

Intratumoral OH2, an oncolytic herpes simplex virus 2, in patients with advanced solid tumors: a multicenter, phase I/II clinical trial

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ABSTRACT

Background OH2 is a genetically engineered oncolytic herpes simplex virus type 2 designed to selectively amplify in tumor cells and express granulocyte-macrophage colony-stimulating factor to enhance antitumor immune responses. We investigated the safety, tolerability and antitumor activity of OH2 as single agent or in combination with HX008, an anti-programmed cell death protein 1 antibody, in patients with advanced solid tumors.

Methods In this multicenter, phase I/II trial, we enrolled patients with standard treatment-refractory advanced solid tumors who have injectable lesions. In phase I, patients received intratumoral injection of OH2 at escalating doses (10^6 , 10^7 and 10^8 CCID₅₀/mL) as single agent or with fixed-dose HX008. The recommended doses were then expanded in phase II. Primary endpoints were safety and tolerability defined by the maximum-tolerated dose and dose-limiting toxicities (DLTs) in phase I, and antitumor activity assessed per Response Evaluation Criteria in Solid Tumors (RECIST version 1.1) and immune-RECIST in phase II.

Results Between April 17, 2019 and September 22, 2020, 54 patients with metastatic cancers were enrolled. Forty patients were treated with single agent OH2, and 14 with OH2 plus HX008. No DLTs were reported with single agent OH2 in phase I. Four patients, having metastatic mismatch repair-proficient rectal cancer or metastatic esophageal cancer, achieved immune-partial response, with two from the single agent cohort and two from the combination cohort. The duration of response were 11.25+ and 14.03+ months for the two responders treated with single agent OH2, and 1.38+ and 2.56+ months for the two responders in the combination cohort. The most common treatment-related adverse event (TRAE) with single agent OH2 was fever (n=18, 45.0%). All TRAEs were of grade 1–2, except one case of grade 3 fever in the 10^8 CCID₅₀/mL group. No treatment-related serious AEs occurred. Single agent OH2 induced alterations in the tumor microenvironment, with clear increases in CD3⁺ and CD8⁺ cell density and programmed death-ligand 1 expression in the patients' post-treatment biopsies relative to baseline.

Conclusions Intratumoral injection of OH2 was well-tolerated, and demonstrated durable antitumor activity in patients with metastatic esophageal and rectal cancer. Further clinical development of OH2 as single agent or

with immune checkpoint inhibitors in selected tumor types is warranted.

BACKGROUND

Oncolytic virotherapy represents a unique antitumor strategy using natural or genetically engineered viruses to infect and replicate in tumor cells. The mechanisms of the tumoricidal effect include direct tumor cell lysis caused by selective infection, and the subsequent release of cell debris and viral antigens, which may overcome immunosuppression in the tumor microenvironment and trigger antitumor immune responses. In addition, the advances in biotechnology have enabled the insertion of therapeutic transgenes into the viral genomes to enhance efficacy.^{1,2}

A diverse range of viruses have been studied as candidates of oncolytic virus, among which the herpes simplex virus type 1 (HSV-1) was the earliest reported oncolytic viruses generated by genetic engineering.³ HSV derived oncolytic viruses may offer a few advantages, including a broad host range allowing clinical application in many different types of cancers, the potential for incorporating a sizeable foreign DNA, and the readily available antiherpetic therapy to control undesired infection and replication.⁴ The significant benefits observed in the phase III randomized trial of the HSV-based talimogene laherparepvec (T-VEC) in patients with advanced melanoma⁵ and its approval in the USA and European Union have shown great promise for this class of anticancer agent and inspired further advances in this field.

OH2 is a novel oncolytic virus derived from the wild-type HSV-2 strain HG52. In the construction of this virus, the ICP34.5 neurovirulence gene was deleted to attenuate the

toxicity and enhance tumor selectivity. The ICP47 gene was removed to help present tumor associated antigens and promote the oncolytic activity. The gene encoding the human granulocyte-macrophage colony-stimulating factor (GM-CSF) was added to potentiate antitumor immunity.⁶ Preclinical studies demonstrated that OH2 was genetically and biologically stable,⁷ and exhibited potent oncolytic activity and safety in animal models.^{6,8} Moreover, a specific antitumor immune response and a long-term effect to inhibit tumor recurrence and metastasis were observed in a murine colon cancer model treated with OH2.⁹ These data validated subsequent clinical investigation of the drug.

There have been limited reports concerning the application of HSV-based oncolytic viruses in solid tumors other than glioma and melanoma. We therefore initiated a phase I/II study in patients with advanced solid tumors to evaluate the safety, biodistribution and antitumor activity of OH2 as single agent or in combination with other anticancer drugs. Meanwhile, with tumor samples obtained from baseline and on-therapy biopsies, we also aim to explore the alterations of the tumor microenvironment after OH2 virotherapy. Here we report the results of treatment with single agent OH2, and OH2 in combination with HX008, an anti-programmed cell death protein 1 (PD-1) antibody.

METHODS

Patients

In this open-label, multicenter, phase I/II trial, we enrolled patients aged between 18 and 75 years with a histologically confirmed advanced solid tumor whose disease had progressed despite standard systemic therapies. Inclusion criteria included having tumor(s) deemed safe to inject the virus; at least one measurable lesion as defined in the Response Evaluation Criteria in Solid Tumors (RECIST), version 1.1; an Eastern Cooperative Oncology Group performance status score of 0 or 1; and adequate organ functions assessed by the complete blood count, blood chemistry and coagulation tests. Patients were excluded if they had symptomatic central nervous system metastases, presentations of active infection or an unexplained fever $>38.5^{\circ}\text{C}$, or treatment with antiviral drugs within 4 weeks before treatment initiation.

Study design and treatment

The phase I part of the study comprised the OH2 single agent cohort and the OH2 with HX008 combination cohort. Dose-escalation of each cohort followed the traditional “3+3” design. Patients were treated with OH2 at three dose levels (10^6 , 10^7 and 10^8 CCID₅₀/mL) every 2 weeks. In the combination cohort, HX008 was administered at the fixed dose of 200 mg every 3 weeks. Dose escalation proceeded if no dose-limiting toxicities (DLTs) were observed during the 3-week DLT assessment period in the first three patients, otherwise another three patients would be enrolled. If two or more DLTs were observed at

one dose level, the previous lower dose would be defined as the maximum-tolerated dose (MTD). The recommended dose level of single agent OH2 was determined on completion of dose-escalation, taking into consideration the MTD, safety, biodistribution parameters and tumor response. In the phase II dose-expansion part of the study, patients were assigned either to receive single agent OH2 at the recommended dose level, or HX008 plus OH2 at dosages proved to be tolerable in phase I of the combination therapy.

The intratumoral injection was performed either directly for cutaneous or subcutaneous lesions, or with ultrasound guidance for deep-located nodes or organ metastases. The volume injected to each lesion was based on the longest diameter of the tumor (≤ 1.5 cm, up to 1 mL; >1.5 to ≤ 2.5 cm, up to 2 mL; >2.5 to ≤ 5.0 cm, up to 4 mL; >5.0 cm, up to 8 mL). Injection of multiple tumors was allowed. There were no limits on the number of lesions that can be injected per patient, but the maximum volume that can be injected during each visit was 8 mL.

Safety and response evaluation

Adverse events (AEs) and DLTs were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (version 5.0). DLT was defined as any of the following events within the first 3 weeks of therapy that was deemed treatment-related by the investigator(s): grade 4 neutropenia lasting over 5 days or febrile neutropenia; grade 3 thrombocytopenia with bleeding; any other grade 4–5 hematological AEs; grade 3 rash over 3 days; grade 3 nausea, vomiting and diarrhea lasting over 3 days despite appropriate supportive care; grade 3 alanine aminotransferase or aspartate aminotransferase level increase lasting over 7 days; grade 2 proteinuria lasting over 7 days; any other grade 3–5 non-hematological AEs. AEs were collected throughout the study treatment and for 60 days after the last dose of OH2.

Tumor assessments were conducted at screening, every 6 weeks during the first 6 months after treatment initiation, every 8 weeks after 6 months and every 12 weeks after 12 months. Radiographic assessments were performed using CT or MRI. Measurement of cutaneous or subcutaneous lesions were conducted with calipers. Evaluation of response was performed by the investigators using both the RECIST version 1.1 and the immune-RECIST (iRECIST) criteria.

Biodistribution and biological activity

In phase I, the biodistribution of single agent OH2 was evaluated by detection of viral loads in the blood, urine and saliva by PCR. The injection sites were swabbed for virus shedding on the next day after each injection. To assess the biological activity of single agent OH2, we detected GM-CSF mRNA in tumor samples by reverse transcription-PCR, and blood GM-CSF protein by ELISA before and after OH2 administration. Serum samples were collected for anti-HSV-2 antibody assays by ELISA until confirmation of HSV-seropositive status or 28 days

after the last dose, whichever occurred first. Presence of blood anti-GM-CSF antibodies was assessed using a validated electrochemiluminescence immunoassay on the MesoScale Discovery platform. After the completion of dose-escalation, monitoring for blood OH2 viral loads, HSV serology and anti-GM-CSF antibodies continued in phase II of the trial.

Biomarker analysis

Biopsies of the injected lesions were performed before the first dose of OH2 and subsequently at each response evaluation. Three biomarkers, including programmed death-ligand 1 (PD-L1), CD8 and CD3, using immunohistochemistry (IHC) were stained to characterize the changes in the tumor microenvironment. PD-L1 expression was assessed with the Ventana SP263 assay, and was reported using both the tumor proportion score (TPS) and the combined positive score (CPS). TPS was defined as the percentage of tumor cells with membranous PD-L1 staining, whereas CPS was defined as the number of PD-L1 staining cells (tumor cells, lymphocytes and macrophages) divided by the total number of viable tumor cells, multiplied by 100. For the quantification of CD8⁺ and CD3⁺ cells, the anti-CD8 mouse monoclonal antibody clone C8/144B was used for CD8 IHC, while the CD3 IHC analysis was conducted using the anti-CD3 (2GV6) rabbit monoclonal antibody. The density of CD8⁺ and CD3⁺ stained cells in the intratumoral area plus the invasive margin, if present in the biopsy, were reported as cells/mm². The definition of the invasive margin was a region of 360 μm width on the frontier between malignant cells and peritumoral stroma, which was identical to that reported by Pagès *et al.*¹⁰

Endpoints and statistical analysis

The primary endpoints were safety and tolerability defined by MTD and DLT in phase I and antitumor activity in phase II. The analyses were descriptive. The Kaplan-Meier method was used to estimate time-to-event variables. SPSS statistics (Version22) was used for data analyses.

RESULTS

Between April 17, 2019 and September 22, 2020, we enrolled 54 patients from seven participating centers, among whom 40 patients were treated with single agent OH2 and 14 received OH2 in combination with HX008. The baseline characteristics are summarized in [table 1](#). Notably, the present study was suspended in two centers in early 2020 due to the impact of the COVID-19 pandemic, causing interruptions of the study treatment and sample collection. Thirteen patients were affected, including three patients from phase I and ten from phase II. As a result, one patient in phase II withdrew consent, and five patients in phase II permanently discontinued treatment with single agent OH2 during the study suspension.

Table 1 Baseline characteristics and best response to the study treatment

	Single agent OH2 (n=40)	OH2 with HX008 (n=14)
Median age at entry, years (range)	55 (27–73)	53 (36–64)
Male	30 (75.0%)	10 (71.4%)
Tumor types		
Esophageal cancer	14	3
Colorectal (including appendix) cancer	12	6
Gastric cancer	5	5
Melanoma and other skin cancer	4	0
Head and neck cancer	2	0
Cholangiocarcinoma	1	0
Ovarian cancer	1	0
Breast cancer	1	0
Prior treatment with anti-PD-1 antibodies	10 (25.0%)	1 (7.1%)
Best response (iRECIST)		
iPR	2	2
iSD	9	4
iUPD	22	6

iPR, immune-partial response; iRECIST, immune-Response Evaluation Criteria in Solid Tumors; iSD, immune-stable disease; iUPD, immune-unconfirmed progressive disease; PD-1, programmed cell death protein 1.

All patients were included in the safety analyses, and 45 patients who had completed at least one tumor assessment were included in the efficacy analysis. As of the data cut-off date (December 1, 2020), the median follow-up was 5.41 months (range: 0.46–19.48+). Forty patients had discontinued the study treatment, and the most common reason for treatment discontinuation was progressive disease (PD) (n=27).

OH2 had a favorable safety profile as no DLTs were observed and the MTD was not reached during single agent dose-escalation. Most treatment-related AEs (TRAEs) observed with single agent OH2 were of grade 1 or 2, except that one patient in the 10⁸CCID50/mL group was injected with 8 mL of the study drug and developed grade 3 fever. No grade 4 TRAEs or treatment-related deaths occurred. The most frequent TRAE with single agent OH2 was fever (n=18, 45.0%), which typically occurred within the first 24 hours after OH2 injection, and could be managed with antipyretics. Other common TRAEs were anemia (n=5, 12.5%) and leukopenia (n=3, 7.5%). Dose-escalation of OH2 in combination with HX008 is ongoing, and no DLTs occurred in the 10⁶CCID50/mL and 10⁷CCID50/mL groups. There were no treatment-related serious AEs throughout the

**Table 2** Treatment-related adverse events

Adverse events	Single agent OH2 (n=40)			OH2 with HX008 (n=14)		
	Grade 1	Grade 2	Grade 3	Grade 1	Grade 2	Grade 3
Fever	10	7	1	1	3	0
Leukopenia	3	0	0	0	0	0
Neutropenia	2	0	0	0	0	0
Thrombocytopenia	1	0	0	0	0	0
Anemia	3	2	0	0	0	0
Hyperbilirubinemia	1	1	0	0	1	0
ALT increased	2	0	0	0	0	0
AST increased	2	0	0	0	0	0
QTc prolongation	0	1	0	0	0	0
Pruritus	1	0	0	0	0	0
Rash	2	0	0	0	1	0
Complications of injection	0	3	0	0	0	0
Abdominal pain	0	2	0	0	0	0
Diarrhea	1	0	0	1	0	0
Nausea	0	1	0	0	0	0
Fatigue	1	0	0	3	0	0
Myalgia	1	0	0	0	0	0
Arthralgia	1	0	0	0	0	0
Proteinuria	0	0	0	1	0	0
Hypothyroidism	0	0	0	0	1	0

No grade 4 or 5 treatment-related adverse events occurred in the study. ALT, alanine aminotransferase; AST, aspartate aminotransferase.

study. The TRAEs observed in the trial are summarized in [table 2](#).

In dose-escalation of single agent OH2, four blood samples were positive for OH2 viral loads (range: 1.43×10^3 – 4.38×10^4 copies/ μ L), between 1 hour and 7 days after injection. These samples were from four different patients, but none reported signs or symptoms of HSV infection. Meanwhile, none of the four patients presented with fever at the timepoints of blood sample collection. The blood viral loads of these four patients and the corresponding timepoints are shown in [table 3](#). Viral copies in the blood did not seem to correlate with clinical benefits, since all the four patients with detectable viral loads did not respond to OH2. Pretreatment and post-treatment saliva, urine and injection site swabs were all negative for OH2 viral DNA. In patients enrolled for single agent dose-expansion, blood OH2 replication was not detected at all timepoints.

In phase I, GM-CSF mRNA was detected in the injected tumors from five patients after treatment with single agent OH2, while two patients had detectable GM-CSF expression in their blood samples. Among the 40 patients treated with single agent OH2, 38 were seronegative and 2 were seropositive for anti-HSV-2 antibodies at baseline; 26 of the seronegative patients received subsequent

HSV serology tests and were all converted to seropositive status after the study treatment. One patient in the single agent dose-escalation cohort developed transient antibodies to GM-CSF, as the antibodies were detected only once at the fifth week from the first dose. The results of GM-CSF mRNA in the tumor, GM-CSF expression and anti-GM-CSF antibodies in the blood of each patient are shown in [table 3](#) (single agent OH2 dose-escalation) and online supplemental table 1 (single agent OH2 dose-expansion). No association was noted between clinical responses and any laboratory endpoints, including OH2 biodistribution, GM-CSF mRNA, GM-CSF expression, HSV serology, or anti-GM-CSF antibodies.

Although the MTD was not reached in dose-escalation of single agent OH2, we found that the incidence of fever seemed dose-related (1/3 with 10^6 CCID50/mL, 1/4 with 10^7 CCID50/mL and 3/4 with 10^8 CCID50/mL), suggesting an increased risk of fever for patients receiving OH2 at 10^8 CCID50/mL. Meanwhile, we did not observe any dose-dependent biodistribution parameters or biological activities induced by OH2. On the other hand, clinical responses were noted in patients from both 10^6 CCID50/mL and 10^7 CCID50/mL groups. Therefore, the recommended dose level for single agent OH2 dose-expansion in phase II was 10^7 CCID50/mL.

Table 3 Biodistribution and biological activity of single agent OH2 in phase I*

Patient	Dose level (CCID50/mL)	Tumor types	Blood OH2 viral loads (copies/ μ L, timepoint)	Tumor GM-CSF mRNA levels (copies/ μ L, timepoint)	Blood GM-CSF expression (pg/mL, timepoint)	Blood anti-GM-CSF antibody (timepoint)	Best response (RECIST version 1.1/iRECIST)
1	10^6	Esophageal cancer	BQL	1.24×10^4 (C1D3)	BQL	Negative	PD/iPR
2	10^6	Esophageal cancer	BQL	BQL	BQL	Negative	PD/iUPD
3	10^6	Breast cancer	BQL	BQL	BQL	Negative	PD/iUPD
4	10^7	Rectal cancer	BQL	BQL	BQL	Negative	PR/iPR
5	10^7	Esophageal cancer	BQL	1.01×10^4 (C1D3)	BQL	Negative	NA
6	10^7	Head and neck cancer	3.38×10^3 (C1D1, 1 hour)	BQL	BQL	Negative	PD/iUPD
7	10^7	Appendix cancer	BQL	BQL	BQL	Negative	SD/ISD
8	10^8	Esophageal cancer	1.43×10^3 (C1D7)	1.41×10^3 (C3D1)	17.37 (C2D1) 19.40 (C3D1)	Negative	PD/iUPD
9	10^8	Esophageal cancer	BQL	1.00×10^4 (C1D3)	BQL	Negative	PD/iUPD
10	10^8	Gastric cancer	3.76×10^3 (C1D2)	1.72×10^4 (C1D3)	30.07 (C1D2) 20.98 (C1D3)	Negative	NA
11	10^8	Ovarian cancer	4.38×10^4 (C1D7)	BQL	BQL	Transient positivity (C3D1)	SD/ISD

*The biodistribution studies included OH2 viral DNA in the blood, saliva, urine, and injection site swabs. The results for saliva, urine and injection site swabs were all negative and not shown in the table. The results for blood OH2 viral loads, with values and timepoints of sample collection, are listed. BQL, below quantifiable limit; C1D1, the first day of cycle 1; 1 hour, one hour (after injection with OH2); iUPD, immune-unconfirmed progressive disease; NA, not available; PD, progressive disease; PR, partial response; SD, stable disease.

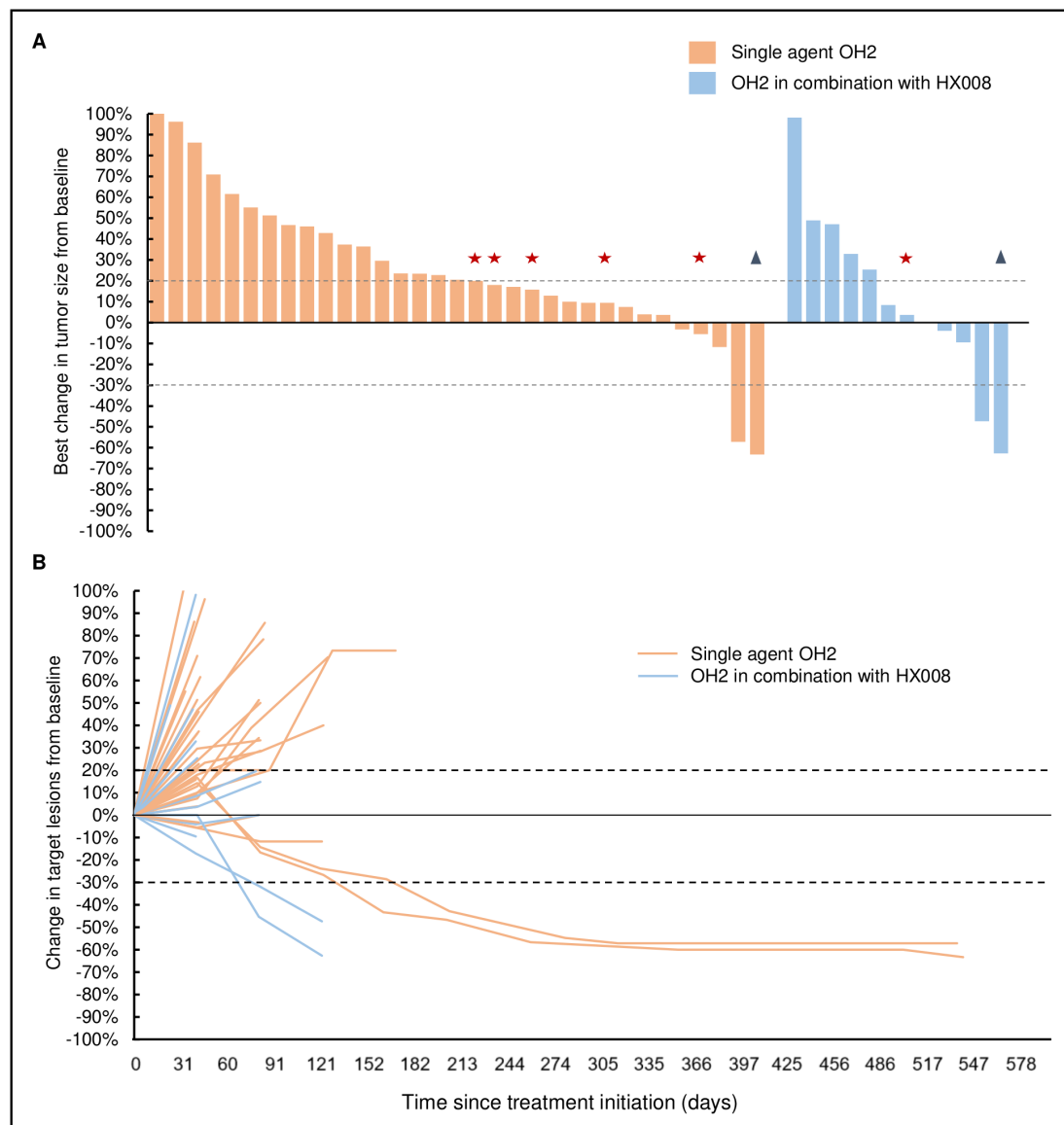


Figure 1 Response to the study treatment. (A) Waterfall plot showing best percentage change in target lesions from baseline. The stars indicate patients with new lesions despite stable target lesions; the triangles indicate patients having pseudo-progression before achieving immune-partial response. (B) Chronological change of target lesions from baseline.

Thirty-three patients receiving single agent OH2 and twelve patients treated with OH2 plus HX008 were assessable for response. According to RECIST version 1.1, one patient in the single agent cohort and one in the combination cohort achieved partial response (PR), nine patients in the single agent cohort and four in the combination cohort had stable disease (SD), while the other 30 patients had PD. Immune-PR (iPR) was observed in four patients by iRECIST, with two in the single agent cohort and two in the combination cohort. The clinical responses as per iRECIST are summarized in table 1. The changes in the size of target lesions from baseline are shown in figure 1.

In the single agent cohort, the patient achieving PR per RECIST had multiple liver metastases from rectal cancer and was treated with OH2 in one of the liver lesions. Notably, both injected and non-injected liver metastasis reduced in size. The other responder with metastatic

esophageal cancer had iPR after initial enlargement of the injected submandibular node and a suspected new lesion in the lung at the first radiological evaluation. The primary tumors of both patients were mismatch repair-proficient (pMMR) as confirmed by immunochemistry. The time to initial response for the two patients were 6.89 and 5.44 months, respectively. Both patients had discontinued treatment with OH2 due to difficulties of injection with increased intratumoral pressure, and absence of tumor cells in their latest post-treatment biopsies. The responses were ongoing, and the duration of response had reached 11.25+ and 14.03+ months. Moreover, the metastatic lymph node in the patient with esophageal cancer continued to regress despite treatment discontinuation. The representative tumor response images of the two responders in the single agent cohort are shown in figure 2A,B.

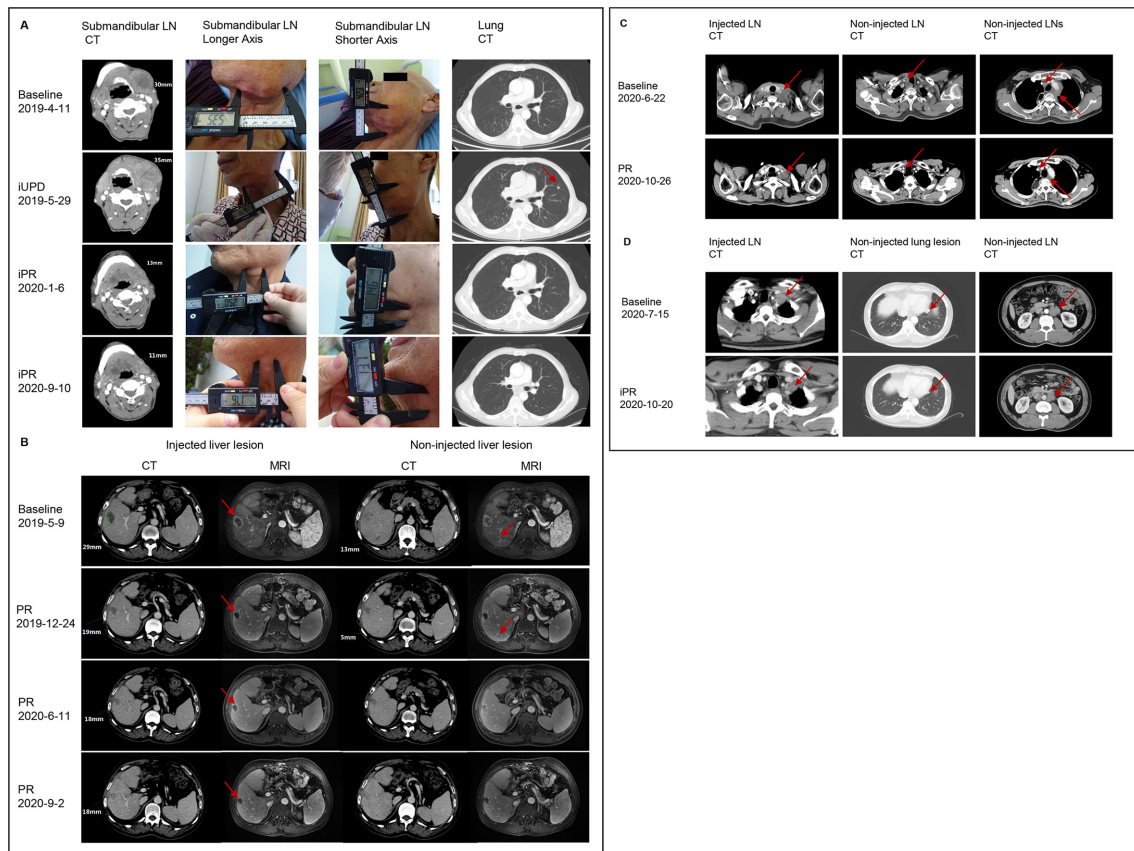


Figure 2 Representative tumor response data of patients who responded. (A) Patient with submandibular lymph node metastasis from esophageal cancer responded to single agent OH2. The red arrow indicates the suspected new lesion in the lung. The injected lymph node continued to regress after discontinuation of the study treatment on January 3, 2020. (B) Patient with liver metastasis from rectal cancer responded to single agent OH2. The red arrows indicate the lesions on MRI. (C) Patient with metastatic esophageal cancer responded to OH2 plus HX008. The red arrows indicate tumor lesions. (D) Patient with metastatic rectal cancer responded to OH2 plus HX008. The red arrows indicate tumor lesions. CT, computed tomography; iPR, immune-partial response; iUPD, immune-unconfirmed progressive disease; LN, lymph node; MRI, magnetic resonance imaging; PR: partial response.

In the combination cohort, one patient with metastatic esophageal cancer achieved PR, and one patient with pMMR metastatic rectal cancer had iUPD after initial immune-unconfirmed PD (iUPD). The time to response was 2.72 months for both patients, and the duration of response were 1.38+ and 2.56+ months, respectively. The representative tumor response images of these two patients are shown in [figure 2C,D](#).

CD8 and CD3 staining of baseline and on-treatment biopsies were performed in 14 patients, and PD-L1 expression was assessed in 17 patients. The results of PD-L1 CPS and TPSs as well as densities of CD8⁺ and CD3⁺ infiltrating cells for each patient are listed in [table 4](#).

We observed an increase in PD-L1 CPS after OH2 injection in 10 out of the 14 patients. Meanwhile, three patients showed an increase in PD-L1 TPS after treatment. We also noted that clinical responses were seen only in patients having a positive PD-L1 TPS with their baseline specimens. Further studies of the potential predictive value of baseline TPS for the treatment with OH2 is justified.

In the immune infiltrates, clear increases in the density of CD8⁺ and CD3⁺ cells after OH2 injection were observed

in 11 and 12 out of the 14 patients, respectively. Responses to OH2 observed in this trial seemed independent of baseline and subsequent changes in CD8⁺ and CD3⁺ cell density. The two patients who responded to OH2 both had increases in PD-L1 CPS, TPS, CD8⁺ and CD3⁺ cell density relative to baseline after treatment. The representative changes in the tumor microenvironment induced by OH2 are shown in online supplemental figure 1.

DISCUSSION

Although the ability of viruses to kill cancer cells has been investigated for over a century, the therapeutic benefit of oncolytic virotherapy has been demonstrated only recently in clinical trials.¹¹ To date, several genetically modified mutants of HSV-1 have been applied for the treatment of malignant brain tumors, melanoma and other solid tumors.^{12–18} Despite their favorable toxicity profiles, convincing evidence of efficacy was noted in a small number of cases in these early trials,^{15 16} and successful clinical development into phase III stage was achieved only with T-VEC.⁵ In the present phase I/II trial,

**Table 4** Results of biomarkers analysis

Patient	Time of biopsy	PD-L1 TPS	PD-L1 CPS	Density of CD8+ cells (cells/mm ²)	Density of CD3+ cells (cells/mm ²)
1*	Baseline	3	6	3.8	22.9
	Week 6	15	28	412.5	575.0
2	Baseline	0	2	116.7	266.7
	Week 6	0	3	106.7	208.3
3	Baseline	0	13	163.9	250.0
	Week 7	0	20	265.4	371.8
4*	Baseline	3	6	38.1	64.3
	Week 3	10	13	62.5	208.3
	Week 6	10	14	104.2	208.3
6	Baseline	0	0	6.7	16.3
	Week 6	0	0	12.5	20.8
7	Baseline	0	1	93.8	145.8
	Week 6	0	6	885.2	944.4
8	Baseline	0	1	83.3	168.8
	Week 6	0	17	366.7	761.9
9	Baseline	5	5	NA	NA
11	Baseline	0	0	25.8	36.9
	Week 12	0	1	81.7	115
12	Baseline	20	20	66.7	84.0
	Week 5	20	20	114.9	147.1
13	Baseline	5	5	62.1	131.8
	Week 3	40	46	570.4	870.4
16	Baseline	0	1	32.1	73.8
	Week 21	0	47	121.4	290.5
17	Baseline	0	0	98.5	50.0
	Week 11	0	15	61.4	233.3
18	Baseline	2	4	NA	NA
19	Baseline	5	6	NA	NA
21	Baseline	1	2	128.0	269.3
	Week 10	0	0	61.1	147.2
24	Baseline	0	0	51.4	105.6
	Week 7	0	0	78.3	200.0

*Patients responded to OH2.

CPS, combined positive score; NA, not available; PD-L1, programmed death-ligand 1; TPS, tumor proportion score.

the oncolytic HSV-2 virus OH2 was well-tolerated with no DLTs observed and thus the MTD was not identified in the prespecified three dose levels. More importantly, durable objective responses in patients with metastatic esophageal cancer and rectal cancer were noted, showing great promise for future clinical studies. To the best of our knowledge, this is the first report on the safety, biodistribution and efficacy of an HSV-2 based oncolytic virus.

The most frequent OH2-associated AE was transient fever, which was also reported with most other oncolytic viruses administered intratumorally, including the HSV-1 based T-VEC,¹³ adenovirus,¹⁹ poxvirus²⁰ and

reovirus,²¹ and might be a class effect. The fever observed in the present trial was associated with higher volumes of the injected OH2, and we suggest the use of preventive antipyretics in these patients. The biodistribution study revealed no shedding of OH2 in swabbing, urine and saliva collections from any patients. Moreover, the transient replication of OH2 detected in the blood in a limited number of patients was not associated with any AEs of clinical significance. Therefore, we may infer that OH2 was generally retained in the injected tumor, and the risk of spreading the virus to the environment or people in close contact were extremely low. The GM-CSF

mRNA detected in tumor samples was not associated with clinical response in our present study. The interpretation of the finding is difficult, since we were unable to distinguish whether the GM-CSF mRNA was derived from OH2 or secreted by immune cells with our current method, and the precise role of GM-CSF expressed by OH2 in altering the tumor microenvironment warrants further investigation.

The antitumor activity was encouraging for this first-in-human study. In the single agent OH2 cohorts, marked tumor regression was observed in patients who were heavily pretreated and had documented disease progression immediately before entry to the present trial. OH2 was effective in both injected and non-injected lesions. The bystander effect proved the mechanism of oncolytic virotherapy as OH2 eradicated distant malignancies through tumor-selective infection or immune responses. The time to response as well as duration of response in patients treated with single agent OH2 were considerably longer than those for cytotoxic drugs. Furthermore, the pattern of pseudo-progression observed in the present trial was consistent with that seen in other immunotherapy trials. The iRECIST criteria is hence helpful in clinical decision making for patients treated with oncolytic viruses. However, it remains a challenging task to define the optimal timing as to confirm true PD and cease the treatment, and still requires further investigation.

We did not observe objective responses in dose-expansion of single agent OH2. In addition to the limited sample size, the suspension of the trial during the peak of COVID-19 pandemic could also have affected the outcomes. The response rate was possibly underestimated, as some patients permanently discontinued the study treatment and might have missed the opportunity of further clinical benefit.

Biopsies taken following single agent OH2 injection showed increases in CD8⁺ and CD3⁺ cell density and PD-L1 expression levels in most patients relative to baseline regardless of clinical responses, suggesting the potential immune-modulating effect of OH2 to enhance the intratumoral infiltration of T cells. The attraction of cytotoxic T cells by injection of OH2 may partly contribute to clinical responses. Specifically, the ability of OH2 to inflame “cold” tumors is especially useful in the setting of combination therapy with immune checkpoint inhibitors. It has been proved that higher numbers of CD8, PD-1 and PD-L1 expressing cells at the invasive tumor margin and inside tumors are associated with responses to anti-PD-1 antibodies in patients with metastatic melanoma.²² Therefore, the addition of PD-1 blockade after priming therapy with an oncolytic virus is of potential synergistic effect. Indeed, the combination of T-VEC with either pembrolizumab or ipilimumab have yielded high response rates in patients with advanced melanoma.^{23 24} In our present study, although preliminary, we have observed a higher proportion of patients achieving an objective response in the OH2 and HX008 combination cohort, and the

time to response seemed shorter, suggesting a trend of improved efficacy. We will seek to confirm the advantage of the combination strategy in specific tumor types with larger sample sizes.

In conclusion, intratumoral injection of the HSV-2 based oncolytic virus OH2 was safe in patients with advanced solid tumors. Encouraging antitumor activities were observed in patients with metastatic rectal cancer and esophageal cancer. The changes in the tumor microenvironment induced by OH2 implied the underlying mechanisms of response, and supported further development of the drug as single agent or in combination with immune checkpoint inhibitors in the future.

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Competing interests BL is the founder of Binhui Biopharmaceutical Co., Ltd. XG is a salaried employee of Binhui Biopharmaceutical Co., Ltd.

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on reasonable request and approval from study sponsor according to available guidelines at time of request.

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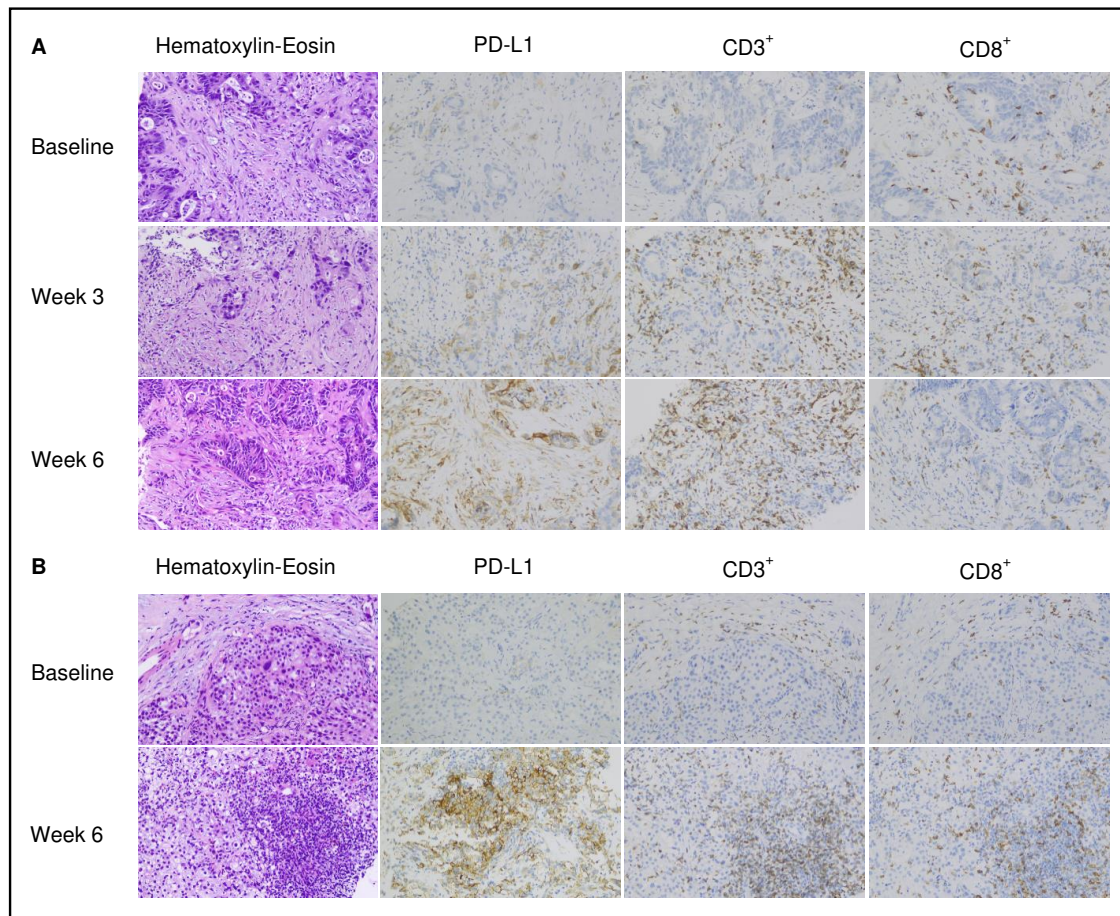
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1. Supplementary Figure



Supplementary Figure 1: OH2 injection increased PD-L1 expression, attracted CD3⁺ and CD8⁺ lymphocytes in the tumor microenvironment regardless of clinical response (magnification ×200).

(A) Patient with liver metastases from rectal cancer who responded to single agent OH2. (B) Patient with liver metastases from esophageal cancer who had progressive disease after treatment with single agent OH2.

2. Supplementary Table

Supplementary Table 1 Biodistribution and activity of single agent OH2 in phase 2

Patient	Dose level (CCID50/mL)	Tumor Types	Blood OH2 viral loads (copies/ μ L, timepoint)	Blood anti-GM-CSF antibody (timepoint)	Best response (RECIST v1.1/iRECIST)
12	10^7	Esophageal cancer	BQL	Negative	PD/iUPD
13	10^7	Esophageal cancer	BQL	Negative	PD/iUPD
14	10^7	Esophageal cancer	BQL	Negative	PD/iUPD
15	10^7	Colon cancer	BQL	Negative	NA
16	10^7	Colon cancer	BQL	Negative	SD/iSD
17	10^7	Rectal cancer	BQL	Negative	PD/iUPD
18	10^7	Colon cancer	BQL	Negative	PD/iUPD
19	10^7	Colon cancer	BQL	Negative	PD/iUPD
20	10^7	Colon cancer	BQL	Negative	PD/iUPD
21	10^7	Esophageal cancer	BQL	Negative	NA
22	10^7	Intrahepatic cholangiocarcinoma	BQL	Negative	PD/iUPD
23	10^7	Colon cancer	BQL	Negative	PD/iUPD
24	10^7	Colon cancer	BQL	Negative	PD/iUPD
25	10^7	Appendix cancer	NA	NA	SD/iSD
26	10^7	Gastric cancer	NA	NA	SD/iSD
27	10^7	Esophageal cancer	NA	NA	SD/iSD
28	10^7	Gastric cancer	NA	NA	NA
29	10^7	Gastric cancer	NA	NA	NA

(Continued on the following page)

Supplementary Table 1 Biodistribution and activity of single agent OH2 in phase 2 (cont'd)

Patient	Dose level (CCID50/mL)	Tumor Types	Blood OH2 viral loads (copies/ μ L, timepoint)	Blood anti-GM-CSF antibody (timepoint)	Best response (RECIST v1.1/iRECIST)
30	10^7	Esophageal cancer	BQL	Negative	NA
31	10^7	Esophageal cancer	BQL	Negative	PD/iUPD
32	10^7	Malignant melanoma	BQL	Negative	NA
33	10^7	Skin cancer	NA	Negative	PD/iUPD
34	10^7	Esophageal cancer	NA	Negative	PD/iUPD
35	10^7	Malignant melanoma	NA	NA	NA
36	10^7	Esophageal cancer	NA	NA	NA
37	10^7	Malignant melanoma	BQL	Negative	PD/iUPD
38	10^7	Head and neck cancer	NA	NA	PD/iUPD
39	10^7	Rectal cancer	NA	NA	NA
40	10^7	Gastric cancer	NA	NA	SD/iSD

Abbreviations: BQL, below quantifiable limit; PD, progressive disease; SD, stable disease; iUPD, immune-unconfirmed progressive disease; NA, not available