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OrienX010, an oncolytic virus, in patients with unresectable stage IIIC–IV melanoma: a phase Ib study

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ABSTRACT

Background Melanoma in people of Asian descent presents primarily in non-sun-exposed areas, such as acral and mucosal melanoma. Compared with the predominant sun-exposed area melanomas in Caucasians, acral and mucosal melanomas do not respond as well to immunotherapy and are associated with a worse prognosis. Hence, there is an urgent need for improved treatment for melanoma in Asians. This phase Ib trial evaluated the safety and efficacy of the modified herpes simplex virus-1 oncolytic virus OrienX010 in Chinese patients with unresectable stage IIIC–IV melanoma.

Methods Patients were treated in two different cohorts. In cohort 08 (n=12), patients received up to 5 mL of 8×10^7 pfu/mL OrienX010 intratumoral injections every 2 weeks until disease progression and responses were evaluated every 6 weeks. In cohort 09 (n=14), patients received up to 10 mL of 8×10^7 pfu/mL OrienX010 intratumoral injections and responses were evaluated every 8 weeks.

Results Between June 2014 and May 2017, 26 patients were enrolled, including 18 (69.2%) patients with acral melanoma. Fever and injection site reaction were the most frequent adverse events. Only one patient experienced a grade ≥3 adverse event and no dose-limiting toxicities were observed. The objective response rate was 19.2% and the disease control rate was 53.8%. The median duration of response was 6.0 months. Antitumor effects were observed in 54.6% of injected lesions and 48.8% of non-injected lesions, including one (16.7%) of six evaluable distant lung metastases. The median progression-free survival was 2.9 months and overall survival was 19.2 months. Compared with patients treated in cohort 08, patients treated in cohort 09 had an improved objective response rate (28.6% vs 8.3%) and a median progression-free survival of 3.0 months vs 2.8 months.

Conclusions OrienX010 oncolytic virotherapy has a tolerable safety profile with antitumor effects in both injected and non-injected metastases and warrants further evaluation in patients with melanoma. Based on these results, the higher cohort 09 dose (up to 10 mL of 8×10^7 pfu/mL every 2 weeks) was selected as the recommended phase II dose for ongoing trials.

Trial registration number CTR20140631 (cohort 08), CTR20150881 (cohort 09).

Key messages

What is already known on this topic

- Acral and mucosal melanoma, the primary types of melanoma in Asians, are associated with worse outcomes than sun-exposed melanoma in Caucasians.
- Oncolytic viral therapy has been associated with improved outcomes in sun-exposed melanomas, whereas little is known about these treatments in Asians with non-sun-exposed melanomas.

What this study adds

- This study demonstrated the safety, tolerability, efficacy, and recommended phase II dose of OrienX010 (a herpes simplex virus-1 oncolytic virus) in Chinese patients with unresectable melanoma.
- At the recommended phase II dose, the objective response rate was 28.6%, the disease control rate was 57.1%, the median progression-free survival was 3.0 months, and the median overall survival was 17.4 months.
- These data will further the knowledge base for investigators and clinicians on cancer immunotherapy and oncolytic viruses.

INTRODUCTION

Melanoma is an aggressive disease with an increasing global burden.[1–3] This is very evident in China, where the incidence rate of melanoma was relatively low (0.4 per 100,000 in 1990); however, there has been a sharp rise (110%) over the past three decades (0.9 per 100,000) to 16,073 new diagnoses in 2017.[3] The prevalence also markedly increased (489%) during this span to 109,316 people affected in 2017. These epidemiology trends in China far surpass the global patterns.

In addition, melanoma in people of Asian descent presents and behaves differently from melanoma in Caucasians.[4 5] Primary melanoma of the sun-exposed skin accounts for about 90% of cases in Caucasians (affecting the back, chest, abdomen,

and lower limbs), yet less than 50% of cases in Asians.[4] In Asians (and other people of color), 58% of all melanomas are acral (palms, soles) or mucosal (rectum, anus, vulva, eye, nasopharynx).[5 6] Because acral and mucosal melanomas occur in non-sun-exposed, 'hidden', and less common locations, Asian patients often remain undiagnosed until the disease is at an advanced stage.[5] In fact, few Caucasians are diagnosed with advanced melanoma (13%),[7] yet nearly half (45%) of Chinese patients with melanoma have regionally advanced or metastatic disease at initial diagnosis.[8 9] Worsening the situation is that acral and mucosal melanomas are generally regarded as more aggressive.[5 10]

Whereas cutaneous melanomas are characterized by novel mutational patterns attributable to ultraviolet radiation, acral and mucosal melanomas (in addition to being in non-sun-exposed areas) are more frequently characterized by DNA structural changes and mutation signatures of unknown etiology.[5 11] Significantly mutated genes include *BRAF*, *NRAS*, and *NF1* in acral melanoma and *SF3B1* in mucosal melanoma compared with *BRAF*, *CDKN2A* (cyclin-dependent kinase inhibitor 2A), *NRAS*, and *TP53* (tumor protein 53) in cutaneous melanoma. Notably, while *BRAF* mutations occur in 45%–50% of melanomas in Caucasians, such mutations are reported to occur in only 25.5% of Chinese patients overall, with the incidence even lower in acral (17.9%) and mucosal (12.5%) melanoma.[12–14]

Preferred treatment options for unresectable melanoma include programmed cell death protein 1-based immunotherapy and *BRAF*-targeted therapy; chemotherapy/cytotoxic agents, other targeted agents, and intralesional immunotherapy are useful in certain circumstances.[4 15 16] However, acral and mucosal melanomas, as well as melanoma in Asian patients, are associated with poorer outcomes, including suboptimal responses to immunotherapy.

Specifically, while the objective response rates (ORRs) were 21%–50% in registrational trials of immune checkpoint inhibitors (ICI) in patients with primarily sun-exposed cutaneous melanoma (≥98% white),[17 18] a retrospective analysis noted that for mucosal melanoma the ORR was only 19%.[19] Another retrospective analysis of ICI in Chinese patients noted ORRs of 20%–25% overall: 26.7% for acral and 20% for mucosal.[10] A prospective study found similarly poor outcomes in Chinese patients with melanoma; the ORR was 16.7% (15.8% for acral, 13.3% for mucosal).[5] The median progression-free survival (PFS) was 2.8 months and the 6-month PFS rate was 20.4%; the median overall survival (OS) was 12.1 months.

Oncolytic virotherapy is a promising immunotherapy that uses genetically modified viruses which can selectively replicate in tumor cells and mediate tumor regression.[20–22] Lysis of cancer cells by oncolytic virotherapy has also been shown to stimulate systemic tumor-specific immune responses, leading to suppression of distant melanoma metastases.[23] Oncolytic viruses can be constructed using herpes simplex virus (HSV)—a highly lytic virus in which deletion of the gene encoding infected cell protein (ICP) 34.5 provides tumor selectivity.[21 24] In 2015, talimogene laherparepvec (T-VEC), which is constructed from a modified HSV, became the first approved oncolytic virus to treat melanoma[25–27] based on its durable response rate (DRR) and good tolerability in the phase III OPTiM clinical trial.[28 29] Oncolytic virotherapy continues to show clinical benefit in real-world analyses.[30] However, because of the notable differences in epidemiology, tumor subtype, anatomical location, genetics, and outcomes between Caucasian and Asian patients with melanoma—all of which indicate a diverse immune microenvironment—there might also be different clinical outcomes of oncolytic virotherapy. Therefore, evidence about oncolytic virotherapy in the Asian population is still needed.

OrienX010, an HSV type 1-derived oncolytic virus modified to express the gene encoding human granulocyte-macrophage colony-stimulating factor (GM-CSF), was designed to be directly injected into melanoma tumor lesions (not published). Previously, initial data from an open-label, dose-increasing, phase I, first-in-human clinical trial (NCT01935453, cohort 07) of OrienX010 intratumoral (IT) injection for adult malignant tumors were reported.[31] Patients were randomized to four dose groups: 10^6 pfu, 10^7 pfu, 10^8 pfu, and 4×10^8 pfu. No dose-limiting toxicities (DLTs) were observed in all cohorts. Based on the safety profile and good tolerance, a phase Ib clinical trial in patients with unresectable stage IIIC–IV melanoma was conducted to evaluate the safety and efficacy of OrienX010 in Chinese patients. Here final data are reported.

METHODS

Study design

This was a single-center, open-label, single-arm, phase Ib clinical trial performed at the Department of Renal Cancer and Melanoma, Peking University Cancer Hospital and Institute, Beijing, China. The study was conducted as two similar, but separate, protocols (08 and 09), which were approved by the hospital's ethics committee. The primary endpoint was the overall safety of repeated IT injection of OrienX010 into dermal, subcutaneous, and lymph node melanoma metastases in patients with advanced melanoma. Secondary endpoints included response rates and survival.

Patients

Chinese patients with histologically confirmed, unresectable, American Joint Committee on Cancer (AJCC) Seventh Edition stage IIIB, IIIC, or IV melanoma that had progressed on standard therapy were enrolled. Patients had at least one measurable lesion for IT injection (long diameter ≥10 mm) as well as adequate hepatic, renal, and hematologic functions, and a life expectancy of at least 3 months (cohort 08) or at least 6 months (cohort 09). Patients were required to be HSV seropositive. The

inclusion and exclusion criteria are listed in online supplemental table 1. All patients provided written informed consent.

Study drug

OrienX010 is an oncolytic virus originally isolated from the oral cavity of a Chinese patient with wild-type HSV-1 (CL1 strain) and designed to be directly injected into melanoma tumor lesions.[32] Specifically, OrienX010 was modified at three HSV-1 viral ICP genes (ICP34.5, ICP47, ICP6) and was also designed to express the gene encoding human GM-CSF. The neurovirulence protein ICP34.5 normally blocks a cell-mediated antiviral response and allows for a productive infection in neurons and other healthy cells.[24 33 34] Deletion of ICP34.5 allows for enhanced viral replication of OrienX010 in cancer cells, but not healthy cells.[24 33] The ICP47 gene normally reduces immune destruction of HSV-1-infected cells.[33 35] Deletion of ICP47 allows for enhanced antigen presentation and T cell priming, leading to tumor cells being more easily recognized by the immune system and thus enhancing the efficiency of OrienX010.[36] An inactivated ICP6 gene was inserted into the OrienX010 genome. Inactivated ICP6 has been shown to decrease the growth of oncolytic HSV-1 viruses in neurons, thus reducing the potential for neurotoxicity of injection.[24] In addition, the GM-CSF gene was inserted into the original location of ICP34.5. GM-CSF promotes dendritic cell accumulation at sites of inflammation and enhances antigen-presenting cell function.[33 37]

Study drug administration

Patients received repeated IT injections of OrienX010 (HSV-1 (CL1)/ICP34.5/ICP47/ICP6/human GM-CSF) into dermal, subcutaneous, and lymph node melanoma metastases based on the size of the injectable metastatic lesions (online supplemental table 2). If the tumor size changed during the course of treatment, the dose was adjusted accordingly.

Patients enrolled into cohort 08 received IT injections of OrienX010 up to 5 mL of 8×10^7 pfu/mL every 2 weeks. Disease status was assessed at baseline and then every 6 weeks. Cycles were defined as every 6 weeks.

Based on preliminary safety, tolerability, and efficacy of OrienX010 in cohort 08, patients were enrolled into cohort 09 to evaluate tolerability and benefits of a higher dose. Patients received IT injections of OrienX010 up to 10 mL of 8×10^7 pfu/mL every 2 weeks. The response evaluation period was also extended to every 8 weeks. Cycles were defined as every 8 weeks.

In both cohorts, treatment continued until progression of disease, intolerant toxicity, or withdrawal of informed consent (online supplemental figure 1).

Assessment of adverse events and efficacy

The primary endpoint of this study was overall safety, including treatment-related adverse events (AEs) according to Common Terminology Criteria for Adverse Events (version 4.0). Secondary endpoints included ORR, disease control rate (DCR), PFS, OS, and DRR at 24 weeks. ORR was defined as the rate of complete response (CR) plus partial response (PR). DCR was defined as the sum of CR, PR, and stable disease. PFS was the interval between the first OrienX010 intralesional treatment and progression of disease, or occurrence of death due to all causes. DRR was defined as the proportion of CR or PR lasting more than 24 weeks continuously.

Efficacy evaluation was based on Response Evaluation Criteria in Solid Tumors (RECIST V.1.1) and immune-related (ir) RECIST. CT scan or MRI was performed every 6 (cohort 08) or 8 (cohort 09) weeks. Visible or palpable lesions that could not be evaluated by CT or MRI were measured clinically using a caliper.

Tumor biopsies and specimen analysis

Core needle biopsies of target lesions were obtained at baseline, at 12 weeks (cohort 08), or at 16 weeks (cohort 09) and when evaluated as immune-related progressive disease (irPD). Specimens were stained using H&E. At baseline, pretreatment specimens were analyzed to pathologically confirm a diagnosis of metastatic melanoma. During treatment and irPD, specimens were analyzed to evaluate the pathologic features of response and progression (including tumor cell morphology and changes to the tumor microenvironment). Specimen collection timepoints are noted in online supplemental table 3.

Biodistribution

Blood, urine, and injection site swabs were collected throughout the study (eg, every 2 weeks as correlated with dosing). OrienX010 nucleic acid (eg, modified HSV-1) was determined for each sample type via fluorescence quantitative PCR using commercial HSV-1 primers (Sun Yat-sen University Daan Gene; https://en.daangene.com/product/product_32.html). Note that these primers cannot distinguish between DNA of wild-type HSV-1 and OrienX010. The concentrations of GM-CSF and HSV-1 antibodies in serum were detected via ELISA (ExCell Bio, EH012; http://www.excellbio.com/product-center/info.aspx?itemid=314&Lcid=262).

Bioinformatic analysis of RNA expression and pathways

Pretreatment and on-treatment tumor biopsies were available from 11 patients (6 patients with stable disease and 5 with progressive disease). Total RNA was extracted from the formalin-fixed, paraffin-embedded tumor biopsies using RNeasy Mini Kit (QIAGEN). NanoString analysis was performed using the nCounter PanCancer Immune Profiling Panel codeset. The results of the paired samples were analyzed using NSolver V.3.0. Pathway and process enrichment analyses were carried out for highly expressed genes by Metascape (https://metascape.org). Terms with $p<0.01$, a minimum count of 3, and an enrichment factor >1.5 were collected and grouped into clusters based on their membership similarities. In addition, Gene Set Enrichment Analysis (GSEA; V.4.1.0) was performed to

elucidate the underlying signaling pathways using Kyoto Encyclopedia of Genes and Genomes sets.

Statistical analysis

SPSS V.22.0 software and R software were used for statistical analysis. Measurement data were described with mean and SD. Median, maximum, and minimum were used to describe partially distributed variables. Count data were described by frequency. A t-test or Kruskal-Wallis test was used for measurement data, and count data were evaluated using an unadjusted Fisher's exact test. PFS and OS were analyzed using the Kaplan-Meier method and Cox regression analysis. Statistical significance was evaluated by log-rank test. A two-sided significance level of 0.05 was used.

RESULTS

Patient baseline characteristics

Between June 2014 and May 2017, a total of 26 patients with melanoma were enrolled into cohort 08 (n=12) and cohort 09 (n=14). The clinical stages, per AJCC Seventh Edition, included stage IIIC (34.6%), IVM1a (42.3%), IVM1b (19.2%), and IVM1c (3.8%). *BRAF*-mutant melanoma, all V600E, was seen in six (23.8%) patients. The mean size of all measurable tumor lesions was 84.6 mm (range: 18.0–220.0 mm). Patients in cohort 09 had greater tumor burden than cohort 08 (mean size: 100.0 mm vs 66.7 mm), with a higher average injection dose. Other baseline characteristics of the patients in the two cohorts are shown in table 1.

Safety results

Safety data were collected from enrollment until 30 days following the end of treatment. Two patients from cohort 09 had their OrienX010 dose adjusted/lowered during treatment based on tumor lesion shrinkage. Two patients from cohort 08 and four patients from cohort 09 did not complete the required OrienX010 injections per protocol due to early disease progression at the end of the first cycle (6 weeks for cohort 08 and 8 weeks for cohort 09).

Treatment-related AEs were mostly grade 1 or 2 (table 2). Only one patient in cohort 09 developed grade ≥3 AEs (grade 4 leukopenia and grade 4 neutropenia). There were no grade ≥3 AEs observed in cohort 08. No treatment-related deaths or DLTs were observed. No AEs led to the discontinuation of treatment.

Treatment-related AEs observed in more than two patients were fever (73.1%), injection site reaction (61.5%), proteinuria (23.1%), neutropenia (23.1%), leukopenia (19.2%), nausea (15.4%), peripheral edema (15.4%), vomiting (15.4%), fatigue (11.5%), vitiligo (11.5%), chills (11.5%), pain in extremity (11.5%), and rash (7.7%). There were no significant differences in the incidence of treatment-related AEs between cohorts 08 and 09, except for proteinuria, which was not seen in cohort 09 (table 2).

Biodistribution results

In 12 patients of cohort 08, none had evidence of OrienX010 viral activity in the 24-hour, postinjection site swab samples. Five (42%) patients had evidence of OrienX010 virus nucleic acid in the blood within 24 hours after injection. After 24 hours, no nucleic acid of the virus could be detected. Only two (16.7%) patients were found to have OrienX010 virus nucleic acid in their urine before the fifth injection (week 9); however, this was at low levels (47 copies and 132 copies as estimated via regression analysis, with an actual detection limit of 1000 copies).

In 14 patients of cohort 09, none had evidence of OrienX010 viral activity in the 24-hour, postinjection site swab samples. Eight (57%) patients had evidence of OrienX010 virus nucleic acid in the blood within 24 hours after injection. Except for one patient, nucleic acid was no longer detected in the blood 48 hours after injection. None of the patients had evidence of OrienX010 nucleic acid in the urine before the fifth injection (week 9).

The concentration of GM-CSF in the blood was evaluated at multiple timepoints prior to injection of OrienX010. Of the 26 patients in cohort 08 and cohort 09, 2 (7.7%) patients in cohort 08 were found to have a substantial increase in GM-CSF of 10.0-fold and 12.6-fold compared with pretreatment levels on cycle 1 day 1 (online supplemental figure 2).

Efficacy results

The median follow-up was 33.2 months (range: 2.5–63.8 months). Response data are listed in table 3. In cohort 09, the ORR was 28.6%, which was numerically much higher than the ORR (8.3%) in cohort 08 (a statistical comparison between cohorts was not planned). The median treatment time was greater in cohort 09 compared with cohort 08: 6.05 months vs 2.45 months.

Responses were seen in both injected and non-injected lesions (figure 1). Among the 97 measurable lesions directly injected with OrienX010, 53 (54.6%) regressed. Regression was seen in 15 (41.7%) of 36 lesions in cohort 08 and in 38 (62.3%) of 61 lesions in cohort 09. Twenty-five (25.8%) of the injected lesions regressed ≥30%; most of which were from cohort 09, with 20 (32.8%) of 61 metastases regressing ≥30%. For the non-injected lesions, responses were seen in both regional (defined as lesions sharing the same lymphatic drainage with the injected lesions) and distant (21 (48.8%) overall) lesions. Among the 37 regional lesions, 20 (54.1%) regressed: 3 in cohort 08 and 17 in cohort 09. Eight (21.6%) of these regional lesions regressed ≥30%, seven of which were from cohort 09. Of the six measurable distant lesions, one lung metastasis in cohort 08 showed a reduction of 58% from baseline (figure 1).

Although some patients experienced disease progression per RECIST V.1.1—either as growth of existing metastases or new metastases—continued treatment was allowed if the investigator deemed that the progression was not clinically substantial. Responses were also

Table 1 Baseline characteristics

Characteristics	Cohort 08 (n=12)	Cohort 09 (n=14)	Total (N=26)
Age, years, mean (range)	55 (42–77)	61 (26–83)	59 (26–83)
Sex, n (%)			
Male	5 (41.7)	8 (57.1)	13 (50.0)
Female	7 (58.3)	6 (42.9)	13 (50.0)
ECOG performance status, n (%)			
0	7 (58.3)	4 (28.6)	11 (42.3)
1	5 (41.7)	10 (71.4)	15 (57.7)
Disease stage*, n (%)			
IIIC	5 (41.7)	4 (28.6)	9 (34.6)
IVM1a	4 (33.3)	7 (50.0)	11 (42.3)
IVM1b	2 (16.7)	3 (21.4)	5 (19.2)
IVM1c	1 (8.3)	0 (0.0)	1 (3.8)
LDH, n (%)			
≤ULN	10 (83.4)	12 (85.7)	22 (84.6)
>ULN	2 (16.7)	2 (14.3)	4 (15.4)
Treatment line, n (%)			
First	7 (58.3)	3 (21.4)	10 (38.5)
Second or later†	5 (41.7)	11 (78.6)	16 (61.5)
DTIC-based chemotherapy	4 (33.3)	7 (50)	11 (42.3)
Platinum-based chemotherapy	1 (8.3)	1 (7.1)	2 (7.7)
Immunotherapy	0	1 (7.1)	1 (3.8)
Targeted therapy	0	2 (14.3)	2 (7.7)
PD-1-based	0	0	0
Melanoma subtype, n (%)			
Cutaneous (sun-exposed)	4 (33.3)	2 (14.3)	6 (23.1)
Acral	7 (58.3)	11 (78.6)	18 (69.2)
Mucosal	0 (0.0)	0 (0.0)	0 (0.0)
Unknown	1 (8.3)	1 (7.1)	2 (7.7)
Ulceration of primary melanoma, n (%)			
Present	7 (58.3)	9 (64.3)	16 (61.5)
Absent	0 (0.0)	2 (14.3)	2 (7.7)
Unknown	5 (41.7)	3 (21.4)	8 (30.8)
BRAF V600E status, n (%)			
Mutation	3 (25.0)	3 (21.4)	6 (23.1)
Wild-type	9 (75.0)	11 (78.6)	20 (76.9)
Total tumor burden (mm±SD)	66.7±28.3	100±56.2	84.6±47.8

M1a: metastases to the skin, soft tissue (including muscle), and/or non-regional lymph nodes; M1b, metastasis to the lung with or without metastasis of M1a; M1c, visceral metastases other than the central nervous system with or without metastasis of M1a or M1b.

*Disease stage based on TNM criteria according to the AJCC Seventh Edition.
†Patients who received multiple lines of prior treatment were counted only once by their first prior therapy received.
AJCC, American Joint Committee on Cancer; DTIC, dacarbazine; ECOG, Eastern Cooperative Oncology Group; LDH, lactate dehydrogenase; PD1, programmed cell death protein 1; TNM, tumor, node, metastasis; ULN, upper limit of normal.

evaluated based on irRECIST criteria to determine the potential benefit of OrienX010 beyond progression based on RECIST V.1.1. Two patients in cohort 09 who initially had disease progression based on RECIST V.1.1 were subsequently found to gain clinical benefit from the OrienX010 treatment. The median time on treatment was 4.0 months (range: 0.5–15.0 months, 95% CI 2.4 to 10.8 months). Patients in cohort 09 had a longer median

Table 2 Treatment-related AEs occurring in >5% patients or grade ≥3

	Cohort 08 (n=12)			Cohort 09 (n=14)			Total (N=26)			
Treatment-related AE, n (%)	**Grades 1–2**	**Grades 3–4**	**All grades**	**Grades 1–2**	**Grades 3–4**	**All grades**	**Grade 1**	**Grade 2**	**Grades 3–4**	**All grades**
All AEs	11 (91.7)	–	11 (91.7)	13 (92.9)	1 (7.1)	14 (100.0)	13 (16.0)	11 (42.3)	1 (3.8)	25 (96.2)
Fever	8 (66.7)	–	8 (66.7)	11 (78.6)	–	11 (78.6)	11 (42.3)	8 (30.8)	–	19 (73.1)
Injection site reaction	7 (58.3)	–	7 (58.3)	9 (62.3)	–	9 (62.3)	15 (57.7)	1 (3.8)	–	16 (61.5)
Proteinuria	6 (50.0)	–	6 (50.0)	–	–	–	6 (23.1)	–	–	6 (23.1)
Neutropenia	3 (25.0)	–	3 (25.0)	2 (14.3)	1 (7.1)	3 (21.4)	2 (7.7)	3 (11.5)	1 (3.8)	6 (23.1)
Leukopenia	3 (25.0)	–	3 (25.0)	1 (7.1)	1 (7.1)	2 (14.3)	3 (11.5)	1 (3.8)	1 (3.8)	5 (19.2)
Nausea	3 (25.0)	–	3 (25.0)	1 (7.1)	–	1 (7.1)	4 (15.4)	–	–	4 (15.4)
Peripheral edema	2 (16.7)	–	2 (16.7)	2 (14.3)	–	2 (14.3)	4 (15.4)	–	–	4 (15.4)
Vomiting	3 (25.0)	–	3 (25.0)	1 (7.1)	–	1 (7.1)	3 (11.5)	1 (3.8)	–	4 (15.4)
Fatigue	1 (8.3)	–	1 (8.3)	2 (14.3)	–	2 (14.3)	3 (11.5)	–	–	3 (11.5)
Vitiligo	1 (8.3)	–	1 (8.3)	2 (14.3)	–	2 (14.3)	3 (11.5)	–	–	3 (11.5)
Chills	2 (16.7)	–	2 (16.7)	1 (7.1)	–	1 (7.1)	3 (11.5)	–	–	3 (11.5)
Pain in extremity	3 (25.0)	–	3 (25.0)	–	–	–	3 (11.5)	–	–	3 (11.5)
Rash	–	–	–	2 (14.3)	–	2 (14.3)	2 (7.7)	–	–	2 (7.7)

AE, adverse event.

Table 3 Response rates and survival times

	n	Treatment duration, months, median (range)	Response*, n (%)				mDOR (months)	Survival, months, median (95% CI)		
			ORR	DCR	SD	PR		PFS	irPFS	OS
All patients	26	4.8 (0.9–15.0)	5 (19.2)	14 (53.8)	9 (34.6)	5 (19.2)	6.0	2.9 (1.8 to 5.7)	2.9 (1.8 to 5.9)	19.2 (10.0 to 27.9)
Cohort 08	12	2.45 (0.9–12.6)	1 (8.3)	6 (50.0)	5 (41.7)	1 (8.3)	3.1	2.8 (1.4 to 5.7)	2.8 (1.4 to 5.9)	19.2 (3.8 to 27.9)
Cohort 09	14	6.05 (1.4–15.0)	4 (28.6)	8 (57.1)	4 (28.6)	4 (28.6)	7.8	3.0 (1.8 to NA)	3.0 (1.8 to NA)	17.4 (9.7 to 40.2)
Patients with ALM	18	–	–	–	–	–	–	3.0 (1.8 to 5.7)	3.0 (1.8 to 5.9)	19.2 (9.7 to 30.0)

*According to RECIST V.1.1.
ALM, acral lentiginous melanoma; DCR, disease control rate; irPFS, immune-related progression-free survival; mDOR, median duration of response; NA, not available; ORR, objective response rate; OS, overall survival; PFS, progression-free survival; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease.

time on treatment compared with patients in cohort 08 (6.0 months vs 2.4 months).

A case example of tumor regression is shown in figure 2, which depicts a 71-year-old female patient from cohort 09 (patient 06) with stage IIIC in-transit metastatic melanoma on her right leg. She had previously received treatment with isolated limb infusion. The photographs demonstrate improvement from week 1 to week 40. In addition, the increased presence of vitiligo indicates immune activation—a desired effect.

Survival outcomes are displayed in table 3 and figure 3. The median PFS for all 26 patients was 2.9 months (95% CI 1.8 to 5.7 months; figure 3A, left). For 18 patients with acral melanoma, the median PFS was 3.0 months (95% CI 1.8 to 5.7 months; figure 3B, left). Although not a statistically significant difference, the median PFS of cohort 08 was 2.8 months, while the median PFS of cohort 09 was 3.0 months (figure 3C, left).

The median immune-related PFS (irPFS; calculated by the Kaplan-Meier method) of all 26 patients was 2.9 months (95% CI 1.8 to 5.9 months; figure 3A, middle). For 18 patients with acral melanoma, the median irPFS was 3.0 months (95% CI 1.8 to 5.9 months; figure 3B, middle). Although not a statistically significant difference, the median irPFS of cohort 08 was 2.8 months, while the irPFS of cohort 09 was 3.0 months (figure 3C, middle).

No factors were found to have a significant influence on PFS either in the univariate or multivariate analysis (online supplemental figure 3). Results from the multivariate analysis showed that cohort 08 was associated with a less favorable PFS, but this did not reach significance.

At the primary analysis of OS, 24 deaths had occurred (11 in cohort 08 and 13 in cohort 09). The median OS for all patients was 19.2 months (95% CI 10.0 to 27.9 months; figure 3A, right). The median OS for 18 patients with acral melanoma was 19.2 months (95% CI 9.7 to 30.0 months; figure 3B, right). The median OS in cohort 08 was 19.2 months (95% CI 3.8 to 27.9 months), while the median OS in cohort 09 was 17.4 months (95% CI 9.7 to 40.2 months); the difference between cohorts was not significant (figure 3C, right). Notably, cohort 09 had a wide 95% CI due to one patient still alive (the long survival data were censored). Two patients from cohort 08 withdrew early and were lost to follow-up (their survival data were also censored and were quite short). Elevated baseline lactate dehydrogenase (LDH) level was an independent factor associated with OS in the univariate analysis, and in the multivariate analysis the LDH level was also demonstrated to be a significant independent factor associated with OS. The acral subtype was not associated with OS both in univariate and multivariate analyses (online supplemental figure 3).

NanoString analysis

Please see online supplemental file 1, including online supplemental figures 4–7, for a description of the genes and pathways associated with outcomes to OrienX010.

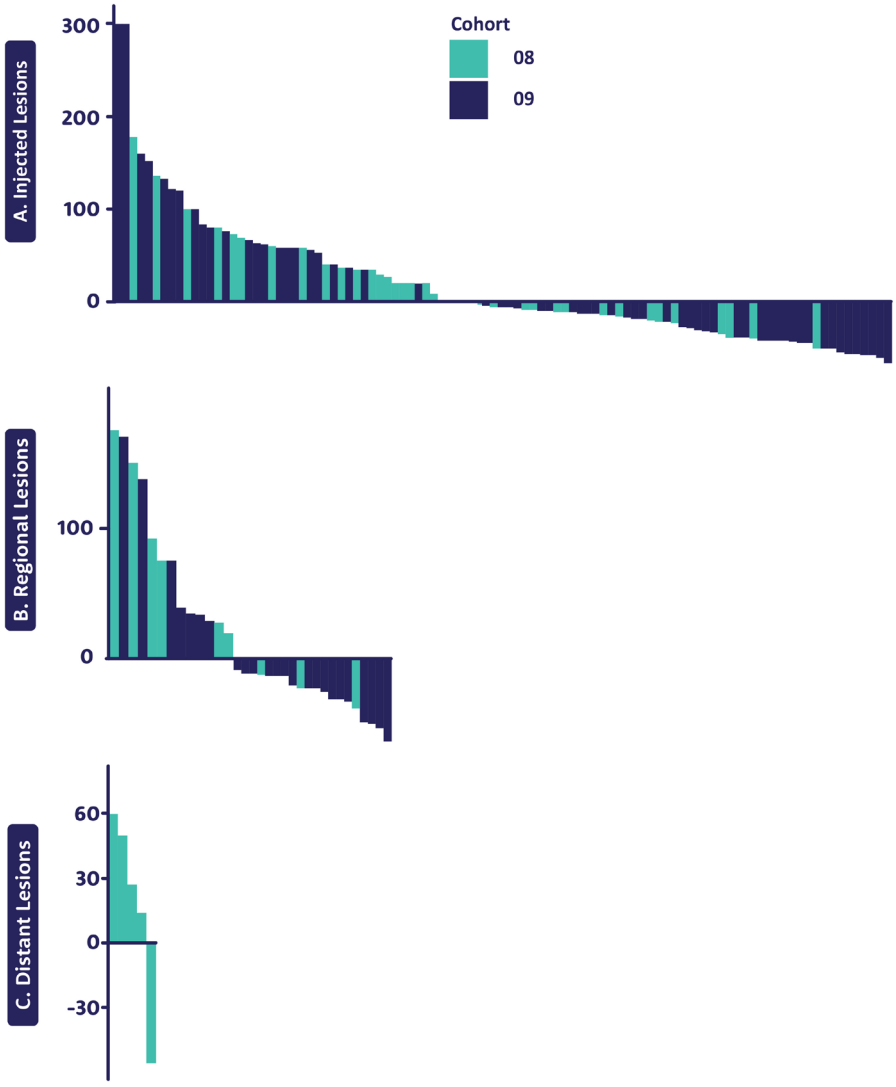

Figure 1 Maximal change in tumor burden from baseline: (A) injected lesions; (B) regional lesions, defined as lesions sharing the same lymphatic drainage with the injected lesions; and (C) distant lesions.

DISCUSSION

This phase Ib study evaluated IT injection of the genetically modified HSV type 1 oncolytic virus OrienX010 in patients with unresectable stage IIIC–IV melanoma. The trial demonstrated an acceptable safety profile and good tolerability for OrienX010, with evidence of response in both injected and non-injected lesions.

Repeated IT injections of OrienX010 were well tolerated overall. AEs occurring in more than 25% of patients included mild to moderate fever (73.1%) and injection site reaction (61.5%). The AEs seen with OrienX010 are indicative of immune activation by oncolytic viruses and consistent with AEs seen with other oncolytic viruses such as T-VEC.[28 30] Only one patient in the higher-dose cohort 09 experienced grade 3–4 AEs of myelosuppression. The low incidence of grade 3 and 4 AEs is also consistent with the safety profile seen with T-VEC.

Melanoma is one of the most genetically mutated cancers, and individual melanoma metastases also exhibit a high level of intratumor heterogeneity.[38 39] The intratumor heterogeneity has been associated with mixed tumor responses often seen in metastatic melanoma treated with immunotherapy. Highly heterogeneous melanoma metastases have been associated with reduced immune cell infiltration and immune response activation, as well as decreased survival.[40] Patients with injectable dermal, subcutaneous, or lymph node injectable melanoma metastases often present with multiple metastases. Injecting as many of these metastases as possible allows for a more robust activation of the immune system against multiple heterogeneous tumor antigens. In the T-VEC OPTiM phase III trial, the injected volume of T-VEC into metastatic melanoma lesions was determined by the size of the metastasis, and the total maximum dose of T-VEC was 4 mL of 1×10^8 pfu/mL (total dose of 4×10^8 pfu). In the evaluation of OrienX010 in Chinese patients, those in cohort 08 were injected up to 5 mL of 8×10^7 pfu/mL (total dose of 4×10^8 pfu). However, many patients had injectable

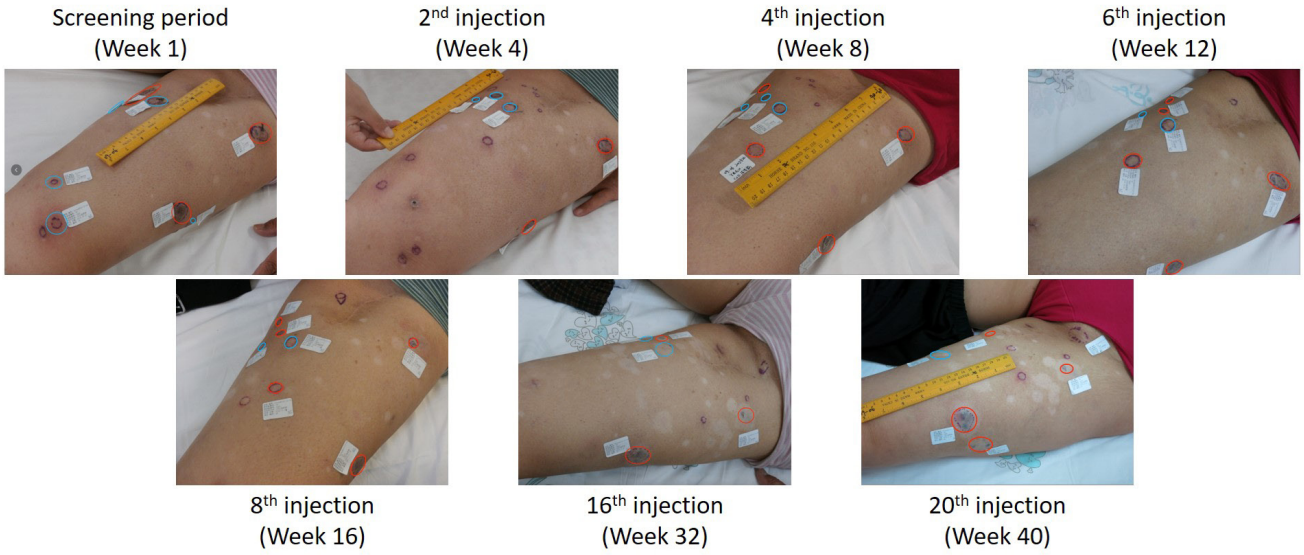

Figure 2 A patient's response to OrienX010. Lesions are shown at screening (week 1) and at every two injections (every 4 weeks) thereafter. The red circles indicate injection lesions, while the blue circles indicate non-injection lesions.

metastases that could not be injected due to the limitation in available injectable volume. To allow for injection of more metastatic lesions and a potentially improved immune activation against a greater set of tumor antigens in heterogeneous metastases, the volume was increased to 10 mL of 8×10^7 pfu/mL (total dose 8×10^8 pfu) in cohort 09. The increased volume was well tolerated, did not result in an increase in AEs, and numerically provided a more robust response in both injected and non-injected metastases.

Patients in cohort 08 and cohort 09 showed clinically beneficial response to OrienX010. The overall ORR was 19.2%, with the higher dose in cohort 09 having an ORR of 28.6%. This ORR is similar to the T-VEC response of 26.4% in the OPTiM trial. In the OrienX010 trial, 69.2% of patients had acral (non-sun-exposed) melanoma, whereas the majority of patients in the OPTiM trial had sun-exposed cutaneous melanoma.[28] It is reassuring to see that OrienX010 is able to generate robust responses in non-sun-exposed melanoma.

Responses were seen in both injected and non-injected lesions, demonstrating that in addition to selectively replicating in tumor cells and mediating local tumor regression, OrienX010, similar to other oncolytic viruses, can also amplify systemic antitumor immune responses to eradicate distant metastases.[41] The 25.8% response rate seen in the injected metastases was higher than the 21.6% rate seen in the regional and distant non-injected metastases. Similarly, T-VEC has also shown to have a lower response rate in non-injected metastases, which could be related to a slowly expanding T cell response primed close to the site of virus injection.[42 43] Mechanistic research (from a phase II trial) on local and distant immunity induced by oncolytic therapy showed that there was significant decrease in T regulatory cells and suppressor T cells in injected lesions compared with non-injected lesions.[41] These results indicate the necessity to combine oncolytic viruses with other therapies to strengthen the control of distant lesions. Dual immunotherapies of T-VEC in combination with pembrolizumab in a phase I trial—but not in a larger phase III trial—and T-VEC in combination with ipilimumab have shown positive results in clinical trials.[44–47]

The OrienX010 trial only enrolled patients who were HSV seropositive. The OPTiM trial enrolled patients who were either HSV seropositive or seronegative, and T-VEC was found to be safe and efficacious in both groups.[28] Thus, the safety and efficacy of OrienX010 in HSV seronegative patients are unknown and need to be determined.

OrienX010 also displayed a good biodistribution profile. Based on the presence of OrienX010 in serum and urine, as well as the concentration of GM-CSF in serum, local injection of OrienX010 elicited viremia (42% in cohort 08, 57% in cohort 09) and might be subsequently excreted through the urinary system. However, this viremia was transient because almost no HSV-1 nucleic acid was detected in the blood after 48 hours following injection. In addition, no severe systemic side effects associated with OrienX010 occurred in patients with viremia and no viral activity of OrienX010 was detected in the swab samples at injection sites after 24 hours of injection.

By comparison, T-VEC was detected in the surface lesions of 100% of patients, blood of 98.3%, and urine of 31.7%.[48] In addition, T-VEC DNA was detected in injected lesions (14% of patients, 5.8% of samples) during the safety follow-up period. Occlusive dressings for lesions and instructions are provided to mitigate the potential for accidental exposure and transmission of the T-VEC virus to close contacts of the patients.

In this study with monotherapy OrienX010, the median PFS was 2.9 months, the median irPFS was 2.9 months,

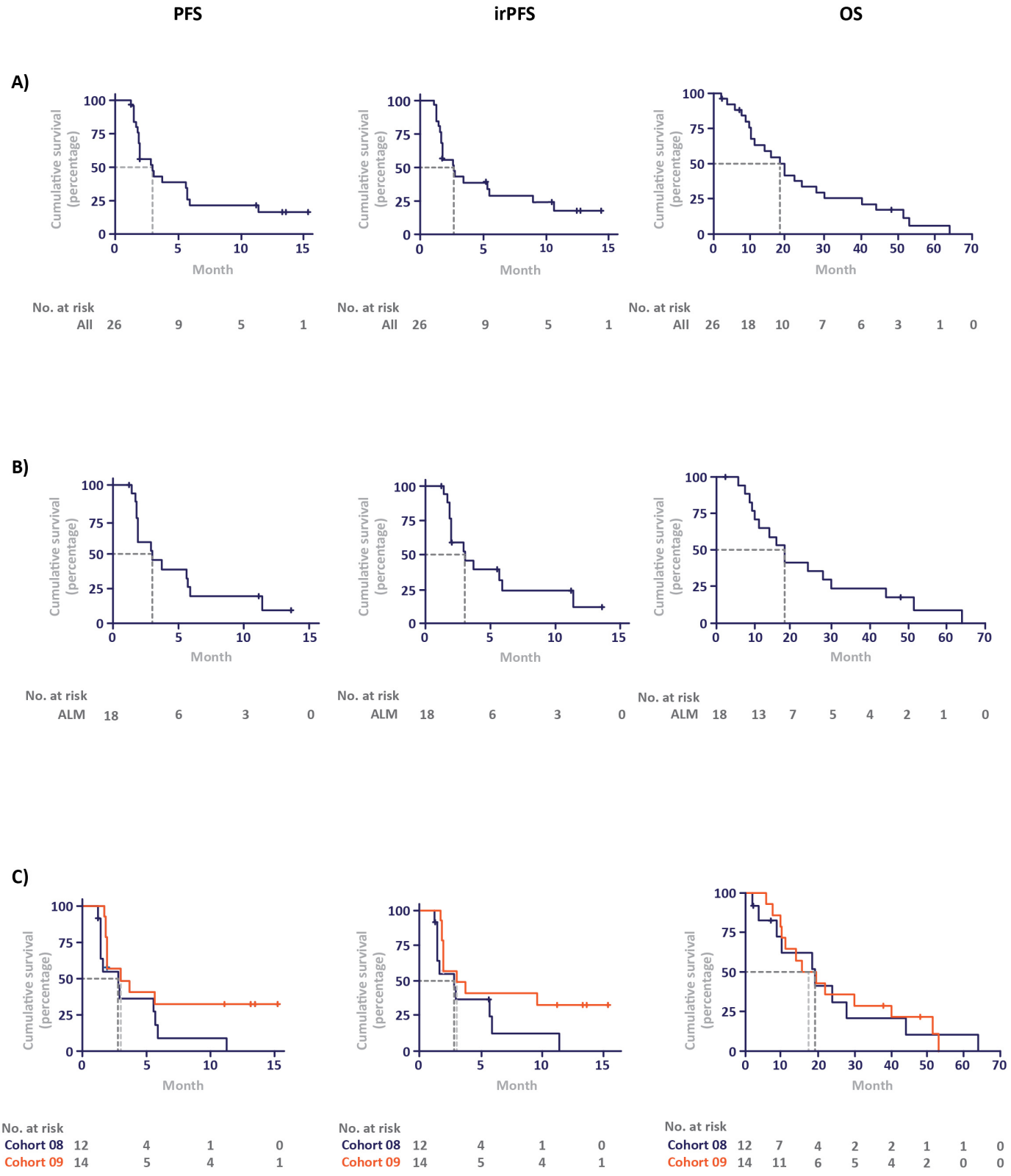

Figure 3 Kaplan-Meier estimates of PFS (left), irPFS (middle), and OS (right): (A) all patients; (B) patients with acral melanoma; and (C) patients in different cohorts. ALM, acral lentiginous melanoma; irPFS, immune-related progression-free survival; PFS, progression-free survival; OS, overall survival.

and the median OS was 19.2 months. Importantly, 69.2% of patients in this trial were diagnosed with acral melanoma, which is the most common subtype of melanoma in Asia and carries a worse prognosis than cutaneous melanoma. These patients with acral melanoma had a median PFS of 3.0 months, a median irPFS of 3.0 months, and an OS of 19.2 months. In addition, the acral subtype was not a risk factor for PFS and OS both in the univariate

and multivariate analyses. It might be concluded that OrienX010 is a promising therapy for acral melanoma.

There were some subtle differences in the survival outcomes between the two cohorts of different treatment doses. The ORR in cohort 09 was better than the ORR in cohort 08, and the median PFS in cohort 09 was also longer than the median PFS in cohort 08. However, no significant differences were shown in the univariate analysis, which might be due to the heavier tumor burden in patients of cohort 09. In addition, an increasingly longer tail was shown in the cohort 09 PFS survival curve, and the multivariate analysis of PFS suggested that a higher treatment dosage might be a meaningful factor, although this hypothesis still needs to be validated further due to the limited sample size of this trial. In general, patients treated with a higher dose of OrienX010 showed a better trend of response and longer PFS while still having tolerable AEs, with only one patient experiencing grade ≥3 AEs of myelosuppression. This is the first study to evaluate the safety and efficiency of oncolytic virus treatment with a higher dose up to 10 mL of 8×10^7 pfu/mL (T-VEC required a loading dose and was used up to only 4 mL of 1×10^8 pfu/mL). The results showed that, for patients with heavy tumor burden, higher dose injection would not increase the incidence of AEs and might bring more benefits. A larger clinical trial is warranted to validate the results of this study.

Tumor samples of patients with different responses were compared to explore the characteristics of patients with better response to OrienX010. Gene expression profile demonstrated significant differences in the expression of 55 genes—46 of which were highly expressed in tumor samples of patients with better responses. Pathway and process enrichment analyses showed most of these genes are associated with inflammatory and immune responses. The results of GSEA analysis revealed that *IL-2_STAT5* signaling pathway gene sets were significantly enriched in the response group, which is critical in controlling the hemostasis and function of regulatory T cells.[49–51] It might be speculated that patients with a more activated immune system are more likely to benefit from oncolytic therapy. From this perspective, the combination of oncolytic and checkpoint immunotherapies might work partly by making the immune system more activated to encourage the oncolytic virus to work better.

In conclusion, this phase Ib trial of unresectable stage IIIC–IV melanoma demonstrated that OrienX010 is safe and well tolerated with a positive trend of antitumor effects and may be a valuable agent for patients with melanoma. Moreover, the higher dosage of OrienX010 (10 mL of 8×10^7 pfu/mL every 2 weeks) might bring more benefits without severe AEs and is recommended for phase II clinical trials.

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Competing interests None declared.

Patient consent for publication Not required.

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