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Phase 1, open-label, multicenter, dose escalation safety and tolerability study of oncolytic virus OVV-01 in advanced solid tumors

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ABSTRACT

Background OVV-01 is a genetically engineered vesicular stomatitis virus oncolytic virus designed to selectively amplify in tumor cells and express tumor-associated antigen NY-ESO-1. This study was designed to evaluate the safety, tolerability, and efficacy of OVV-01 in patients with advanced solid tumors.

Methods This is a phase 1, first-in-human, open-label, multicenter study of OVV-01 in patients with advanced solid tumors. OVV-01 was intratumorally injected biweekly (every two weeks, Q2W), 3 weeks after the first dose for a total of six doses. Dose escalation follows a 3+3 design at four doses of 6×10^7 Plaue-Forming Unit (PFU), 6×10^8 PFU, 6×10^9 PFU, and 1.2×10^{11} PFU. The primary endpoints were safety and tolerability. The second endpoints included overall response rate (ORR) and disease control rate (DCR) of OVV-01, by investigators per Response Evaluation Criteria in Solid Tumors V.1.1.

Results 18 patients were enrolled into four dose groups, among whom 6 were soft tissue sarcoma (STS). No dose-limiting toxicities and treatment-related severe adverse events were observed. 11 patients were evaluated for efficacy, and the ORR was 27.3%, and the DCR was 63.6%. Among the four evaluable patients with advanced STS, the ORR was 75%. Two patients with STS achieved CR at doses above 6.0×10^9 PFU.

Conclusions The intratumor injection of OVV-01 was safe and well-tolerated in patients with advanced solid tumors. A significant response was observed in patients with STS.

Trial registration number NCT04787003.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Oncolytic viruses (OVs) are emerging as a promising class of immunotherapy, designed to selectively replicate in and destroy tumor cells while stimulating antitumor immunity. Genetically modified OVs expressing immune-modulating molecules or bispecific/trispecific T-cell engagers have shown promising efficacy in various tumors.

WHAT THIS STUDY ADDS

⇒ We designed OVV-01, an OV engineered to express NY-ESO-1, and conducted a phase 1, first-in-human study in patients with advanced solid tumors. We found this strategy was safe and well-tolerated, achieving an overall response rate (ORR) of 27.3%, and a disease control rate of 63.6%. Notably, an ORR of 75% and a complete response rate of 50% were achieved in patients with soft-tissue sarcoma (STS).

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ In this phase 1 study, OVV-1 administered intratumorally showed manageable toxicity, and responses were promising in patients with advanced solid tumors, especially for patients with STS.

INTRODUCTION

Cancer remains one of the leading causes of death globally, with advanced solid tumors posing significant therapeutic challenges.[1] While traditional therapies such as chemotherapy, targeted therapy and immune-checkpoint inhibitors have shown effectiveness, resistance frequently develops. Sarcomas, characterized by their diverse histological subtypes, pose additional therapeutic challenges due to their inherent resistance to standard treatments.[2] Consequently, there is an urgent need for innovative therapeutic approaches.

Oncolytic viruses (OVs) offer a novel option for antitumor therapy for patients resistant to traditional treatments. OVs are viruses that can selectively replicate in and destroy tumor cells, inducing immunogenic cell death (ICD), and enhancing antitumor immunity.[3–5] OVs can be categorized into naturally existing viruses and genetically modified viruses. Although naturally existing viruses have shown antitumor activities in preclinical studies and clinical trials, the infection specificity and immune response have often been

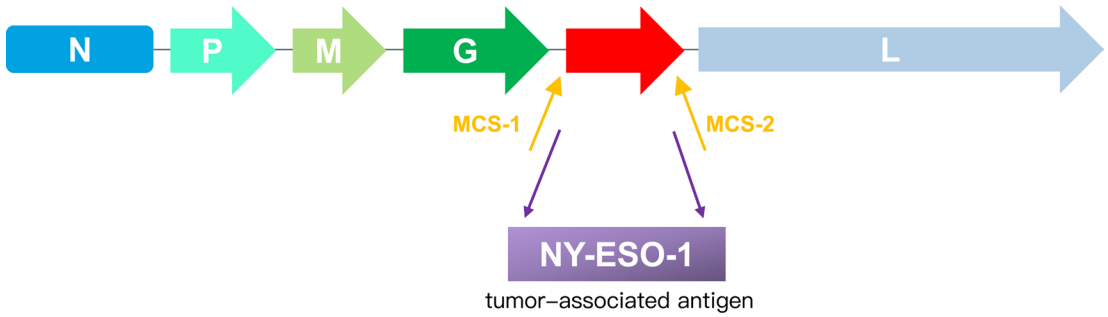

Figure 1 Structure of OVV-01. The genome of VSV is approximately 11.2 kilobases (kb) in length and comprises five genes that encode the five proteins N, P, M, G, and L. The genes are tightly arranged with no overlapping regions and contain conserved promoter sequences at both ends. OVV-01, a genetically engineered VSV oncolytic virus, was constructed by inserting the tumor antigen NY-ESO-1 gene between the viral G and L protein genes through molecular biology techniques. VSV, vesicular stomatitis virus.

unsatisfactory.[6–8] To address these limitations, genetic engineering strategies have been employed. These strategies include attenuating viral pathogenesis,[9] arming OVs with immune-modulating molecules such as cytokines or chemokines,[10 11] and modifying OVs to express bispecific/trispecific T-cell engagers.[12 13]

Cancer testis antigens (CTAs) are tumor-associated antigens known for their high immunogenicity.[14] Among CTAs, NY-ESO-1 has been considered one of the most immunogenic antigens, capable of eliciting both humoral and cellular immunity.[15 16] Currently, strategies targeting NY-ESO-1 are under active investigations, including NY-ESO-1 vaccines and engineered T cell receptor-T cell (TCR-T).[17–20] However, NY-ESO-1 expression is often heterogeneous and may be downregulated under treatment pressure,[21] highlighting the need for new anti-NY-ESO-1 strategies.

Vesicular stomatitis virus (VSV) is an attractive candidate as OV due to its high specificity for tumor cells and non-pathogenicity.[22 23] Additionally, humans are not natural hosts of VSV, thus lacking pre-existing immunity.[24] The relatively small VSV genome allows for the accommodation of functional transgenes. Based on such futures, we developed OVV-01, a genetically engineered VSV OV designed to selectively amplify in tumor cells and express NY-ESO-1 (figure 1). This design aims to harness the antitumor properties of VSV while simultaneously enhancing NY-ESO-1 expression in tumor cells and boosting the antigen-specific immune response. In this first-in-human, phase 1 trial, we evaluated the safety and tolerability of OVV-01 administrated intratumorally and explored the antitumor efficacy of OVV-01 in patients with advanced solid tumors, including sarcomas.

METHODS

Patients

Eligibility criteria included an age between 10 and 75; histologically or cytologically confirmed advanced solid tumor; failed standard treatment or standard treatment unsuitable for medical reasons; measurable disease according to Response Evaluation Criteria in Solid Tumors (RECIST), V.1.1; an Eastern Cooperative Oncology Group performance status of 0–2; and adequate organ functions.

Key exclusion criteria included untreated active brain metastases, radiotherapy of the target lesion within 2 months before OVV-01 injection, systemic antitumor therapy within 4 weeks before OVV-01 injection, active infection or unexplained fever >38.5°C prior to first dose. Patients with active autoimmune diseases were excluded as well.

Study design and intervention

This is a phase 1, first-in-human, open-label, multicenter dose escalation trial of OVV-01, designed to evaluate the safety, tolerability, and efficacy of OVV-01 in patients with advanced solid tumors. Dose escalation follows a "3+3" design. The planned dose levels were 6.0×10^{7} PFU/subject, 6×10^{8} PFU/subject, 6×10^{9} PFU/subject, and 1.2×10^{11} PFU/subject. OVV-01 was administered intratumorally biweekly (every two weeks,Q2W) 3 weeks after the first dose for a total of six doses. If none of the first three patients experienced dose-limiting toxicities (DLTs), the dose would be escalated to the next level. Otherwise, three more patients would be enrolled to further evaluate the safety and tolerability of OVV-01 injection. Full study design is provided in the protocol (online supplemental file 1).

The volume of injection was determined by the size of the injection lesion (≤0.5 cm, up to 0.1 mL; >0.5 to ≤1.5 cm, up to 0.5 mL; >1.5 to ≤3.0 cm, up to 1 mL; >3.0 to ≤5.0 cm, up to 1.5 mL; >5.0 cm, up to 3 mL). The injection lesion size should be evaluated 24 hours before each dosing. Injection of multiple lesions was allowed, with a maximum of 10 lesions.

Endpoints

The primary endpoints were safety and tolerability, including the exploration of DLT, maximum tolerated dose, adverse events (AEs) and treatment-related AEs. The AEs were graded with the Common Terminology Criteria for Adverse Events, V.5.0. DLT was defined as any of the following events within 3 weeks after the single dose or 2 weeks after multiple doses, that is, within 5

weeks of the first administration that was considered to be treatment-related: grade 4 neutropenia lasting over 7 days; neutrophil count <1.0×10^9/L, with a single temperature measurement >38.3°C or ≥38.0°C for more than 1 hour; grade 4 thrombocytopenia; grade 3 thrombocytopenia with bleeding; any other grade 4 hematologic toxicity resulting in treatment delay >14 days or requiring permanent discontinuation of the study drug; grade 3 rash over 3 days or grade 4 rash; grade 3 vomiting or diarrhea over 3 days; grade 3 or higher influenza-like symptoms over 7 days; any other grade 3 or higher non-hematologic toxicity; treatment interruption due to toxicity for more than 14 days.

The secondary endpoints included objective response rate (measured at week 6, 16, and 24), disease control rate (DCR) (measured at week 24), duration of response, progression-free survival rate at 24 weeks, and overall survival rate at 24 weeks. The response was measured based on CT or MRI images and assessed by investigators according to RECIST V.1.1.

Biodistribution and virus shedding assessment

The biodistribution of OVV-01 was evaluated by detecting the virus complementary DNA concentration in the peripheral blood by quantitative PCR (qPCR). Virus shedding was measured in patients' serum, saliva, urine and stool samples by median tissue culture infective dose ($TCID_{50}$) or qPCR. Serum samples were collected 15 min, 1 hour, 3 hours, 12 hours, 24 hours after each dosing, while saliva, urine and stool samples were collected 15 min, 1 hour, 3 hours, 12 hours, 24 hours, 48 hours after each dosing.

Immune phenotype assessment

Peripheral whole blood samples were processed for immunophenotyping using a standardized flow cytometry protocol. 30 µL of whole blood was incubated with anti-CD45 FITC, anti-CD3 BV650, anti-CD8 BV510, anti-CD45RO BV421, anti-CD45RA PE, anti-CCR7 PE-Cy7, and anti-CD27 APC fluorochrome-conjugated antibodies (BD Biosciences) at room temperature in the dark for 17±2 min. Erythrocyte lysis was performed using 2 mL of Lysing Solution with 12±2 min of dark incubation, followed by centrifugation (250 g, 4°C, 5 min) and two phosphate-buffered saline (PBS) washes under identical conditions. The final cell pellet was resuspended in 300 µL PBS. The flow cytometer was configured with fluorescence channels FITC, Violet660, KO525, PB450, PE, PC7, and APC.

Lymphocyte populations were initially gated on $CD45^+$ events (FITC vs SSC plot), followed by sequential identification of $CD3^+CD8^+$ T cells within the Violet660 (CD3) versus KO525 (CD8) scatter plot. Within CD3+CD8+ T cells, memory T-cell subsets were resolved using a dual-step strategy: $CD45RO^+CD45RA^-$ cells were gated in the PB450 (CD45RO) versus PE (CD45RA) quadrant, and $CD27^-CCR7^-$ subsets were further defined within the APC (CD27) versus PC7 (CCR7) plot. Gating accuracy was validated using Immuno-Trol control cells (Beckman Coulter), ensuring proper identification of CD3+CD8+ T cells and fluorescence compensation. Data analyses were carried out with CytExpert software.

Statistical analysis

The statistical analysis population sets were defined as below:

Safety set (SS): all subjects who received at least one dose of the study drug and have post-dose safety evaluation data were included in this set. Full analysis set (FAS): all subjects who received at least one dose of the study drug were included in this set. The cut-off date for the data analysis was March 14, 2024. The statistical analysis was performed using SAS V.9.4.

Ethical approval

This trial was conducted in accordance with the International Council for Harmonization—Good Clinical Practice guidelines, with applicable local regulations and with the ethical principles laid down in the Declaration of Helsinki. The study protocol was approved by the institutional review board or ethics committee for each participating center.

RESULTS

Patient characteristics

From June 14, 2021, through January 2, 2024, a total of 22 patients were screened, and 18 patients were enrolled in the study (figure 2). The patients were treated in four dose groups: 6×10^7 PFU/subject (n=4), 6×10^8 PFU/subject (n=4), 6×10^9 PFU/subject (n=7), and 1.2×10^{11} PFU/subject (n=3). Of the 18 patients, 10 were women and 8 were men, with a median age of 55.5 years (range 13–69 years). Tumor types included breast cancer (n=3),

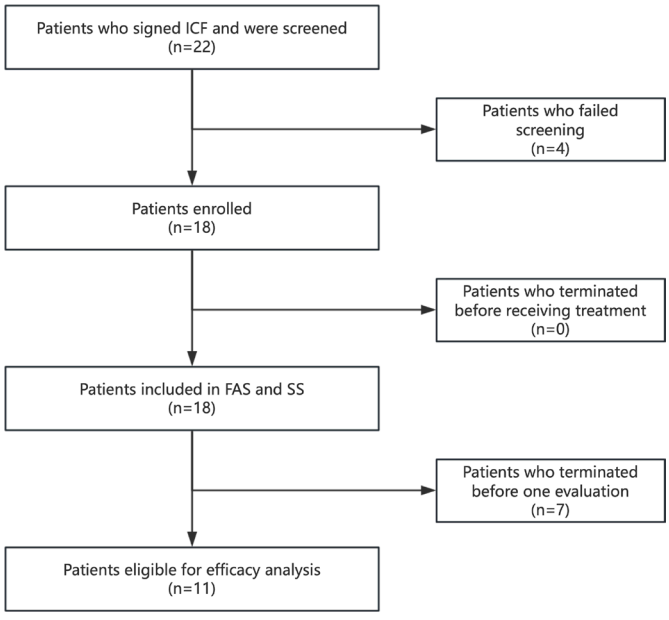

Figure 2 Study profile. FAS, full analysis set; ICF, informed consent form; SS, safety set.

Table 1 Baseline character of patients

	6×10^7 PFU (N=4)	6×10^8 PFU (N=4)	6×10^9 PFU (N=7)	1.2×10^{11} PFU (N=3)	Total (N=18)
Age, years, median (range)	50 (33–69)	60 (46–65)	52 (13–69)	55 (50–59)	55.5 (13-69)
Gender, No. (%)					
Male	1 (25.0)	2 (50.0)	2 (28.6)	3 (100.0)	8 (44.4)
Female	3 (75.0)	2 (50.0)	5 (71.4)	0	10 (55.6)
Tumor type, No. (%)					
Breast cancer	2 (50.0)	1 (25.0)	0	0	3 (16.7)
Colorectal cancer	1 (25.0)	1 (25.0)	0	0	2 (11.1)
Hepatocellular carcinoma	0	1 (25.0)	1 (14.3)	0	2 (11.1)
Cholangiocarcinoma	1 (25.0)	0	0	0	1 (5.6)
Uterine leiomyoma	0	0	1 (14.3)	0	1 (5.6)
Soft-tissue sarcoma	0	1 (25.0)	3 (42.9)	3 (100)	7 (38.9)
Endometrial stromal sarcoma	0	0	1 (14.3)	0	1 (5.6)
High-grade soft tissue sarcoma	0	1 (25.0)	0	0	1 (5.6)
Dedifferentiated liposarcoma	0	0	1 (14.3)	0	1 (5.6)
Myxoid liposarcoma	0	0	1 (14.3)	0	1 (5.6)
Epithelioid sarcoma	0	0	0	1 (33.3)	1 (5.6)
Rhabdomyosarcoma	0	0	0	1 (33.3)	1 (5.6)
Myxofibrosarcoma	0	0	0	1 (33.3)	1 (5.6)
Chondrosarcoma	0	0	1 (14.3)	0	1 (5.6)
Osteosarcoma	0	0	1 (14.3)	0	1 (5.6)
Stage					
I	0	0	0	1 (33.3)	1 (5.6)
III	1 (25.0)	1 (25.0)	0	0	2 (11.1)
IV	3 (75.0)	3 (75.0)	6 (85.7)	2 (66.7)	14 (77.6)
UK	0	0	1 (14.3)	0	1 (5.6)

PFU, Plaque-Forming Unit.

colorectal cancer (n=2), hepatocellular carcinoma (n=2), cholangiocarcinoma (n=1), uterine leiomyoma (n=1), soft-tissue sarcoma (STS) (n=7), chondrosarcoma (n=1), and osteosarcoma (n=1). 14 patients were diagnosed at stage IV (77.6%), while other patients were at stage I (n=1) and stage III (n=2), respectively. The tumor stage of one patient with endometrial stromal sarcoma was unknown (table 1).

Adverse events and safety

All 18 patients were evaluable for therapeutic safety. No DLTs were observed in the four dose groups. A total of two severe AEs were reported in dose groups 1 and 2 (abdominal fluid and blood creatine phosphokinase increased), both of which were determined to be unrelated to the study drug. No AEs leading to death occurred.

A total of 141 treatment-related AEs were reported in 17 patients (94.4%) (table 2). The most common events (incidence >20%) were fever (n=12, 66.7%), lymphocyte count decreased (n=5, 27.8%), and anemia (n=4, 22.2%). 10 treatment-related AEs above grade 3 were reported in six patients (33.3%). Grade 3 or 4 treatment-related AEs included lymphocyte count decreased (n=5, 27.8%), fever (n=2, 11.1%), white blood cell count decreased (n=1, 5.6%), and neutrophil count decreased (n=1, 5.6%). All treatment-related AEs were manageable and could recover after appropriate treatment.

The occurrence of AEs did not show a strong correlation with the dose of OVV-01. However, the frequency of local reactions at the injection site appeared to be higher in dose group 4, which might have been caused by the subcutaneous leakage of OVV-01. No treatment-related AEs above grade 3 were reported in dose group 4.

Efficacy analysis

Seven patients terminated the study before receiving the first evaluation. The remaining 11 patients were evaluable for efficacy. At the time of the data cut-off, one patient achieved a complete response (CR), two patients achieved partial response (PR), and five patients experienced stable disease. Thus, the overall response rate (ORR) was 27.3%, and the DCR was 63.6% (figure 3A).

Table 2 Overview of adverse events related to OVV-01

	6×10^7 PFU (N=4)		6×10^8 PFU (N=4)		6×10^9 PFU (N=7)		1.2×10^{11} PFU (N=3)		Total (N=18)	
Events	**All grade**	**Grade 3–4**	**All grade**	**Grade 3–4**	**All grade**	**Grade 3–4**	**All grade**	**Grade 3–4**	**All grade**	**Grade 3–4**
Adverse events related to OVV-01 that occurred during treatment										
Any	3 (75.0)	0 (0)	4 (100.0)	3 (75.0)	7 (100.0)	3 (42.9)	3 (100.0)	0 (0)	17 (94.4)	6 (33.3)
Adverse events related to OVV-01 that occurred during treatment in ≥10% of patients										
General disorders and administration site conditions										
Fever	0 (0)	0 (0)	3 (75.0)	2 (50.0)	6 (85.7)	0 (0)	3 (100.0)	0 (0)	12 (66.7)	2 (11.1)
Injection site pain	0 (0)	0 (0)	0 (0)	0 (0)	1 (14.3)	0 (0)	2 (66.7)	0 (0)	3 (16.7)	0 (0)
Fatigue	0 (0)	0 (0)	2 (50.0)	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	3 (16.7)	0 (0)
Chest discomfort	0 (0)	0 (0)	1 (25.0)	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	2 (11.1)	0 (0)
Injection site edema	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (66.7)	0 (0)	2 (11.1)	0 (0)
Investigations										
Lymphocyte count decreased	0 (0)	0 (0)	2 (50.0)	2 (50.0)	3 (42.9)	3 (42.9)	0 (0)	0 (0)	5 (27.8)	5 (27.8)
White blood cell count decreased	0 (0)	0 (0)	1 (25.0)	0 (0)	2 (28.6)	1 (14.3)	0 (0)	0 (0)	3 (16.7)	1 (5.6)
Platelet count decreased	0 (0)	0 (0)	1 (25.0)	0 (0)	2 (28.6)	0 (0)	0 (0)	0 (0)	3 (16.7)	0 (0)
Gamma-glutamyltransferase increased	0 (0)	0 (0)	1 (25.0)	0 (0)	2 (28.6)	0 (0)	0 (0)	0 (0)	3 (16.7)	0 (0)
Aspartate aminotransferase increase	0 (0)	0 (0)	1 (25.0)	0 (0)	2 (28.6)	0 (0)	0 (0)	0 (0)	3 (16.7)	0 (0)
Alanine aminotransferase increase	0 (0)	0 (0)	1 (25.0)	0 (0)	2 (28.6)	0 (0)	0 (0)	0 (0)	3 (16.7)	0 (0)
Neutrophil count decreased	0 (0)	0 (0)	0 (0)	0 (0)	2 (28.6)	1 (14.3)	0 (0)	0 (0)	2 (11.1)	1 (5.6)
C-reactive protein increased	0 (0)	0 (0)	1 (25.0)	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	2 (11.1)	0 (0)
Calcitoninogen increase	0 (0)	0 (0)	1 (25.0)	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	2 (11.1)	0 (0)
Blood bilirubin increased	0 (0)	0 (0)	1 (25.0)	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	2 (11.1)	0 (0)
Blood unconjugated bilirubin increase	0 (0)	0 (0)	1 (25.0)	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	2 (11.1)	0 (0)
Blood triglycerides increase	1 (25.0)	0 (0)	0 (0)	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	2 (11.1)	0 (0)
Metabolism and nutrition disorders										
Hypokalemia	0 (0)	0 (0)	1 (25.0)	0 (0)	2 (28.6)	0 (0)	0 (0)	0 (0)	3 (16.7)	0 (0)
Gastrointestinal disorders										
Vomiting	1 (25.0)	0 (0)	1 (25.0)	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	3 (16.7)	0 (0)
Nausea	0 (0)	0 (0)	1 (25.0)	0 (0)	1 (14.3)	0 (0)	0 (0)	0 (0)	2 (11.1)	0 (0)
Blood and lymphatic system disorders										
Anemia	0 (0)	0 (0)	1 (25.0)	0 (0)	3 (42.9)	0 (0)	0 (0)	0 (0)	4 (22.2)	0 (0)
Immune system disorders										
CRS	2 (50.0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (11.1)	0 (0)

CRS, cytokine release syndrome; PFU, plaque-forming unit.

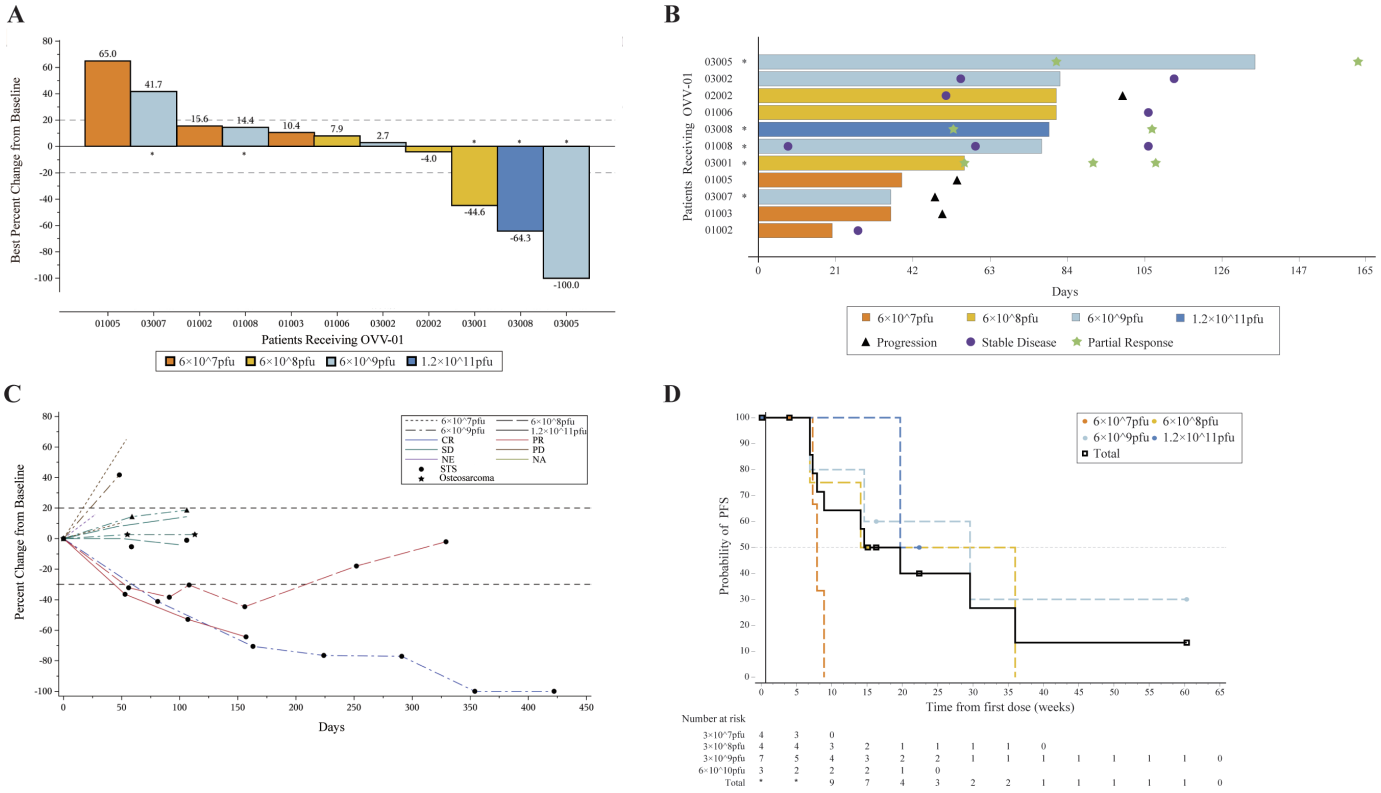

Figure 3 Response to OVV-01. (A) Waterfall plot showing best percentage change from baseline in the sum of the diameters for all target lesions. The asterisks indicate patients diagnosed as STS. (B) The time to response, the duration of treatment, and patient status by 16 weeks after first dosing. The asterisks indicate patients diagnosed as STS. (C) The percentage of change from baseline in the sum of the diameters for all target lesions over time in FAS. (D) The Kaplan-Meier curve of progression-free survival in FAS. CR, complete response; FAS, full analysis set; NA, not available; NE, not evaluable; PD, progression disease; PFU, plaque-forming unit; PPS, per-protocol set; PR, partial response; SD, stable disease; STS, soft-tissue sarcoma.

Specifically, the ORRs for the four dose groups were 0%, 33.3%, 25.0%, and 100% in the 6×10^7 PFU/subject, 6×10^8 PFU/subject, 6×10^9 PFU/subject, and 1.2×10^{11} PFU/subject dose groups, respectively, and the DCRs were 66.6%, 100%, 75%, and 100%. Among the five patients in the dose groups above 6×10^9 PFU, 40% (n=2) had a response, and 80% (n=4) had disease control. For patients observed with response, persistent tumor control could be observed after the end of treatment (figure 3B,C). Nine non-targeted lesions were present in six patients. Of these, seven lesions of four patients achieved non-CR/non-progression disease (PD) status, while the other two showed progression. This indicates the potential of OVV-01 in controlling non-injected lesions, although most non-targeted lesions were physically close to the injected lesions.

For the 18 patients in the FAS, 10 patients showed progression, and 5 patients died due to disease progression. The progression-free survival (PFS) rates at 24 weeks ($PFSR_{24w}$) were 0% (6×10^7 PFU/subject), 50% (6×10^8 PFU/subject) and 60% (6×10^9 PFU/subject), respectively. The $PFSR_{24w}$ was not evaluable for the dose level of 1.2×10^{11} PFU due to data censoring. For all patients in FAS, the median PFS (mPFS) was 17.2 weeks (figure 3D, with the mPFS as 7.9 weeks (6×10^7 PFU/subject), 25.1 weeks (6×10^8 PFU/subject), 29.6 weeks (6×10^9 PFU/subject) and not reached (NR) (1.2×10^{11} PFU/subject) for each dose group. The median overall survival (OS) was 29.6 weeks.

Notably, among the four evaluable patients with advanced STS, 75% (n=3) had a response. The ORR at 16 weeks for patients with STS was 100% (6×10^8 PFU/subject), 50% (6×10^9 PFU/subject), and 100% (1.2×10^{11} PFU/subject), respectively (table 3).

Patient 03005 was a patient in their 40s with myxoid liposarcoma who had received six surgical resections and adjuvant chemotherapies. The patient was assigned to the dose level of 6×10^9 PFU/patient and achieved PR at the first evaluation. 1 year after treatment, the patient achieved CR. Until August 31, 2024, the patient has remained cancer-free for 20 months (figure 4A). Patient 03008 was diagnosed with epithelioid sarcoma, underwent nine surgeries and perioperative chemotherapy and radiation therapy. Following the administration of 1.2×10^{11} PFU of OVV-01, a PR was achieved at the first evaluation, and continuous tumor shrinkage was observed. At the most recent follow-up (August 31, 2024), which occurred beyond the data cut-off date, this patient achieved a CR (figure 4B). The duration of response reached 14 months. Of note, these two patients did not receive any other anticancer treatment other than the six doses of OVV-01.

Table 3 Treatment response of OVV-01 in patients with soft-tissue sarcoma

	6×10^7 PFU (N=0)	6×10^8 PFU (N=1)	6×10^9 PFU (N=2)	1.2×10^{11} PFU (N=3)	Total (N=6)
Best overall response — no. (%)					
Complete response	0	0	0	0	0
Partial response	0	1 (100.0)	1 (50.0)	1 (33.3)	3 (50.0)
Stable disease	0	0	0	0	0
Progression disease	0	0	1 (50.0)	0	1 (16.7)
Not available	0	0	0	2 (66.7)	2 (33.3)
Overall response rate 16 weeks after first dosing	0	1 (100.0)	1 (50.0)	1 (100.0)	3 (75.0)
95% CI (%)	(-, -)	(2.5, 100.0)	(1.3, 98.7)	(2.5, 100.0)	(19.4, 99.4)
Disease control rate 16 weeks after first dosing	0	1 (100.0)	1 (50.0)	1 (100.0)	3 (75.0)
95% CI (%)	(-, -)	(2.5, 100.0)	(1.3, 98.7)	(2.5, 100.0)	(19.4, 99.4)

PFU, plaque-forming unit.

We further analyzed the relationship between the size of the injected lesions and both AEs and treatment response. While it could be expected that smaller lesions might lead to increased viral leakage, potentially resulting in more AEs and a reduced overall response, no clear trends were observed in serum OVV-01 detection, AEs, or treatment response (online supplemental table S2). This lack of correlation may be attributed to the complex factors affecting the viral leakage from the injected lesion, as well as the multiple tumor-killing mechanisms of OVV-01.

Virus shedding and VSV antibody assessment

For the 18 patients in the FAS, qPCR was performed to determine the systemic dissemination of OVV-01, and $TCID_{50}$ or qPCR assay was performed to detect virus shedding. No viral genome was detected by qPCR after OVV-01 dosing. However, low levels of the virus were detected in the serum of seven patients at 0.25 hours post-injection using the $TCID_{50}$ assay, which may be related to the high infectivity of OVV-01. Among the seven, one was in the dose level of 6.0×10^7 PFU/subject, two were in the dose level of 6×10^8 PFU/subject, and four were in the dose level of 6×10^9 PFU/subject. The virus was undetectable at 1 hour in four patients, and the other three patients became undetectable after 1 hour, 3 hours, and 12 hours, respectively. The patients with detectable serum OVV-01 showed a trend toward increased administration site reactions, including pain and edema at the injection site, as well as a higher incidence of fever and chest discomfort (online supplemental table S1). No other clear patterns were observed, indicating that the transient viremia did not contribute to increased overall toxicity. However, due to the limited sample size, this observation should be interpreted with caution, and further studies are warranted to explore this potential association. Neither live virus particles nor OVV-01 fragments were detected in all samples of patients' saliva, urine, or stool at any time.

Neutralizing antibody against VSV-G protein (anti-VSV-G) in 18 patients was tested to evaluate the immune response to OVV-01. Before OVV-01 injection, 15 out of 18 patients were negative for VSV-G antibody, 2 patients were positive with a low titer (<2.0), and only patient 02004 showed a high titer of VSV-G antibody at pre-dose (21.4). Among the three with anti-VSV-G at baseline, patient 02004 withdrew from the trial before receiving radiological assessment, while among the other two patients, one achieved PR in the dose level of 6×10^9 PFU/subject and the other one achieved PD in the dose level of 6.0×10^7 PFU/subject. An elevation of anti-VSV-G was observed after dosing, with the magnitude of titer increase correlating with the dose level (figure 5A). In the 1.2×10^{11} PFU dose group, the titer showed a declining trend between weeks 6 and 12 due to missing data points, and may not reflect an actual reduction in anti-VSV-G. No clear correlation between the anti-VSV-G titer and therapeutic response was identified (online supplemental table S2).

Immune phenotype assessment

Peripheral blood samples were collected at multiple time points to investigate the immune response to OVV-01. The counts and proportions of CD3+, CD3+CD8+, and CD3+CD4+ T cells were analyzed by flow cytometry. Three patients with complete data were analyzed for the trend of T-cell dynamics. These three patients were all in the 6.0×10^9 PFU/subject dose group. No significant trends were observed for T-cell proportions across the time points. A transient decline in the number of T cells was noted at 24 hours post-dosing, which may reflect changes in T-cell distribution or T-cell consumption (figure 5B). Additionally, the proportion of effector memory CD8+T cells (CD27−CCR−/CD3+CD8+CD45RO+CD45RA−) was also assessed, with no significant changes detected over time either.

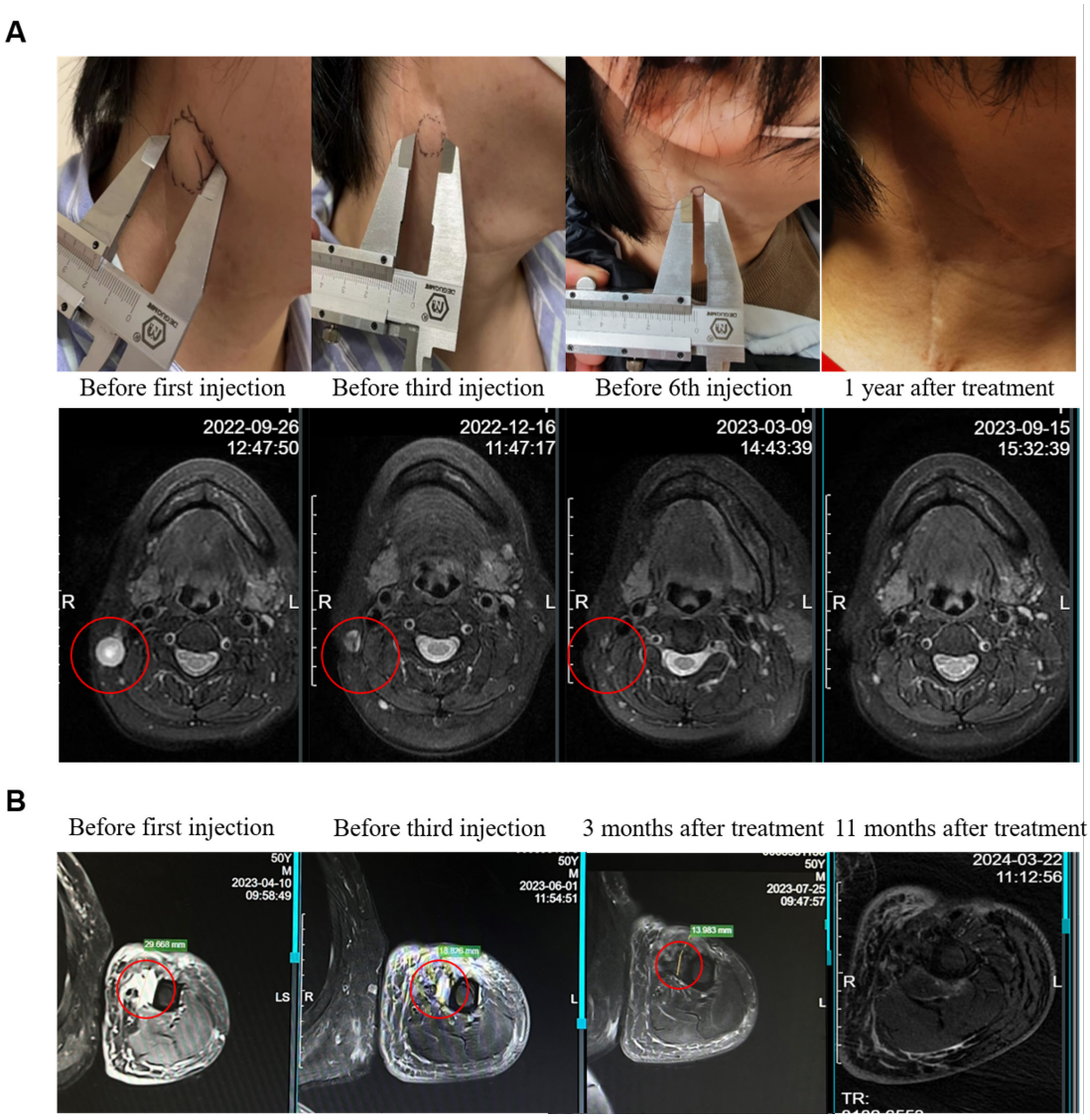

Figure 4 Target lesion images of the two patients achieved complete response (CR). (A) Patient 03005 with myxoid liposarcoma responded to OVV-01 injection and achieved CR 1 year after treatment. The red circles indicate the target lesion. (B) Patient 03008 with epithelioid sarcoma achieved CR 11 months after treatment. The red circles indicate the target lesion.

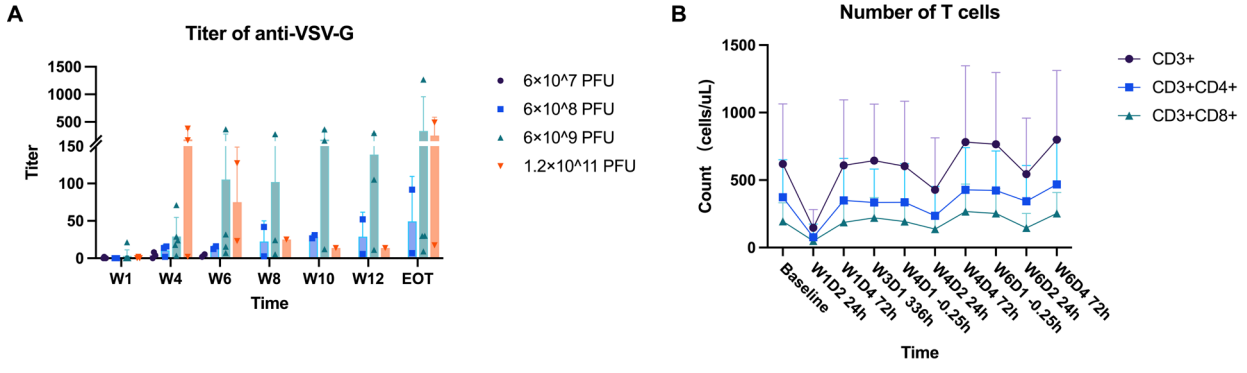

Figure 5 Immunological evaluations of patients receiving OVV-01. (A) The titer of neutralizing antibody against vesicular stomatitis virus in different dose groups at baseline and after each dosing. (B) The number of CD3+, CD3+CD4+, and CD3+CD8+ T cells in the peripheral blood of patients with complete data at different time points. EOT, end of treatment; PFU, plaque-forming unit.

DISCUSSION

OVs have emerged as one of the promising therapeutics against tumors. Genome editing has been widely used in the development of OV therapies to further enhance their efficacy and reduce toxicity. Common strategies for modifying OVs often focus on expressing immune activators or immune checkpoint inhibitors, such as granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon (IFN)-β, interleukin (IL)-12, IL-15, or programmed cell death protein 1/programmed death-ligand 1 (PD-1/PD-L1), with fewer reports on antigen expression strategies.[25–27] Voyager-V1, another VSV-based OV, is engineered to express IFN-β to enhance therapeutic efficacy and encodes human sodium iodide symporter (Na+/I– symporter, NIS) for virus tracking.[28] Here, we modified the VSV to express the highly immunogenic endogenous antigen NY-ESO-1 to construct an OV vaccine, OVV-01. OVV-01 can enhance tumor-specific immune activation and simultaneously induce the expression of NY-ESO-1 antigen on tumor cells with limited immunogenicity. Further, tumor cells expressing NY-ESO-1 can be targeted by NY-ESO-1-specific TCR-T cells or other specific therapies, offering a potentially synergistic treatment approach.

We conducted a first-in-human, open-label, multicenter, dose-escalating study to assess the safety, tolerability, and efficacy of OVV-01 for advanced solid tumors. To our knowledge, this is the first study evaluating the clinical efficacy of an OV modified to express NY-ESO-1. Overall, intratumoral injection of OVV-01 was well tolerated. No patient experienced DLTs. The most common adverse effects were fever, decreased lymphocyte count and anemia, which are consistent with the AE reports from other OV therapies.[29 30] Clinically significant abnormalities in lymphocyte, white blood cell and neutrophil count were observed, all of which could be controlled and recovered after appropriate symptomatic treatment. The occurrence of adverse effects did not correlate with the dose level, which has also been observed in other intratumorally administered OV studies.[31] At the highest dose level of 1.2×10^{11} PFU, AEs were primarily localized reactions, with no severe systemic adverse reactions observed. Additionally, live virus particles were rapidly cleared from the blood, and no virus shedding was detected in patients' saliva, urine, or stool. These results indicate that OVV-01 injection has a generally good safety profile.

The antitumor activity observed in this first-in-human study of OVV-01 is promising. The ORR was 27.3%, and the DCR was 63.6%. Notably, for doses above 6.0×10^{9} PFU, the ORR reached 40%, and the DCR reached 80%. In the FAS population, the median PFS was 17.2 weeks, and the $PFSR_{24w}$ achieved 50% in the 6×10^{9} PFU/subject dose group. Such efficacy is superior to that reported for current OV therapies in advanced solid tumors. In the phase I/II trial of OH2, an oncolytic herpes simplex virus (HSV) two genetically engineered to express GM-CSF, the ORR of 5% and DCR of 27.5% was observed in the single agent cohort.[30] T3011 is an oncolytic HSV inserted with IL-12 and anti-PD-1 antibody. An ORR of 11% and DCR of 49% was observed in the patients treated under RP2D.[32]

The efficacy observed in patients with advanced STS is particularly noteworthy in our study. STS is a class of rare tumors that are highly heterogeneous and normally resistant to chemotherapy. Current treatment options include anthracyclines, ifosfamide, dacarbazine, and other agents. In the first-line treatment, doxorubicin-based systemic therapy generates a response rate of 12–24%.[33 34] However, beyond the first-line setting, the benefit of systemic chemotherapy appears to be moderate, with a response rate even lower than 10%.[35] Recently, immunotherapy has been investigated for STS but has shown limited efficacy. In the Alliance A091401 trial, an ORR of 5% was observed in patients with STS who received nivolumab, and the combination of nivolumab and ipilimumab reached an ORR of 16%.[36] Similar results were reported for patients with STS treated with pembrolizumab, achieving an ORR of 18% and a DCR of 55%.[37] Therefore, new effective treatments are needed for patients with STS. In our study, the ORR of patients with STS reached 75%, with two patient achieving CR (up to August 31, 2024). Therefore, OVV-01 demonstrated superior antitumor activity in STS, and may offer a new therapeutic option for these patients.

NY-ESO-1 expression is restricted in germ cells and placental cells and is re-expressed in tumor cells. NY-ESO-1 expression has been reported in multiple tumor types, including metastatic melanoma, synovial sarcoma, bladder cancer, esophageal cancer, hepatocellular carcinoma, non-small cell lung cancer, ovarian cancer, and breast cancer.[38–43] The frequency of NY-ESO-1 expression varies greatly among these tumor types, with the most common being myxoid/round cell liposarcoma (MRCL) (89–100%), neuroblastoma (82%), SS (80%), melanoma (46%), and ovarian cancer (43%).[43 44] The expression of NY-ESO-1 in other tumor types ranged from 20% to 40%. Given the high expression, strategies targeting NY-ESO-1 are being investigated in STS, including vaccination and adoptive cell therapy. CMB305 is a prime-boost vaccine designed to induce NY-ESO-1-specific immune response. In the phase 1 study of CMB 305, stable disease was observed in 61% of the patients with SS/MRCL, indicating the potential of targeting NY-ESO-1 in STS treatment.[17] OVV-01 is a VSV engineered to express NY-ESO-1, as a combination of OV therapy and vaccination. Theoretically, infection with OVV-01 would upregulate the expression of NY-ESO-1 in tumor cells and induce ICD of tumor cells. Subsequently, the NY-ESO-1-specific immune response would be enhanced. Therefore, tumors with high NY-ESO-1 expression may potentially benefit more from OVV-01. In our study, one of the two patients who achieved CR was diagnosed as myxoid liposarcoma, which supported our assumption. However, tumor biopsies pretreatment and post-treatment were not collected in this study, therefore unable to interpret the NY-ESO-1 expression in the tumors and how OVV-01 may have changed that. Further exploration is warranted in future studies.

To better understand the systemic immune response to OVV-01, the neutralizing antibody against VSV-G protein was measured. As expected, most of the patients were negative for anti-VSV-G at baseline. Among the patients who were positive for anti-VSV-G at baseline, the majority exhibited low titers, and no trend in response difference was observed. Theoretically, pre-existing anti-VSV-G antibodies could influence the efficacy of OVV-01. However, their impact might be influenced by the antibody titer. Low-titer antibodies may not necessarily neutralize OVV-01 at clinically relevant levels. To better understand this, future studies with larger sample sizes will be needed to further evaluate the clinical significance of pre-existing immunity, and establishing a threshold titer for potential impact may be necessary.

Moreover, we assessed the change in count and proportion of T cells, CD8+T cells, CD4+T cells and CD8+effector memory T cells in peripheral blood by flow cytometry. However, no trend was observed over treatment. This may possibly be related to insufficient classification of immune cell clusters. Besides, a decline in T-cell count 24 hours after injection was observed. This may be due to the transient redistribution and consumption of T cells to the injection site. Further investigation of both local regional and systemically immune response is needed.

In conclusion, intratumoral injection of OVV-1 was safe and well-tolerated in patients with advanced solid tumors. The efficacy of OVV-01 was encouraging, especially at doses higher than 6×10^9 PFU/subject. A significant response rate was observed in patients with STS, which warranted further investigation.

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