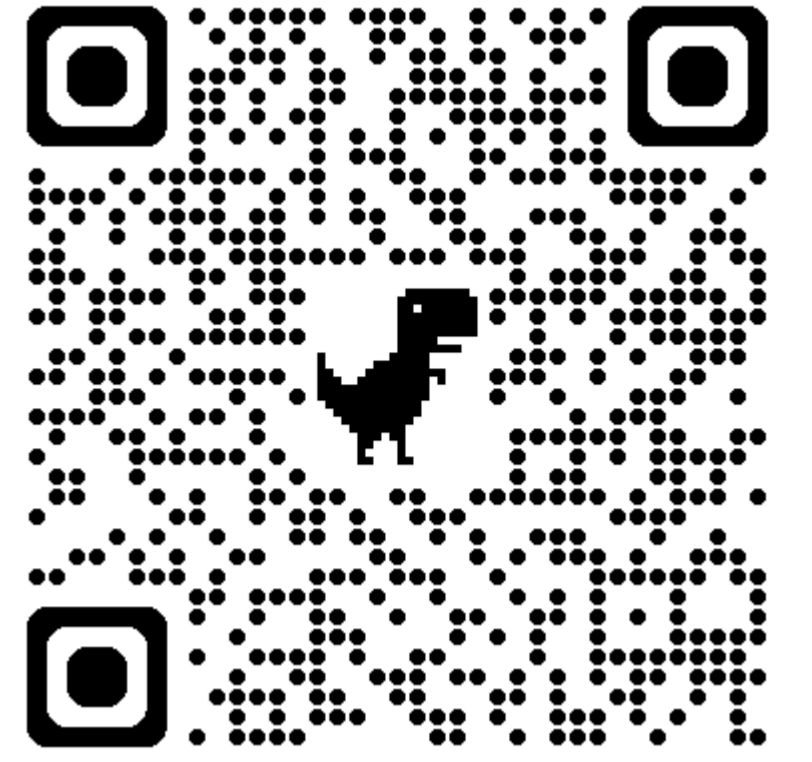
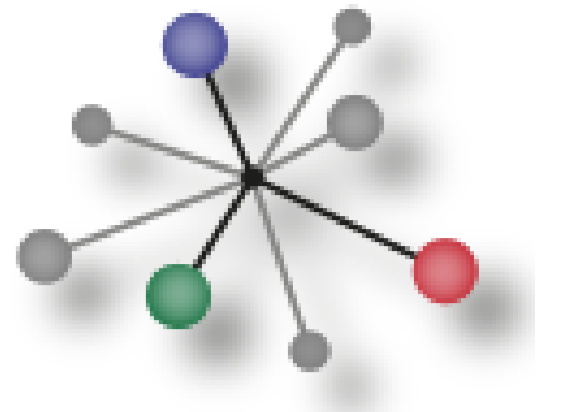


# AN UNCONVENTIONAL CASE STUDY OF NEOADJUVANT ONCOLYTIC VIROTHERAPY FOR RECURRENT BREAST CANCER

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## INTRO

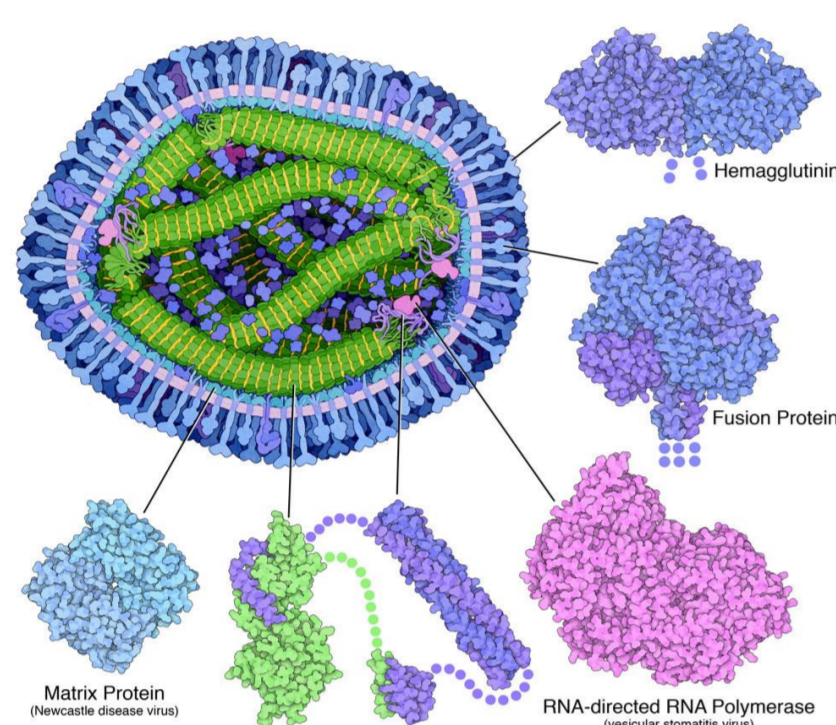
Oncolytic virotherapy (OVT) has been considered for almost 100 years a promising experimental modality for the treatment of cancer, but has only recently gained acceptance in clinical practice. The slow progress of OVT in clinical testing is partially due to inappropriateness of preclinical models to fully predict effectiveness and safety in humans. In addition, clinical trials have typically been focused on patients with metastatic cancer who have already passed many rounds of chemotherapy, radiotherapy and/or immunotherapy, and who therefore often have a compromised immune system and overall health status. Recently, the development of OVT has been directed also towards its use as neoadjuvant therapy before the surgery, in the early-stage cancer patients. Several clinical trials have been initiated, some of them targeting also breast cancer (BC).

## AIM

The aim is to present the human case of successful self-treatment of a localized breast cancer recurrence by experimental OVT, which may offer important insights for overcoming some of the challenges in OVT development: sequential administration of different viruses to avoid potential OVT inhibition mediated by antiviral immune response and an intensive schedule of repeated intratumoral virus administrations to enhance antitumour effect.

## VIRUSES FOR OVT

enveloped, non-segmented, negative-stranded RNA viruses capable to infect epithelial cells

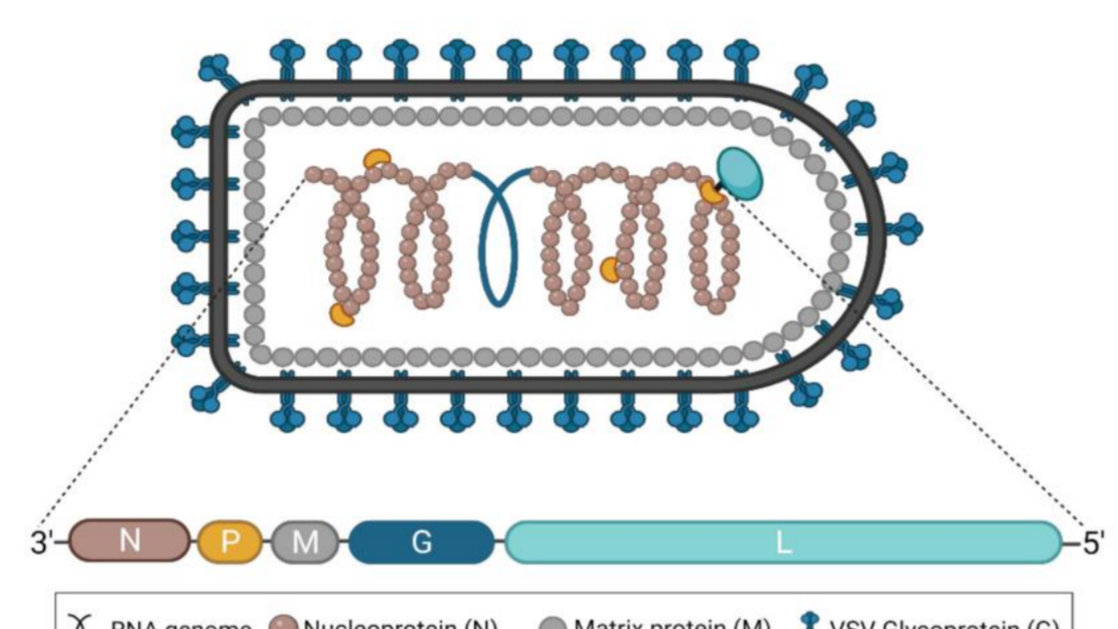


By Gotsell D. Molecule of the Month 2019 at PDB-101

### Measles virus (MeV)

Edmonston-Zagreb vaccine strain  
family *Paramyxoviridae*, genus *Morbillivirus*  
human virus, attenuated, non-pathogenic  
recombinant variant in clinical trials for BC

MeV was propagated in MRC-5 or Vero cell culture (as indicated in Figure 1B). Infections were performed by Edmonston-Zagreb vaccine strain working seed (Institute of Immunology, Inc.) at a cell density of 75,000 cells/cm<sup>2</sup> for MRC-5 or 100,000 cells/cm<sup>2</sup> for Vero cells, with a MOI of 0.01 in MEM + 10% FBS. After 24 hours of cultivation at 36 °C, the medium was replaced with MEM without FBS and cultivated at 32 °C, 5% CO<sub>2</sub> until the appearance of cytopathic changes. Cell culture supernatant was collected, clarified by centrifugation (10 min, 1400 g) and filter (0.45 µm)-sterilized (**OncoMeV**).



From Liu et al. Pathogens 2021

### Vesicular stomatitis virus (VSV)

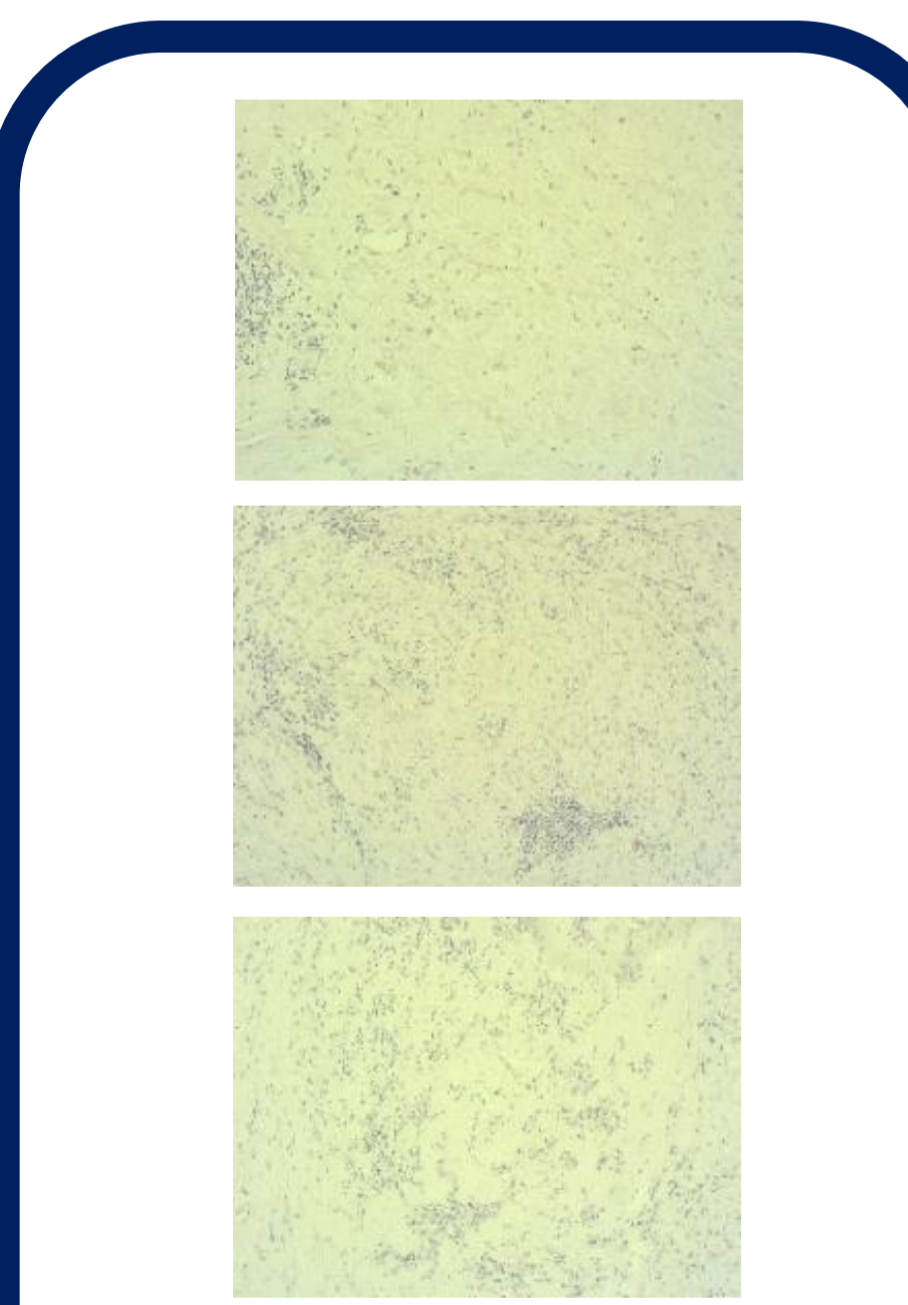
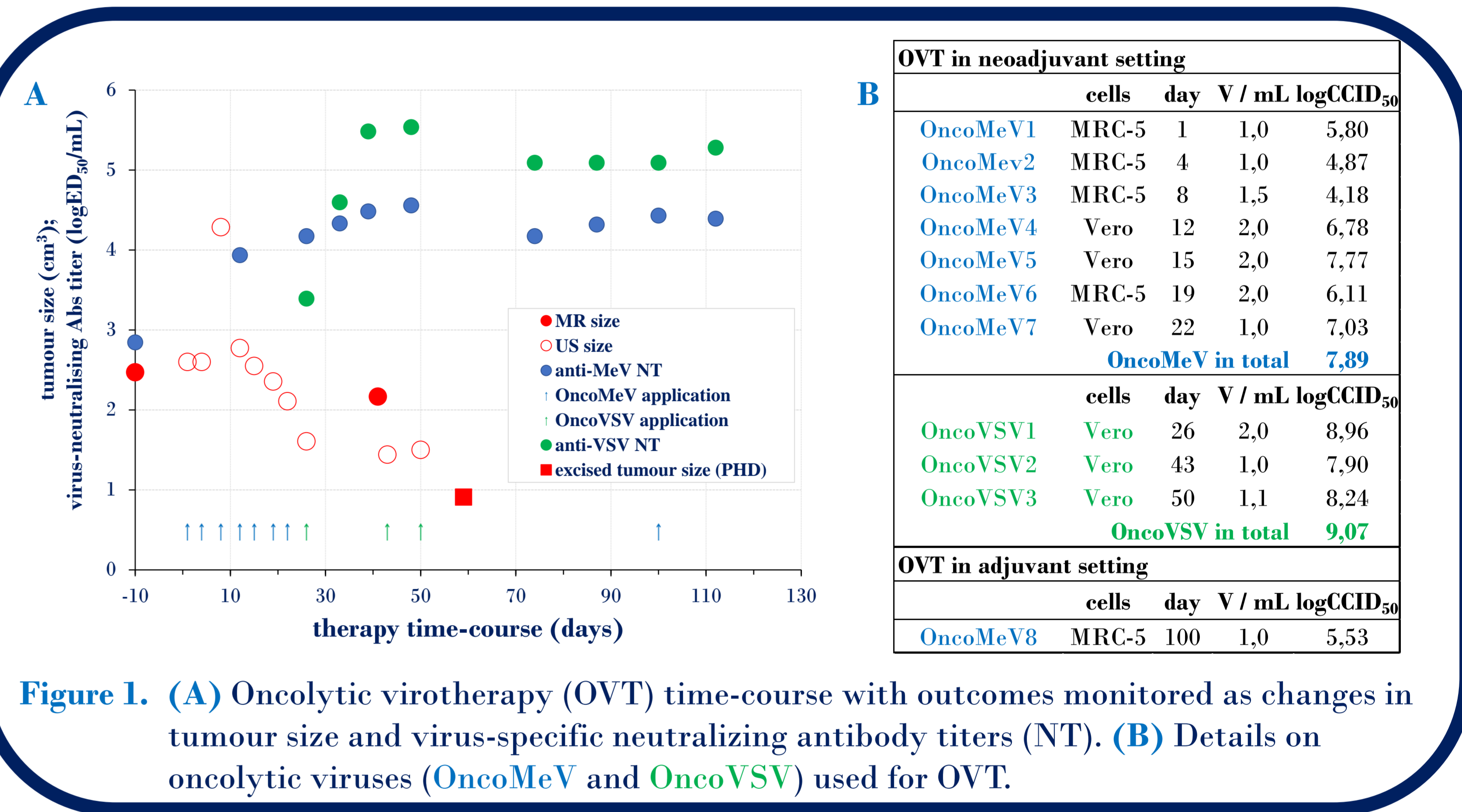
Laboratory-adapted Indiana strain  
family *Rhabdoviridae*, genus *Vesiculovirus*  
animal virus, mildly pathogenic in humans  
effective in murine model of BC

VSV was propagated in Vero cell culture. Infections were performed by laboratory-adapted Indiana strain working seed (Institute of Immunology, Inc.) at a cell density of 100,000 cells/cm<sup>2</sup>, with a MOI of 0.5 in MEM + 10% FBS. After 4 hours of cultivation at 37 °C, the medium was replaced with MEM without FBS and cultivated for 24 hours and 37 °C, 5% CO<sub>2</sub> until the appearance of cytopathic changes. Virus suspension was collected from the cell culture supernatant after centrifugation (10 min, 1400 g) and was sterilized by 0.45 µm filtration (**OncoVSV**).

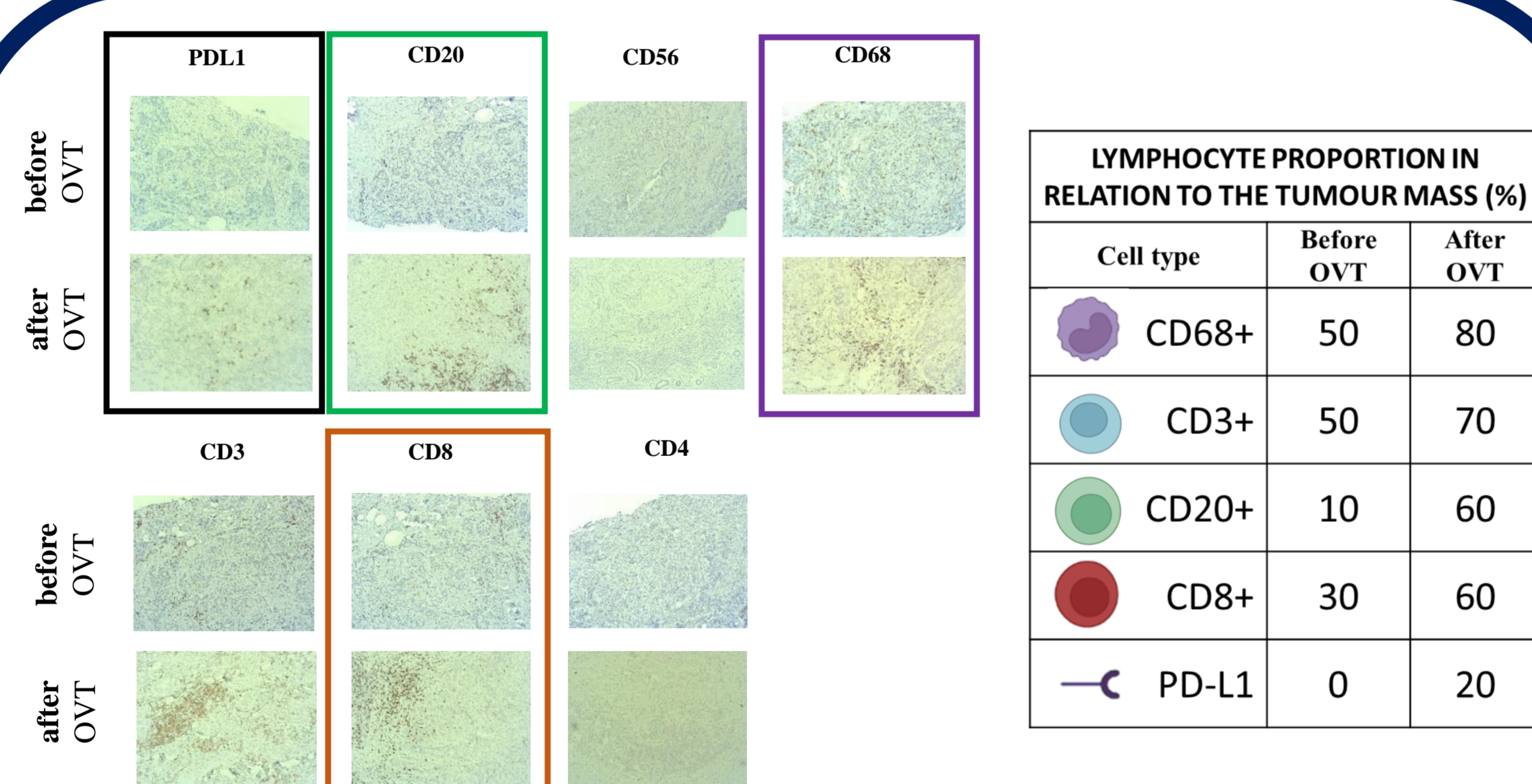
## CASE HISTORY

- 08/2016.** the whole breast was hardened, painful, swollen (overnight)  
Dg. several foci of invasive breast cancer, small, largest 0.7 cm  
triple-negative breast cancer  
80% of channels filled with DCIS  
Th. mastectomy plus sentinel lymph node (negative) → **Local recurrence after 20 months**  
DC chemotherapy in adjuvant setting
- 09/2018.** small recurrence (below 0.5 cm) on the site of surgical suture  
Dg. local recurrence, invasive triple-negative breast cancer  
Th. surgical excision → **Local recurrence after 21 months**  
small seroma remained on the site of the excision  
no chemotherapy or radiotherapy (per the wish of the patient)
- 08/2020.** seroma became carcinoma, increased in size from 1.2 cm to 2 cm in 8 months  
skin infiltration and spreading into pectoral muscle  
Dg. Locoregional recurrence, invasive HER3+++ breast cancer  
Th. Experimental neoadjuvant OVT using **OncoMeV** and **OncoVSV** → **Disease-free after 48 months**

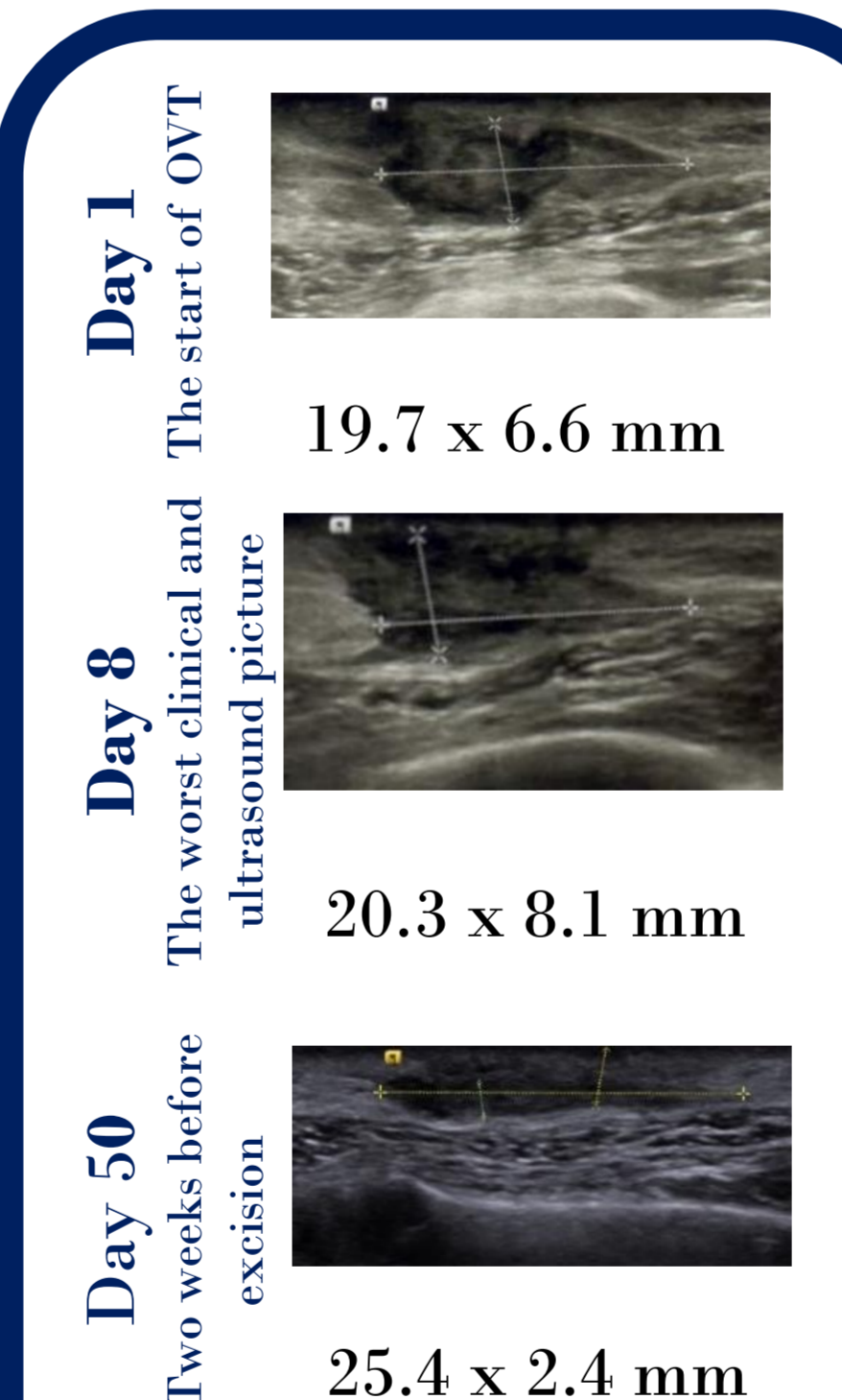
## RESULTS



**Figure 3.** PH analysis of the tumour bed after OVT, demonstrating places emptied from malignant cells.



**Figure 4.** Immunohistochemistry staining of tumour slices before and after OVT with anti-PDL1, anti-CD20, anti-CD3, anti-CD8, and anti-CD4, anti-CD56 and anti-CD68 antibodies. Brownish, black dots demonstrate positive staining.



**Figure 2.** US monitoring of tumour changes at indicated days during OVT

**Table 1. Tumour properties before and after intratumoural OVT**

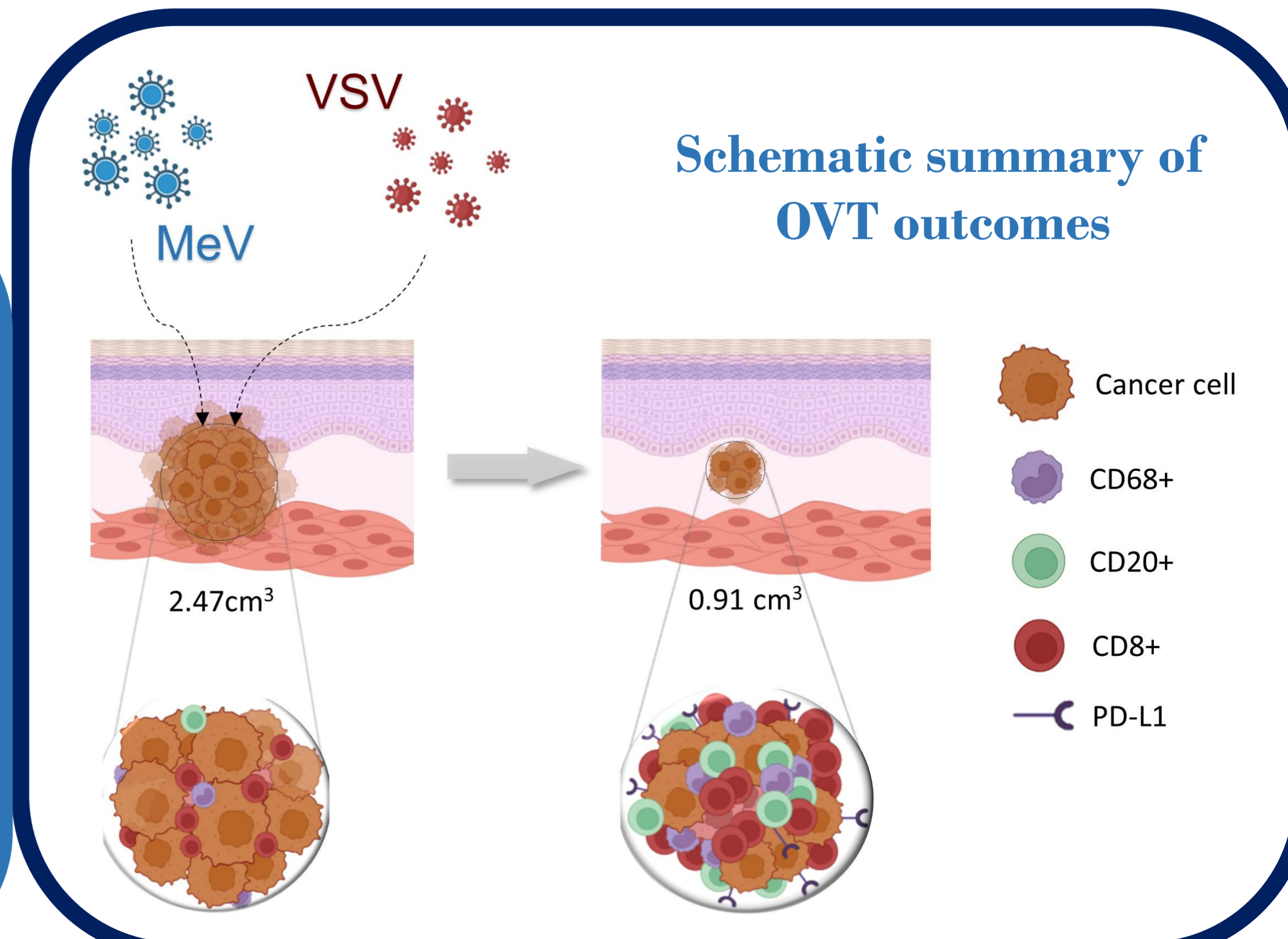
	before	after
Size	19.7 x 6.6 mm	25.4 x 2.4 mm
Texture	Hard, fixed nodule with overlying inflamed skin	Much smaller and softer mobile nodule with no skin inflammation
Ultrasound appearance	Hypoechoic, spiculated mass	Less hypoechoic, less spiculated, better circumscribed and substantially flattened
Location	Skin infiltration (US, MR) and spreading into pectoral muscle suspected (MR diagnosed, PET-CT suspected; US denied)	Tumour only in subcutis; no skin or muscle infiltration by tumour cells (PHD)

## SIDE EFFECTS

At the beginning tolerable painful administration  
Occasionally transient local erythema that resolved spontaneously  
Single episode of systemic side effects fever and rigors with onset 12 hrs after first VSV administration

## CONCLUSIONS

**Eight weeks of neoadjuvant OVT, devoid of any significant toxicity, resulted in:**  
**Short-term benefits**  
tumour shrinkage from 2.5 to 0.9 cm<sup>3</sup> (Figures 1 & 2) and improved clinical picture (Table 1)  
elimination of majority of cancer cells (Figure 3)  
adaptive immunity generation as evidenced by strong lymphocyte (CD8+ and CD20+) infiltration (Figure 4)  
**Middle-term benefits**  
at least 48 months in remission, after two previous recurrences in 20-month intervals  
**OVT might be attractive neoadjuvant therapy for the treatment of early-stage solid cancers, due to its efficacy and tolerability**



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