Clinical Trial Protocol

An Open-Label, Single-Arm Compassionate Use Study of VSV-02 Given by Intravenous and Intratumoral Injection in Patients with Advanced Solid Tumors

Protocol Number:	**VSV-02A01tqyy**
Version Number:	**v 1.0**
Version Date:	**June 25, 2025**
Principal Investigator:	**Liuzhong Yang**
Group leader unit:	**The First Affiliated Hospital of Xinxiang Medical University**
Sponsor:	**Shanghai Rong Rui Pharmaceutical Technology Co., Ltd.**
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List of Abbreviations

Abbreviations and Terms	Full name in English
AE	Adverse Event
ADA	Anti-drug Antibody
ADR	Adverse Drug Reaction
ALT	Alanine Aminotransferase
ALP	Alkaline Phosphatase
ANC	Absolute Neutrophil Count
APTT	Activated Partial Thromboplastin Time
AST	Aspartate Aminotransferase
CCr	Creatinine Clearance
CR	Complete Response
CRF	Case Report Form
CRO	Contract Research Organization
CRS	Cytokine Release Syndrome
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CVA	Cerebralvascular Accident
DCR	Disease Control Rate
DLT	Dose Limited Toxicity
DMP	Data Management Plan
DNA	Deoxyribonucleic Acid
DoR	Duration of Response
ECOG	Eastern Cooperative Oncology Group
ECG	Electrocardiogram
EDC	Electronic Data Capture System
FAS	Full Analyse Set
FIB	Fibrinogen
GCP	Good Clinical Practice
HBcAb	Hepatitis B Core Antibody
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B virus
HCV	Hepatitis C virus
HED	Human Equivalent Dose
HIV	Human Immunodeficiency Virus

HNSTD	Highest Non-severely Toxic Dose
HSV	Herpes Simplex Virus
IB	Investigator's Brochure
ICF	Informed Consent Form
IEC	Independent Ethics Committee
IgG	Immunoglobulin G
IL	Interleukin
INR	International Normalized Ratio
IFN-γ	Interferon-γ
irAE	Immune-Related Adverse Events
IRB	Institutional Review Board
ITT	Investigator-initiated Trial
LVEF	Left Ventricular Ejection Fraction
MedDRA	Medical Dictionary for Regulatory Activities
MRI	Magnetic resonance imaging
MTD	Maximum Tolerated Dose
NY-ESO-1	New York esophageal squamous cell carcinoma 1
NYHA	New York Heart Association
ORR	Objective Response Rate
OS	Overall Survival
OV	Oncolytic Viruses
PD-L1	Programmed cell death 1 ligand 1
PFS	Progression-Free-Survival
PFU	Plaque Forming Unit
PPS	Per Protocol Set
PR	Partial response
PT	Prothrombin Time
PT	Preferred Terms
QD	Quaque Die
qPCR	Quantitative PCR Detecting System
RECIST	Response Evaluation Criteria in Solid Tumours
RNA	Ribonucleic Acid
RP2D	Recommended Phase 2 Dose
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SD	Stable Disease
SMC	Safety Monitoring Committee

SOC	System Organ Classification
SOP	Standard Operation Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
SS	Safety Analysis set
TAA	Tumor-associated Antigen
TBIL	Total bilirubin
$TCID_{50}$	50% Tissue Culture Infective Dose
TNF-α	Tumor Necrosis Factor
TPO	Thrombopoietin
TIA	Transient Ischemic Attack
TLS	Tumor Lysis Syndrome
ULN	upper limit of normal
VSV	Vesicular stomatitis virus
WBC	white blood cell

Catalog

Program Summary

Summary

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<tr><td>• To evaluate the preliminary efficacy of intravenous and intratumoral administration of VSV-02 Injection in subjects with advanced solid tumors;</td><td>• Objective remission rate (ORR), disease control rate (DCR), duration of response (DoR), progression-free survival (PFS), and overall survival (OS);</td></tr>
<tr><th>Secondary endpoints</th><th>Secondary Endpoints</th></tr>
<tr><td>• To evaluate the safety of VSV-02 Injection administered intravenously and intratumorally in subjects with advanced solid tumors.</td><td>• Incidence and characterization of adverse events (AE);
• Changes in safety indicators such as laboratory findings, physical examination, electrocardiogram and vital signs compared to baseline;</td></tr>
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</td></tr>
<tr><td>Experimental design.</td><td>This study is an open, single-arm, dose-escalation clinical study designed to evaluate the preliminary efficacy and safety of VSV-02 Injection intravenously and intratumorally in subjects with advanced solid tumors for compassionate use in patients lacking effective treatment modalities.
Patients eligible for enrollment will be screened and given a fixed dose: $3\times10^{(1)}$ $^{(0)}$PFU/mL (it.) + 3×10^{11}PFU (iv.); systematic safety observations will be made within 6 weeks of the first dose, and if no DLTs are observed in three patients, the dose will be determined to be the Maximum Tolerated Dose (MTD) and the Recommended Phase II Dose (RP2D). If DLT is observed in 1 patient, 3 additional subjects will be added to the cohort, If DLT is observed in $\geqslant$2 patients, the new cohort will be added at a lower dose as recommended by the SMC (recommended dose is: 3×10^{10}PFU/mL(it.) + 3×10^{10}PFU (iv.)). Upon completion of the dose group, the SMC assessed whether to newly continue the dose escalation based on outcome assessment.
After the first dose, subjects entered a 28-day DLT observation period, and if no DLT occurred during this period, they continued to be given subsequent treatment.
The study included a screening period, a treatment period and a follow-up period.
Screening period:was from the date the subject signed the informed consent form to the time of the first dose (D-28 to D-1).
TREATMENT PERIOD: Screening-eligible subjects were administered starting on Week 1 Day 1 (W1D1), with treatment cycles every 3 weeks, with intravenous and intratumoral injections administered on the same day, and adjusted as necessary to account for the patient's condition. Subjects were administered according to the dose group in which they were placed until disease progression, intolerable toxicity in the subject, withdrawal of informed consent by the subject, death of the subject, loss to follow-up, termination of treatment as deemed by the investigator to be in the best interest of the patient, or the study drug had been administered for the full 6 cycle, whichever occurred earliest. Some subjects may experience transient tumor growth and pseudo-progression during the first few months after initiation of immunotherapy, followed by disease remission. After the first imaging progression in a subject, as determined by the investigator according</td></tr>
</table>

	to RECIST 1.1 criteria, subjects will be allowed to continue treatment with VSV-02 injection if the investigator determines that the subject is in a clinically stable state and it is believed that there may be a clinical benefit to continue treating the subject. If the imaging assessment shows disease progression as determined according to RECIST 1.1 and there is no clinical deterioration, another tumor assessment should be performed at least 4 weeks later to confirm disease progression according to iRECIST. Clinical stability is defined as the absence of clinically significant signs and symptoms suggestive of disease progression (including abnormal laboratory test values), no reduction in Eastern Cooperative Oncology Group (ECOG) Physical Status Score, no rapid disease progression, and the absence of progressive tumors (e.g., spinal cord compression) at important anatomical sites that would require other urgent medical intervention. Subjects judged to be clinically unstable should terminate trial treatment after the first imaging disease progression evaluated by the investigator and without the need for repeat imaging to confirm disease progression. Follow-up Period: The follow-up period consists of a safety follow-up and a survival follow-up. If a subject terminates treatment for any reason, he/she is required to return to the study center for a safety follow-up within 28 days (±7 days) of the last dose. Subjects then enter a survival follow-up period, which occurs every 12 weeks until the subject withdraws informed consent, dies, loses the visit, receives another antitumor therapy other than that specified in the protocol, or the study ends, whichever occurs earliest. After subjects have completed 6 cycles of dosing, if the investigator judges that subjects may still benefit from continuing treatment with VSV-02 Injection, each patient should be consulted, and if the subject requests to continue treatment, he or she may apply to continue treatment, but must sign a separate informed consent form, the specific content of which should be based on the actual condition of the patient, and formulated after communication with the investigator.
Dose-Limiting Toxicity (DLT)	Sympathetic to patients on the drug, **it is not necessary to observe the emergence of DLT. In the event of an adverse reaction requiring treatment in clinical judgment, medication may be given at any time and discontinued at any time if necessary**.
Trial Population	**Inclusion Criteria:** Subjects **need to** meet the following enrollment criteria to be enrolled in this trial: 1. Voluntarily sign an informed consent form, understand the study and be willing to follow the protocol and complete all trial procedures; 2. Age ≥ 18 years at the time of signing the ICF and gender. 3. Patients with advanced solid tumors, including but not limited to: melanoma, squamous cell carcinoma of the head and neck, cervical cancer, osteosarcoma, nasopharyngeal carcinoma, breast cancer, lung cancer, colorectal cancer, hepatocellular carcinoma, gastric carcinoma, etc., that have been diagnosed by histologic/cytologic examination of the primary and/or metastatic lesion pathology. 4. Patients who have failed standard therapy and lack standard therapy in the endline or are not candidates for standard therapy for medical reasons. Patients need to have progressed on at least two standard therapies (including but not limited to targeted therapies). For example, subjects with metastatic or unresectable advanced melanoma will need to have failed standard therapy such as PD-1 antibody (or BRAF and MEK inhibitors if carrying a BRAF mutation); subjects with recurrent or metastatic advanced squamous cell carcinoma of the head and neck will need to have failed standard therapy such as anti-PD-1 monoclonal antibody as well as platinum-containing chemotherapy; and patients with recurrent or metastatic osteosarcoma will need to have failed standard of care with chemotherapeutic agents, including high-dose methotrexate, doxorubicin, cisplatin, isocyclophosphamide, etc.); colorectal cancer patients who have received standard chemotherapy with fluoropyrimidines, oxaliplatin, bevacizumab, and irinotecan, wild-type KRAS patients with anti-EGFR therapy, and patients with high microsatellite instability who have received at least one immune checkpoint inhibitor; and breast cancer patients (including HR positive, HER2 + and triple-negative breast cancer), need to have received at least 2 prior treatments, which should include paclitaxel and/or anthracycline therapy, as appropriate, and an approved checkpoint inhibitor. **5.** In principle, subjects have a lesion judged measurable according to RECIST 1.1 criteria, i.e., a non-lymph node lesion with a long diameter of ≥10 mm and a lymph node lesion

	with a short diameter of ≥15 mm based on CT or MRI. and need to have an injectable tumor lesion, including a superficial lesion, as well as a deeper lesion that can be injected under ultrasound/CT/ or endoscopic guidance**.** 6. Subjects with an ECOG physical status score of 0-2 and an expected survival of not less than 12 weeks. **7.** Adequate organ and hematopoietic function, **if the following criteria are not met, safety may be judged on a clinical basis:** • Absolute Neutrophil Count (ANC) ≥ 1.5×10^9/L; • Platelets ≥ 75×10^9/L (no platelet transfusion therapy or thrombopoietin (TPO) therapy within 2 weeks prior to first dose); • Hemoglobin ≥ 90 g/L (no blood transfusion within 2 weeks); • Serum creatinine ≤ 1.5× upper limit of normal (ULN) or endogenous creatinine clearance (CCr) ≥ 50mL/min; • Glutamine transaminase (AST) and alanine transaminase (ALT) ≤ 3.0 × ULN; AST and ALT < 5 × ULN if patients with liver metastases; • Serum total bilirubin (TBIL) ≤2×ULN; • International Normalized Ratio (INR) ≤ 1.5×ULN, or Activated Partial Thromboplastin Time (APTT) ≤ 1.5×ULN; 8. Females of childbearing potential must have had a pregnancy test with a negative result within 7 days prior to initiating treatment. 9. Male and female subjects of childbearing potential must agree to use a reliable method of contraception for the duration of the trial and for at least 6 months after the last dose. **Exclusion Criteria:** Patients with any of the following are not eligible for enrollment in this study. **Sympathetic medication patients may be treated sympathetically on a case-by-case basis at the joint judgment of the PI and the investigator:** 1. Known brain metastases and/or clinical suspicion of brain metastases from the tumor (however, patients with asymptomatic brain metastases or who have been clinically stable for more than 3 months with local therapy may be enrolled); 2. Subjects who have had radiotherapy within 2 months of the target lesion; 3. Subjects with other active malignancies within the previous 5 years. Subjects who have been completely cured and do not require follow-up therapy are excluded, except for subjects whose malignancy is within the indications; 4. The longest diameter of the lesion used for injection is >100 mm; 5. Subjects who have participated or are participating in a clinical trial of another drug or medical device within the previous 4 weeks; 6. Subjects who are preparing for or have previously received a tissue/organ transplant; 7. Subjects with human immunodeficiency virus (HIV) infection and AID-related opportunistic infections within 12 months, or CD4+ T-cell (CD4+) count < 350 cells/uL; Screening Hepatitis B Surface Antigen (HBsAg) and/or Hepatitis B Core Antibody (HBcAb) positivity with HBV-DNA above the lower limit of measurability, Screening HCV antibody Positive subjects with HCV-RNA above the lower measurable limit; subjects with positive syphilis spirochete serology; 8. Subjects requiring antiviral medication during the study or within 5 half-lives of antiviral medication at the time of first dose. 9. Subjects requiring therapeutic anticoagulant medication during the study. 10. Subjects with an uncontrolled grade ≥3 active infection of significant clinical relevance according to CTCAE v5.0; 11. Received antitumor agents such as chemotherapy, radiotherapy, biotherapy, endocrine therapy, immunotherapy, etc. within 4 weeks prior to the first dose; Received small molecule targeted therapies and oral fluorouracil analogs within 2 weeks or 5 half-lives (whichever is longer) prior to the first dose; Received herbal or proprietary Chinese medicines with antitumor indications within 2 weeks prior to the first dose; Received nitrosoureas or mitomycin C; palliative radiotherapy to non-target lesions permitted (≥2 weeks prior to first dose); 12. Medication-uncontrolled hypertension or pulmonary hypertension or unstable angina pectoris; myocardial infarction or bypass or stent surgery within 6 months prior to

	dosing; history of chronic heart failure in New York Heart Association (NYHA) criteria grade 3-4; severe arrhythmias requiring treatment (except atrial fibrillation and paroxysmal supraventricular tachycardia, which, in the judgment of the investigator, have no effect on the trial), including QTcF ≥450 ms in men and ≥450 ms in women. 450ms in men and ≥470ms in women (calculated using the Fridericia formula); cerebrovascular accident (CVA) or transient ischemic attack (TIA) within 6 months prior to enrollment; 13. Patients with active autoimmune disease or a history of autoimmune disease with possible relapse, but patients with the following diseases were not excluded and could be screened further • Type 1 diabetes mellitus; and • Hypothyroidism (if controllable with hormone replacement therapy alone) ;and • Controlled celiac disease ; • Skin conditions that do not require systemic treatment (e.g., vitiligo, psoriasis, alopecia areata) ; • Any other condition that will not reoccur in the absence of an external trigger ;and 14. Subjects requiring treatment with systemic corticosteroids (>10 mg /day prednisone or equivalent dose) or other immunosuppressive medications within 14 days prior to the first dose or during the study period, except as follows: • Adrenergic replacement steroids (prednisone ≤ 10 mg / day or equivalent dose of a similar drug) ;. • Topical, ophthalmic, intra-articular, intranasal, or inhaled corticosteroids. • Prophylactic short-term (≤ 7 days) use of corticosteroids (e.g., allergy to contrast media) or for the treatment of non-autoimmune disorders (e.g., delayed hypersensitivity reactions caused by contact allergens); 15. Subject has a tumor located in a high-risk location (including in a mucosal area, or in close proximity to the airway, major blood vessels, or spinal cord) that may result in occlusion or compression due to tumor enlargement, or erosion into major blood vessels due to necrosis, or encasement of major vascular structures (e.g., carotid arteries), tumors adjacent to important neurovascular structures, or other tumors deemed unsuitable for intratumoral injections; 16. Subjects will be required to receive any live vaccine during the Screening and Treatment Periods; 17. Subject is allergic to any component of the investigational drug, immunotherapy or related medications; 18. Subjects with concomitant mental illness, alcoholism, inability to quit smoking, drug abuse or substance abuse; 19. Pregnant or lactating females; 20. Adverse effects of prior antineoplastic therapy that have not recovered to (CTCAE 5.0) Grade 1 (except alopecia); 21. Serious uncontrollable disease, as determined by the investigator, or the presence of other conditions that may interfere with the receipt of treatment under this study and are considered unsuitable for participation in this study; 22. Other conditions deemed unsuitable for enrollment by the investigator.
Basis for Starting Dose Selection	In non-clinical studies, rhesus monkeys were injected intravenously with 8.0×10^{9}PFU/kg and 8.0×10^{10}PFU/kg OVV-01 for 2 cycles (QD of 7 days with a 5-day cycle interval), followed by a 4-week recovery period after a toxicology study that showed a maximum non-severe toxicity dose (HNSTD) of 8.0×10^{10}PFU/kg. after conversion, the Human Equivalent Dose (HED) was 2.58×10^{10}PFU/kg, which corresponds to a dose of 1.55×10^{12}PFU/subject. In a GLP toxicology study in tumor-bearing mice, repeated intratumoral and intravenous injections of OVV-01 for 2 cycles (1 cycle in 7 days, with 1 day off in between), followed by a 7-day recovery period, showed a HNSTD of 3.0×10^{9}PFU/mouse (intratumoral) + 1.2×10^{9}PFU/mouse (intravenous). Converted from 20 g of mouse body weight, the HED was 1.22×10^{10}PFU/kg (intratumoral) + 4.88×10^{9}PFU/kg, which

<table>
<tr><td></td><td>corresponds to 7.32×10^{11}PFU/subject (intratumoral) + 2.93×10^{11}PFU/mg (intravenously).
In the completed dose-escalation study (RR-001), 18 patients with advanced solid tumors received intra-tumoral injections of OVV-01 Injection in four dose groups (6×10^{7}PFU, 6×10^{8}PFU, 7×10^{9}PFU, and 1.2×10^{11}PFU) administered every two weeks in multiple doses (1 to 6 doses). No serious adverse events or DLTs related to the study drug occurred in any of the dose groups, and no MTDs were achieved.
In the ongoing dose-escalation study (RR-008), four patients received OVV-01 Injection via intra-tumor injection at a dose of 1.2×10^{11}PFU once daily for 6 consecutive days for 2 weeks. As of the cut-off date (December 16, 2024), there were no DLT events or serious adverse events, and the treatment demonstrated a favorable safety and tolerability profile. Taken together, these data support the proposed clinical IV starting dose of 6×10^{10}PFU/subject and the combined starting dose of 6×10^{10}PFU/mL (intratumoral) + 6×10^{10}PFU/mL (IV) administered multiple times by once-daily dosing.</td></tr>
<tr><td>Study Intervention</td><td>VSV-02 Injection, provided by Shanghai Rongrui Pharmaceutical Technology Co. Specification: 1mL/strike. Labeled dose: viral titer of 3.0×10^{10}PFU/mL, administered intravenously, intratumorally.
VSV-02 injection is administered on Day 1 (D1) of each cycle, every 3 weeks for one treatment cycle, up to a maximum of 6 cycles, and IV and intratumoral injections are administered on the same day. Subjects were enrolled in fixed dose groups.
Dose administered: 6×10^{10}PFU/mL (it.) + 6×10^{11}PFU (iv.).
If DLT occurs during the DLT observation period, VSV-02 administration will be discontinued. If DLT does not occur, treatment will continue until disease progression, the subject develops intolerable toxicity, the subject withdraws informed consent, the subject dies, is lost to follow-up, the investigator determines that it is in the patient's best interest to terminate treatment, or 6 consecutive cycles of administration have been completed, whichever occurs earliest.
Dosing regimen
The volume of VSV-02 injected intratumorally was determined based on the size of the tumor lesion and was given to the maximum volume possible according to the guidelines in the table below. The investigator evaluated the size of the subject's injected lesion within 24 h prior to each injection to determine the volume of study drug to be injected. The total injection volume of all lesions per subject per dosing day was not to exceed 10 mL.
Guidelines for Injection Volume of VSV-02 Injection Based on Tumor Size
<table>
<tr><th>Lesion size (longest diameter)</th><th>Injection volume</th></tr>
<tr><td>≤0.5 cm</td><td>0.5 mL</td></tr>
<tr><td>>0.5 cm and ≤1.0 cm</td><td>1.0 mL</td></tr>
<tr><td>>1.0 cm</td><td>For every 1.0 cm increase in lesion size (rounded) from 1.0 cm, increase the injection volume by 1.0 mL from 1.0 mL (e.g., 3.0 mL for a 3.0 cm lesion).</td></tr>
</table>
Injection:
（1） It may not be possible to inject all lesions at a time; prioritize the largest lesion and inject other lesions according to the size of the lesion until the maximum delivery volume is reached. The same lesion should be injected each time unless the injected lesion shrinks to the point where it is unacceptable to inject.
（2） If there is residual drug due to shrinkage of the tumor lesion, the new lesion may be selected for injection.</td></tr>
<tr><td>Sample size</td><td>3-6 cases according to clinical needs</td></tr>
<tr><td>Study Suspension Rules</td><td>SMC will require suspension of enrollment and drug administration until the situation can be assessed when the following occurs
• Death occurring within 30 days of study drug administration (unless clearly due to disease progression)
• Deaths that the investigator believes are at least potentially related to the study drug</td></tr>
</table>

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<tr><td></td><td>• Two Grade ≥4 DLTs in 2 subjects
• Any grade 4 hypersensitivity reaction, immediate onset severe allergic reaction</td></tr>
<tr><td>Safety/tolerability evaluation</td><td>Adverse events occurring throughout the study period were evaluated based on the safety analysis set, looking at DLT and MTD according to CTCAE v5.0.Safety indicators included: adverse events (CTCAE v5.0), laboratory tests (routine blood, blood biochemistry, coagulation function, and urinalysis, etc.), electrocardiograms, vital signs, and physical examination.</td></tr>
<tr><td>Efficacy Evaluation</td><td>Tumor efficacy evaluation was assessed by the investigator according to RECIST 1.1 criteria. In this study, after the first imaging progression as determined by the investigator according to RECIST 1.1 criteria, subjects were allowed to continue treatment with VSV-02 Injection if the investigator determined that no clinical deterioration had occurred; however, subsequent tumor evaluations should be performed again after at least 4 weeks to confirm disease progression according to iRECIST. Clinical stability was defined as the absence of clinically significant signs and symptoms suggestive of disease progression (including abnormal laboratory test values), no reduction in Eastern Cooperative Oncology Group (ECOG) physical status score, no rapid disease progression, and the absence of progressive tumors (e.g., spinal cord compression) at important anatomical sites requiring other urgent medical intervention. Subjects judged to be clinically unstable should terminate trial treatment after the first imaging disease progression evaluated by the investigator and without the need for repeat imaging to confirm disease progression.
Efficacy endpoints include ORR, DCR, DoR, PFS, and OS.</td></tr>
<tr><td>Evaluation of biodistribution and viral shedding</td><td>Biodistribution and viral shedding will be evaluated by detection of viral gDNA in blood samples and shed samples (urine, feces, saliva and injection sites) using quantitative polymerase chain reaction (qPCR) analysis. If positive viral gDNA is detected in biodistribution and shedding samples using qPCR, the $TCID_{50}$will be used to assess viral infectivity, and it is recommended that testing be performed according to protocols, but not mandatory if circumstances do not permit.</td></tr>
<tr><td>Immunogenicity Evaluation</td><td>Evaluate the time, titer, and subject rate of anti-VSV-G antibody (IgG) production in treated subjects. Evaluated only in subjects who received at least one dose of 0VV-01 and provided a baseline sample and at least one post-treatment sample, and testing is recommended as specified in the protocol or not mandatory if not allowed.</td></tr>
<tr><td>Biomarker and Cytokine Evaluation</td><td>Biomarker and cytokine assays will evaluate the expression of NY-ESO-1 antigen, PD-L1, and lymphocytes in tissue samples, and will detect levels of NY-ESO-1 antigen, cytokines, and lymphocytes in blood samples. Specific assays may be performed according to study needs and study realities.</td></tr>
<tr><td>Statistical Analysis</td><td>Analysis Sets
<table>
<tr><td>Full Analysis Set (FAS)</td><td>Includes all subjects enrolled who have used the test drug at least once. The FAS will be used for demographic data, baseline descriptive analysis, medication adherence, medication combinations during the trial, and efficacy analysis.</td></tr>
<tr><td>Efficacy Evaluable Set (EEAS)</td><td>A subset of the FAS that includes subjects with at least one post-treatment tumor assessment. The EEAS will be used for efficacy analysis.</td></tr>
<tr><td>Safety Set (SS)</td><td>Includes all subjects who have received at least one dose of the investigational drug; SS will be used for safety and tolerability analyses.</td></tr>
<tr><td>Biodistribution Analysis Set</td><td>This analysis set will include all enrolled subjects who have received at least one dose of the test drug and have completed at least one post-dose biodistribution blood collection. This analysis set will be used to assess biodistribution.</td></tr>
<tr><td>Stool Assessable Analysis Set</td><td>This analysis set will include enrolled subjects who have received at least one trial drug and completed at least one fecal swab collection. This analysis set will be used to assess the level of VSV-02 Injection gDNA and active virus detected in fecal samples.</td></tr>
<tr><td>Urine Assessable</td><td>This analysis set will include enrolled subjects who have</td></tr>
</table></td></tr>
</table>

Analysis Set	received at least one trial drug and have completed at least one urine swab collection during the treatment period. This analysis set will be used to assess the level of VSV-02 Injection gDNA and active virus detected in urine swab samples during the Treatment Period.
Saliva Assessable Analysis Set	This analysis set will include enrolled subjects who have received at least one trial drug and have completed at least one saliva swab collection during the treatment period. This analysis set will be used to assess the level of VSV-02 injectable gDNA and active virus detected in saliva swab samples during the treatment period.
Injection Site Assessable Analysis Set	This analysis set will include enrolled subjects who have received at least one trial drug and have completed at least one injection site swab collection. This analysis set will be used to assess the levels of VSV-02 Injection gDNA and active virus detected on the surface of the injection site.
Pharmacodynamic Analysis Set	This analysis set will enroll subjects who have received at least one trial drug and have at least one assessable biomarker or cytokine parameter. This analysis set will be used to assess biomarker and cytokine levels of VSV-02 Injection.
Immunogenicity Analysis Set	This analysis set will include all subjects who have received at least one trial drug and have data from at least one post-baseline immunogenicity sample. This analysis set will be used to assess the immunogenicity of VSV-02 Injection.

Statistical analysis will be performed using SAS 9.4 (or newer) statistical software. Measurement information is described statistically by the number of cases, mean, standard deviation, median, maximum and minimum values. Count data or grade data were statistically described by the number of cases and percentage.

Safety Evaluation

Safety evaluation will be analyzed on the safety analysis set.

Abnormalities of all examination items will be summarized in the form of pre- and post-treatment cross-tabulation (according to the clinician's judgment) for the physical examination, 12-lead electrocardiogram (ECG), and clinical laboratory examination indexes in a categorical manner. Descriptive summaries of the 12-lead electrocardiogram (ECG), laboratory tests, and vital signs, including the observed values at each time point and their changes relative to baseline.

All adverse events were coded according to System Organ Classification (SOC) and Preferred Terminology (PT) using the International Medical Dictionary of Terms (MedDRA) version 22.0 or above. Categorize and summarize the coded adverse events: summarize the number and percentage of occurrences describing all adverse events, treatment-emergent adverse events (TEAE), serious adverse events, treatment-emergent serious adverse events, adverse events leading to termination of treatment, and deaths associated with adverse events; summarize all treatment-emergent adverse events and serious adverse events by SOC and PT.

List all adverse events, including subject number, age, dose group, adverse event name, description, start date, stop date, NCI-CTCAE 5.0 level, whether serious adverse event, relationship to study drug, effect on study drug, regression, etc.

Efficacy Evaluation

Efficacy evaluation will be based on FAS and EEAS. ORR and DCR will be assessed based on RECIST V1.1 and iRECIST. ORR is defined as the percentage of subjects achieving CR or PR. DCR is defined as the percentage of subjects achieving CR, PR or SD. 95% confidence intervals (CI) for ORR and DCR were calculated using the Clopper-Pearson method.

DoR, PFS and OS: The median survival time and its 95% CI will be estimated using the Kaplan- Meier method, and the survival rate and 95% CI will be estimated for each time point.The standard error of the Kaplan-Meier curve estimates will be calculated using the Greenwood formula. Kaplan- Meier curves will also be plotted.

	Evaluation of biodistribution and viral shedding The proportion of subjects meeting the criteria for detectable gDNA at each time point will be calculated. Statistics will be presented by dose level. **Compassionate medication patients will not be mandatorily invited for testing if circumstances do not permit.** **Immunogenicity Evaluation** Descriptive statistical analysis of changes in the production of anti-VSV-G antibodies (IgG) in subjects at each time point after treatment. **Compassionate medication patients were not compulsorily invited for testing if circumstances did not permit.** **Evaluation of biomarkers and cytokines** Biomarkers and cytokines and their changes relative to baseline were statistically characterized according to their profile. Exploratory analyses of pharmacodynamic parameters were performed as needed. **Compassionate medication patients are not mandatorily invited for testing if circumstances do not permit.**
Duration of the test:	The study will consist of the following phases: a screening period (28 days prior to dosing), a treatment period (administration of VSV-02 injection until disease progression, intolerable toxicity in the subject, withdrawal of informed consent by the subject, death of the subject, loss to follow-up, termination of treatment as deemed by the investigator to be in the best interest of the patient, or multiple administrations of VSV-02 have completed 6 cycles, whichever occurs earliest.) and follow-up periods (safety follow-up (28 days after the last dose) and survival follow-up (every 12 weeks until the subject withdraws informed consent, dies, is lost to follow-up, receives another antitumor therapy other than that specified in the protocol, or the study is completed, whichever occurs earliest).) .

1 INTRODUCTION

1.1 BACKGROUND

1.1.1 Disease background

In 2022, there will be an estimated 20 million new cancer cases and 9.7 million deaths. The number of people surviving within 5 years of a cancer diagnosis is estimated to be 53.5 million. About one in five people develop cancer in their lifetime, and about one in nine men and one in twelve women die from cancer.In 2022, lung cancer was the most common cancer worldwide, accounting for 12.4% of all new cases; female breast cancer ranked second (2.3 million cases, 11.6%), followed by colorectal cancer (1.9 million, 9.6%), prostate cancer (1.5 million. 7.3%) and stomach cancer (970,000 cases, 4.9%). Lung cancer is also the leading cause of cancer deaths (1.8 million deaths, 18.7% of all cancer deaths), followed by colorectal cancer (900,000 deaths, 9.3%), liver cancer (760,000 deaths, 7.8%), breast cancer (670,000 deaths, 6.9%) and stomach cancer (660,000 deaths, 6.8%). Malignant tumors (cancer) have become one of the major public health problems that seriously threaten the health of the population[1] .

Although cancer treatment has made great progress in multidisciplinary integrated therapy such as surgery, chemotherapy, radiotherapy and molecular targeted therapy, recurrence and metastasis of tumors still lack effective treatment so far. The direction of tumor treatment has shifted from traditional treatments (e.g., surgery, chemotherapy, radiotherapy) to more targeted and personalized interventions. Among these, the emergence of immunotherapy has brought about significant changes in the field of cancer treatment. Immune checkpoint inhibitors, such as antibodies to programmed cell death protein 1 (PD-1) and programmed cell death ligand 1 (PD-L1), activate the body's immune system, allowing it to recognize and attack cancer cells[2] . Considering the heterogeneity of solid tumors, the use of combination therapies is essential to improve treatment outcomes. Researchers have attempted to improve treatment efficacy and break through resistance mechanisms by combining many different treatments, such as chemotherapy, targeted therapy and immunotherapy[3] . Despite the favorable results of anti-PD-1/PD-L1 antibody therapy, most patients are not sensitive to the treatment and some sensitive patients do not achieve complete remission after treatment[4][5] . Anti-PD-1/PD-L1 antibody therapy has the strongest antitumor effect in tumors with high levels of tumor-infiltrating lymphocytes, high mutational burden, and increased PD-L1 expression[6] . These responsive tumors are referred to as immunologically "hot tumors" as opposed to "cold tumors" that lack response. Oncolytic viruses (OVs) represent an ideal therapeutic pathway for enhancing the response of patients and certain tumor types to anti-PD-1/PD-L1 antibody therapy.

The emergence of oncolytic virus (OV) therapy has provided a promising new therapeutic

approach in the field of cancer treatment, offering a new strategy for the treatment of a wide range of solid tumors. Oncolytic viral therapy employs specific types of viruses to give full play to their selective infection and lysis of cancer cells without harming normal healthy cells. By using the replication ability of viruses in host cells, lysing the cells and releasing the progeny viruses, the progeny viruses re-infect and lyses the new tumor cells, and finally destroys the tumor foci . Currently, five lysogenic virus products have been approved and marketed worldwide: Rigvir (ECHO-7 virus), Ancoris (recombinant human adenovirus type 5), Imazalil (human adenovirus type 5), T-Vec (HSV) and Delytact (HSV). With the deepening of medical research, the application of oncolytic viruses in the field of anti-tumor therapy is increasing, bringing more hope to patients with malignant tumors. Several preclinical and clinical studies have demonstrated the significant therapeutic potential of lysosomal viruses in solid tumors.A study conducted by Kaufman et al. in subjects with advanced melanoma confirmed the efficacy of the lysosomal viral drug, T-VEC.T-VEC not only directly destroys tumor cells, but also has an immune-stimulating effect that activates the anti-tumor immune response[8] . Lysoviral therapy can also be combined with other therapeutic approaches to achieve better results. Studies have shown that the combination of T-VEC and PD-1 antibodies can enhance tumor regression and achieve durable remission, confirming the potential of such combinations to improve treatment outcomes[9] . The selective tumor killing and immunostimulatory effects of lysosomal viral therapies offer a new avenue for more effective and targeted cancer treatment strategies.

1.1.2 Investigational Drugs and Therapeutic Context

Oncolytic viral products offer many advantages and are therefore a promising area of research in the field of cancer therapy. Derived from natural or genetically modified or modified classes of viruses, lysoviruses are versatile and novel therapeutic agents. Common oncolytic viruses include mutant adenovirus ONYX-015 (dl1520), HSV-1 mutant G207, human retrovirus 3, vesicular stomatitis virus (VSV) and EBV. It is also known as conditional replication virus or selective replication virus, which can selectively infect tumor cells and replicate in them, and eventually lyses and kills the tumor cells and releases daughter virus particles to further infect the surrounding tumor cells, while there is no replication or killing effect in the cells of normal tissues, and it can also induce specific anti-tumor immune response and so on.

In order to obtain lysogenic adenoviruses with good tumor-targeting properties, many modification strategies have been developed in the past decades. As early as 2003 Giedlin MA proposed that Vesicular stomatitis virus (VSV) could be used as an oncolytic virus for tumor treatment. The rationale is that it cannot interact with endogenous IFN-β in normal cells, but can only selectively amplify and grow in tumor cells[10] . In 2009, McMaster University, Canada, demonstrated that VSV

can be used as a new tumor drug carrier to promote immune response[11] . In recent years, researchers have paid more attention to the study of VSV, a non-pathogenic, enveloped negative-stranded RNA elastovirus that mainly infects rodents, cattle, swine, and horses, with a very low prevalence in the population, and only a few cases of infection in animal breeders and laboratory personnel with mild or no symptoms after infection. VSV is characterized by rapid replication and trans-synaptic speed, and ultra-high gene expression. Compared to other tumor lysing cytovirus platforms currently under development, VSV has a small genome and is easy to manipulate; has a shorter replication time; has an independent cell cycle; can grow rapidly in a wide range of cell lines with high titers, allowing for large-scale production; and has no translational risk of cytoplasmic replication of the host cell. This lysosomal cytotoxic virus does not integrate into DNA and has been modified to avoid the neurologic inflammation caused by wild-type viruses.

In recent years, with the continuous development of the VSV lysovirus platform, many recombinant variants of lysoviruses based on VSV modification have emerged, which are mainly aimed at improving tumor targeting, prolonging in vivo retention time, and enhancing the effect of lysis of tumors, etc., which have enhanced the safety and efficacy of VSV lysoviruses for tumor therapy, and a series of VSV-based lysoviral agents have gradually entered the stage of clinical trials. VSV-IFNβ has entered phase I clinical trials for the treatment of patients with advanced or relapsed refractory melanoma, hepatocellular carcinoma, endometrial carcinoma, leukemia, lymphoma, etc. (NCT03865212, NCT01628640, NCT03017820 and NCT03120624). Combination regimens of VSV+CTLA-4 antibody, TIM3 antibody or LAG3 antibody are also being evaluated. VSV-IFNβ-NIS (thyroid sodium iodide homotransporter protein) in combination with a PD-1/PD-L1 antibody such as Pembrolizumab/Avelumab is currently undergoing a Phase I clinical trial in the treatment of patients with relapsed, refractory solid tumors, including non-small-cell lung cancer and squamous carcinoma of the head and neck (NCT02923466 and NCT03647163). On July 20, 2020, Vyriad announced the completion of enrollment of the first subject in a clinical Phase II trial of its lysosomal virus product, Voyager-V1. The study is an open study of Voyager-V1 in combination with a PD-1 inhibitor (Cemiplimab) for the treatment of subjects with four relapsed refractory progressive tumors, with 152 subjects planned to be enrolled. The study covers four tumor types, including non-small cell lung cancer and melanoma that have progressed after the application of checkpoint inhibitors, as well as hepatocellular carcinoma and endometrial cancer (NCT04291105).VSV has been successful in the field of lysosomal viruses, and has a very promising application.

CD3/PD-L1 bispecific antibody is an emerging and highly promising strategy for tumor immunotherapy. It combines two different immunotherapeutic mechanisms in one, aiming to activate the immune system more effectively and overcome the immune evasion mechanism of tumors. Its core

mechanism of action and advantages are described below:

Core mechanism of action

1. Simultaneous targeting of two key molecules:
   - CD3: A key signaling molecule located on the surface of T cells and part of the T cell receptor complex. Binding to CD3 activates T cells, whether or not their TCR recognizes tumor antigens.
   - PD-L1: Programmed death ligand-1, usually highly expressed on a variety of tumor cells and other cells in the tumor microenvironment (e.g., myeloid cells.) Binding of PD-L1 to PD-1 on T-cells transmits inhibitory signals that lead to depletion, inactivation, or apoptosis of T-cells, and is an important mechanism of immune escape from tumors.
2. "Bridging" effect:
   - One arm (Fab) of the bispecific antibody binds PD-L1 on tumor cells or cells in the tumor microenvironment.
   - The other arm binds CD3 on T cells.
   - In this way, the bispecific antibody acts as a "bridge", physically pulling and anchoring the T-cells next to the PD-L1-expressing tumor cells.
3. Dual action:
   - Forced T-cell activation: By binding to CD3, a strong activation signal is delivered directly to the T-cells. These T cells can be reactivated even if they have low affinity for tumor antigens or are in a resting/depleted state.
   - Blocking Immune Checkpoints: By binding to PD-L1, the interaction of PD-L1 with PD-1 on T cells is blocked. This removes the "brakes" (inhibitory signals) that the tumor applies to the T-cells, unsuppressing T-cell function.
   - Synergistic effect: Based on the physical connection, the simultaneous provision of T-cell activating signals (CD3) and removal of inhibitory signals (PD-L1 blockade) creates a powerful, localized immune-activating effect within the tumor microenvironment. This significantly enhances the ability of T cells to recognize and kill tumor cells.

Role and advantages for the treatment of tumors

1. Overcome immunosuppression in the tumor microenvironment: Directly target PD-L1, a key immunosuppressive factor in the tumor microenvironment, while activating T cells to effectively counteract the tumor's immune escape strategy.
2. Recruitment and activation of bystander T-cells: It does not require T-cells to be specifically activated by tumor antigens or to recognize tumor antigens beforehand (traditional TCR-dependent approach). It is able to recruit any T cells in the tumor microenvironment or in

circulation ("bystander T cells"), regardless of their TCR specificity, forcing them to recognize and attack nearby PD-L1-positive tumor cells. This addresses the lack of sufficient tumor-specific T cell infiltration within the tumor.

3. Enhanced T-cell killing potency: Dual action (activation + disinhibition) puts recruited T-cells in a highly activated state, significantly enhancing their cytotoxicity against tumor cells.
4. Potentially Overcome PD-1/PD-L1 Inhibitor Resistance: For patients who are ineffective or resistant to PD-1/PD-L1 inhibitors alone, CD3/PD-L1 dual antibody provides a different mechanism. It not only blocks the PD-1/PD-L1 pathway, but also actively pulls T-cells to the tumor and forces activation, which may be effective in some resistant patients.
5. Targets tumor locally, potentially reducing systemic toxicity: Theoretically, its activity relies on binding to both PD-L1-expressing tumor cells and T-cells, so that activation of T-cells and cytokine release occurs primarily at the tumor site (although systemic toxicity remains a concern), and is likely to be more controllable than toxicity of systemic T-cell activating drugs (e.g., anti-CD3 monoclonal antibodies).
6. Applicable to PD-L1-positive tumors: Provides a new therapeutic option for a wide range of solid tumors with positive PD-L1 expression.

Challenges and Potential Risks

1. Cytokine Release Syndrome: This is the primary safety concern. Strong activation of T cells may lead to rapid release of large amounts of inflammatory cytokines (e.g., IL-6, IFN-γ, TNF-α, etc.) into the bloodstream within a short period of time, resulting in systemic inflammatory reactions such as fever, hypotension, and respiratory distress, which may be life-threatening in severe cases. Close monitoring and management strategies (e.g., stepwise dosing, prophylactic dosing) are required.
2. Neurotoxicity: Some patients may experience neurologic side effects such as headache, tremor, confusion and even seizures, the mechanism of which is not fully understood.
3. "Target-dependent" off-target toxicity: T cells may be recruited and attack cells that express low levels of PD-L1 in normal tissues, causing tissue damage (e.g., hepatotoxicity, pneumonia, etc.).
4. T-cell depletion: Continued strong stimulation may lead to eventual depletion of activated T-cells, affecting long-term efficacy.
5. Complex production process: Bispecific antibodies are more complex than monoclonal antibodies, making production and quality control more challenging.

Summary

CD3/PD-L1 bispecific antibody is an innovative immunotherapeutic weapon. It generates a

powerful anti-tumor immune response locally by physically connecting T-cells to PD-L1-positive tumor cells, simultaneously forcibly activating T-cells and lifting PD-1/PD-L1-mediated immunosuppression. Its core advantage lies in its ability to actively recruit and activate "bystander" T cells to overcome the inhibition of the tumor microenvironment, and is expected to resolve the problem of resistance to some PD-1/PD-L1 inhibitors. However, its strong immune activation ability also brings significant risk of side effects, especially CRS, which is a key challenge to be managed and overcome in clinical applications. Several CD3/PD-L1 dual antibodies are currently in clinical trials (e.g., phase I/II) and have shown preliminary anti-tumor activity in a variety of solid tumors (e.g., non-small cell lung cancer, head and neck squamous carcinoma, gastric cancer, ovarian cancer, etc.); however, their long-term efficacy and safety still need to be validated in larger clinical trials.

1.1.3 Overview of preclinical studies

Information on preclinical studies is detailed in the Investigator's Manual.

1.1.3.1 Pharmacodynamic Studies

1.1.3.1.1 In Vitro Pharmacodynamics

VSV-M3-BITE P5 synergizes tumor killing by PBMC cells

To verify whether BITE activates PBMC cells to synergize VSV virus for tumor killing, 2 E4 cells were spread into 96-well culture plates for group experiments using .A549 cell digestion and counting.

Viral infection for 24.5h

A549+PBMC+VSV-M3-BITE group elevated tumor killing by 51.81% relative to A549+VSV-M3-BITE

The A549+PBMC+VSV-M3-EGFP group showed a 31.7% increase in tumor killing relative to the A549+VSV-M3-EGFP group.

This result proves that any VSV virus can activate PBMC cells to achieve synergistic tumor killing effect

A549+PBMC+VSV-M3-BITE group showed a 13.13% increase in tumor killing compared to A549+PBMC+VSV-M3-EGFP group.

This result demonstrates that VSV-M3-BITE can specifically activate PBMC cells, thus enhancing the tumor killing effect.

Viral infection for 28 h

A549+PBMC+VSV-M3-BITE group increased tumor killing by 31.72% compared to A549+VSV-M3-BITE group.

The A549+PBMC+VSV-M3-EGFP group showed a 13.68% increase in tumor killing relative to the A549+VSV-M3-EGFP group.

This result proves that any VSV virus can activate PBMC cells to achieve synergistic tumor killing effect

A549+PBMC+VSV-M3-BITE group showed a 15.81% increase in tumor killing compared to A549+PBMC+VSV-M3-EGFP group.

This result demonstrates that VSV-M3-BITE can specifically activate PBMC cells, thus enhancing tumor killing.

Conclusion: Any VSV virus can activate PBMC cells to synergistically kill A549 tumor cells, and VSV-M3-BITE can specifically activate PBMC cells on this basis to increase the tumor killing effect by about 15%.

1.1.3.1.2 In vivo pharmacodynamics and toxicology

Pharmacodynamic experiments of lysovirus VSV-02 in subcutaneous transplantation tumor model of LLC mouse lung cancer cell line C57BL/6 mice

In this study, the antitumor activity of the test substance VSV-02 blistering stomatitis virus injection (hereinafter referred to as VSV-02) was evaluated in vivo in animals using an animal model of subcutaneous transplantation tumor in LLC mouse lung cancer cell line C57BL/6 mice.

Method: Approximately 5×10^6 cells/mL of LLC cells (with consecutive passage generations less than 10) were suspended in PBS and subcutaneously inoculated into the right dorsal region of mice at a volume of 100 μL per mouse. On day 8 after LLC cell inoculation, the mice were randomly divided into 4 groups based on body weight and tumor volume:

G1: Vehicle control group, i.t., QOD × 7 times;

G2: VSV-02 3×10^8 PFU/mouse, i.t., QOD × 7 times;

G3: VSV-02 3×10^8 PFU/mouse, i.v., QOD × 7 times;

G4: VSV-02 $3\times10^8 + 3\times10^8$ PFU/mouse, i.t. + i.v., QOD × 7 times.

Each group consisted of 6 animals. The day of grouping and administration was defined as Day 0. During the administration period, tumor volume was measured and recorded twice per week. Body weight was measured and recorded twice per week before the first administration and during the administration period. Body weight was also recorded when animals became moribund and were euthanized. At the experimental endpoint, all mice were euthanized.

RESULTS: In this experiment, compared with the G1 Vehicle control group during the same period, the tumor volume of the G2 VSV-02 3×10^8PFU/only intratumorally administered group was significantly lower than that of the control group at Day 17, with a TGI% of 85.67%, and that of the G3 VSV-02 3×10^8PFU/only intravenously administered group was significantly lower than that of the control group, with a TGI% of 50.15 %. TGI% was 50.15%; G4 VSV-02 $3\times10^8+3\times10^8$PFU/only Intratumoral + intravenous administration group had a significantly lower tumor volume than that of the control group, with a TGI% of 92.50%.

The TGI% was 92.50% in the control group. G2: VSV-02 3×10^8PFU/only, i.t., QOD×7times; G3: VSV-02 3×10^8PFU/only, i.v., QOD×7times; G4: VSV-02 $3\times10^8+3\times10^8$PFU/only, i.t. + i.v., QOD×7times; G4: VSV-02 $3\times10^8+3\times10^8$PFU/only, i.t. + i.v., QOD×7times. 7times None of the average body weights of the animals on Day 17 were significantly reduced compared to Day 0. Overall the mice were tolerant to the test drug VSV-02.

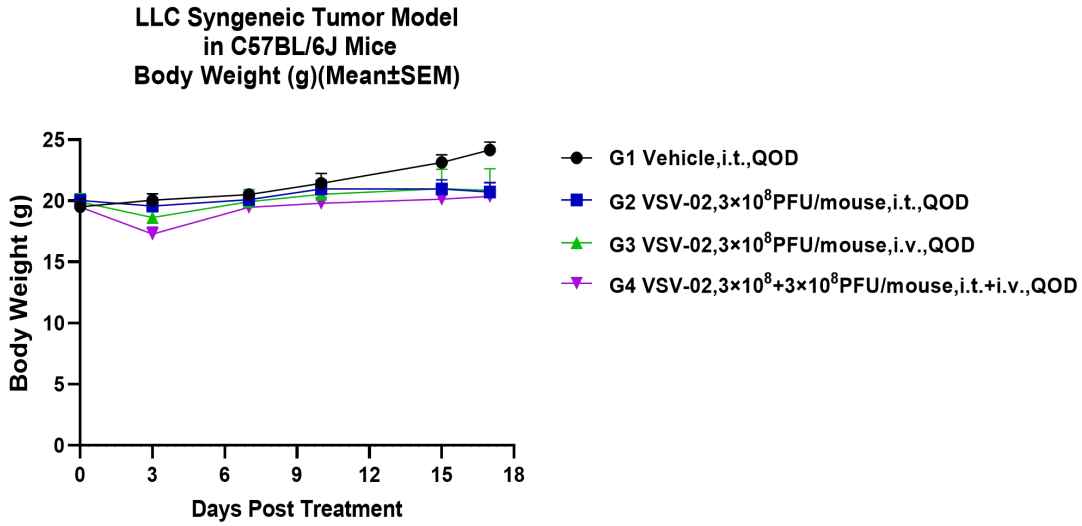
Effect of Subject VSV-02 on Body Weight of Animals in the Lung Cancer Homograft Tumor Model in LLC Mice

During the experimental period, the overall body weight of the mice in each group continued to increase and their mental status was good. The lysate control group, G2 VSV-02 3×10^8PFU/unit, i.t., QOD×7times administration group, G3 VSV-02 3×10^8PFU/unit, i.v., QOD×7times administration group, and G4 VSV-02 $3\times10^8+3\times10^8$PFU/unit, i.t. + i.v., QOD×7times administration group. 7times dosing groups Day 17 mean body weight of animals were not significantly toxic compared to Day0.

Effect of Subject VSV-02 on Body Weight of Hormonal Animals in the Lung Cancer Homograft Tumor Model in LLC Mice

Group	Number of animals N	Dosing regimen	Mean body weight of animals (g, Mean± SEM)		Mean % change in body weight Day17vs Day0	Number of drug-related animal deaths
			Day 0	Day 17		
1	6	Vehicle,i.t., QD×7times	19.5±0.28	24.16±0.28	24.34% (+ 4.66g)	0/6
2	6	VSV-02,3×10^8PFU/only, i.t.,QOD×7times	20.02±0.16	20.72±0.31	3.49% 0.70g)	0/6
3	6	VSV-02, 3 × 10^8PFU/only, i.v., QOD × 7times	19.91±0.28	20.86±0.87	6.41 % (0.96g)	0/6

4	6	VSV-02,3×10^8+3×10^8PFU/only,i.t.+i.v., QOD×7times	19.49±0.16	20.37±0.47	4.43% (0.88g)	0/6



The effect of subject VSV-02 on the body weight of LLC murine lung cancer homograft tumor model animals

CONCLUSION: Under the conditions of this experiment, the growth of LLC cell lines was normal and the tumor growth curves conformed to the pattern of historical data. Compared with the control group G1 Vehicle, i.t., QOD×7 times, the G2 VSV-02 3×10^8PFU/only, i.t., QOD×7 times group, the G3 VSV-02 3×108 PFU/only, i.v., QOD×7 times group, the G4 VSV-02 3×10^8+3×10^8PFU/only, i.t. + i.v., QOD×7 times group all demonstrated a significant inhibitory effect on tumor growth ($p<0.05$), with TGI% of 85.67%, 50.15% and 92.5% at Day 17, respectively. Among them, 3/6 tumors disappeared in the G4 intratumoral + intravenous group on Day 17, which showed the most obvious tumor suppression effect.

The lysate control group, G2 VSV-02 3×10^8PFU/only, i.t., QOD×7times administration group, G3 VSV-02 3×10^7PFU/only, i.v., QOD×7times administration group and G4 VSV-02 3×10^8+3×10^8PFU/only, i.t. + i.v., QOD× There was no significant toxic response in any of the 7times dosing groups up to Day 17 mean animal body weight compared to Day0 ($p > 0.05$).

Lysovirus VSV-CD3-PDL1 Efficacy Experiment in MC38 Mouse Colon Cancer Cell Line C57BL/6J Mouse Subcutaneous Transplant Tumor Model

In this study, we evaluated the antitumor activity of the subject VSV-CD3-PDL1 blistering stomatitis virus injection (hereinafter referred to as VSV-CD3-PDL1) using the mouse colon cancer MC38 syngeneic transplantation tumor model and compared the effects of different doses on the antitumor effects of the subject VSV-CD3-PDL1.

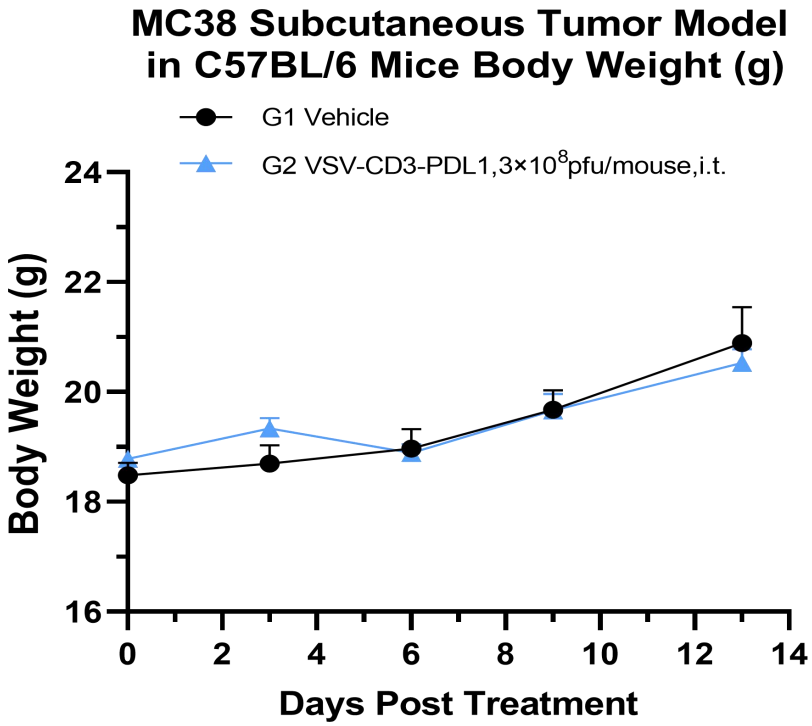
METHODS: About 2 × 106 cells /mL MC38 cells (consecutive culture generations less than 10 generations) were suspended in PBS and inoculated into the right dorsal subcutaneous area of mice by subcutaneous injection, with an inoculation volume of 100 μL/only. Mice were randomly divided into 2 groups according to body weight and tumor volume, G1: Vehicle control group, i.t., Q2D×7times; and G2 VSV-CD3-PDL1 3×108 pfu/one, i.t., Q2D×7times administration group with 6 animals in each group. The day of group administration was defined as Day 0.

Effect of subject VSV-CD3-PDL1 on body weight of MC38 mouse colon cancer homograft tumor model animals

During the experimental period, the mice in each group showed a continuous increase in body weight and good mental status. The average body weight of animals in the lysate control group, G2 VSV-CD3-PDL1 3×10^8pfu/only, i.t., Q2D×7times administration group on Day13 did not show any significant toxicity response compared with that on Day0.

Effect of Subject VSV-CD3-PDL1 Administered Alone on Body Weight of Hormonal Animals in MC38 Mouse Colon Cancer Homograft Tumor Model

Group	Number of animals N	Dosing regimen	Mean body weight of animals (g, Mean SEM)		Mean body weight % change Day 13vs Day 0	Number of drug-related animal deaths
			Day 0	Day 13		
1	6	Vehicle, i.t., Q2D×7	18.48±0.23	20.89±0.65	0.13% (+ 2.41g)	0/6
2	6	VSV-CD3-PDL1. 3×10^8pfu/only, i.t., Q2D × 7	18.78±0.14	20.53±0.25	0.09 % (+1.75g)	0/6



Effect of subject VSV-CD3-PDL1 on body weight of MC38 mouse colon cancer homograft tumor model animals

RESULTS: In this experiment, Day13, G2 VSV-CD3-PDL1 3×10^8pfu/only, i.t., Q2D×7times administration group %T/CRTV was 52.67; the group of the test substance G2 VSV-CD3-PDL1 had better inhibition of the tumor growth of the mouse colon cancer MC38.

During the experimental period, the weight of mice in each group continued to increase and their mental status was good. There was no significant toxicity reaction in both the lysate control group and the G2 VSV-CD3-PDL1 3×10^8pfu/only, i.t., Q2D×7times administration group of the average body weight of animals on Day13 compared with that on Day0.

1.1.3.2 Pharmacokinetic study

1.1.3.2.1 Distribution.

Consistent with OVV-01, which has received FDA Clinical I and II study approval.

Repeated Dose Biodistribution Assay in C57BL/6J Hormonal Mice (Study Nos. ALFT008M and ALFT013M, GLP)

Repeated dosing toxicity in 2 GLP loaded mice, there were differences in the dosing intervals between the 2 experiments, but the results of the biodistribution of the 2 experiments were consistent. The results of the biodistribution conducted along with the repeated dosing toxicity test in tumor-

bearing mice showed that, for vector VSV, viral copy number could be detected in the blood samples of the animals in each dosing group mainly during the dosing period, but not at the end of the recovery period; basically, viral copy number could be detected in the tissues of the animals in each dosing group mainly in tumors and spleens after dosing, and also at the end of the recovery period, with a decreasing trend with the extension of time. At the end of the recovery period, viral copy number could also be detected, and the copy number basically showed a decreasing trend with the prolongation of time. For the tumor antigen NY-ESO-1, only tumor tissues were positive after administration of the drug to animals in each dosing group, and the incidence or intensity of staining was basically positively correlated with the dose, and the intensity of staining tended to decrease from strong to weak with the prolongation of time, while no significant positivity was observed in other organs.

Repeated intravenous administration of rhesus monkeys for 2 cycles (once a day for 7 days and 5 days off), 4 weeks off recovery toxicity test accompanied by viral shedding, and biodistribution evaluation (Study No. AX2401P, GLP)

The results of biodistribution evaluation showed that after receiving repeated intravenous administration of OVV-01, OVV-01 was visible in whole blood and most tissues, mainly in the spleen, followed by the lungs: Day 20 after administration of OVV-01 in the high and low dose groups, OVV-01 virus was detected in all tissues, except for adrenal glands and thymus, which were lower than the lower limit of quantification in the low dose group; Day 48 after administration of OVV-01 in the high and low dose groups. OVV-01 virus was detected in the heart, liver, spleen, and lungs in the low-dose group and in the heart, spleen, lungs, kidneys, thymus, and sciatic nerves in the high-dose group, and was predominantly found in the spleen, all of which showed a significant decrease from Day 20.

The results of viral shedding evaluation showed that after receiving repeated intravenous administration of OVV-01, OVV-01 virus was detected in saliva, nasal mucus and fecal samples of the high and low dose groups, and only in urine samples of the high dose group; at the end of the recovery period (Day 46/47), virus was detected in only one saliva sample and one nasal mucus sample of the high dose group, and in the rest of the dose groups each of the Virus was not detected in any of the virus shedding samples in the remaining dose groups.

1.1.3.2.2 Metabolism

Consistent with OVV-01, which has received FDA approval for clinical studies I and II.

1.1.3.2.3 Excretion

Consistent with OVV-01, OVV-01 has received FDA Clinical I and II study approval.

1.1.4 Overview of Clinical Studies

OVV-01 Injection has completed 1 investigator-initiated study (IIT) (RR-001) in patients with advanced solid tumors in which subjects received a single dose and 2-weekly sequential doses for a total of 6 doses.

In RR-001, a total of 18 subjects received study drug in 4 dose groups (6×10^7PFU/person: 4; 6×10^8PFU/person: 4; 6×10^9PFU/person: 7; $1.2\times10^{(1)}$ $^{(1)}$PFU/person: 3).3 weeks after the initial administration of the OVV-01 injection, a 2-weekly serial dosing was initiated administration, with 6 overall administrations.

The results of this study showed that the study drug did not cause any DLT events during single and multiple dosing in a total of 18 subjects in the low dose group of 3×10^7PFU/person (4 cases), the medium dose group of 3×10^8PFU/person (4 cases), the high dose group of 3×10^9PFU/person (7 cases), and the even higher dose group of 6×10^{10}PFU/person (3 cases), and did not serious adverse events related to the study drug occurred, and the maximum tolerated dose (MTD) was not reached. The overall safety profile was favorable, with no unanticipated new safety issues identified.

The most common (incidence ≥ 20.0%) TEAEs associated with OVV-01 Injection were fever (12 cases, 66.7%), decreased lymphocyte count (5 cases, 27.8%), and anemia (4 cases, 22.2%). Subjects received administration of the study drug all subjects were immunogenic positive. Live virus particles were detected in the peripheral blood (serum) of only some of the subjects at the 0.25h, 1h, 3h, 12h, and 24h time points after subjects received study drug administration, and were not detected in saliva, urine, or feces. No live virus particles were detected in whole blood (plasma) after subjects received study drug administration.

Based on compliance with the protocol set, the objective remission rate (ORR_{16w}) assessed at 16 weeks after the first treatment was 27.3%, $ORR_{8w}$was 0, $ORR_{24w}$was 27.3%, $DCR_{24w}$was 63.6%, $PFSR_{24w}$was 43.08%, the median $DoR_{24w}$was NA, and the median overall survival was NA; in advanced sarcoma $ORR_{16w}$was 50.0% and $DCR_{16w}$was 83.3% in subjects with advanced sarcoma, and $ORR_{16w}$was 75.0% and $DCR_{16w}$was 75.0% in subjects with advanced soft-tissue sarcoma.OVV-01 Injection demonstrated efficacy in addition to preliminary efficacy in an advanced solid tumor population.

1.2 Risk/Benefit Assessment

VSV-02 is an attenuated VSV virus carrying CD3/PD-L1 bispecific antibodies that selectively infects tumors and displays potent tumor lysis efficacy against tumors.VSV-02, due to its lysogenic viral properties, specifically replicates and expresses insertion of bispecific antibodies in tumor cells,

which elicits spontaneous humoral and cellular immune responses, and, coupled with its restricted expression pattern , is a good candidate target for cancer immunotherapy.

Currently, there are no similar products (VSV-vectored lyssaviruses) on the market in China or abroad. A single intravenous dose-escalation study of VSV-IFNβ -NIS in clinical development was conducted in patients with relapsed/refractory hematologic malignancies (NCT03017820).Fifteen patients received VSV-IFNβ -NIS administration, and none of the four dosage groups (0.05, 0.17 , 0.5, and 1.7×10^{11}TCID50) had DLT occurred, the most common AEs observed were hematologic and usually returned to baseline levels within 2 to 10 days, and most patients had a transient febrile response to the viral infusion that began a few hours after the infusion and subsided overnight and was usually associated with nausea, vomiting, hypotension, and/or mild hypoxemia. Transient elevations of several cytokines were observed 4h after lysosomal virus infusion and returned to baseline levels at 24 or 48 hours. Various concentrations of viral particles were detected immediately, 2h, 4h, 24h, 48h, and 72h post-infusion, but no viral persistence was observed. Anti-VSV neutralizing antibodies were detected at 29 days post-infusion. T cells, NK cells, myeloid-derived suppressor cells (MDSC), and myeloid cells in the peripheral blood were measured at baseline, day 3, day 8, day 15, and day 29 post-injection, and a trend toward increased PD-1 expression on the surface of CD8+ T cells was observed[12] . The overall safety profile of OVV-01 injection in the RR-001 study that has been conducted with this product was favorable, with no DLT events and a total of 2 SAEs, both unrelated to the study drug. The most common adverse reactions were fever, decreased lymphocyte count and anemia.

In terms of efficacy, published clinical trial results for a foreign marketed lysosomal virus product (T-Vec) are very encouraging. Other VSV-based oncolytic viral products have also been studied in a variety of solid tumor indications. 3 of 7 patients with T-cell lymphoma (TCL) demonstrated remission in a study of VSV-IFNβ -NIS in patients with relapsed/refractory hematologic malignancies (NCT03017820). In the completed RR-001 study with this product, with an $ORR_{16w}$of 27.3%, $ORR_{8w}$of 0, $ORR_{24w}$of 27.3%, $DCR_{24w}$of 63.6%, and $PFSR_{24w}$of 43.08%, OVV-01 Injection demonstrated efficacy beyond preliminary efficacy in an advanced solid tumor population.

Based on results from non-clinical studies, results from approved and in-development lysosomal viral products, and results from the clinical RR-001 study, OVV-01 has the potential to improve therapeutic outcomes in subjects with advanced solid tumors, and the overall safety profile of OVV-01 is manageable. Taken together, the data suggest that OVV-01 is expected to be an effective interventional therapy that provides clear clinical benefit to subjects with advanced solid tumors, while maintaining manageable safety risks.

2 Trial Objectives and Endpoints

Study Objectives	Study Endpoints
Primary Endpoint	**Primary Endpoint**
• To evaluate the preliminary efficacy of intravenous and intratumoral administration of VSV-02 Injection in subjects with advanced solid tumors;	• Objective Remission Rate (ORR), Disease Control Rate (DCR), Duration of Response (DoR), Progression Free Survival (PFS) and Overall Survival (OS);
Secondary endpoints	**Secondary Endpoints**
• To evaluate the safety of VSV-02 Injection administered intravenously and intratumorally in subjects with advanced solid tumors.	• Incidence and characterization of adverse events (AE); • Changes in safety indicators such as laboratory findings, physical examination, electrocardiogram and vital signs compared to baseline;

3 Experimental design

3.1 Overall design.

This is an open-label, single-arm, dose-escalation Phase I clinical study designed to evaluate the preliminary efficacy and safety of VSV-02 Injection intravenously and intratumorally in subjects with advanced solid tumors for compassionate use in patients lacking effective treatment modalities.

Screening-eligible subjects must be subjects with histologically or cytologically confirmed advanced malignant solid tumors and must have measurable and injectable tumor lesions, including superficial lesions as well as deep lesions that are amenable to injection under ultrasound/CT guidance.

The study consists of a screening period, a treatment period, and a follow-up period. The screening period lasted from the day the subject signed the ICF (Day -28) to the day before the first dose (Day -1). Screening-eligible subjects will receive IV therapy (Part 1) or IV + intratumor therapy (Part 2) from administered on Days 1 and 3 (D1, D3) of each cycle, with one treatment cycle every 3 weeks for a maximum of 6 cycles, with IV and intratumor administered on the same day. If DLT occurs during the DLT observation period, VSV-02 administration will be discontinued. If DLT does not occur, treatment will continue until disease progression, the subject develops intolerable toxicity, the subject withdraws informed consent, the subject dies, is lost to visitation, the investigator determines that it is in the patient's best interest to terminate treatment, or 6 consecutive cycles of administration have been completed, whichever occurs earliest.

Some subjects may experience transient tumor growth and pseudo-progression during the first few months after initiation of immunotherapy, followed by disease remission. After the first disease progression in a subject, as determined by the investigator according to RECIST 1.1 criteria, subjects will be allowed to continue treatment with VSV-02 if the investigator determines that the subject is in

a clinically stable state and it is believed that continued treatment of the subject may be of clinical benefit. If the imaging assessment shows disease progression as determined according to RECIST 1.1 and there is no clinical deterioration, the tumor should be evaluated again at least 6 weeks later to confirm disease progression according to iRECIST for Cancer Immunotherapy Trials. Clinical stability is defined as the absence of clinically significant signs and symptoms suggestive of disease progression (including abnormal laboratory test values), no reduction in ECOG Physical Status Score, no rapid disease progression, and the absence of progressive tumors (e.g., spinal cord compression) at important anatomical sites that would require other urgent medical intervention. Subjects judged to be clinically unstable should be terminated from trial treatment since the first imaging disease progression evaluated by the investigator, and repeat imaging is not required to confirm disease progression.

The follow-up period consisted of safety follow-up and survival follow-up, with subjects required to return to the study center for safety follow-up within 28 days (±7 days) of the last dose if treatment was discontinued for any reason. Subjects then enter a survival follow-up period with survival follow-up assessments every 12 weeks until the subject withdraws informed consent, dies, loses the visit, receives other antitumor therapy other than that specified in the protocol, or the study is completed, whichever occurs earliest. After subjects have completed 12 cycles of multiple dosing, if the investigator judges that subjects may still benefit from continued treatment with VSV-02 Injection, each patient should be consulted, and if the subject requests to continue treatment, he or she may apply for continued treatment, but must sign a separate informed consent form, the specific content of which should be based on the actual condition of the patient, and formulated after communication with the investigator.

3.1.1 Dose-limiting toxicity

In accordance with the internationally recognized CTCAE v5.0 toxicity evaluation criteria, the study DLT was defined as the following toxic reactions related to the study drug VSV-02 occurring within 4 weeks after the first dose. This study DLT includes, but is not limited to, the following provisions (fulfillment of any of the following conditions was judged to be a DLT event):

- Hematologic toxicity

（1） Grade 4 neutropenia (ANC) lasting ≥ 7 days;

（2） Febrile neutropenia (ANC <1000/mm^3, accompanied by a single measurement of body temperature >38.3°C or persistent ≥38.0°C for more than 1 hour);

（3） Grade 4 thrombocytopenia;

（4） Grade 3 thrombocytopenia with bleeding or bleeding events requiring platelet transfusion;

（5） Any ⩾ grade 3 hematologic toxicity that does not improve to grade 2 or below within 14 days after standard interventions and supportive measures.

- Non-hematologic toxicity

（1） Grade 3 vital organ toxicity, including cardiac, pulmonary, gastrointestinal, hepatic, renal, and neurologic toxicity; and any other Grade ≥4

（2） Concomitant occurrence of aspartate aminotransferase (AST) or alanine aminotransferase (ALT) >3 × upper limit of normal (ULN) and total bilirubin (TBIL) >2 × ULN (according to Hay's Law) without any other reason to explain the elevation; and

（3） Grade ≥3 influenza-like symptoms (including fever, etc.) lasting longer than 7 days after optimal supportive care;

（4） ≥ grade 3 cytokine release syndrome

（5） Other ≥ Grade 3 non-hematologic toxicity (except simple laboratory test abnormalities that, in the judgment of the investigator, are not clinically symptomatic and do not require intervention);

（6） Grade 3 TLS with clinical symptoms such as cardiac arrhythmias;

（7） Any death that cannot be clearly attributed to tumor progression or other external causes (Grade 5 toxicity)

- The following are not considered DLT:

• Grade 3 rash, nausea, vomiting, diarrhea, or electrolyte disturbances that recover to ≤ grade 2 within 72 hours of optimal medical management;

• Grade 3 fatigue ≤ 7 days;

• Grade 3 endocrine disorders adequately controlled with hormone replacement therapy;

• Grade 3 tumor lysis syndrome or associated electrolyte disturbances that resolve to ≤ grade 2 within 7 days;

• Grade 3 ALT elevation, AST elevation, or alkaline phosphatase (ALP) elevation that resolves to ≤ grade 2 within 7 days;

If a subject develops an investigational drug-related adverse event outside of the above DLT

criteria, any adverse event judged by the investigator to require termination of treatment will be discussed by the SMC to determine if it is a DLT.

3.1.2 Maximum tolerated dose

A total of 6 subjects will be required to determine the MTD. if ≤1/6 subjects develop a DLT, that dose will be considered the MTD; if >1/6 subjects develop a DLT, the previous dose level will be considered the MTD.

3.1.3 Starting Dose Based on

Toxicology studies in non-clinical studies in rhesus monkeys following 2 cycles of intravenous administration of 8.0×10^{9}PFU/kg and 8.0×10^{10}PFU/kg OVV-01 (QD 7 days, cycle interval 5 days) followed by a 4-week recovery period showed a maximum non-severe toxicity dose (HNSTD) of 8.0×10^{10}PFU/kg. converted. Human Equivalent Dose (HED) was 2.58×10^{10}PFU/kg, which corresponds to a dose of 1.55×10^{12}PFU/subject.

In a GLP toxicology study in tumor-bearing mice, repeated intratumoral and intravenous injections of OVV-01 for 2 cycles (1 cycle in 7 days, with a 1-day stop in between), followed by a 7-day recovery period, showed a HNSTD of 3.0×10^{9}PFU/mouse (intratumoral) + 1.2×10^{9}PFU/mouse (intravenous). Converted from 20 g of mouse body weight, the HED was 1.22×10^{10}PFU/kg (intratumoral) + 4.88×10^{9}PFU/kg, which corresponds to 7.32×10^{11}PFU/subject (intratumoral) + 2.93×10^{11}PFU/mg (intravenously).

In the completed dose-escalation study (RR-001), 18 patients with advanced solid tumors received intra-tumoral injections of OVV-01 Injection in four dose groups (6×107 PFU, 6×10^{8}PFU, 7×10^{9}PFU, and 1.2×10^{11}PFU) administered every two weeks in multiple doses (1 to 6 doses). No serious adverse events or DLTs related to the study drug occurred in any of the dose groups, and no MTDs were achieved.

In the ongoing dose-escalation study (RR-008), four patients received OVV-01 Injection via intra-tumor injection at a dose of 1.2×10^{11}PFU once daily for 6 consecutive days for 2 weeks. As of the cut-off date (December 16, 2024), there were no DLT events or serious adverse events, and the treatment demonstrated a favorable safety and tolerability profile. Taken together, these data support the proposed clinical IV starting dose of 3×10^{10}PFU/subject and the combined starting dose of 3×10^{10}PFU/mL (intratumoral) + 6×10^{11}PFU/mL (IV) administered multiple times by once-daily dosing.

3.1.4 Rationale for the dose-escalation rule

Dose group for this study: 3×10^{10}PFU/mL (it.) + 3×10^{11}PFU/subject (iv.). Referring to the preclinical data, the dose range has covered the clinically effective dose. Upon completion of the dose group, the SMC assessed whether the dose escalation was newly continued based on outcome assessment.

The investigator determines the highest dose level that can be tolerated by the subject by closely monitoring the DLT.

3.1.5 Dose Escalation Settings

The DLT observation period was 4 weeks after the first dose. The first 3 subjects enrolled in each dose group were incremented to the next dose group if none developed DLT during the DLT observation period. If ≥2 DLTs occurred in the initial 3 subjects in a dose group, the dose escalation was stopped or an additional cohort was added to the lower dose group, as recommended by the Safety Monitoring Committee (SMC). If 1 DLT occurred in the initial 3 subjects, enrollment of 3 additional subjects at the same dose was continued. If no DLT occurs in the other 3 subjects, the dose is escalated to the next higher dose group. If ≥1 DLT occurs in 3 additional subjects, dose escalation will be stopped.

A total of 6 subjects will be required to determine the MTD. if ≤1/6 of the subjects develop a DLT, the dose will be considered the MTD; if >1/6 of the subjects develop a DLT, the previous dose level will be considered the MTD.

Dose escalation and stopping rules are summarized in Table 3 of .

Table3 Dose Escalation and Stopping Rules

Number of cases of subjects experiencing a DLT	Measure
0/3	Escalation to next dose group
1/3	3 additional subjects at the same dose level
1/3 + 0/3	Increment to next dose group
1/3 + ≥1/3	Stop dose escalation and recommend using previous dose level as MTD

NOTE: Treatment of each subject must not raise or lower the dose.

Escalation to the next higher dose group may only occur when the SMC confirms that the current dose group is sufficiently safe and tolerable. As recommended by the SMC, an intermediate dose level (3×10^{10}PFU/mL) may be explored between the dose group in which 2 DLTs were seen and the adjacent lower dose group.The SMC will review the safety and tolerability data for each of the dose groups of VSV-02, as well as other available data, including biodistribution, viral shedding, pharmacodynamics, and immunogenicity, and then may discuss the appropriate dose escalation and duration of treatment, and recommend whether to include additional dose groups (which may include

downward/upward adjustments to the planned dosing, adding additional dose groups, etc.). If both dose levels proposed in this study are shown to be safe, higher dose levels (e.g., 6×10^{11}PFU/mL) may be explored, and the exact dose level will be determined by the SMC.

3.2 Study Suspension Rules

The SMC will request that enrollment and dosing be suspended until the situation can be assessed when the following conditions occur:

- Deaths occurring within 30 days of study drug administration (unless clearly due to disease progression)
- Deaths considered by the investigator to be at least potentially related to the study drug
- Two Grade ≥4 DLTs in 2 subjects
- Any grade 4 hypersensitivity reaction, rapid onset severe allergic reaction

3.3 Definition of study completion

A subject was considered to have completed the study if the subject completed all phases of the study, including the final visit or the last planned procedure listed in the study schedule.

End of study was defined as completion of the last visit by the final subject, withdrawal of informed consent by the subject, death, loss of visit, or termination of the study (whichever occurred earliest).

4 Study Population

4.1 Enrollment Criteria

Subjects must meet all of the following enrollment criteria to be enrolled in this trial:

1. Voluntarily sign an informed consent form, understand the study and be willing to follow the protocol and be willing to complete all trial procedures;
2. Age ≥ 18 years old at the time of signing the ICF, regardless of gender.
3. Patients with advanced solid tumors diagnosed after histologic/cytologic examination of the pathology of the primary and/or metastatic site .
4. Patients with advanced disease who have failed standard therapy and lack standard treatment.
5. In principle, subjects must have at least one lesion judged measurable according to RECIST 1.1 criteria, i.e., non-lymph node lesions with a long diameter of ≥ 10 mm and lymph node lesions with a short diameter of ≥ 15 mm according to CT or MRI. and must have injectable tumor lesions, including superficial lesions, as well as deeper lesions that can be injected under

ultrasound/CT/ or endoscopic guidance.

6. Subjects with an ECOG physical status score of 0-2 and an expected survival of not less than 12 weeks.
7. Adequate organ and hematopoietic function:
   - Absolute Neutrophil Count (ANC) ≥ 1.5×10^9/L;
   - Platelets ≥ 75×10^9/L (no platelet transfusion therapy or thrombopoietin (TPO) therapy within 2 weeks prior to first dose);
   - Hemoglobin ≥ 90 g/L (no blood transfusion within 2 weeks);
   - Serum creatinine ≤ 1.5 × upper limit of normal (ULN) or endogenous creatinine clearance (CCr) ≥ 50mL/min;
   - Glutamine transaminase (AST) and alanine transaminase (ALT) ≤ 3.0 × ULN; AST and ALT < 5 × ULN if patients with liver metastases;
   - Serum total bilirubin (TBIL) ≤2×ULN;
   - International Normalized Ratio (INR) ≤ 1.5 × ULN, or Activated Partial Thromboplastin Time (APTT) ≤ 1.5 × ULN;
8. Females of childbearing potential must have had a pregnancy test with a negative result within 7 days prior to initiating treatment.
9. Male and female subjects of childbearing potential must agree to use a reliable method of contraception for the duration of the trial and for at least 6 months after the last dose.

4.2 Exclusion Criteria

Patients with any of the following are not eligible for enrollment in this study (compassionate use patients may be treated compassionately on a case-by-case basis at the joint discretion of the PI and investigator):

1. Known brain metastases and/or clinical suspicion of brain metastases from the tumor (however, patients with asymptomatic brain metastases or who have been clinically stable for more than 3 months with local therapy may be enrolled);
2. Subjects who have had radiotherapy within 2 months of the target lesion;
3. Subjects with other active malignancies within the previous 5 years. Subjects who have been completely cured and do not require follow-up therapy are excluded, except for subjects whose malignancy is within the indications;
4. The longest diameter of the lesion used for injection is >100 mm;

5. Subjects who have participated or are participating in a clinical trial of another drug or medical device within the previous 4 weeks;
6. Subjects who are preparing for or have previously received a tissue/organ transplant;
7. Subjects with human immunodeficiency virus (HIV) infection and AID-related opportunistic infections within 12 months, or CD4+ T-cell (CD4+) count < 350 cells/uL; Screening Hepatitis B Surface Antigen (HBsAg) and/or Hepatitis B Core Antibody (HBcAb) positivity with HBV-DNA above the lower limit of measurability, Screening HCV antibody Positive patients with HCV-RNA above the lower measurable limit; subjects with positive syphilis spirochete serology;
8. Subjects requiring antiviral medication during the study or within 5 half-lives of antiviral medication at the time of first administration.
9. Subjects requiring therapeutic anticoagulant medication during the study.
10. Subjects with an uncontrolled grade ≥3 active infection of significant clinical relevance according to CTCAE v5.0;
11. Received antitumor agents such as chemotherapy, radiotherapy, biotherapy, endocrine therapy, immunotherapy, etc. within 4 weeks prior to the first dose; Received small molecule targeted therapies and oral fluorouracil analogs within 2 weeks or 5 half-lives (whichever is longer) prior to the first dose; Received herbal or proprietary Chinese medicines with antitumor indications within 2 weeks prior to the first dose; Received nitrosoureas or mitomycin C; palliative radiotherapy to non-target lesions allowed (≥2 weeks prior to first dose);
12. Medication-uncontrolled hypertension or pulmonary hypertension or unstable angina pectoris; myocardial infarction or bypass or stent surgery within 6 months prior to dosing; history of chronic heart failure in New York Heart Association (NYHA) criteria grade 3-4; severe arrhythmias requiring treatment (except atrial fibrillation and paroxysmal supraventricular tachycardia, which, in the judgment of the investigator, have no effect on the trial), including QTcF ≥450 ms in men and ≥450 ms in women. 450ms in men and ≥470ms in women (calculated using the Fridericia formula); cerebrovascular accident (CVA) or transient ischemic attack (TIA) within 6 months prior to enrollment;
13. Patients with active autoimmune disease or a history of autoimmune disease with possible relapse, but patients with the following diseases were not excluded and could be screened further
    - Type 1 diabetes mellitus; and
    - Hypothyroidism (if controllable with hormone replacement therapy alone) ;Â
    - Controlled celiac disease .
    - Skin conditions that do not require systemic therapy (e.g., vitiligo, psoriasis, alopecia

areata) ;and

- Any other condition that will not reoccur in the absence of external triggers ;Â

14. Subjects requiring treatment with systemic corticosteroids (>10 mg /day prednisone or equivalent dose) or other immunosuppressive medications within 14 days prior to the first dose or during the study period, except as follows:
    - Adrenergic replacement steroids (prednisone ≤ 10 mg / day or equivalent dose of a similar drug) ;.
    - Topical, ophthalmic, intra-articular, intranasal, or inhaled corticosteroids.
    - Prophylactic short-term (≤ 7 days) use of corticosteroids (e.g., allergy to contrast media) or for the treatment of non-autoimmune disorders (e.g., delayed hypersensitivity reactions caused by contact allergens);
15. Subject has a tumor located in a high-risk location (including in a mucosal area, or in close proximity to the airway, major blood vessels, or spinal cord) that may result in occlusion or compression due to tumor enlargement, or erosion into major blood vessels due to necrosis, or encasement of major vascular structures (e.g., carotid arteries), tumors adjacent to important neurovascular structures, or other tumors deemed unsuitable for intratumoral injections;
16. Subjects will be required to receive any live vaccine during the Screening and Treatment Periods;
17. Subject is allergic to any component of the investigational drug, immunotherapy or related medications;
18. Subjects with concomitant mental illness, alcoholism, inability to quit smoking, drug abuse or substance abuse;
19. Pregnant or lactating females;
20. Adverse effects of prior antineoplastic therapy that have not recovered to (CTCAE 5.0) Grade 1 (except alopecia);
21. Serious uncontrollable disease, as determined by the investigator, or the presence of other conditions that may interfere with receiving treatment in this study and are considered unsuitable for participation in this study;
22. Other conditions deemed by the investigator to be unsuitable for enrollment.

4.3 Screening Failure

Screening failure is defined as a subject who signed the ICF but did not receive study treatment for any reason. Necessary information will be collected on subjects with screening failures, including, but not limited to, demographics, reasons for screening failure/non-compliance with enrollment criteria, any SAEs and SAE medications that occurred between signing the ICF and withdrawal from

the study due to screening failure.

Subjects who do not meet the entry criteria may be rescreened. If the rescreening occurs more than 30 days after the last signed ICF, a new ICF must be signed.The rescreened subject will receive a new screening number in place of the initial screening number.

4.4 Subject Replacement

Subjects who terminate the study and do not have any imaging after Screening Period imaging through the end of treatment will be replaced with a new subject, except that subjects who develop Grade ≥3 toxicity that is definitely or probably related to the study drug as determined by the investigator will not be replaced.

The decision to replace will be made after discussion and agreement by the Principal Investigator (PI), SMC.

4.5 Termination of Treatment for Subjects

Subjects will be considered for permanent termination of treatment upon occurrence of any of the following conditions

- Subject develops DLT during the DLT observation period.
- Subject develops clinically unstable disease progression as determined in accordance with RECIST 1.1.
- The subject develops any AE or complication that may result in discontinuation of study treatment for more than 6 weeks, or the investigator deems it necessary to terminate study treatment.
- The subject experiences a clinically significant deterioration in health status that warrants discontinuation of treatment.
- The subject is unfit to continue treatment for other medical reasons (e.g., pregnancy).
- The subject requests termination of treatment for personal reasons.
- The subject misses a visit.

Reason for treatment termination must be documented in the eCRF. Termination of treatment does not mean withdrawal from the study and all remaining study processes must be completed in accordance with the study protocol.

4.6 Subject Withdrawal from Study

Subjects have the right to request withdrawal from the study at any time and may be withdrawn from the study for safety, behavioral, or administrative reasons at the discretion of the investigator.

Subjects must terminate study medication and withdraw from the study when any of the following conditions occur

1. Pregnancy
2. Obvious noncompliance with the study intervention
3. Development of any clinical AE, laboratory test abnormality, or other disease or condition that makes continued participation in the study contrary to the subject's best interest
4. Need for anticancer treatment outside of the study protocol
5. Subject is unable to receive VSV-02 administration for 6 consecutive weeks. If the disappearance of an injectable tumor lesion results in termination of study dosing, in which case it is the investigator's decision whether the subject will continue to receive VSV-02 and/or continue to participate in the study
6. Occurrence of what the investigator, after discussion, unanimously recognizes as a significant protocol violation
7. Withdrawal of informed consent
8. Termination of the study by the investigator
9. Termination of the study by the local health regulatory authority, regulatory agency or Institutional Review Board/Independent Ethics Committee (IRB/IEC)

If the subject decides to withdraw from the study, or if the investigator believes that the subject should withdraw from the study: the collaborating research medical monitor must be notified within 24 hours, and the reason for withdrawal must be documented in the subject's medical record and CRF. The investigator must try to follow all subjects for safety until the follow-up assessment or study drug-related toxicity resolves, returns to baseline, or is judged to be irreversible, whichever occurs latest. Reasons for subject termination or withdrawal from the study must be documented in the eCRF.

4.7 Research Intervention Abort/Terminate

This trial has the potential for early termination under certain circumstances; circumstances that may terminate a clinical trial include, but are not limited to:

（1） The occurrence of a serious adverse event due to the test drug at the subject dose that, in the judgment of the investigator, may result in a serious safety risk to other subjects, shall result in termination of the trial;

（2） If the Investigator or Collaborator becomes aware of new research evidence suggesting that there is a serious safety concern with the trial drug product that is not included in the Drug Formulary/IB and that may result in harm to subjects if the clinical trial is continued, the Collaborator and the Investigator jointly evaluate and decide whether to terminate the trial;

（3） Significant deviations or human errors are found at the end of the trial implementation process, which seriously affects the quality of the trial and makes it difficult to achieve the purpose of the trial;

（4） The administrative authority requires the termination of the trial;

（5） The partner requests to terminate the trial under the premise of fully protecting the rights and safety of the subjects (e.g., financial reasons, management reasons, etc.).

When the research intervention is discontinued, the follow-up study procedures should be completed in accordance with the provisions of the trial protocol. In the event of a clinically meaningful change in a subject after enrollment (including, but not limited to, a change that deviates from baseline levels), the investigator or qualified designee will determine whether a change in subject management is warranted. Any clinically relevant new findings will be reported as an adverse event (AE).

4.8 Loss of Subjects to Visit

A subject was considered "lost" if he or she was unable to attend a scheduled visit and could not be contacted by Research Center staff. A lost visit was defined as a situation in which the subject could not be reached after 2 or more non-consecutive phone calls or through other contact information provided by the subject. A subject is considered a lost visit if he or she cannot be contacted after taking the above steps. These contact records should be documented in the subject's medical record or study documentation.

5 Research Intervention

5.1 Study Drug

5.1.1 Description of study drug

Study Drug	VSV-02 Injection
Specification	1mL/vial, 3.0×10^{10}PFU/mL
Storage and transportation conditions	≤-70°C Storage and transportation.
Manufacturer	Shanghai Rongrui Pharmaceutical Technology Co.

Supplier	Shanghai Rongrui Pharmaceutical Technology Co.

5.1.2 Study drug dosing regimen

VSV-02 injection was administered intravenously + intratumorally at a dose of 3×10^{10}PFU/mL (it.) + 3×10^{11}PFU (iv.) on day 1 (D1) of each cycle, with a maximum of 6 cycles per 3-week treatment cycle, and intravenous and intratumorally on the same day.

Patients who are screened for eligible enrollment will undergo systematic safety observations within 6 weeks of the first dose, and if the patient does not develop a DLT, the dose will be determined to be the Maximum Tolerated Dose (MTD) and the Recommended Phase II Dose (RP2D). If DLT is observed in 1 patient, 3 subjects will be added to the cohort, and if DLT is observed in ≥2 patients, the new cohort will be added at a lower dose as recommended by SMC.

If DLT occurs during the DLT observation period, VSV-02 administration will be discontinued. If DLT does not occur, treatment will continue until disease progression, the subject develops intolerable toxicity, the subject withdraws informed consent, the subject dies, is lost to visitation, the investigator determines that it is in the patient's best interest to terminate treatment, or 6 consecutive cycles of administration have been completed, whichever occurs earliest.

Intratumoral injection administration:

The volume of intratumorally injected VSV-02 was determined based on the size of the tumor lesion and was given to the maximum volume possible according to the guidelines in the table below. The investigator evaluated the size of the subject's injected lesion within 24 h prior to each injection to determine the volume of study drug to be injected. The total injection volume of all lesions per subject per dosing day was not to exceed 10 mL.

Table4 Guidelines for Injection Volume of VSV-02 Injection Based on Tumor Size

Lesion size (longest diameter)	Injection volume
≤0.5 cm	0.5 mL
>0.5 cm and ≤1.0 cm	1.0 mL
>1.0 cm	For every 1.0 cm increase in lesion size (rounded) from 1.0 cm, increase the injection volume by 1.0 mL from 1.0 mL (e.g., 3.0 mL for a 3.0 cm lesion).

Injection:

（3） It may not be possible to inject all lesions at a time; prioritize the largest lesion and inject other lesions according to the size of the lesion until the maximum delivery volume is reached. The same lesion should be injected each time unless the injected lesion shrinks to the point where it cannot receive the injection.

If there is residual drug available because the tumor lesion has shrunk, a new lesion may be selected for injection.

Intravenous Administration:

Each subject will receive a fixed dose intravenously, prepared as a 100 ml solution using an intravenous bag of 100 ml saline (0.9% sodium chloride injection), and administration can be completed in 30 minutes (±10 minutes).

5.1.3 Precautions for administration

1) Inject a lesion that is measurable and capable of receiving the injection either directly or under ultrasound or CT guidance or endoscopy;
2) Full dose uniformity and complete dispersion is achieved by using a single insertion point, pulling the needle back within the lesion without withdrawing from the lesion, along 2-4 tracks as far as the radial extent of the needle allows the lesion to be reached. If the lesion is larger than 5 cm, 2 insertion points of the needle radial may be used;
3) When the needle is removed, it is withdrawn slowly from the lesion and should be held briefly before completely withdrawing from the lesion to avoid leakage of the injected fluid at the insertion point;
4) Use a new needle any time the needle is completely withdrawn from a lesion and when injecting in a different lesion;
5) Injections at special sites follow clinical practice or are at the discretion of the investigator.

5.1.4 Principles of dosing adjustments

VSV-02 dose should not be adjusted during the DLT observation period. VSV-02 administration should be terminated if any DLT occurs.

During the study period, the investigator will take necessary safety measures for subjects based on AE level (as determined by NCI-CTCAE v5.0) and clinical presentation. Possible measures to be taken are shown below:

- For ≤ Grade 2 AE, continue VSV-02 administration whenever possible. The investigator will consider administering the appropriate interventional therapy based on the clinical situation.
- Suspend VSV-02 administration for Grade 3 AE or Grade 2 AE of ≥ 7 days duration. The investigator will decide to continue or terminate treatment based on the subject's recovery.

- For Grade 4 AE, terminate VSV-02 administration immediately.
- For grade ≥3 asymptomatic laboratory test abnormalities, close observation will be given if the investigator determines that there is no clinical significance. The investigator will decide whether to suspend dosing or provide appropriate interventional therapy based on the outcome of these abnormalities.

5.2 Preparation/Handling/Storage/Duties of Study Drugs

5.2.1 Receipt and inventory of drug

The partner is responsible for shipping the study drug to the study center and the investigator/pharmacist signs to acknowledge receipt.

The study medication will only be used for this study and will only be administered by a person authorized by the investigator to do so. The investigator/pharmacist responsible for drug administration dispenses, recovers, and destroys the study drug according to study procedures and keeps accurate records.

5.2.2 Dosage form, appearance, packaging and labeling

VSV-02 Injection (for clinical research use only)

Study protocol number: Bottle no:

Product lot number:

Product Specification: 3×10^{10}PFU/mL, 1 mL/branch

Production date: MM/DD/YYYYY

Expiry date: 36 months (tentative)

Storage condition: ≤-70 ℃, keep away from light.

Mode of administration: Intratumoral injection, Intravenous injection

Manufacturer: Shanghai Rongrui Pharmaceutical Technology Co.

5.2.3 Product storage and stability

The research center should strictly comply with the provisions of GCP "Management of experimental drugs", and at the same time, store and manage the research drug according to the storage conditions provided by the sponsor, ≤ -70 ℃ for storage and transportation.

The investigational drug must be stored in a safe area and accessible only to authorized personnel before distribution to subjects.

5.3 Destruction and Recall of Investigational Drug

Destruction and retrieval of investigational drugs will be conducted in accordance with the relevant Standard Operating Procedures (SOPs) of the research center. The investigator must ensure that all trial medications are used only for the subjects enrolled in the clinical trial, that any remaining medications after opening should be destroyed, and that any remaining medications that have not been opened for use should be returned to the sponsor, and that the trial medications should not be passed on to any non-clinical trial participant. Supervisors are responsible for supervising the process of supplying, using, storing, and disposing of leftover drugs for clinical trials.

5.4 Adherence to the study intervention

To ensure treatment adherence, dosing will be supervised by the investigator or his/her assistant. The exact time of administration and dose of medication will be recorded on the eCRF. Reasons for delayed dosing, medication reduction, omission, and termination should also be recorded in the eCRF. Adherence will be further confirmed based on study drug dispensing, drug preparation, drug return, study case and eCRF records. Circumstances affecting subject compliance (e.g., side effects of treatment, perceived lack of antitumor activity, subject's unwillingness to comply with study requirements, etc.) affecting the decision to discontinue treatment should also be noted in the eCRF at that time. Subject compliance with the treatment and regimen refers to voluntary compliance with all aspects of the regimen, including compliance with all tests required for safety assessment and imaging for tumor assessment. Subjects who do not return for follow-up visits may be terminated from the study at the discretion of the Principal Investigator.

5.5 Combination of medications and treatments

All comorbid medications and treatments from the time the subject signs the informed consent to 28 days after the last dose (or until the subject starts a new antitumor therapy) are to be documented in detail in the electronic case report form (eCRF) with the reason for the medication.

5.5.1 Dispensing of Study Drugs

The study drug is provided by the sponsor and distributed to the research center on a scheduled basis. Upon receipt of the study drug, the designated personnel at the research center will be required to verify the shipping temperature of the study drug, count the drug, sign for the drug after verifying that it is correct, and the relevant records should be retained in the investigator's folder, as well as record the storage temperature of the drug on a daily basis, and record the items in the inventory sheet: date of receipt of the study drug, number of doses, batch number, number of doses dispensed, and the amount of the drug remaining in the inventory. Dispensing of medication during the study will be

conducted by a member of the study team. This member will be required to ensure and document that the subject receives the medication as scheduled and record the number and date of medications dispensed and recalled in the original medical record. Investigators are expected to use study medications within the framework of the clinical study as required by the protocol.

5.5.2 Prohibited Foods, Drugs, and Treatments

1) Concomitant use of any anticancer therapy (chemotherapy, immunotherapy, biologics, extensive radiotherapy, hormonal therapy, targeted therapy, surgery, interventional therapy, device therapy), investigational therapy, or approved therapy.
2) Immunosuppressive agents, except for the treatment of immune-related adverse events (irAE).
3) Systemic corticosteroid therapy with immunosuppressive effects, except for the short-term treatment of injection site reactions, irAE, or adrenal insufficiency requiring hormone replacement therapy. If systemic corticosteroids are used, they must be tapered prior to the next study treatment and the daily dose must not exceed 10 mg prednisone or equivalent. If the daily dose exceeds 10 mg prednisone, study drug administration should be suspended.
4) No herbal medications approved for the treatment of cancer should be used.
5) Live or live attenuated vaccines should not be administered throughout the study period.

5.5.3 Permitted medications and treatments

The following medications are permitted during the study, as appropriate

（1） Long-term medications required by co-morbidities (e.g., hypertension, diabetes, etc.);

（2） Topical, ocular, intra-articular, intranasal administration, and inhaled corticosteroids (minimal systemic absorption) are allowed for subjects during the study. Short-term (<1 week) use of corticosteroids is allowed for prophylaxis (e.g., contrast allergy) or for treatment of non-autoimmune conditions (e.g., delayed allergic reactions due to contact allergens);

（3） Symptomatic supportive treatment for toxic reactions to the drug or for control of tumor symptoms, in a manner that protects the interests and safety of the subject, by the investigator.

（4） Patients with bone metastases may receive bisphosphonate therapy. Palliative small-area (radiotherapy area must be <5% bone marrow area) radiation therapy is permitted if painful bone metastatic lesions are not effectively controlled by systemic therapy or local analgesia. Local radiation therapy should be avoided as much as possible during the DLT observation period.

6 Study Steps

6.1 Screening Period

Unless otherwise specified, screening assessments will be completed within 28 days prior to the first dose of VSV-02. For the protection of the subject, if the required screening or assessment has been completed prior to the signing of the ICF and is completed within the time frame specified in the protocol, it does not need to be repeated.

To ensure that subjects meet the enrollment criteria for this study, the following study processes must be completed during the Screening Period (D-28 through D-1, including the Baseline Period [D-7 through D-1]):

1) Informed Consent: sign name and date on ICF.
2) Review of Inclusion/Exclusion Criteria: if the first dose is more than 7 days from the Screening Period, reassessment will be done at the Baseline Period.
3) Demographic information contains year of birth, gender, ethnicity.
4) Collection of medical, prior treatment, allergy, personal, and family history: includes history of tumor-related medical conditions and prior treatments, non-tumor-related medical conditions and prior treatments, food and drug allergies, smoking, alcohol consumption, and substance abuse.
5) Physical examination: including general condition, head and neck, lymph nodes, skin, chest, abdomen, musculoskeletal system (including extremities and spine), height and weight. Receive the results of an examination at our study center within 7 days prior to the first dose.
6) Vital signs exam: including blood pressure, pulse/heart rate, respiration, and temperature. Accept results from this study center within 7 days prior to the first dose.
7) ECOG score: reassessed at baseline if the first dose was given more than 7 days before the screening period.
8) Laboratory tests, including routine blood tests, blood biochemistry tests, coagulation tests, thyroid function tests, and routine urine tests. Receive results of routine blood, blood biochemistry tests, coagulation function tests, and routine urine tests from our study center within 7 days prior to the first dose of drug. Accept results of thyroid function tests from this study center within 14 days prior to the first dose.

9) Virologic examination, including Hepatitis B V (HBsAg, HBsAb, HBeAg, HBeAb, HBcAb), HCV antibody, HIV antibody, CD4+ T-cell count, and syphilis serologic test; if HBsAg (+) and/or HBcAb (+), and/or HCV antibody (+) are examined in the screening period, continued examination of HBV-DNA and/or HCV-RNA for further screening.

10) Perform a blood pregnancy test in female subjects of childbearing potential within 7 days prior to the first dose.

11) 12-lead electrocardiogram: repeat at baseline if first dose is more than 7 days from screening.

12) Echocardiography: performed within 28 days prior to first dose.

13) Enhanced CT or MRI is preferred for tumor imaging, and plain CT is available for contrast-allergic subjects, but the method of examination should be consistent, and whether to apply PET-CT examination is decided by the investigator; the sites of imaging examination in the Screening Period include the chest, abdomen, pelvis, brain, and systemic bone, and the method of imaging examination in the subsequent cycles should be the same as that in the Baseline Period. If the patient has already had an imaging study within 28 days prior to the first dose and meets the requirements, the study may not be repeated.

14) Paraffin sections of tissue specimens will be provided by the subjects, or if this is not possible, the investigator will obtain tumor tissue and normal tissue adjacent to the tumor by fine-needle puncture before the first treatment. Tissue sample collection was voluntary.

15) AEs/SAEs and concomitant medications were recorded from the time the ICF was signed.

6.2 Treatment Period

Screening-eligible subjects were administered starting on Week 1, Day 1 (W1D1) and every 3 weeks as a treatment cycle, with intravenous and intratumoral injections administered on the same day. Subjects were administered according to a fixed dose until disease progression, intolerable toxicity in the subject, withdrawal of informed consent by the subject, death of the subject, loss to follow-up, termination of treatment as deemed by the investigator to be in the best interest of the patient, or 6 cycles of study drug administration had been completed, whichever occurred earliest.

1) VSV-02 injection was administered directly or under ultrasound/CT guidance at single or multiple sites throughout the tumor body, with a maximum injection volume of no more than 10 mL.The investigator should select the appropriate injection lesion according to the injection volume guidelines.

2) Physical Examination: one guided physical examination prior to the first dose of each cycle.

3) Vital signs: within 2 h before, 2 h (±15 min) and 4 h (±30 min) after administration of C1D1, within 2 h before administration of C1 other, within 2 h before the first dose of each cycle of C2~C6.

4) ECOG score: once before the first dose of each cycle.

5) Laboratory tests (including blood routine, blood biochemistry test, coagulation function test, urine routine):

- Blood routine: within 48h prior to the first dose of each cycle. The investigator may increase the frequency of tests according to clinical indications.
- Blood biochemistry, routine urine and coagulation tests: within 48h before the first dose of each cycle.

6) Urine pregnancy every 4 weeks during the treatment period. In case of a positive urine pregnancy test, a blood pregnancy test will be performed.

7) 12-lead electrocardiogram: within 48 h prior to the first dose of each cycle.

8) Biodistribution and time points for viral shedding, biomarker and cytokine blood sample collection are listed in Table 2 .

9) Immunogenic blood collection within 2 h prior to the first dose of each cycle.

10) Tumor efficacy evaluation: Tumor efficacy was assessed every 6 weeks (±7 days) after the first dose of drug, with examination of the lesion site only (except in cases of suspected metastasis) and bone scan (only during the treatment period when relevant signs were present). Unscheduled imaging may be performed if disease progression is suspected (e.g., worsening of symptoms).

11) Tissue specimen collection is performed after the last treatment session. Tissue specimen collection is voluntary.

12) AE and concomitant medications are recorded.

6.3 End of Treatment (EOT) Visit

Subjects ending treatment or withdrawing early from the study should have an EOT visit within 7 days of being informed of the end of treatment. If the EOT visit is within the safe follow-up time window, no re-examination is required. For safety reasons, the investigator may conduct additional visits as needed (i.e., unplanned visits) if the subject develops any AEs, has abnormal laboratory test results, or has any discomfort during the study. The investigator should document the details of each

unplanned visit in the unplanned visit section of the original medical record and in the eCRF.

Processes to be completed for end of treatment, early withdrawal from the study, or unplanned visits include but are not limited to

1) Physical examination
2) Vital signs
3) ECOG score
4) Laboratory tests (including routine blood tests, blood biochemistry tests, coagulation tests, thyroid function tests, and urinalysis)
5) Pregnancy test
6) 12-lead electrocardiogram
7) Echocardiogram
8) Imaging: Imaging does not need to be repeated at the EOT visit if the interval between the EOT visit and the previous imaging is no more than 4 weeks.
9) Biodistribution and viral shedding sampling
10) Immunogenic blood collection
11) Documentation of AE and concomitant medications

Note: Unscheduled visits may be scheduled by the investigator depending on the status of the subject.

6.4 Safety Follow-Up

A safety follow-up visit is required 28 days (±7 days) after the last dose. For subjects who discontinued treatment due to disease progression, safety data were collected within 7 days of notification/establishment of subject discontinuation. If the subject receives other antitumor therapy outside of the protocol within the 28-day (±7-day) timeframe of safety follow-up, no subsequent study data will be collected for safety follow-up and survival follow-up.

The following process will be performed:

1) Physical examination
2) Vital signs
3) ECOG score

4) Laboratory tests (including routine blood tests, blood biochemistry tests, coagulation tests, thyroid function tests, urine routine)

5) Pregnancy test

6) 12-lead electrocardiogram

7) Immunogenic blood collection

8) Evaluation of biodistribution and viral shedding

9) Documentation of AE and concomitant medications

If the proposed end-of-treatment visit is close to the safety follow-up visit, the investigator may assess whether to perform additional safety assessment tests.

Note: ADRs are to be followed until ADRs stabilize or return to ≤ Grade 1, or baseline levels, unless the subject withdraws informed consent, dies, is lost to follow-up, or receives other antitumor therapy other than that specified in the protocol.

6.5 Survival Follow-Up

Subjects who discontinued treatment were followed every 12 weeks until the subject withdrew informed consent, died, lost to visit, received other antitumor therapy than that specified in the protocol, or the study was completed (whichever occurred earliest), with telephone follow-up allowed. After the subject has completed 12 cycles of multiple dosing, if the investigator judges that the subject may still benefit from continuing VSV-02 treatment, he/she may apply to the collaborator to continue the treatment, but he/she must sign a separate informed consent form, which is based on the actual condition of the patient, and is prepared after communication with the investigator.

Subjects who have terminated treatment and have not progressed (and have not been treated with any other antitumor therapy other than that specified in the protocol) will be required to return to the hospital every 12 weeks for oncologic efficacy assessment follow-up; subjects who have progressed (and have not been treated with any other antitumor therapy other than that specified in the protocol) will be required to undergo survival follow-up only once every 12 weeks with an option of telephone follow-up; if they receive any other antitumor therapy other than that specified in the protocol, follow-up visits will be discontinued. discontinue follow-up. The time window for each assessment is ± 7 days.

7 Study Evaluation

7.1 Safety Evaluation

7.1.1 General information

Demographics: age, gender, ethnicity.

Medical/treatment history: including history of prior illnesses and history of other illnesses and treatments; prior medical history includes histologic/pathologic diagnosis, date of diagnosis, staging, and prior therapeutic measures taken. Ask and record the subject's history of other diseases and treatments.

7.1.2 Adverse events

All AEs and SAEs, whether or not related to the study drug, occurring from the start of ICF signing to 28 days (±7 days) after the last dose will be recorded. AEs will be graded using CTCAE v5.0 and recorded throughout the study period.

7.1.3 ECOG Physical Fitness Score

The ECOG Physical Fitness Status Rating Scale is shown below.

ECOG Physical Fitness Status[13]

Level	ECOG
0	Complete normal mobility, no difference in mobility from before the onset of the disease
1	Able to walk freely and engage in light physical activities, including general housework or office work, but unable to engage in heavier physical activity
2	Able to walk freely and take care of oneself, but incapable of work, and can get up and move around not less than half of the time during the day.
3	Can only partially take care of themselves, and are bedridden or wheelchair-bound for more than half of the day.
4	Completely incapacitated, bedridden and unable to take care of themselves.
5	Death

7.1.4

Laboratory tests specifically include the following:

1. Routine blood: including white blood cell count, neutrophil count, eosinophil count, basophil count, lymphocyte count, erythrocyte count, reticulocyte, erythrocyte hematocrit, hemoglobin, platelet count.
2. Blood biochemistry: including (liver function) serum alanine aminotransferase, aspartate aminotransferase, albumin, total bilirubin, direct bilirubin, alkaline phosphatase, gamma-glutamyltransferase, (renal function) creatinine, urea/urea nitrogen, uric acid, (electrolytes) potassium, sodium, chloride, calcium, magnesium, phosphorus.
3. Urine routine: including protein, glucose, white blood cells, red blood cells, occult blood. If there are clinical indications, 24 h urine protein quantification should be added.

4. Coagulation function: including international normalized ratio, activated partial thromboplastin time, prothrombin time, fibrinogen;
5. Thyroid function: including T4, T3, FT3, FT4, TSH, TG, TGAb, TPOAb;
6. Virological testing: the test items include Hepatitis B two-half pairs, which include Hepatitis B Virus Surface Antibody (HBsAb), Hepatitis B Virus Surface Antigen (HBsAg), Hepatitis B Virus e Antigen (HBeAg), Hepatitis B Virus e Antibody (HBeAb), and Hepatitis B Virus Core Antibody (HBcAb), and are required for HBsAg positivity and/or HBcAb positivity. Hepatitis B Virus Deoxyribonucleic Acid (HBV DNA); Hepatitis C Virus (HCV) Antibody, plus HCV-RNA if HCV antibody positive; HIV Antibody, CD4+ T-cell count; Syphilis serologic testing.

7.1.5 Vital Signs

Tests included: including temperature, pulse/heart rate, respiratory rate, and blood pressure (systolic and diastolic), which was checked and recorded in the seated position starting after the subject had rested for at least 5 minutes. The site of blood pressure measurement at baseline and after drug administration needs to be on the same side of the arm as far as possible. The investigator may decide to increase the frequency of vital sign examinations based on the subject's clinical condition.

7.1.6

Physical examination includes a complete physical examination (general condition, head and neck, lymph nodes, skin, chest, abdomen, musculoskeletal system (including limbs and spine), height and weight) and a symptom-oriented physical examination.

7.1.7 12-lead electrocardiogram

The ECG was completed according to the flow of the protocol, and it was necessary to verify that the system was calibrated and that the time recorded on the ECG corresponded to the true time before starting the ECG.

Subjects should rest for at least 10 minutes before performing the ECG and complete the examination in a flat lying position. The ECG report is required:

- Heart rate
- Rhythm (sinus rhythm or pacing rhythm)
- PR interval (msec), RR (msec), QRS (msec)

- QT interval (msec), QTcF (msec)

During the entire study period (including the screening period, treatment period, safety observation period, etc.), those with abnormal tests may be retested at the judgment of the investigator, and the next retest is required to be performed at intervals of 10 (±5) min, for a total of 3 times, and the average value is calculated.

7.1.8 Echocardiography

Echocardiography was performed during the screening period and at the end of treatment or early exit visit to measure left ventricular ejection fraction and related indices of cardiac function. Unscheduled examinations can be arranged if required during the treatment period. Examination reports are required to provide: output per beat (SVmL), left ventricular ejection fraction (LVEF%), cardiac output (CO L/min), and left ventricular short-axis shortening (FS%).

7.2 Validity Evaluation

7.2.1 Criteria for effectiveness evaluation

Tumor response will be assessed every 6 weeks after the first dose and at the end of study (EOT) visit. Assessed by the investigator according to RECIST 1.1[14] . Lesions are preferably followed throughout the study period using the same imaging modality as at baseline evaluation.

Pseudoprogression may be observed in some subjects on their first few imaging examinations after receiving immunotherapeutic agents such as PD-1/PD-L1 inhibitors, mainly because of the following reason: immunotherapeutic agents are able to elicit immune cells infiltrating into and around the tumor to respond to the drugs, resulting in the simultaneous detection of both the tumor as well as the tumor-surrounding immune cells on the imaging examinations. As a result, the examination results may show a significant increase in tumor size or the appearance of new tumor foci, but in reality, the tumor size may be decreasing. Therefore, in this study, after the first imaging disease progression in a subject, as determined by the investigator according to RECIST 1.1 criteria, subjects were allowed to continue to receive VSV-02 if the investigator determined that no deterioration in the subject's clinical status had occurred and it was believed that there might be a clinical benefit to continue treating the subject. However, for such subjects, another tumor evaluation should be performed at least 4 weeks later to confirm disease progression according to iRECIST[15] . After the investigator assesses a subject for first disease progression, if the subject develops grade ≥3 toxicity, the subject may not continue on study treatment. Upon confirmation of disease progression, the subject should discontinue treatment and receive an end-of-treatment visit.

Clinical stability is defined as the absence of clinically significant signs and symptoms suggestive of disease progression (including abnormal laboratory test values), no reduction in Eastern Cooperative Oncology Group (ECOG) Physical Status Score, absence of rapid disease progression, and absence of progressive tumors (e.g., spinal cord compression) at important anatomical sites that would require other urgent medical intervention. Subjects judged to be clinically unstable should terminate trial treatment since the first imaging disease progression evaluated by the investigator and not require repeat imaging to confirm disease progression according to iRECIST. At the end of study treatment, if the last tumor imaging evaluation was performed within the last 4 weeks, it may be omitted upon evaluation by the investigator.

7.2.2 Definition of efficacy evaluation endpoints

The efficacy endpoints for this study will be evaluated according to RECIST 1.1 and are defined below:

1) Objective remission rate (ORR): proportion of subjects achieving CR or PR.
2) DCR: Proportion of subjects achieving CR, PR or SD.
3) DOR: For subjects achieving objective remission, DOR is the time between the first recorded objective tumor remission (CR or PR) and the first recorded disease progression or death from any cause, whichever occurs earlier.
4) PFS: time from first study treatment to the date of first documented disease progression or death due to any cause, whichever occurs earlier.
5) OS: time from first study treatment to date of death from any cause.

7.3 Biodistribution and viral shedding evaluation

Biodistribution of VSV-02 was assessed by detecting viral gDNA levels in blood using a real-time quantitative poly synthase chain reaction (qPCR) assay. Viral shedding was evaluated by detecting viral gDNA levels in urine, feces, saliva and injection site swabs. Samples will first be analyzed by qPCR to determine the detectability of viral gDNA. If the qPCR assay is positive, the biodistribution and shedding samples will be tested for TCID50 to determine viral infectivity.

7.4 Immunogenicity Assessment

To assess changes in the production of anti-VSV-G antibody (IgG) antibodies in subjects following treatment. The potential immunogenicity of VSV-02 was assessed by determining the concentration of ADA before and after administration. Evaluate only subjects who have received at

least one dose of VSV-02 and have provided a baseline sample and at least one post-treatment sample.

7.5 Biomarker and Cytokine Evaluation

7.5.1 Analysis of Tissue Samples

During the screening period, paraffin sections of tissue specimens will be provided by the subjects, or the investigator will obtain specimens of tumor tissues and normal tissues adjacent to the tumors by fine-needle puncture before the first treatment; during the treatment period, the above tissue specimen collection will be performed after the last treatment for the following items: (the process of tissue specimen collection is voluntary for the subjects, and the specific items can be carried out according to the study needs and the actual situation of the study).

1) Analysis of NY-ESO-1 antigen expression by immunohistochemistry (IHC);

2) Analyze the expression of PD-L1 by IHC method;

3) After the tissue cells were resuspended, flow cytometry was used to detect the expression of immune cells infiltrating CD4+ T cells (CD3+ CD4+), CD8+ T cells (CD3+ CD8+), NK cells (CD3- CD16+CD56+), monocytes (CD14 high CD16 low) and macrophages (CD14 low CD16 high). The expression of

4) Detect the distribution of VSV-02 virus particles in tissues by qPCR.

7.5.2 Blood sample analysis

- Test items and sample requirements (specific test items can be performed according to the needs of the study and the actual situation of the study):
- Detection of NY-ESO-1 antigen by ELISA;
- Plasma cytokines: IL-6, IL-2, IL-8, IL-10, IL-12p70, TNF-α, IFN-γ, GM-CSF, IL-5, IL-15 by MSD;
- Lymphocyte subpopulations were analyzed by flow cytometry: CD3+ T cells, the proportion of CD4+ T cells (CD3+ CD4+), CD8+ T cells (CD3+ CD8+) and the absolute cell number;
- Expansion of lymphocyte subsets was analyzed by flow cytometry: proportion of CD27-, CCR7- subsets expanded in CD3+, CD8+, CD45RO+, CD45RA- cell subsets; blood samples were shared with lymphocyte subsets.

8 Adverse Events and Serious Adverse Events

8.1 Adverse Events

8.1.1 Definition of adverse events

Adverse events (AEs) are all adverse medical events that occur after a subject receives an investigational medication, which may be manifested as signs and symptoms, disease, or abnormal laboratory tests, but are not necessarily causally related to the investigational medication.

AEs include, but are not limited to, the following: (1) exacerbation of a pre-existing (prior to entry into the clinical trial) medical condition/disease (including exacerbation of symptoms, signs, laboratory test abnormalities); (2) new onset of any adverse medical condition (including signs, symptoms, newly diagnosed disease); and (3) abnormal clinically significant laboratory test values/results.

Definite signs or symptoms of tumor progression should not be recorded as an adverse event unless they are more severe than expected or the investigator believes that the tumor progression is related to the test drug or study procedures. If a new primary malignancy develops, such events should be considered adverse events.

8.1.2 Severity of Adverse Events

Grading refers to the severity of the adverse event. Determine the severity of the AE based on the U.S. Department of Health and Human Services CTCAE 5.0 criteria. If an AE not listed in the table occurs, grade the severity of the AE according to Table 5 .

Table5 Adverse Event Severity

Grade	CTCAE Situation
1	Mild; asymptomatic or mild; seen only clinically or diagnostically; no treatment required.
2	Moderate; requires minor, localized or non-invasive treatment; age-appropriate instrumental daily living activity limitations*.
3	Severe or medically significant but not immediately life-threatening; results in hospitalization or prolonged hospitalization; disabling; self-instrumented limitations in activities of daily living**.
4	Life-threatening; requires urgent treatment.
5	Death associated with an adverse event.

CTCAE = Common Criteria for the Evaluation of Adverse Events

*:Instrumental activities of daily living refer to cooking, purchasing clothing, using the telephone, and managing money.

**:Autonomous activities of daily living refers to bathing, dressing and undressing, eating, toileting, taking medications, etc., and was not bedridden.

8.1.3 Determination of Adverse Event Relevance to Trial Medications

The investigator must assess the relationship between the event and the study intervention based on the temporal relationship between the AE and the study intervention and clinical judgment. The following categories will be used to rate the degree of certainty of causality. The causal relationship

between the investigational product and the event must always be viewed with skepticism in clinical trials. Drug-related AEs will be defined as probably related, probably related, or definitely related.

- **Positively Related** - Clear evidence of a causal relationship and other possible factors can be ruled out. Clinical events (including abnormal laboratory test results) have a reasonable temporal relationship to the administration of the study intervention that cannot be explained by complications or other drugs or chemicals. The response after cessation of the investigational intervention (de-stimulation) should be reasonably explainable from a clinical standpoint. The event must have a clear pharmacological or phenomenological profile and should be confirmed by a re-priming procedure if necessary.
- **Likely related** - evidence of causality, unlikely to be influenced by other factors. The clinical event (including abnormal laboratory test results) occurs within a reasonable time after the implementation of the study intervention, is unlikely to be attributable to complications or to other drugs or chemicals, and produces a response that can be reasonably explained from a clinical perspective after discontinuation of the medication (de-priming). There is no need to provide re-priming information in order to meet this definition.
- **Possibly Related** - There is some evidence of causation (e.g., the event occurred within a reasonable period of time after administration of the test drug). However, other factors may have contributed to the event (e.g., the subject's clinical condition and other comorbid events). Although an AE may only be rated as "possibly related" shortly after discovery, it may be flagged as requiring more information and subsequently upgraded to "probably related" or "definitely related" as appropriate. ".
- **Possibly unrelated** - The temporal relationship between the clinical event (including abnormal laboratory test results) and the study intervention suggests that a causal relationship is unlikely (e.g., the event did not occur within a reasonable time after the study intervention) and that other drugs or chemicals or the underlying disease provide a reasonable explanation (e.g., the subject's clinical condition, other comorbid treatments).
- **Definitely Unrelated** - The AE is completely unrelated to the implementation of the study intervention and/or there is evidence that the event is definitely related to another etiology. There must be another clear etiology documented by the investigator.

8.1.4 Collection of Adverse Events

A consistent, non-induced questioning method should be used when collecting information on

subjects' adverse events.AEs will be collected for all adverse events from the time the subject signs the informed consent form until 28 days after the last dose or until the subject starts a new antineoplastic therapy (safety follow-up period).The investigator must monitor the patient's health status for the duration of the trial.AEs will be collected from the time of the signing of the informed consent form until 28 days after the last dose or until the subject starts a new antineoplastic therapy (safety follow-up period). Clinical adverse events occurring after signing the informed consent and prior to the first dose are recorded in the CRF as history/concomitant disease and are not recorded as AEs unless one of the following conditions is met:

- Injury/damage resulting from any clinical laboratory test manipulation;
- Adverse events resulting from discontinuation of medication associated with the trial protocol;
- Adverse events caused by medications other than the trial medication taken as part of the treatment regimen.

AEs will no longer be actively collected at the end of the safety follow-up period; however, investigators will still be required to report to the Collaborator (or Collaborator-appointed CRO) if they become aware of an SAE that is reasonably causally related to the trial medicinal product.

Sources of AEs include:

- Patient responses to questions about his/her health status (standardized non-leading questions such as "How have you felt since your last visit?" asked at each visit). The patient's self-reported symptoms.
- The patient's self-reported symptoms.
- Findings or tests that the investigator assesses as clinically significant changes or abnormalities.
- Other information relevant to the patient's health (e.g., hospitalization) that comes to the researcher's attention.

8.1.5 Documentation of adverse events

The Investigator must document all adverse events on the Adverse Events form provided in each patient's eCRF which should include the information below:

- Name of adverse event
- Date and time of initiation (time may be omitted if not applicable)

- Severity
- Relationship to the test drug
- Measures taken with respect to the test drug
- Other measures taken
- Date and time of regression (time may be omitted if not applicable)
- Diversion
- Whether serious adverse event and corresponding severity criteria

➢ Name of the adverse event

The name of the adverse event should be in medical terminology and the medical diagnosis should be used in preference to the medical diagnosis, or if the diagnosis cannot be clarified, separate symptoms/signs should be recorded separately, and the diagnosis updated when the subsequent diagnosis is clarified, replacing the previous symptoms/signs with the diagnosis . If the same adverse event occurs more than once in a patient and the patient has recovered between adverse events, this adverse event should be recorded separately according to the number of occurrences.

It is important to note: the measure taken is not the adverse event, the reason for the measure is the adverse event. Hospitalization is not an adverse event; the cause of the hospitalization is an adverse event. Death is not an adverse event, the cause of death is an adverse event (sudden unexplained death can be recorded as "unexplained death").

➢ Date and time of onset

The onset of an adverse event is the date when the first sign or symptom is first observed. If the adverse event is an abnormal laboratory test or examination result of clinical significance, the start time is the date of sampling or examination. In the case of progression from an adverse event to a serious adverse event, the start of the date on which the adverse event is judged to have been upgraded to a serious adverse event is taken as the time of the serious adverse event.

➢ Measures taken on the test drug

Measures taken on a test drug product to address an adverse event must be categorized as one of the following:

- Permanent discontinuation-The trial medicinal product is permanently discontinued because of a specific AE.

- Dose unchanged-The specific AE does not require a change in the dosing regimen of the investigational medicinal product, which continues to be administered.
- Dose reduction-Dose reduction because of the specific AE.
- Suspension-Temporary discontinuation (suspension) (including subject-initiated discontinuation) of the Test Drug because of a specific AE, followed by resumption of dosing.
- Unknown-Used only when there is no certainty about the action taken.
- Not applicable-Discontinuation of the Test Medicinal Product for reasons other than the specified AE, e.g., the study was terminated, the subject died, the Test Medicinal Product was discontinued before the AE occurred.

➢ Other measures taken

Adverse events that require treatment to be given must be treated using recognized standards of care to protect the health and interests of the patient. Appropriate CPR equipment and medications must be available to ensure that the best possible treatment is provided in an emergency.

If medication is used to treat an adverse event, the medication used should be recorded in the combined medication record.

➢ End date and time

The end date of the AE needs to be completed in accordance with the requirements for completion of the CRF and SAE report form.

➢ Categorization

The attribution of an adverse event must be categorized as one of the following:

- Recovery/Recovered: the "(serious) adverse event termination date" should be indicated.
- Improved/remitting: status recovering/improving or stabilized.
- Not recovered/not cured/continuing: event in progress.
- Recovered/recovered with sequelae: only if the subject will have sequelae that last for a prolonged period of time or for life, e.g., blindness due to diabetes mellitus, hemiplegia due to stroke. The "(Serious) Adverse Event Termination Date" should be indicated.
- Death: when the adverse event is fatal the time of death needs to be recorded.
- Unknown: The adverse event is not known to the investigator, e.g., subject lost to

follow-up.

8.1.6 Follow-up of adverse events

After the initial AE report, the investigator is required to initiate follow-up with each subject and provide further information to the collaborator regarding the subject's status. The frequency of follow-up visits should be determined based on the severity of the adverse event, diagnostic and therapeutic routines, and trial protocol requirements. If the adverse event is not completed at the current visit, it should be asked and recorded again at the next visit; if there is a combination of medication, it should be collected and recorded; if the patient is treated in the local hospital, the local hospital treatment records and medication information should be collected as much as possible. Specific requirements should be in accordance with the relevant SOPs of the healthcare organization.

AEs (regardless of causality) that have not fully recovered or stabilized at the end of the safety follow-up period must be followed until recovery/cure (return to baseline level or cure) with or without sequelae, clinical stability, reasonable explanation, death of the subject, or loss to follow-up.

8.2 Serious Adverse Events (SAE)

8.2.1 Definition of Serious Adverse Event

Serious Adverse Event (SAE) means an adverse medical event such as death, life-threatening, permanent or severe disability or loss of function, subject requiring hospitalization or prolonged hospitalization, and congenital anomalies or birth defects that occur after the subject has received the test drug.

Table6 Definitions of Serious Adverse Events

SAE Definition	Guideline
Death	AE is the direct or primary cause of a subject's death.
Life-threatening	The occurrence of an adverse event that immediately poses a risk of death to the subject. Excludes those adverse events that may result in death after serious progression, e.g., drug-induced hepatitis without liver failure.
Permanent or Serious Disability or Loss of Function	Any adverse event that results in an injury that impairs or destroys a subject's function, physiology, or both, physical activity, or quality of life.
Subject requires hospitalization or prolonged hospitalization	An event requiring extended treatment for an already hospitalized subject shall be recorded as an SAE Examples of such events include transfer from routine hospital care to an intensive care unit (ICU) or if the event results in an extension of an existing planned hospital stay. The following hospitalizations should not be considered SAEs: ● Retention in the emergency department or other hospital unit for no more than 24 hours that does not result in hospitalization (unless considered a medically significant event or life-threatening); ● Elective surgery that was planned prior to signing the informed consent form; ● Hospitalization required for routine health screening (e.g., routine colonoscopy);

	● Hospitalizations that are not for the purpose of disease treatment and are planned prior to enrollment and need to be documented; ● Admissions that are not related to a health condition and do not require medical/surgical intervention (e.g., family reasons, etc.).
Adverse medical events such as congenital anomalies or birth defects	The presence of congenital anomalies, malformations, etc. in the offspring of the subject.
Other significant medical events	Medical and scientific judgment must be used to determine whether to expedite reporting of other conditions, such as important medical events that may not be immediately life-threatening, fatal, or hospitalizing, but are also generally considered serious if medical measures are needed to prevent one of the above conditions from occurring. Examples include critical treatment in the emergency room or allergic bronchospasm at home, malaise or convulsions without hospitalization, development of drug dependence or addiction, etc.

8.2.2 Reporting of Serious Adverse Events

Any serious adverse event that occurs during the trial, regardless of whether it is related to the trial drug or not, the investigator should provide prompt rescue treatment and report it to the partner (or CRO appointed by the partner) by completing, signing, and dating the "Serious Adverse Event (SAE) Report Form" provided by the partner, within 24 hours of notification. The investigator should provide other required information, such as autopsy report and final medical report, to the collaborator and the Ethics Committee. In particular, if the SAE is fatal or life-threatening, the Collaborators must be informed immediately, regardless of the amount of AE information available. This timeframe also applies to additional new information from previous SAE reports (follow-up visits), as well as initial and follow-up reports for pregnancy exposures. In rare instances where the investigator is not immediately aware of the occurrence of an SAE (e.g., if an outpatient study patient initially seeks treatment elsewhere), the investigator should report the event within 24 hours of learning of the event and document the time when he or she first became aware of the SAE.

Investigators must provide their assessment of causation when reporting serious adverse events. If the Investigator's assessment of causation is missing or unavailable, the Collaborator or Collaborator-appointed CRO shall immediately contact the Investigator and send an Urgent Medical Challenge requesting that assessment; if the Investigator is temporarily unable to provide an assessment of causation, it will be left to the judgment of the Collaborator until such time as the Investigator's assessment can eventually be obtained.

If the investigator is unable to determine whether the adverse event is a Serious Adverse Event, it will be considered a Serious Adverse Event until its nature can be demonstrated.

The investigator should sign and read the relevant safety information of the clinical trial provided by the collaborator in a timely manner after receiving it and consider the treatment of the subjects, whether to adjust it accordingly, and communicate with the subjects as early as possible if necessary,

and should report the suspected and unintended serious adverse events provided by the collaborator to the Ethics Committee.

8.2.3 Follow-up of Serious Adverse Events

The follow-up requirements of SAE are the same as those of AE, and the investigator should fill in the Serious Adverse Event (SAE) Report Form in a timely manner and report it to the collaborator and the CRO assigned by the collaborator in accordance with the SAE reporting process.

8.3 Suspected Unexpected Serious Adverse Reactions

8.3.1 Definition of Suspected Unanticipated Serious Adverse Reaction

Suspected Unexpected Serious Adverse Reaction (SUSAR) refers to a suspected and unanticipated serious adverse reaction whose clinical manifestations are of a nature and severity that exceeds the information already available in the investigator's manual of the study drug, the specification of the marketed drug, or the summary of product characteristics. Unintended Adverse Reaction means an Adverse Reaction whose nature, severity, consequences, or frequency are different from the expected risk described in current information about the test drug (e.g., documents such as the Investigator's Manual). The investigator's brochure serves as the primary document that provides the safety reference information used to determine whether an adverse reaction is anticipated or unanticipated. For example, (1) acute renal failure is listed as an adverse reaction in the investigator's brochure, but the development of interstitial nephritis during the course of the trial should be judged as an unintended adverse reaction, and (2) hepatitis is listed as an adverse reaction in the investigator's brochure, but the development of acute severe hepatitis during the course of the trial should be judged as an unintended adverse reaction.

8.3.2 Reporting of Suspected Unintended Serious Adverse Reactions

Any safety-related information received by the Collaborator from any source should be analyzed and evaluated immediately, including severity, relevance to the test drug, and whether it is an expected event. The Collaborator shall make a SUSAR Rapid Report to all investigators participating in the clinical trial, the clinical trial site, the ethics committee, and the drug regulatory and health authorities.

（1） Start/end time of the rapid report: the start time is the date of approval of the clinical trial/start date of the implied license of the national drug review agency, and the end date is the end date of the follow-up of the last case of subjects in China (unanticipated serious adverse reactions occurring after the end of the follow-up of the clinical trial and before obtaining the conclusion of the review and approval should also be reported rapidly).

（2） Duration of the rapid report

- Fatal or life-threatening SUSAR: first report within 7 days, and report and improve follow-up information within the following 8 days.
- Non-fatal or life-threatening SUSAR: first report within 15 days.
- Day 0 is the day the partner is first notified of a signed version of a valid report.

8.3.3 Follow-up for Rapid Reports

After the initial report (SUSAR), the collaborator should continue to follow up on serious adverse reactions and report relevant new information or changes to the previous report, etc., in a timely manner in the form of a follow-up report, with a reporting timeframe of 15 days from the date of obtaining the new information;

8.4 Reporting of other potential serious safety risk information

（1） Other potentially serious safety risk information should also be reported to the national drug review organization by the partner as soon as possible, while medical and scientific judgments need to be made in each case.

（2） In general, this is the case for information that clearly affects the assessment of the risk-benefit of a drug product or information that may contemplate a change in drug usage or affect the overall drug development process.

I. For known, serious adverse reactions with an increased incidence judged to be clinically important;

II. For obvious hazards to exposed populations, such as ineffectiveness of the drug in the treatment of life-threatening diseases;

III. significant safety findings (e.g. carcinogenicity) in newly completed animal tests.

IV. The latest global safety alerts for similar drugs or the latest safety concerns disclosed in newly completed epidemiologic investigations or academic conference papers.

8.5 Adverse Events of Special Concern

An Adverse Event of Special Concern is an event for which close monitoring is particularly warranted to enhance the understanding of the safety of the trial drug. Adverse events of special concern were reported by the investigator to the collaborator within 24 hours. Adverse events of special concern for this study include:

8.5.1 Cytokine release syndrome (CRS)

Oncolytic virus therapy may result in the release of inflammatory factors into the bloodstream, resulting in the formation of cytokine syndrome, which usually peaks 5-7 days after infusion. Severe CRS may result in acute respiratory distress syndrome, multi-organ failure, or even life-threatening. Once serious cytokine release syndrome is suspected or considered, it is recommended that the clinic should intensively test body temperature, blood pressure, oxygen saturation and neurological examination, and should draw blood for cytokine testing in a timely manner. The initial symptoms of cytokine syndrome are often nonspecific and may manifest as nonspecific symptoms in all major systems of the body, including respiratory, gastrointestinal, cardiovascular, renal, and neurologic.

CRS will be graded according to the American Society for Transplantation and Cellular Therapy (ASTCT) Consensus for Grading Immune Effector Cell-Related CRS and Neurotoxicity.

Table7 ASTCT CRS Grading Consensus

CRS parameters	Grade 1	Grade 2	Grade 3	Grade 4
Fever[a]	Body temperature ≥38°C	Body temperature ≥38°C	Body temperature ≥38°C	Body temperature ≥38°C
		Combined		
Hypotension	No	No need for antihypertensive medication	Need for one antihypertensive medication with or without vasopressin	Need for multiple antihypertensive medications (excluding vasopressin)
		Combined (or) [b]		
Hypoxia	None	Needs low oxygen flow nasal cannula[c]or oxygen blowing therapy	Requires high oxygen flow nasal cannula[c], face mask, non-rebreathing mask, or venturi mask oxygen therapy	Need for positive pressure ventilation (e.g., CPAP, BiPAP, tracheal intubation mechanical ventilation)

Reference: ASTCT CRS grading consensus

Organ toxicity leading to CRS may be graded according to CTCAE v5.0 without affecting CRS grading.

[a]Fever is defined as a temperature ≥38°C that cannot be reasonably explained by any other cause. For subjects who develop CRS and receive antipyretic or anticytokine therapy (e.g., tolizumab or steroids), fever need not be considered when grading the severity of subsequent CRS. In such cases, CRS is graded based on hypotension and/or hypoxia.

[b]CRS grading is based on a more severe event (e.g., hypotension or hypoxia) that cannot be reasonably explained by any other cause. For example, a subject with a temperature of 39.5°C, hypotension requiring treatment with 1 antihypertensive medication, and hypoxia requiring treatment with a low oxygen flow nasal cannula would be classified as having a CRS of grade 3.

[c]A low oxygen flow nasal catheter was defined as an oxygen delivery rate of ≤6 L/min. low oxygen flow also included blown oxygen delivery, sometimes used in pediatric subjects. (d) High oxygen flow nasal cannulae are defined as oxygen delivery at a rate of >6 L/min.

If a subject develops any of the above symptoms, the investigator should immediately perform cytokine testing (including but not limited to IL1a, IL2, IL6, TNF-α, and IFN-γ). If a subject develops persistent mild CRS or moderately severe CRS, tolizumab (monoclonal antibody to IL-6) and/or corticosteroids should be promptly administered, along with appropriate supportive therapeutic measures such as oxygen, antipyretic and analgesic agents, and antihypertensive medications.

Table8 Generalized Management of CRS*

1. For the first presentation of fever, a thorough evaluation should be performed immediately and the infection should be treated empirically with broad-spectrum antibiotics. There should be no unwarranted assumption that fever is caused by CRS.
2. Currently, prophylactic tolizumab is not required except in clinical trials.
3. Repeated IV fluid pushes should be avoided whenever possible, as this may exacerbate the complication of capillary leakage.
4. In subjects who develop recurrent or refractory hypotension associated with CRS, tolizumab should usually be used and should be administered before CRS reaches grade 3 or worse.
5. Tolizumab is the only drug approved by the FDA for the treatment of CRS and is the first-line treatment for CRS.
6. Multiple doses of tolizumab may be needed, but usually no more than 2 doses should be given without the addition of corticosteroids.
7. Tolizumab reduces the severity of CRS and does not interfere with the efficacy of immunotherapy.
8. If there is no response to tolizumab, corticosteroids should be added. Corticosteroids may also be given at the same time as the first dose of tolizumab.

IV = intravenous

*The items in the table above are suggested measures or generic considerations or points. The investigator should prescribe medications and take appropriate measures for the subject based on medical judgment, considering the subject's medical care and safety. The investigator should also review the complete prescribing information for all medications (e.g., tolizumab) that have been prescribed.

8.5.2 Tumor Lysis Syndrome (TLS)

Tumor lysis syndrome (TLS) is a group of clinical syndromes characterized by hyperuricemia, hyperkalemia, hyperphosphatemia, hypokalemia, and acute renal failure caused by the release of potassium, phosphate, and uric acid into the blood circulation during the administration of a test drug, which can occur rapidly due to the rapid destruction and lysis of a large number of tumor cells under the action of the drug or spontaneously, and is a potentially serious complication of the early induction of treatment for malignant tumors. TLS occurs most often in patients with tumors associated with high proliferation rate, high tumor load, and high drug sensitivity. The clinical manifestations of TLS depend on the severity of metabolic abnormalities, and mainly include nausea, vomiting, shortness of breath, congestive heart failure, cardiac arrhythmia, cloudy urine, edema, and muscle spasms. The severity of TLS is differentiated according to renal function, cardiac arrhythmias, seizures, etc. Patients with TLS may be treated with hydration, diuresis (preferred to labeled diuretics such as furosemide, etanercept, bumetanide), alkalinization of the urine (bicarbonate is not recommended), lowering of the uric acid (labrynthase is recommended) and correction of electrolyte disturbances.

Table9 TLS grading system

	Grade I	Grade II	Grade III	Grade IV
Impaired renal function	SCr 1.5mg/dl or CCr30~45 mL/min	SCr 1.5~3.0 mg/dl or CCr 10~30mL/min	SCr 3.0~6.0 mg/dl or CCr 10~20mL/min	SCr>6.0 mg/dl or CCr<10 mL/min
Nature of arrhythmia	No indication for intervention	No indication for urgent intervention	Symptomatic, incompletely controlled, or manageable with a device	Life-threatening, e.g., arrhythmia with congestive heart failure, hypotension, syncope, shock
Seizures	None	A brief generalized seizure that is well controlled by medication or an occasional focal motor seizure that does not interfere with daily life.	Seizures with altered consciousness; poorly controlled epilepsy, generalized explosive seizures despite drug intervention	Prolonged, recurrent, difficult-to-control epilepsy (e.g., status epilepticus and intractable epilepsy).

Note: SCr: serum creatinine, CCr: endogenous creatinine clearance.

Table10 Recommendations for management of electrolyte disorders

Electrolyte abnormalities	Recommendations for management of electrolyte abnormalities
Hyperphosphatemia	
Moderate (≥2.1 mmol/L)	Avoid phosphorus-containing drugs intravenously, aluminum hydroxide (50~150 mg/kg/d) 1ci/6h, use 1~2d
Severe	Dialysis (HD, PD) CAVH, CVVH, CAVHD, CVVHD
Hypocalcemia (≤ 1.75 mmol/L)	
Asymptomatic	No treatment required
Accompanying symptoms	Calcium gluconate under cardiac monitoring 50~100mg/kg slow IV push
Hyperkalemia	
Moderate (≥6.0 mmol/L) and asymptomatic	Avoid intravenous or oral potassium supplementation, give ECG and cardiac rhythm monitoring.
Severe (>7.0 mmol/L) with or without symptoms.	In addition to the above: calcium gluconate 100-200 mg/kg IV and regular insulin (0.1 U/kg) + D25 2 mg/kg IV and 5% sodium bicarbonate 2-4 mL/kg IV for arrhythmias; dialysis.
Acute renal insufficiency	Fluid and electrolyte balance therapy Uric acid and phosphate therapy Adjustment of renal drug metabolism dose Dialysis or hemofiltration

Note: HD: hemodialysis, PD: peritoneal dialysis CAVH: continuous arterial-venous hemofiltration, CVVH: continuous venous-venous hemofiltration, CAVHD: continuous arterial-venous hemodialysis, CVVHD: continuous venous-venous hemodialysis, D25: diclofenac sulfide

8.5.3 Adverse events related to intratumoral injection

During the intratumoral injection administration of the test drug to the subjects, depending on the location of the tumor, some risks may occur during the puncture process: for example, the needle may puncture the lung tissue and the pleura of the dirty layer during the administration of the drug to the

thoracic malignant tumors to form a pneumothorax; gastrointestinal malignant tumors: it may puncture the wall of the stomach or the wall of the intestines to cause a gastrointestinal perforation; or it may puncture blood vessels and lead to hemorrhage during the injection of the test drug to the tumor, and so on.

Therefore, when lysosomal virus is administered, it should be performed under endoscopic, CT or ultrasound guidance to avoid the above events. If pneumothorax or gastrointestinal perforation occurs during drug administration, the clinical condition of the subject should be closely observed and timely and appropriate treatment measures should be taken:

Pneumothorax: Untimely or inappropriate treatment of persistent pneumothorax will affect the subject's cardiopulmonary function, causing serious damage to circulatory and respiratory functions, and may even threaten life, requiring early and aggressive treatment. The diagnosis can be confirmed by clinical symptoms, signs and imaging. Typical symptoms are sudden chest pain, followed by chest tightness and dyspnea, and irritating cough. Pneumothorax subjects should have absolute bed rest and adequate oxygen intake to minimize lung activity, which is conducive to gas absorption and lung reexpansion. Consultation with relevant departments should be requested in time, and operations such as pleural cavity puncture and aspiration or closed chest drainage should be performed to promote the early reexpansion of lung tissues. Surgery can be performed if drainage fails or if necessary.

Gastrointestinal perforation: The seriousness of perforation is that a large amount of gastrointestinal fluid flows into the abdominal cavity after perforation, causing chemical or bacterial peritonitis and toxic shock. Clinical manifestations: acute abdominal pain, severe cutting or burning persistent pain, may be accompanied by fever, nausea and vomiting, and in severe cases, infectious shock may occur. Significant signs of peritoneal irritation can be seen on examination, manifested by abdominal pressure, rebound pain and muscle tension, and abdominal refusal to be pressed. The diagnosis is confirmed by clinical symptoms, physical signs and X-ray/CT examination. Treatment of GI perforation begins with terminating the continued leakage of gastrointestinal contents into the abdominal cavity and allowing the acute peritonitis to resolve. Conventional conservative treatment includes water fasting, placement of a nasogastric tube to suction the gastric contents for continuous gastrointestinal decompression, application of proton pump inhibitors to reduce digestive fluid secretion, nutritional support and correction of electrolyte disorders, and systemic anti-infective treatment with antibacterial agents. If the symptoms are not relieved or worsened after conservative treatment, emergency surgery is needed.

If the syringe punctures a blood vessel during the operation and causes internal bleeding, the

operation should be suspended in time, and the clinical manifestations and vital signs of the subject should be closely observed. Symptomatic treatment should be carried out according to the actual situation in serious cases: including the application of hemostatic drugs, intravenous rehydration, and intravenous blood transfusion treatment can be given when there is massive bleeding with progressive decrease of hemoglobin and obvious anemia symptoms.

If VSV-02 is inadvertently injected into a vein and enters the systemic circulation, acute toxicity or anaphylaxis may occur. Anaphylactic reactions during administration may be characterized by chills, fever (usually around 38 ° C in mild cases and 40 ° C or more in severe cases), and may be accompanied by headache, nausea, vomiting, rash, itching, urticaria, tachypnoea, general malaise, arthralgia, hypotension, hypertension, bronchospasm, or other anaphylactic reactions. In severe cases, angioneurotic edema, laryngospasm, asthma, anaphylaxis, heart failure, and pulmonary edema may occur. Allergic reactions usually occur rapidly and may be life-threatening in severe cases. Some subjects may experience delayed hypersensitivity reactions, mostly within 1 week after administration, which may be characterized by local or generalized itching and rash.

Clinical manifestations and vital signs will be closely monitored during administration. If anaphylaxis occurs, the investigational product will be immediately discontinued and symptomatic treatment will be initiated. Symptomatic treatment may include antihistamines and/or glucocorticoids.

8.6 Pregnancy.

Male and female subjects of childbearing potential must agree to use effective, medically approved contraception from the time of first dose until 6 months after discontinuation. The investigator should emphasize the time frame requirement for the use of contraception to the investigator at the time of the subject's informed consent and advise the subject of the possible effects of the application of the test drug on the fetus.

Female subjects should discontinue the study drug and notify the investigator as soon as they become pregnant during study treatment. Female subjects must withdraw from the study. The investigator should complete the Pregnancy Report Form within the same timeframe as the Serious Adverse Event Report and report it to the Collaborator and the Collaborator-appointed CRO within 24 hours and follow the pregnancy outcome (e.g., termination of pregnancy, labor and delivery) until the end of the pregnancy/third trimester of the newborn. Male subjects should also notify the investigator immediately if their heterosexual partner becomes pregnant during treatment. The investigator will obtain a separate informed consent form from their female partner for the collection of pregnancy-related information and follow up with their heterosexual partner until three months after the end of

pregnancy/delivery.

Pregnancy is not an SAE, but abnormal pregnancy outcomes (spontaneous abortion, medically selected abortion, fetal/neonatal congenital anomalies, malformations, or deaths, etc.) will need to be documented and reported as an SAE at the same time as the pregnancy report is completed. Subjects should be appropriately monitored and cared for until the end of the pregnancy.

8.7 VSV-02 Exposure Report

VSV-02 is a herpetic stomatitis virus that may cause infection in subjects. VSV-02 should not be given to subjects who have significant immune disorders in addition to the underlying cancer or who are receiving prohibited immunosuppressive medications.Due to the potential for transmission of VSV-02 and vesicular stomatitis virus as a result of unintentional exposures, healthcare workers, close contacts (family members, caregivers, sexual or bed partners), pregnant women, and newborns should avoid contact with injected lesions of subjects receiving treatment, dressings or body fluids. Caregivers should wear protective gloves when assisting subjects in applying or changing occlusive dressings and follow safety precautions for handling used dressings, gloves, and cleaning materials. If accidental exposure to VSV-02 occurs during preparation or administration, the exposed area should be rinsed with water for at least 15 minutes. If broken skin or needle punctures come into contact with VSV-02, the affected area should be thoroughly cleaned with soap and water and/or disinfectant.

If a family member, caregiver, or healthcare worker in close contact with the subject is suspected of being exposed to VSV-02 during the subject's treatment with VSV-02, report the exposure event to the Collaborator as specified below. In addition to reporting unanticipated exposure events during study treatment, investigators should monitor potential exposure events that occur within 28 (±7) days of the last dose of VSV-02.

For any potential or known unintended exposure, the investigator should report the exposure event to the Collaborator within 24 hours of being informed of the exposure event. If necessary, the Collaborator will attempt to follow up with the Exposed Individual to gather additional information about the Exposed Individual's contact with clinical trial subjects, signs and/or symptoms associated with the exposure, medical history, and/or exposure outcomes. If an exposed individual reports signs or symptoms suspected to be related to VSV-02 exposure, a swab may be taken from the exposed individual within 3 days of the onset of symptoms or signs to confirm the presence of VSV-02 in the lesion.

9 Statistical Considerations

Detailed summary and statistical analysis methods for the data collected for this study will be

included in a Statistical Analysis Plan (SAP), which will be developed after the protocol and CRF are finalized and finalized prior to database locking and data unblinding.The SAP will specify and describe in detail all elements of the planned statistical analyses based on the key features of the protocol.

9.1 Sample size estimation

This study is expected to enroll 3 to 6 subjects.

9.2 Analytic Population

Full Analysis Set (FAS)	The Full Analysis Set (FAS) will include all enrolled subjects who have used the trial drug at least once. The FAS will be used for demographic data, baseline descriptive analysis, medication adherence, trial coadministration, and efficacy analysis.
Efficacy Evaluable Set (EEAS)	A subset of the FAS that includes subjects with at least one post-treatment tumor assessment. The EEAS will be used for efficacy analysis.
Safety Set (SS)	Includes all subjects who have received at least one dose of the investigational drug; SS will be used for safety and tolerability analyses.
Biodistribution Analysis Set	This analysis set will include all enrolled subjects who have received at least one dose of the test drug and have completed at least one post-dose biodistribution blood collection. This analysis set will be used to assess biodistribution.
Stool Assessable Analysis Set	This analysis set will include enrolled subjects who have received at least one trial drug and completed at least one fecal swab collection. This analysis set will be used to assess the level of VSV-02 Injection gDNA and active virus detected in fecal samples.
Urine Assessable Analysis Set	This analysis set will include enrolled subjects who have received at least one trial drug and have completed at least one urine swab collection during the treatment period. This analysis set will be used to assess the level of VSV-02 Injection gDNA and active virus detected in urine swab samples during the Treatment Period.
Saliva Assessable Analysis Set	This analysis set will include enrolled subjects who have received at least one trial drug and have completed at least one saliva swab collection during the treatment period. This analysis set will be used to assess the level of VSV-02 injectable gDNA and active virus detected in saliva swab samples during the treatment period.
Injection Site Assessable Analysis Set	This analysis set will include enrolled subjects who have received at least one trial drug and have completed at least one injection site swab collection. This analysis set will be used to assess the levels of VSV-02 Injection gDNA and active virus detected on the surface of the injection site.
Pharmacodynamic Analysis Set	This analysis set will enroll subjects who have received at least one trial drug and have at least one assessable biomarker or cytokine parameter. This analysis set will be used to assess biomarker and cytokine levels of VSV-02 Injection.
Immunogenicity Analysis Set	This analysis set will include all subjects who have received at least one trial drug and have data from at least one post-baseline immunogenicity sample. This analysis set will be used to assess the immunogenicity of VSV-02 Injection.

9.3 Statistical Analysis

9.3.1 General Methods

Measurement information is statistically described by the number of cases, mean, standard deviation, median, maximum and minimum values. Count or grade information is described statistically by the number of cases and percentages. Survival data will be presented at the 25th, 50th (median), and 75th percentile if estimation is possible. In addition, the number and percentage of subjects with events and censored subjects will be presented. A list of individual subject data will be

provided to support the provision of summary tables. If there are any deviations in the actual statistical methods used from those provided in the study protocol, these will be described and justified in the SAP. All statistical analyses will be performed using Statistical Analysis Software (SAS®) version 9.4 or later.

9.3.2 Distribution of subjects

The number and percentage of subject cases in each analyzed population will be summarized, and in addition, the primary reasons for discontinuation of VSV-02 will be summarized.

9.3.3 Demographic and Baseline Characteristics

Baseline characteristics and demographic data for FAS will be summarized.

9.3.4 Safety Analysis

Safety analyses will be performed based on the Safety Analysis Set.

9.3.5 Validity Analysis

Efficacy analysis will be based on FAS and EEAS.This study will initially evaluate the antitumor activity of VSV-02. Efficacy endpoints, including ORR and DCR, will be evaluated using RECIST version 1.1 and iRECIST.

ORR is defined as the percentage of subjects achieving CR or PR for overall remission and DCR is defined as the percentage of subjects achieving CR, PR or SD. ORR and DCR will be presented by dose level at each program plan time point. 95% CIs for ORR and DCR will be calculated using the Clopper-Pearson method.

In addition, other efficacy parameters such as DoR, PFS and OS will be evaluated.

For the subset of subjects who experience remission, DoR (defined as the time from the date of the first documented remission [CR or PR] to disease progression or death from any cause [whichever is earlier]) will be summarized using Kaplan-Meier curves. For subjects who experienced remission during the study period but did not subsequently experience disease progression or death or were lost to follow-up, the DoR will be censored at the date of the final tumor assessment.

PFS, defined as the time from the date of first administration of study drug to disease progression or death from any cause (whichever is earlier), will be summarized using Kaplan-Meier curves. For subjects who do not progress or die or are lost to follow-up during the study period, PFS will be censored at the date of the final tumor assessment.

OS is defined as the time from first study treatment to the date of death due to any cause, and

will be censored at the date of last contact obtained for subjects who have not experienced death or loss to visit as of the time of study analysis.

Kaplan-Meier estimates of median DOR, PFS and OS, first and third quartiles will be presented by dose level at study entry, and 95% CIs will also be calculated for subjects who progressed or died if a sufficient number of subjects progressed or died.Survival and 95% CIs will be calculated at each time point.The Greenwood formula will be used to calculate Kaplan-Meier curve standard error of the estimates. In addition, Kaplan-Meier plots of DOR, PFS, and OS will be plotted by dose level at the time of study enrollment.

9.3.6 Biodistribution and viral shedding analysis

The biodistribution of VSV-02 will be evaluated by detecting the viral gDNA level in blood samples using real-time quantitative polymerase chain reaction (qPCR) method. Viral shedding will be evaluated by detecting viral gDNA levels in urine, feces, nasopharyngeal fluid and injection site swabs. Biodistribution and viral shedding analyses will be based on the biodistribution analysis set and associated viral shedding analysis set defined in Section9.2 . The proportion of subjects meeting the criteria for detectable DNA at each time point will be calculated. Summary statistics will be presented by dose level.

9.3.7 Immunogenicity Assessment

All available immunogenicity data at each VSV-02 concentration level will be presented by dose level and immunogenicity status.

9.3.8 Biomarkers and Cytokines Assessment

Biomarkers and cytokines and their changes relative to baseline are statistically characterized according to their profile. Exploratory analyses of the parameters were performed as needed.

10 Data Processing and Record Keeping

10.1 Data collection and management responsibilities

Prior to clinical trial data management, a Data Management Plan (DMP) is developed by the data management department based on the actual situation of the project. The DMP is a dynamic document written by the data management personnel based on the clinical trial protocol, which specifies and records in detail and comprehensively the data management tasks for a particular clinical trial, including personnel roles, work content, operation specifications, etc.

10.2 Data Collection and Methods

In this trial, the Electronic Data Capture System , EDC, was used for data collection, and data administrators/database programmers created accounts and granted different privileges to access the EDC system according to different identities.

The data recorded in the Electronic Case Report Form (eCRF) should come from the source files and be guaranteed to be consistent with the source data. All source documents should be kept clear and neat to ensure that the data can be accurately identified. Permanent copies of study visit records will be considered source documents for recording enrolled subject data. Data from source documents such as study medical records should be entered into the eCRF by the entry personnel in a timely and accurate manner.

10.3 Data Cleaning and Challenge Resolution

Data cleansing involves the process of data verification (system logic checking and manual logic checking), triggering of queries, investigator/research assistant answering of queries, data updating, until the queries are resolved.

Data cleanup is regularly performed by data managers and supervisors through the EDC system, and medical supervisors regularly perform medical audit work through the EDC system. For EDC queries, investigators/research assistants were online to give answers and/or correct erroneous data. The query initiator confirms the answered data and can repeat the query if necessary.

10.4 Correction and review of data

Data can be modified by the data entry staff or by the investigator after verification of the data, and the reason for the modification must be filled out on eCRF. The researcher has the authority to review all final data.

10.5 Data Lock and Export

The data administrator locks the data after all the data have been reviewed and approved. Any modification of data after locking must be signed by the collaborator, researcher, statistician, medical personnel, data management personnel, etc. before execution. All data were finally exported from the EDC database by the data manager and handed over to the statistician for analysis.

At the end of the study, the eCRF is printed and archived as needed. The Data Management Center will maintain the eCRF.

Data management related tasks not exhaustively specified in the protocol are performed with reference to the data management plan for this trial.

10.6 Study Record Keeping

In order to ensure the evaluation and supervision by the State Drug Administration and the sponsor, the investigator should keep all the study data according to the GCP, including all the subject source data record information, such as study charts or original transcripts, signed informed consent, and detailed records of drug distribution. After the completion of the trial, the PDF version of the eCRF of all subjects in each study center shall be saved on CD-ROM to the corresponding study center. The study data should be kept at least until 5 years after the termination of the clinical trial or 5 years after the approval of the test drug to be marketed, and the destruction of any data is not allowed without the written consent of the sponsor.

The ownership of all the data of this clinical study belongs to the sponsor, and the investigator is not allowed to provide them to a third party in any form without the written consent of the sponsor, except for the requirement of the State Drug Administration.

10.7 Study Publication and Data Sharing Policy

Information provided to the investigator by the sponsor (including this clinical trial protocol) is non-public information and must be kept confidential.

The research organization shall obtain written consent from the funder to communicate the results of this clinical study at academic conferences or in journals. The publication of articles in academic journals by the Research Organization shall be made after the Grantor has filed the relevant patent application and shall be made with the written consent of the Grantor.

All published papers of this clinical research/clinical trial program will be signed by the principal investigator as the first corresponding author (listed last among the co-corresponding authors and showing his/her email address), and the ranking of the other authors will be determined by agreement between the research institution and the funder. Page charges were borne by the first corresponding author.

11 Code of Ethics and Informed Consent

11.1 Ethical norms

This trial was conducted in accordance with GCP, the Declaration of Helsinki, relevant regulations and ethics committee review. The investigator should ensure that this trial is reviewed and approved by a qualified ethics committee that meets the requirements of GCP. At the time of review, the sponsor and the investigator should provide the ethics committee with the trial protocol, the informed consent form, and other required materials. The sponsor can only supply the trial drug after

receiving the approval from the ethics committee. The ethics committee must also be informed of subsequent protocol additions and serious adverse events that may affect the safety and continued participation of subjects in the trial. It is the responsibility of the investigator to report to the ethics committee on the progress of the trial. When reviewing and approving the trial protocol, the Ethics Committee must confirm the title of the protocol, the protocol number, and indicate the reviewed protocol document and the date of review. During the trial, if there are any new amendments to the trial protocol, informed consent, etc., the written approval of the relevant regulatory unit should be obtained again in accordance with the regulations.

11.2 Informed Consent

Subjects must give informed consent for participation in this trial prior to receiving treatment in this trial in order to protect the legal rights of the subjects. It is the responsibility of the investigator to provide the subject or his/her designated agent with a complete and comprehensive description of the purpose of the study, the manner in which the study will be conducted, the action of the drug, the reasonably expected benefits, the possible toxicities and side-effects, and the possible risks. Subjects shall be made aware of their rights, the risks to be borne and the benefits, and shall be informed in a timely manner of any new information regarding the drug to be used in the trial. Subjects should be informed that the clinical trial is conducted on a voluntary basis, that they may withdraw unconditionally from the trial at any time during the trial, and that they will not be penalized for withdrawing from the trial. Subjects should be informed that the investigator and sponsor have the right to read, save and statistically process the subject's trial data in accordance with the provisions of relevant regulations. The informed consent form should indicate the version and date of development or revision. The informed consent form must be signed and dated by the subject (and the subject's legal guardian), and the investigator performing the informed consent process must also sign and date the informed consent form, which may also be signed by an independent witness who can attest to the subject's consent to participate in the trial. One copy of the informed consent form should be retained by the investigator and one by the subject. If significant new information regarding the trial drug is discovered, the informed consent form must be revised in writing and sent to the Ethics Committee for approval before consent is obtained from the subject again.

11.3 Confidentiality and privacy

The confidentiality and privacy of the subjects were kept strictly confidential by the participating investigators, their staff, and the collaborators and their interventions. This confidentiality will extend to tests covering biological sample testing and genetic testing in addition to clinical information

related to subjects. Therefore, study protocols, documents, data and all other information generated in the study will be kept strictly confidential. No relevant study information or data will be released to unauthorized third parties without the written permission of the sponsor.

All research activities will be conducted in a private setting whenever possible.

The Study Monitor, other authorized representatives of the Collaborator, representatives of the Ethics Review Board (IRB), regulatory agencies, or representatives of the pharmaceutical company providing the investigational product may inspect all documents and records required to be maintained by the Investigator, including, but not limited to, the medical records (office, clinic, or hospital) and pharmacy records of the subjects in this study. The Clinical Research Center should allow access to these records.

Subject contact information will be securely stored at each clinical research center for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for longer than the period of time required by IRB review, institutional regulations, or partner requirements.

12 Quality Assurance and Quality Control

All parties to the clinical trial such as collaborators, research centers and CROs shall take appropriate quality control measures to ensure that the clinical trial complies with the requirements of the Declaration of Helsinki, GCP, and appropriate laws, regulations and SOPs.

12.1 Requirements for Collaborators

（1） Collaborators or their representatives must provide the most current version of the Investigator's Brochure (IB) for each unregistered clinical trial compound. For marketed products, provide the IB and/or the most current product information.

（2） The Partner or its representative develops the final version of the clinical trial protocol with the investigator and statistical expert. After all parties have reached an agreement, the clinical trial protocol is signed and submitted to the Ethics Committee.

（3） The partner or its representative provides all investigators with a sufficient number of case report forms and instructs the investigators to complete and keep them.

（4） The partner or its representative provides all investigators with sufficient trial materials to support and allow the investigator to conduct the trial according to the defined protocol.

（5） The Partner or its representative reserves the right to terminate the clinical trial prematurely due to recurring protocol violations, or other well-founded ethical concerns. If this is the

case, both parties will review and consult with each other and take necessary measures to ensure the rights of patients.

12.2 Duties of the Monitor Appointed by the Partner or its Representative

The clinical trial monitor is the primary contact between the collaborator and the investigator. The monitor must follow GCP and SOP, visit the research center regularly or according to the actual situation to carry out clinical monitoring, supervise the conduct and progress of the clinical trial, check and confirm that all data records and reports, eCRF input are correct and complete, and consistent with the original data, to ensure that the clinical trial is carried out in accordance with the clinical trial protocol, and that the investigator should actively cooperate with the work of the monitor. The specific content of the monitor includes:

（1） Confirming before the trial that the trial undertaking unit has appropriate conditions, including staffing and training, laboratory equipment is complete and in good working order, with a variety of test-related inspection conditions, estimated to have a sufficient number of subjects, and the participating trial personnel are familiar with the requirements in the trial protocol.

（2） Monitor the investigator's implementation of the trial protocol during the trial, confirm that informed consent has been obtained from all subjects prior to the trial, understand the enrollment rate of subjects and the progress of the trial, and confirm that the enrolled subjects are qualified.

（3） Confirmed that all data were recorded and reported correctly and completely, and that all case report forms were entered correctly and agreed with the original data. All errors or omissions were corrected or noted, signed and dated by the investigator. Dose changes, therapeutic alterations, comorbidities, intercurrent illnesses, missed visits, and missed tests should be recognized and documented for each subject.

（4） Confirm that all adverse events are documented and that serious adverse events are reported and documented within the required time frame; verify that the test drug is supplied, stored, dispensed, and withdrawn in accordance with the relevant regulations and documented accordingly.

（5） Assist the investigator to carry out the necessary notification and application matters, and report the trial information and results to the partner.

（6） Keep clear and accurate records of visits, tests not performed, and inspections not made by the investigator, and whether or not corrections have been made for errors and omissions.

（7） Complete a written monitoring report after each visit, which should state the date and time of the monitoring, the name of the monitor, and the findings of the monitoring.

12.3 Requirements for Investigators

（1） The investigators participating in the clinical trial must have the professional expertise, qualification and ability of the clinical trial, after qualification examination, and the personnel requirements are relatively fixed;

（2） Before patient enrollment, the investigator should explain the significance of the clinical study to the enrollee or family members, obtain their consent and sign the informed consent form;

（3） Personally execute and supervise the conduct of the clinical study;

（4） Carefully completing the case report form (eCRF) as required;

（5） Cooperate with the regular visits of the clinical supervisor appointed by the collaborator or his/her representative;

（6） Keeping complete records of laboratory tests, clinical records, and original medical records of patients;

（7） In the event of a serious adverse event, the investigator must determine the cause and deal with it accordingly, and report it to the study leader;

（8） Follow-up of serious adverse events.

12.4 Audits and inspections

When the partner or a third party is commissioned to conduct an audit or inspection of the study by the pharmacy authorities according to the needs of the trial, the original documents of the trial can be directly accessed, and the data in the eCRF should be directly derived from the original data.

12.5 Protocol Deviation

All requirements set forth in the study protocol must be strictly enforced. Any intentional or unintentional deviation or violation of the trial protocol and GCP principles can be categorized as protocol deviation. The supervisor should fill in the protocol deviation record by the investigator or the supervisor if protocol deviation is found in the course of supervision, recording in detail the time of discovery, the time and process of the event, the reason and the corresponding treatment measures, which should be signed by the researcher and notified to the Ethics Committee and the collaborators. In the statistical analysis report and summary report, the impact of the occurred protocol deviations on the final data and conclusions was analyzed and reported.

When serious protocol deviations occur, they should be evaluated. If necessary, the collaborators may terminate the study early.

13 References

[1]. Global cancer burden growing, amidst mounting need for services, WHO, 1 February 2024.

[2]. Borghaei H, Paz-Ares L, Horn L, et al. Nivolumab versus Docetaxel in Advanced Nonsquamous Non-Small-Cell Lung Cancer. the New England Journal of Medicine. 2015;373(17):1627-1639.

[3]. Ribas A, Dummer R, Puzanov I, et al. Oncolytic Virotherapy Promotes Intratumoral T Cell Infiltration and Improves Anti-PD-1 Immunotherapy. cell. 2017. 170(6):1109-1119.e10.

[4]. Harrington, K., et al., Optimizing oncolytic virotherapy in cancer treatment. Nat Rev Drug Discov, 2019. 18(9): p.689-706.

[5]. Puzanov, I., et al., Talimogene Laherparepvec in Combination With Ipilimumab in Previously Untreated, Unresectable Stage IIIB-IV Melanoma. j Clin Oncol, 2016. 34(22): p. 2619-26.

[6]. Chesney, J., et al., Randomized, Open-Label Phase II Study Evaluating the Efficacy and Safety of Talimogene Laherparepvec in Combination With Ipilimumab Versus Ipilimumab Alone in Patients With Advanced, Unresectable Melanoma. J Clin Oncol, 2018. 36(17): p. 1658-1667.

[7]. Msaouel P, Dispenzieri A, Galanis E. Clinical testing of engineered oncolytic measles virus strains in the treatment of cancer: an overview. current Opinion in Molecular Therapeutics. 2009;11(1):43-53.

[8]. Kaufman HL, Kohlhapp FJ, Zloza A. Oncolytic viruses: a new class of immunotherapy drugs. Nature Reviews Drug Discovery. 2015;14(9):642-662.

[9]. Ribas A, Dummer R, Puzanov I, et al. Oncolytic Virotherapy Promotes Intratumoral T Cell Infiltration and Improves Anti-PD-1 Immunotherapy. cell. 2017. 170(6):1109-1119.e10.

[10]. Giedlin, M.A., D.N. Cook, and T.W. Dubensky, Jr. Vesicular stomatitis virus: an exciting new therapeutic oncolytic virus candidate for cancer or just another chapter from Field's Virology? Cancer Cell, 2003. 4(4): p. 241-3.

[11]. Bridle, B.W., et al., Vesicular stomatitis virus as a novel cancer vaccine vector to prime antitumor immunity amenable to rapid boosting with adenovirus . Mol Ther, 2009. 17(10): p. 1814-21.

[12]. Cook J, Peng KW, Witzig TE, et al. Clinical activity of single-dose systemic oncolytic VSV virotherapy in patients with relapsed refractory T-cell lymphoma. lymphoma. Blood Adv. 2022 Jun 14;6(11):3268-3279.

[13]. Oken MM, Creech RH, Tormey DC, et al. Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

[14]. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). Eur J Cancer. 2009 Jan;45(2):228-47.

[15]. Seymour L, Bogaerts J, Perrone A, et al. iRECIST: guidelines for response criteria for use in trials testing immunotherapeutics. Lancet Oncol. 2017 Mar; 18(3):e143-e152.