nature communications

Article

https://doi.org/10.1038/s41467-024-53041-7

Non-secreting IL12 expressing oncolytic adenovirus Ad-TD-nsIL12 in recurrent high-grade glioma: a phase I trial

Received: 11 December 2023

Accepted: 30 September 2024

Published online: 08 November 2024

Check for updates

Weihai Ning[1,5], Xiao Qian[1,5], Louisa Chard Dunmall[2], Funan Liu[3], Yuduo Guo[1], Shenglun Li[1], Dixiang Song[1], Deshan Liu[1], Lixin Ma[1], Yanming Qu[1], Haoran Wang[1], Chunyu Gu[1], Mingshan Zhang[1], Yaohe Wang[2] ✉, Shengdian Wang[4] ✉ & Hongwei Zhang[1] ✉

Malignant glioma is a highly fatal central nervous system malignancy with high recurrence rates. Oncolytic viruses offer potential treatment but need improvement in efficacy and safety. Here we describe a phase I, dose-escalating, single arm trial (ChiCTR2000032402) to study the safety of Ad-TD-nsIL12, an oncolytic adenovirus expressing non-secreting interleukin-12, in patients with recurrent high-grade glioma that connects with the ventricular system. Eight patients received intratumoral treatment via stereotaxis or an Ommaya reservoir, with doses ranging from 5×10^9 to 5×10^{10}vp. The primary end point was to determine the maximal tolerated dose. Secondary endpoints included toxicity and anti-tumour ability. Minimal adverse events were observed at doses of 5×10^9 and 1×10^{10}vp. Grade 3 seizure was observed in two patients from Cohort 3 (5×10^{10}vp). Therefore, the maximum tolerated dose was determined to be 1×10^{10}vp. Four patients developed hydrocephalus during follow-up. Among them, symptoms in two patients were relieved after placement of a ventriculo-peritoneal shunt, and the other two only showed ventriculomegaly on MRI scan without neurological deterioration. Complete response (according to Response Assessment in Neuro-Oncology Criteria) in one patient, a partial response in one patient and post-treatment infiltrations of CD4+ and CD8 + T cells into the tumour were documented during this trial. In conclusion, Ad-TD-nsIL12 has demonstrated safety and preliminary efficacy in patients with recurrent high-grade glioma.

High-grade gliomas (HGG) are the most frequent and fatal type, accounting for more than half of all malignant primary brain tumours[1]. Although some pathological subtypes, such as grade III oligodendroglioma, have relatively favourable survival times, the median survival for grade IV tumours is only 14-16 months[2]. The survival rate of glioblastoma (GBM) is even worse, with only about a quarter of patients surviving two years after diagnosis and only 5% to 14% living beyond five years[3–5] Current treatment strategies include maximal tumour resection combined with radiotherapy and temozolomide chemotherapy. More recently, the addition of tumour treating fields to

[1]Department of Neurosurgery, Sanbo Brain Hospital, Capital Medical University, Beijing, China. [2]Centre for Cancer Biomarkers & Biotherapeutics, Barts Cancer Institute, Queen Mary University of London, London, UK. [3]Department of Surgical Oncology and General Surgery, The First Hospital of China Medical University, Shenyang, China. [4]National Laboratory of Biomacromolecules, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China. [5]These authors contributed equally: Weihai Ning, Xiao Qian. ✉e-mail: yaohe.wang@qmul.ac.uk; sdwang@ibp.ac.cn; zhanghongwei@ccmu.edu.cn

standard of care has been shown to improve outcomes in patients[6]. Standard of care (SOC) is often able to reduce tumour progression, but recurrence is common and the prognosis of recurrent HGG (rHGG) is very poor, with median survival of only 6–8 months after tumour progression[7,8]. Tumour location can be key to prognosis as tumours growing close to lateral ventricles are associated with poorer survival rates and higher incidence of recurrence[9]. These outcomes dictate a clear and urgent need for more successful treatment options for these patients to improve clinical outcomes.

Tumour-targeted replicating oncolytic viruses (TOVs) have emerged as extremely attractive candidates for cancer therapeutics and are synergistic with conventional therapeutic approaches and other immunotherapeutic modalities. TOVs are wild-type or genetically modified viruses that selectively replicate in tumour cells. They have multiple mechanisms of action that ultimately lead to a remodelling of the suppressive tumour microenvironment (TME) to produce robust anti-tumour immune responses[10].

Replicating oncolytic Adenoviruses (AdV) are popular TOV candidates due to their ease of production, ease of genetic manipulation and their favourable safety profiles. Oncorine (H101), was the first licenced AdV in China in 2005, for treatment of head and neck cancer. However, while safe, clinical efficacy across multiple tumour types, including malignant glioma, has not been demonstrated and treatment with H101 has resulted in few objective responses[11,12].

Evaluation of the functional impact of genetic modifications introduced into the Adenovirus type 5 (Ad5) genome to create H101, demonstrated that the deletions in the E1B55K and E3 gene regions, introduced to promote tumour-selective replication, had a significant impact on the ability of these viruses to replicate efficiently within tumour cells[13]. H101 was cleared more rapidly after infection and replication and cytotoxicity in cells was poor compared with wild-type Ad5. Poor efficacy was associated with marked macrophage infiltration into tumours consequent to the E3B region deletion[14], a phenotype associated with worse efficacy in brain tumour models[15].

Similar low efficacy issues have been noted with second generation oncolytic AdV such as the *dl*922-947/Delta-24 viruses. These viruses improved on first generation iterations by retention of the E1B55K gene that is vital for modulating transport and stabilisation of viral mRNAs. Instead, a 24 base pair deletion in the early E1A gene was introduced to impart tumour selectivity. A second-generation AdV modified for integrin binding (DNX-2401/tasadenoturev) has shown promise in pre-clinical glioma models[16] and phase I trials with these agents indicated treatment could induce positive anti-tumour effects in recurrent glioma[17]. More recent phase I/II investigating DNX-2401 in combination with pembrolizumab in recurrent glioblastoma was able to replicate the safety endpoints of the phase I trials and evidence of oncolysis and tumour immune cell infiltration was seen, however efficacy endpoints were not met[18]. Retention of the deleterious E3 gene region deletion from first-generation viruses may be a contributing factor to the compromised clinical efficacy of second-generation AdV.

Based on the clear need for improvements in AdV and our improved knowledge of its biology[13,19], we reported a new-generation replicating AdV with three gene deletions (E1A CR2, E1B19K, and E3gp19K), Ad-TD. Of importance is the intact E3B region, preserved to overcome the limitations of previous AdV candidates. Inclusion of an immunostimulatory payload is considered an essential facet of new generation oncolytic therapies, required to achieve stronger and long-term anti-tumour efficacy. In this regard, interleukin-12 (IL-12) has been recognised as one of the most potent and pleiotropic cytokines of the immune system with multiple associated anti-tumour functions[20]. Successful clinical application of IL-12, however, is precluded by its significant toxicity[21–23]. To overcome the toxicity associated with IL-12 expression and capitalise on its potent anti-tumour activity, we reported a system for achieving durable, low-level IL-12 expression within the TME by modification of the signal peptide of the cytokine to create a non-secreting IL-12 molecule. When expressed in Ad-TD, unmodified IL-12 was toxic after systemic delivery, but modified IL-12 (nsIL12) was consistently safe. Moreover, efficacy of Ad-TD-nsIL12 was vastly superior to efficacy of H101 in different tumour models[24,25].

In this work, we present data on the clinical safety and preliminary anti-tumour efficacy of Ad-TD-nsIL12 in rHGG patients with tumours that connect to the ventricular system. We have demonstrated consistent safety upon 1×10^{10}vp administration and found that repeated injections are viable and do not lead to accumulating toxicity. Although four patients developed hydrocephalus, this could not be conclusively attributed to the viral injection, since, as the tumour itself was exposed to the ventricle, development of hydrocephalus may be expected[26,27]. Two of these patients only showed ventriculomegaly on imaging. We have also shown the clinical safety of a modified IL-12 cytokine payload, which can improve outcomes by initiating potent immune responses.

Results

Patient characteristics

In this trial, a total of eight patients were enroled and received Ad-TD-nsIL12 between September 2020 and September 2022 at Sanbo Brain Hospital of Capital Medical University. The inclusion and exclusion criteria are listed in Table S1 of the Supplementary Information. Detailed characteristics for each patient are listed in Table 1. Briefly, the median age of patients was 53 years (45–71 years). Only one patient (No. 2) experienced two recurrences before virotherapy. The KPS score was 90 in three patients (37.5%), 80 in two patients (25.0%) and 70 in three patients (37.5%) at base line (Figure S1). All patients had confirmed rHGG as determined by stereotactic biopsy before virus

Table 1 | . Baseline Clinical Characteristics of Patients

No	Age	Number of recurrences before virotherapy	Extent of tumour resection	RT(Gy)	CTX	Time between initial surgery and Ad-TD-nsIL12 (months)	KPS	Tumour size (mm^2)	Number of Ad-TD-nsIL12 administrations	Gene Mutation		Serum adenovirus antibody at baseline
										IDH1	MGMT	
1	51	1	GTR	60	TMZ	13.3	90	1868.8	1	WT	-	Negative
2	53	2	GTR	60	TMZ	159.6	90	1728.0	4	MUT	+	Negative
3	45	1	GTR	60	TMZ	2.6	90	430.5	2	WT	UC	Negative
4	52	1	Biopsy	60	TMZ + DDP	14.9	70	475.1	2	MUT	+	Negative
5	71	1	GTR	60	TMZ	12.0	70	1794.5	1	WT	+	Negative
6	48	1	GTR	60	TMZ	8.3	80	832.5	5	MUT	+	Negative
7	66	1	PR	60	TMZ	5.4	80	1068.9	1	WT	UC	Negative
8	53	1	PR	60	TMZ	6.0	70	2559.5	1	WT	-	Negative

Abbreviations: WT, wild type; MUT, mutant; UC, unclear; KPS, Karnofsky Performance Scale; GTR, Gross total resection (>95% of the tumour resected); PR, partial resection; TMZ, temozolomide; DDP, cis-diamminedichloroplatinum; RT, Radiotherapy; CTX, chemotherapy.

https://doi.org/10.1038/s41467-024-53041-7

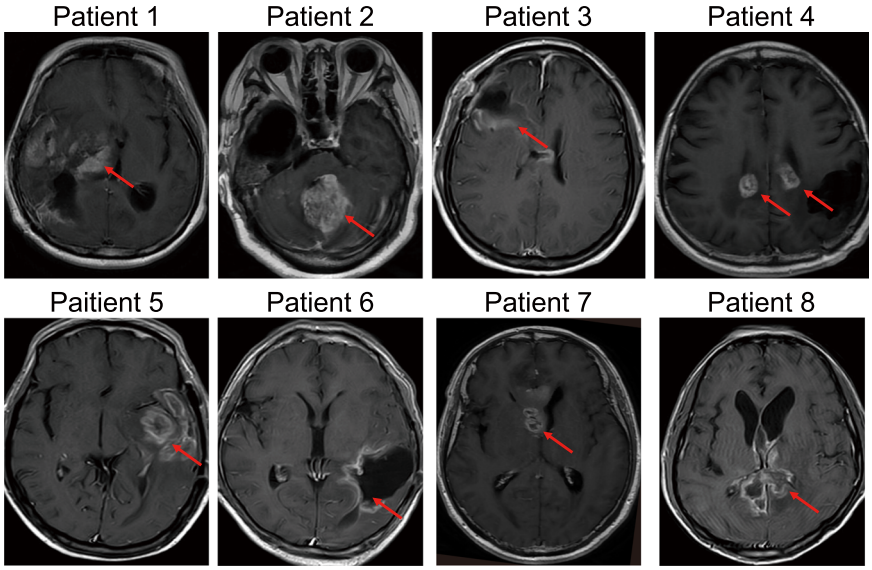

Fig. 1 | MRI determination of the tumour site and the location of the tumour communication with the ventricular system (red arrow). Patient 1: right lateral ventricle; Patient 2: fourth ventricle; Patient 3: right lateral ventricle; Patient 4: bilateral ventricle; Patient 5: left lateral ventricle; Patient 6: left lateral ventricle; Patient 7: right lateral ventricle and third ventricle; Patient 8: bilateral ventricle. Patients 1, 4, 5 and 7 received an intratumoral injection (i.t) by stereotaxic puncture as the tumour lesion could be clearly identified on the image, and the location was superficial. Patients 2, 3, 6 and 8 received virus injection via pre-planted Ommaya reservoir as tumour masses were not accessible by i.t injection.

injection. Mutation of IDH1R132H was documented in three patients (37.5%), and MGMT expression in four patients (50.0%). The serum adenovirus antibodies were negative in all 8 patients at baseline testing. In addition, the connection of the tumour to the ventricle was also verified. In six patients, recurrent tumours connected the ventricular system mainly via the lateral ventricle. In other two patients, one tumour connected through the fourth ventricle and one partially through the third ventricle. After virus treatment, 3 patients used glucocorticoids (methylprednisolone sodium succinate), and the usage of other treatments are listed in Table S2.

Patients 1, 4, 5 and 7 received Ad-TD-nsIL12 by i.t injection using stereotaxic puncture as the tumour lesion could be clearly identified on the image and the location was superficial (Fig. 1). Patients 2, 3, 6 and 8 received virus injection via an Ommaya reservoir (i.O injection). The recurrent tumour was mainly located within the resection cavity in patients 3, 6 and 8. The position of the mass in patient 2 was deep and located in the fourth ventricle (Fig. 1). The median volume of tumour at baseline MRI was 1398.5 mm^2 (430.5-2559.5 mm^2).

Safety and pharmacokinetics

Three dose-escalation cohorts were assigned in this trial; 5×10^9 (cohort 1), 1×10^{10} (cohort 2) and 5×10^{10}vp (cohort 3) in 1 ml volumes. AEs related to treatment were recorded and listed in Table 2, sorted by assigned dose. The most frequent AEs were fever (Grade 1 in 7 patients) and cognitive disturbance (Grade 1 in 3 patients and Grade 2 in 4 patients). Nausea and vomiting were also frequently recorded (in 6 patients), with Grade 3 occurring in cohort 3. In addition, fatigue, dizziness and headache were documented early after virotherapy. These AEs happened mostly within 24 h after virus injection and lasted less than 48 h. Simple symptomatic treatment was effective. In addition, hydrocephalus was documented in four patients (Table S3). Two of these patients (patient 2 and 6) received a ventriculoperitoneal (VP) shunt. In patient 2, the lesion was mainly located in the fourth ventricle, whereas in patient 6, the resection operation resulted in the ventricular opening. These two patients were at high risk for the development of hydrocephalus, and it cannot simply be attributed to virus injection therapy. Patient 1 and patient 7 showed only ventriculomegaly on imaging and did not experience significant symptoms.

Four patients received a single injection of the assigned dose, two received two injections, one received four injections and one received five injections (Table 1). The interval between each injection was 28 days. After a patient received repeated treatments, the same AE was recorded only once at the highest level. With dose escalation, AEs related to treatment occurred similarly. We did not observe an accumulation of viral toxicity from multiple injections.

Minor seizures were documented in patients from cohort 1 and 2 within 48 h after treatment, without recurrence during injection intervals and follow-ups. When the dose reached 5×10^{10}vp, severe general seizures (Grade 3) occurred in both patient 7 and 8. Anti-seizure medication including diazepam and sodium valproate were initiated immediately. While we could not achieve direct evidence of virus infection of the ependymal cells and virus dissemination in the ventricular system, this AE was defined as DLT because the time of onset was closely related to treatment. Therefore, the dose used in Cohort 2 (1×10^{10}vp) was taken as the MTD (primary endpoint of the trial) from this study. The primary objective has been reached.

Before and after virus administration, blood and cerebrospinal fluid (CSF, collected from lumbar puncture) samples were tested to examine Ad-TD-nsIL12 by qPCR. No viral DNA of Ad-TD-nsIL12 was detected in CSF or blood samples (Table S4).

Clinical and imaging outcomes

Intratumoural injection of virus via stereotaxis was performed in four patients, and the other four via a pre-placed Ommaya reservoir. Injection routes, frequency and outcomes for each patient are noted in Table 3. Injection route was determined using baseline MRI scans (T1 gadolinium enhancing phase). The median overall survival was 5.1 months (range 3.1-21.2 months) after the first Ad-TD-nsIL12 injection and 18.9 months (range 8.6-162.7 months). Kaplan–Meier survival curves are presented in Figure S2. As secondary objective, tumour response was tested and evaluated by RANO criteria. A complete reduction of the tumour occurred in patient 6, which was designated as a CR. In addition, a PR was seen in one patient, SD in four patients and PD in two patients (Table 3).

Complete Response: Patient 6. Patient 6 initially presented with dizziness, decreased left visual acuity and anomic aphasia for eight

https://doi.org/10.1038/s41467-024-53041-7

Table 2 | Adverse Events Related to Ad-TD-nsIL12

Assigned dose (vp)	Adverse Events	Grade 1 n(n%)	Grade 2	Grade 3	Grade 4
5×10^9 ($n=3$)	Nausea	0 (0%)	3 (100%)	0 (0%)	0 (0%)
	Vomiting	1 (33%)	2 (66%)	0 (0%)	0 (0%)
	Fatigue	0 (0%)	1 (33%)	0 (0%)	0 (0%)
	Fever	2 (66%)	0 (0%)	0 (0%)	0 (0%)
	Upper respiratory infection	0 (0%)	1 (33%)	0 (0%)	0 (0%)
	Cognitive disturbance	2 (66%)	1 (33%)	0 (0%)	0 (0%)
	Dizziness	2 (66%)	0 (0%)	0 (0%)	0 (0%)
	Headache	0 (0%)	3 (100%)	0 (0%)	0 (0%)
	Seizure	1 (33%)	1 (33%)	0 (0%)	0 (0%)
1×10^{10} ($n=3$)	Nausea	2 (66%)	0 (0%)	0 (0%)	0 (0%)
	Vomiting	2 (66%)	0 (0%)	0 (0%)	0 (0%)
	Fatigue	2 (66%)	0 (0%)	0 (0%)	0 (0%)
	Fever	3 (100%)	0 (0%)	0 (0%)	0 (0%)
	Upper respiratory infection	0 (0%)	1 (33%)	0 (0%)	0 (0%)
	Cognitive disturbance	1 (33%)	2 (66%)	0 (0%)	0 (0%)
	Headache	1 (33%)	0 (0%)	0 (0%)	0 (0%)
	Seizure	0 (0%)	1 (33%)	0 (0%)	0 (0%)
5×10^{10} ($n=2$)	Nausea	0 (0%)	0 (0%)	1 (50%)	0 (0%)
	Vomiting	0 (0%)	0 (0%)	1 (50%)	0 (0%)
	Fatigue	1 (50%)	0 (0%)	0 (0%)	0 (0%)
	Fever	2 (100%)	0 (0%)	0 (0%)	0 (0%)
	Cognitive disturbance	0 (0%)	1 (50%)	0 (0%)	0 (0%)
	Dizziness	0 (0%)	1 (50%)	0 (0%)	0 (0%)
	Headache	0 (0%)	1 (50%)	0 (0%)	0 (0%)
	Seizure	0 (0%)	0 (0%)	2 (100%)	0 (0%)
	Abdominal pain	1 (50%)	0 (0%)	0 (0%)	0 (0%)

1. Adverse events were graded according to Common Terminology Criteria for Adverse Events (CTCAE, version 5.0)
2. Laboratory abnormalities are not included.

months. An MRI revealed a T1 hyperintense mass in the left temporal lobe, which was resected after diagnosis. Pathology indicated rHGG with (IDH1 MUT, MGMT +). After radiation therapy and concurrent temozolomide treatment, new progressions on the edge of the resection cavity were identified, with sections communicating with the left lateral ventricle. Baseline MRI scans demonstrated that the recurrent mass was located at the medial wall of the resection cavity (Fig. 2). This patient received a stereotactic biopsy to confirm rHGG (IDH1 MUT, MGMT +) and an Ommaya reservoir was implanted. 1×10^{10}vp of Ad-TD-nsIL12 was injected into the Ommaya reservoir and injections were repeated 4 more times with an interval of 28 days. After the first and second injections, transient seizures occurred (defined as Grade 1), followed by temporary fatigue and dizziness. In addition, a low-grade fever after each injection was documented (≤ 38°C, Grade 1), associated with vomiting. The post-operative MRI scans initially showed a mild decrease of contrast-enhancing tumour, followed by total regression by the 7th month after the first Ad-TD-nsIL12 injection. KPS scores improved to 90. 5 months after the 5th injection, the patient began to experience unsteady walking and a diminished state of consciousness. A CT scan showed hydrocephalus, and this patient received a VP shunt 14.4 months after first virus. The patient remained CR at 18-month imaging follow-up after virotherapy. However, this

Table 3 | . Summary of Patient Outcomes

No	Dose	Ad-TD-nsIL12 access	Times of Ad-TD-nsIL12 administration	Response assessment	Survival time (months)		Final outcome
					After surgery	After first Ad-TD-nsIL12	
1	Cohort1 (5×10^9)	i.t	1	SD	16.9	3.6	Dead (Tumour Progression)
2	Cohort1 (5×10^9)	i.O	4	SD	162.7	3.1	Dead (Tumour Progression)
3	Cohort1 (5×10^9)	i.O	2	PD	11.2	8.6	Dead (Tumour Progression)
4	Cohort2 (1×10^{10})	i.t	2	PR	20.8	5.9	Dead (Tumour Progression)
5	Cohort2 (1×10^{10})	i.t	1	PD	24.3	12.3	Dead (Tumour Progression)
6	Cohort2 (1×10^{10})	i.O	5	CR	29.5	21.2	Dead (CNS infection)
7	Cohort3 (5×10^{10})	i.t	1	SD	8.6	3.1	Dead (Tumour Progression)
8	Cohort3 (5×10^{10})	i.O	1	SD	10.2	4.2	Dead (Tumour Progression)

Abbreviations: i.t, intratumoral injection via stereotaxis; i.O, Ommaya reservoir injection; CR, complete response; PR, partial response; SD, stable disease; PD, progressive disease; CNS, central nerve system.

https://doi.org/10.1038/s41467-024-53041-7

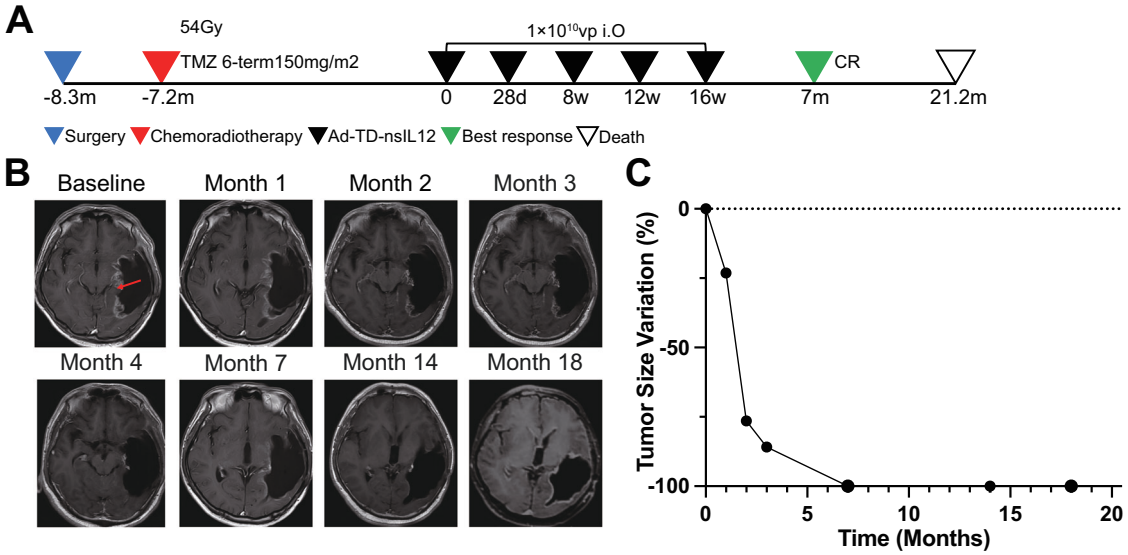

Fig. 2 | Presentation of treatment course and tumour response of patient 6. **A** Patient 6 received surgery and chemoradiotherapy to treat the primary tumour, but tumour recurrence was identified.This patient was then treated with five consecutive administrations of Ad-TD-nsIL12 8.3 months after the initial resection surgery (1×10^{10}vp) and achieved complete response (CR) 7 months from baseline. However this patient died 21.2 months from baseline due to central nerve system infection. **B** Baseline imaging revealed that the recurrent tumour was located inside the surgical resection site (red arrow) and communicated with left lateral ventricle. For this reason, virus was administered through an Ommaya reservoir (i.O). The follow-up scans after treatment showed that the enhancement gradually faded, and the tumour enhancement image disappeared in the 7th month after the first treatment. No obvious tumour enhancement was observed at 18 months. The time shown in the figure is the time of the MRI scan after baseline. **C** The line graph illustrates the percentage change of tumour size over the time course. Source data are provided as a Source Data file.

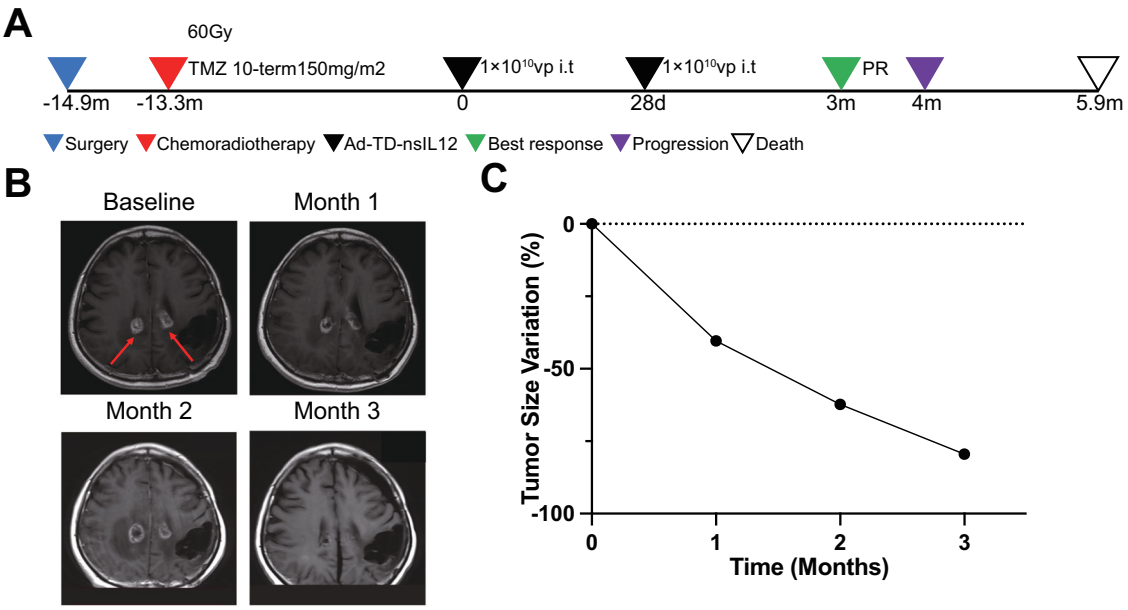

Fig. 3 | Presentation of treatment course and tumour response of patient 4. **A** After the initial surgery, the patient underwent chemoradiotherapy. 14.9 months after surgery, rHGG was identified and this patient received two administrations of Ad-TD-nsIL12 (1×10^{10}vp). The patient achieved partial response (PR) at 3 months after treatment, but tumour progression occurred at 4th month. The patient died 5.9 months after the initial virotherapy due to tumour progression. **B** Baseline MRI images revealed that the recurrent tumour was located in the bilateral lateral ventricles (red arrows). Considering the distribution length of the cephalic foramen of the Ommaya reservoir canal and the location of the tumour, this administration method was deemed unsuitable. Instead, we performed stereotactic virus injection. Central necrosis in the tumour was observed at 1 and 2 months after treatment, and the tumour almost disappeared at 3 months after treatment. **C** The line graph illustrates the percentage change of tumour size before progression. Source data are provided as a Source Data file.

patient died 21.2 months after the treatment (29.5 months after diagnosis) due to a central nervous system infection (bacterial culture of CSF indicating baumanii) rather than tumour progression.

Partial Response: Patient 4. Patient 4 was first diagnosed with HGG (IDH1 MUT, MGMT +) 14.9 months before the first virus treatment. The patient received a total resection followed by concurrent radiation therapy and temozolomide (Fig. 3). After a 10-term-chemotherapy cycle, symptoms including dizziness, right limb weakness and dysarthria reoccurred, indicating tumour re-progression. The baseline MRI scan showed a recurrent mass located at bilateral ventricles. This patient received two i.t injections of Ad-TD-nsIL12 after a stereotactic

biopsy. The collected specimen showed rHGG (IDH1 MUT, MGMT +). Grade 1 fever (≤ 38°C, less than 24 h) followed by headache and dizziness was recorded after the first and second therapies. No other drug-related AEs were identified. 1 month after the second injection, the volume of tumour was significantly reduced. The patient also reported improvement in symptoms including consciousness, limb strength and dysarthria. However, recurrent tumour progression was observed by MRI again 4 months after baseline and this patient died 5.9 months after the first virus injection. This patient received glucocorticoids and bevacizumab after progression to control oedema and improve neurologic dysfunction (Table S2). Although either agent may have had an effect on the imaging findings, in the context of the patient's symptoms, progression was evident.

Histological and immunological responses

For exploring the mechanism of anti-tumour ability of Ad-TD-nsIL12 (secondary objective), samples gathered in the trial are analysed. Patient 2 underwent a second resection surgery one month after the third virus treatment, and tumour samples were used to identify the virus infection and immune cell infiltration. Collected samples showed higher infiltration of CD4+ and CD8+ cells were presented within the tumour compared with pre-treatment tumour samples. Macrophage (CD68) infiltration increased slightly, although the difference was not statistically significant. The increase in M2-like macrophages (CD163) after treatment was more pronounced, though not statistically significant, whereas M1-like macrophages (CD86) were nearly undetectable both before and after treatment (Figure S3). However, a more robust analysis in future trials is required to delineate macrophage phenotype pre- and post-treatment. Viral EIA and hexon proteins were both observed in post-treatment tumours indicating the infection of Ad-TD-nsIL12 to the tumour and subsequent replication. 30 days after treatment, there was a increase in the expression of IL-12 in tumour tissue, although it was not significant (Fig. 4). These results suggest that the virus effectively replicates within the tumour tissue and induces immune response. To investigate the systemic immunological responses to the treatment of Ad-TD-nsIL12, pre- and post-treatment CSF in Patient 4 and 5 were collected to test Ad-TD-nsIL12-associated inflammatory cytokines (Figure S4). While an increase was seen in expression of some cytokines including: IFN-γ, IL-10 and IL-21 in both patients and IL-6 in patient 5, no significant changes were seen in IL-12, IL-1β, IL-2 and IP-10 in either patient, and IL-6 in patient 4, demonstrating that Ad-TD-nsIL12 did not markedly elevate the inflammatory response after i.t administration to rHGG.

Discussion

Oncolytic viruses (OVs) as therapies for malignancies including glioblastoma have been investigated for over a century, but recent advances in molecular virology techniques have advanced the field significantly[10,28]. OVs can target the tumour in multiple ways, through virus-mediated oncolysis, angiogenic collapse, induction of immunogenic cell death and potent, robust activation of the immune system against tumour antigens. They are non-cross resistant with standard therapies and can augment the success of conventional and immunotherapies in patients. Our ability to rationally modify the genome of these viruses has improved safety and efficacy associated with virus administration in numerous pre-clinical models. However, the translation of these results into the clinic has been elusive.

Lessons from the application of first- and second-generation adenoviruses into clinical tumour models demonstrated that genetic modification of the viruses must be applied with care, as both generations contained debilitating mutations that impaired efficacy in a clinical setting. We have also learned that viruses can be modified to express foreign genes to augment their activity, and selection of these payloads is likely to be critical for long-term success of treatment. In this regard, we have developed Ad-TD-nsIL12, a third generation Ad5 virus, which we recently reported as safe and effective in multiple pre-clinical tumour models[24,25]. This virus is deleted in the E1ACR2 gene region, E1B19K gene region and the E3gp19K gene region, while retaining native gene regions identified in first- and second-generation oncolytic AdV as deleterious for activity if absent (E1B55K and E3)[13,14]. These deletions were engineered to impart tumour specificity to the virus (E1ACR2 and E3gp19K), ensuring tumour-specific replication and gene expression, and to increase anti-tumour immune activation (E1B19K).

Interleukin-12 is an extremely potent cytokine that is considered one of the strongest candidates for immunotherapeutic interventions due to its pleiotropic nature. It can act to increase Th1 T cells, CD8 + T cells, natural killer (NK) cells, dendritic cells and M1-like macrophages in the tumour microenvironment (TME), changing the TME from 'cold' (pro-tumour) to 'hot'(anti-tumour)[29]. Clinical use is, however, precluded by its strong toxicity that can lead to the development of lethal inflammatory syndrome in patients[22]. Numerous studies have investigated mechanisms to minimise the toxicity of IL-12 in cancer patients. Recently, phase-1 trials in recurrent high-grade glioma reported that administration of IL-12 using a replication incompetent adenovirus vector Ad-RTS-IL-12 in combination with an oral activator of IL-12 expression via the RheoSwitch Therapeutic System (RTS), veledimex, was well tolerated. Toxicities were as predicted but reversed upon withdrawal of veledimix[30,31].

We have recently reported a modification to the IL-12 cytokine that allows full functionality in terms of immune activation and anti-tumour activity, while preventing systemic toxicity associated with wild-type IL-12[24]. Incorporation of this molecule, nsIL12, into our replicating third-generation virus can be predicted to be more powerful compared to non-replicating versions, with in built safety as the dissemination of IL-12 is limited to within the TME during oncolysis.

Here, we have presented a dose-escalating clinical trial to study the safety of our oncolytic adenovirus, Ad-TD-nsIL12, in rHGG. After surgical resection, recurrent lesions often connect with the ventricles in the brain[32]. The prognosis of these patients is poor and novel treatment options are urgently sought. However, many trials studying OVs have excluded patients with brain tumours as there exists a risk of virus ventricular entry. This risk can be negated by careful manipulation of the virus to prevent replication in healthy cells within the brain, while allowing treatment of intraventricular disseminated cancers in the CNS. In this trial, eight patients with rHGG connecting to the ventricular system received one of 3 assigned doses of Ad-TD-nsIL12. Based on the tumour location, Ad-TD-nsIL12 was delivered into patients in one of two ways, by direct intratumoural (i.t) injection (four patients) or injection via pre-inserted Ommaya reservoir (four patients). I.T injection is a commonly used delivery route in OV research, but can be difficult to apply to patients in whom the recurrent lesion is located at the resection cavity. In contrast, a pre-planted Ommaya reservoir can gain access to these tumours and also makes OV injection at the bedside possible. It is also especially suitable for patients with tumours positioned deep within the brain, including patients with diffuse intrinsic pontine glioma (DIPG). However, because of the variation of resection cavity volume in patients with rHGG, use of the Ommaya reservoir can make determination of the number of viral particles acting on the lesion difficult to determine.

No AEs > Grade 2 concerning Ad-TD-nsIL12 were documented until the dose reached 5×10^{10}vp. Therefore, the Cohort 2 dose (1×10^{10}vp) was determined as our MTD for this study. Fever was most commonly observed at all dose levels (seven patients), followed by cognitive disturbance, nausea and vomiting. These AEs have also been documented in patients receiving other OVs. Drug-associated fevers were initially recorded within 6 h after virus injection, and relieved within 48 h and this condition was considered acceptable by the investigator. The virus was administered repeatedly to four patients with an interval of 28 days between each virus administration. The incidence of early AEs after subsequent treatment was the same as that after the first injection of virus. No cumulative viral toxicity was

https://doi.org/10.1038/s41467-024-53041-7

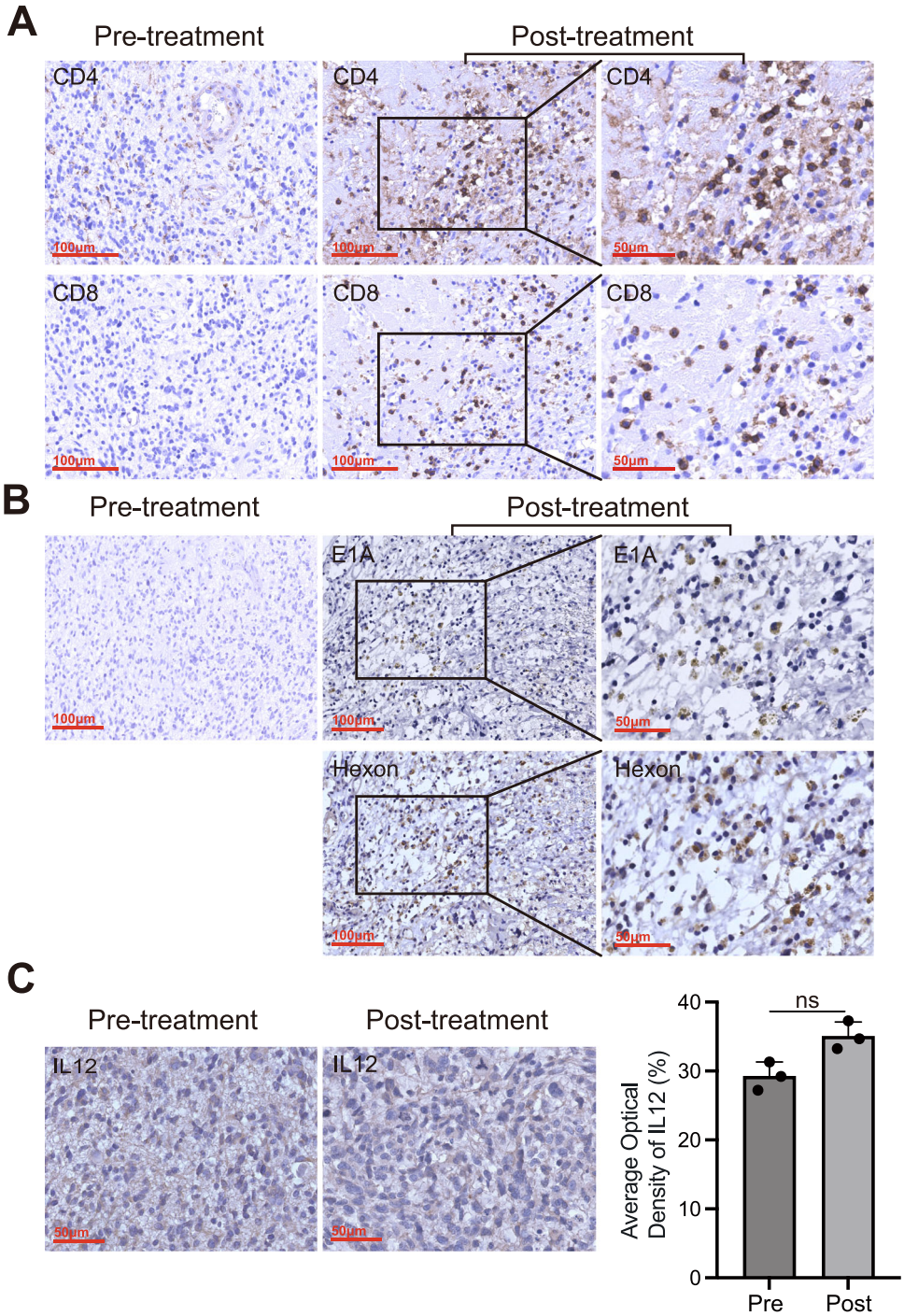

Fig. 4 | Immune cell infiltration and viral protein expression within the tumours pre or post-treatment of Ad-TD-nsIL12. A Represent immunohistochemical staining of pre and post-treatment (one month after the third virus treatment) samples of patient 2 demonstrated that infiltration of CD4+ and CD8+ cells occurred consequent to treatment. **B** In the post-treatment samples of patient 2, EIA and hexon were both observed, indicating the virus infection and replication after the third virus treatment. **C** There was an increase in the expression of IL-12 in post-treatment tumour tissue, although it is not significant (3 random fields under 20x magnification, bar indicates SD, paired *t* test, $p = 0.10$). Source data are provided as a Source Data file.

observed in patients with multiple doses of the virus. Hydrocephalus was recorded in four patients, two of whom received a V-P shunt. The high incidence of hydrocephalus led us to consider a correlation between viral infection and a disturbance of the cerebrospinal fluid circulation for viruses are recognised for their potential to increase risk of hydrocephalus[33]. However, it is believed that the causes of hydrocephalus are complex and cannot be solely attributed to viral injection. Firstly, from the baseline scan of patient 6, the resection surgery linked the surgical cavity and left lateral ventricle, which might increase the risk of hydrocephalus[34]. Secondly, tumour progression obstructing the ventricular system was determined by imaging in patient 2, which lead to a diagnosis of tumour-induced obstructive hydrocephalus. The other two patients who did not receive a VP shunt only presented with ventriculomegaly on imaging without significant associated symptoms. Therefore, the causes of hydrocephalus are diverse and the relationship with viral treatment may not be significant.

In the immunohistochemical staining of post-treatment tissue from patient 2, the presence of E1A and HEXON proteins confirmed viral

infiltration and replication on the 30th day after treatment. In addition, elevated infiltration of CD4+ and CD8+ cells were detected, confirming that adaptive immunity was induced following oncolytic virus therapy. However, the results also showed a higher presence of CD163+ macrophages rather than CD86+ macrophages, indicating that M2-type macrophages are predominant in the later stages of the disease, rather than M1-type. The polarisation of macrophages to the M2 type is currently believed to promote tumour cell proliferation and survival by secreting cytokines and growth factors such as IL-10, TGF-β, and VEGF. It also inhibits the activity of T cells and NK cells, thereby weakening the host's anti-tumour immune response[35]. This suggests that targeting of the M2 cell population may be a useful strategy to improve the overall therapeutic effectiveness of Ad-TD-nsIL12 in future applications.

In terms of efficacy, the median survival after viral intervention in this study was 5.1 months, which is lower than the historical median survival for rHGG. However, it is worth noting that tumour responses were recorded in two patients: a CR was observed in patient 6, and a PR was observed in patient 4. Although efficacy was not the primary objective of this phase 1 trial, these results are still encouraging. Promising results were both documented in Cohort 2 (1×10^{10}vp) and these two patients received multiple injections. However, it is noteworthy that the two patients who achieved CR or PR, both had IDH1 mutations and MGMT expression, indicative of a relatively good prognosis[36,37]. This result also leads us to consider whether glioma patients with IDH mutations are more sensitive to oncolytic virus therapy, providing direction for future research.

In conclusion, Ad-TD-nsIL12 is a safe agent for patients with rHGG that is connecting with the ventricles, as commonly occurs in recurrent cases after resection. Moreover, Ad-TD-nsIL12 could be administered repeatedly without safety concerns. Administration via either an Ommaya reservoir or by i.t injection were both effective and tolerable and an MTD of 1×10^{10}vp was identified. This data demonstrates the safety of both the third generation AdV vector and of a modified IL-12 cytokine for treatment of complex brain tumours. We also see a potential for Ad-TD-nsIL12 to control tumour growth and achieve therapeutic efficacy, but conclusions on efficacy are limited by the small sample size and pandemic-related restrictions to follow-ups. The data presented suggests that larger trials are warranted and that Ad-TD-nsIL12 may present a realistic treatment option for these patients.

Methods

Patients and treatment

This is a single-centre, dose-escalating trial to study safety of an oncolytic adenovirus, Ad-TD-nsIL12, in patients with rHGG that is connecting with the ventricular system. Immune and inflammatory responses induced by Ad-TD-nsIL12 were evaluated in the tumour, cerebral spinal fluid (CSF) and peripheral-blood samples of treated patients. Detailed protocol can be referred to Supplementary information.

Eligible patients were enroled between September 2020 and September 2022, aged over 18 years with a Karnofsky performance scale (KPS) score ≥ 70. Before enrolment, patients underwent a screening process, including historical confirmation of rHGG (based on IHC staining results) and confirmation of a connection between the tumour and the ventricle. Inclusion and exclusion criteria are listed in Table S1. The GMP standard Ad-TD-nsIL12 virus was provided by Beijing Bio-Targeting Therapeutics Technology Co., Ltd (BioTTT). Before treatment, enroled patients underwent stereotactic biopsy to exclude pseudo progression. This trial used 3 + 3 dose-escalating design (assigned doses: 5×10^9, 1×10^{10} and 5×10^{10}vp).

Ad-TD-nsIL12 was delivered in two ways: intratumoural injection via stereotaxis (i.t) and injection into a pre-inserted Ommaya reservoir (i.O). Delivery routes were determined based on the baseline MRI scans. I.t routes were applied to patients with an easily injectable solid mass determined by T1 gadolinium enhancing imaging. i.O was used for patients with a recurrent mass located at the wall of the resection cavity, no easily injectable solid lesion and/or deep positioning of the tumour posing a risk of stereotaxic puncture injury. Where possible (according to the patients' preference and limitations imposed by the COVID-19 pandemic), repeated injections were administered at 28 day intervals. Repetitive injections are not limited to the initial method of viral injection. After evaluation by researchers, repetitive injections can also be performed through stereotactic tumour-directed viral injection. In addition, treatment with glucocorticoids at the lowest dose required was allowed in patients showing neurological deterioration.

The 2016 WHO definition of glioblastoma was used when the trial protocol was drafted. However, IDH-mutant GBM has been classified as grade 4 astrocytoma according to the 2021 WHO guidelines. The patients enrolled in this study were classified as HGG according to the latest guidelines.

Surveillance and follow-up

At baseline, screens included medical history, vital indexes, physical examination (including nervous system), KPS score, head MRI using gadolinium containing contrast agent (GBCA) and blood tests. After Ad-TD-nsIL12 administration, the patients were closely monitored for 1–2 weeks for the occurrence of adverse events (AEs) before discharge. After discharge, patients were followed every four weeks until two months, and then every two months for two years. Collection of all AEs began from day 0 to 2 months after the last treatment. Evaluation included KPS score, vital indexes, MRI of head with or without GBCA (if available), blood tests, AEs and further medications required after the first virus injection. In addition, KPS score was also recorded. Time to disease progression (as a secondary endpoint of the trial) was not analysed in this trial because MRI could not be performed continuously to obtain an accurate time of disease progression, which might lead to bias in survival analysis.

Safety evaluation

AEs were graded using Common Terminology Criteria for Adverse Events (CTCAE, version 5.0) and recorded during both postoperative and follow-up periods by investigators. Grade ≥ 3 AEs associated with Ad-TD-nsIL12 identified the dose-limiting toxicity (DLT), assessed during the interval of virus injection and within 2 months after last virus injection. The preceding dose level was defined as the maximum tolerated dose (MTD) in this study.

Imaging assessment

Tumour response was evaluated based on the Response Assessment in Neuro-Oncology (RANO) criteria, characterised briefly as complete response (CR): no detection of enhanced area on MRI image with no use of corticosteroids and improved clinical status; partial response (PR): ≥ 50% decrease in areas of T1 gadolinium enhancement with stable or decreased corticosteroids usage and stable or improved clinical status; stable disease (SD): < 50% decrease and < 20% increase in areas of T1 gadolinium enhancement with stable or decreased corticosteroids usage and stable or improved clinical status; progressive disease (PD): increased areas of T1 gadolinium enhancement with worsened clinical status[38] Baseline MRI imaging is performed within 15 days before treatment. Subsequent imaging evaluations are conducted at the 4th, 8th, and 12th weeks after the initial treatment. Thereafter, imaging is conducted every 3 months up to one year. After one year, imaging evaluations are conducted every 6 months until two years. Additionally, if the patient undergoes any unscheduled head MRI scans, they are also included in the analysis. Researchers may request additional imaging examinations. The image assessment in this study was evaluated by two senior imaging experts who were blinded to cohort assignment.

Quantitative polymerase chain reaction (q-PCR)

Post-treatment cerebral spinal fluid (CSF) were tested using q-PCR to assess Ad-TD-nsIL12 copy numbers. Primers; Forward 5′-TGTACCGGA

GGTGATCGATC; Reverse 5′-CATCCTCGTCGTCACTGGG). DNA was extracted from whole blood using the TIANamp Genomic DNA Kit (DP304, TIANamp). The reaction was examined using the Thermo Fisher Scientific Quant Studio 6 Pro Real-time qPCR Instrument. All samples were run in triplicate.

Immunohistochemical staining

Immunohistochemistry (IHC) was performed in tumour samples collected from biopsy at baseline or surgery follow by paraffin embedding, deparaffinization, antigen retrieval, incubation with primary antibody. The following antibodies were used: a-hexon (polyclonal, AB1056, Merck, 1:1000), a-E1A (monoclonal, sc-25, Santa Cruz Biotech; 1:200), a-CD4 (4B12, CD4-368-L-CE, Leica Biosystems, 1:80), a-CD8 (C8/144B, MS-457s, Thermo-Scientific, 1:25), a-CD20 (monoclonal, M0755, DAKO, 1:200), a-CD68 (monoclonal, KP1, eBioscience, 1:200), a-CD138 (monoclonal, M7228, DAKO, 1:100), a-CD163 (monoclonal, ab182422, Abcam, 1:500), a-CD86 (monoclonal, B7-2, eBiosciences, 1:200) and a-IL12 (monoclonal, EP5737, abcam, 1:800). After incubation with biotin-labelled second antibody, the streptavidin-peroxidase complex (Dako) was added, followed by colour development and counterstaining with Hematoxylin, the sections were mounted and imaged by light microscopy (20x, Zeiss Axio Imager M2). The quantification of immunohistochemical images was conducted under 20x magnification with three replications. The quantification of immunohistochemical images was conducted under 20x magnification, with 3 random fields.

Anti-adenovirus antibody titre

To determine anti-hexon IgG titers, patient serum samples were subjected to ELISA using the Adenovirus IgG ELISA Kit (DEIA309; Creative Diagnostics) according to the manufacturer's instructions.

Luminex cytokine analysis

Cytokine assessment was performed by Laizee Biotech, using Luminex cytokines technique. The assay process followed the recommended protocol (Kit: EPX340-12167-901). Plates were read using the Luminex 200 instrument. The standard curve was fitted by five-parameter nonlinear regression to calculate the concentration value.

Ethics

This trial was approved by Ethics Committee of Sanbo Brain Hospital, Capital Medical University in December 2019 and registered in April 2020 on Chinese Clinical Trial Registry (ChiCTR2000032402). The first patient was enroled on September 29th, 2020. This study adhered to the following ethical guidelines: the World Medical Association Declaration of Helsinki (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/), the International Council for Harmonisation Good Clinical Practice guidelines (ICH-GCP) (https://ichgcp.net/zh), the Measures for the Ethical Review of Life Sciences and Medical Research Involving Humans (https://www.gov.cn/zhengce/zhengceku/2023-02/28/content_5743658.htm), and the Measures for the Ethical Review of Biomedical Research Involving Humans (https://www.gov.cn/zhengce/2016-10/12/content_5713806.htm). Prior to enrolment, written consent (approved by the Ethics Committee of Sanbo Brain Hospital) was obtained from enrolled patients or their legal representatives.

Statistical analysis

Safety. All patients receiving Ad-TD-nsIL12 were included in the safety analysis. Subjects who did not complete the study had all available data prior to termination of the study included in the safety analysis. AEs were pooled by population and dose group, and tabulated by severity, relationship to Ad-TD-nsIL12, and causation. When an AE occurred more than once, the maximum severity and causation were recorded. Concurrent disorders were noted as possible confounders in the treatment-response relationship.

Vital Signs Measurement: Vital signs measurements (blood pressure, heart rate, respiratory rate, and temperature) results were displayed in the form of a data list based on visits, dose cohorts, and time intervals (as appropriate). Summary data includes univariate statistics for mean, standard error, and median. MRI scans were evaluated by the investigator. Clinical laboratory results were descriptive for selected laboratory parameters based on population, subjects, study dates, and dose groups. The group mean, median, and standard error for each laboratory parameter were calculated.

Validity

In this study, tumour responses to treatment were assessed according to RANO criteria. The response to treatment was determined according to the size of the change from baseline imaging data. When assessing changes in tumour size, changes in clinical disease status and steroid use were considered. The size of the brain tumour was calculated using a T1 enhancement MRI scan. Changes in the Karnofsky performance scale (KPS) score was also summarised using Wilcoxon-signed rank test. All immunohistochemistry images were sampled randomly three times under a 20x objective lens. Paired *t*-test was used to compare the quantitative changes in immunohistochemical staining before and after treatment. Data analysis was performed using GraphPad Prism 10.

Reporting summary

Further information on research design is available in the Nature Portfolio Reporting Summary linked to this article.

Data availability

Due to patient privacy concerns, certain data can be shared after review by the Ethics Committee of Sanbo Brain Hospital and by contacting the corresponding author. We welcome inquiries from interested non-profit organisations. An evaluation will be conducted, and a response will be provided within two weeks of receiving the request. If approved, the duration for which the data can be used will be defined, typically within one year. The study protocol is available in the Supplementary Information file. The remaining data are available within the Article, Supplementary Information, or Source Data file. Source data are provided with this paper.

References

1. Ostrom, Q. T. et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2015–2019. *Neuro Oncol.* **24**, v1–v95 (2022).
2. Gupta, T. et al. Updated systematic review and meta-analysis of extended adjuvant temozolomide in patients with newly diagnosed glioblastoma. *Neurooncol Adv.* **5**, vdad086 (2023).
3. Segura, P. P. et al. SEOM-GEINO clinical guidelines for high-grade gliomas of adulthood (2022). *Clin. Transl. Oncol.* **25**, 2634–2646 (2023).
4. Tan, A. C. et al. Management of glioblastoma: state of the art and future directions. *CA: a Cancer J. Clinicians* **70**, 299–312 (2020).
5. Brain, G. B. D. :and C. N. S. C. C. Other, global, regional, and national burden of brain and other CNS cancer, 1990-2016 a systematic analysis for the global burden of disease study 2016. *Lancet Neurol.* **18**, 376–393 (2019).
6. Stupp, R. et al. Effect of tumor-treating fields plus maintenance temozolomide vs maintenance temozolomide alone on survival in patients with glioblastoma: a randomized clinical trial. *Jama* **318**, 2306–2316 (2017).
7. Ostrom, Q. T. et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2012–2016. *Neuro. Oncol.* **21**, v1–v100 (2019).
8. Li, J., Di, C., Mattox, A. K., Wu, L. & Adamson, D. C. The future role of personalized medicine in the treatment of glioblastoma multiforme. *Pharmgenomics Pers. Med.* **3**, 111–127 (2010).

9. Ripari, L. B. et al. Glioblastoma proximity to the lateral ventricle alters neurogenic cell populations of the subventricular zone. *Front Oncol.* **11**, 650316 (2021).
10. Wang, L., Chard Dunmall, L. S., Cheng, Z. & Wang, Y. Remodeling the tumor microenvironment by oncolytic viruses: beyond oncolysis of tumor cells for cancer treatment. *J. Immunother. Cancer* **10**, e004167 (2022).
11. Hecht, J. R. et al. A phase I/II trial of intratumoral endoscopic ultrasound injection of ONYX-015 with intravenous gemcitabine in unresectable pancreatic carcinoma. *Clin. Cancer Res.: J. Am. Assoc. Cancer Res.* **9**, 555–561 (2003).
12. Mulvihill, S. et al. Safety and feasibility of injection with an E1B-55 kDa gene-deleted, replication-selective adenovirus (ONYX-015) into primary carcinomas of the pancreas: a phase I trial. *Gene Ther.* **8**, 308–315 (2001).
13. Wang, Y. et al. E3 gene manipulations affect oncolytic adenovirus activity in immunocompetent tumor models. *Nat. Biotechnol.* **21**, 1328–1335 (2003).
14. Spurrell, E. et al. STAT1 interaction with E3-14.7K in monocytes affects the efficacy of oncolytic adenovirus. *J. Virol.* **88**, 2291–2300 (2014).
15. Fulci, G. et al. Depletion of peripheral macrophages and brain microglia increases brain tumor titers of oncolytic viruses. *Cancer Res.* **67**, 9398–9406 (2007).
16. Fueyo, J. et al. Preclinical characterization of the antiglioma activity of a tropism-enhanced adenovirus targeted to the retinoblastoma pathway. *J. Natl Cancer Inst.* **95**, 652–660 (2003).
17. Lang, F. F. et al. Phase I study of DNX-2401 (Delta-24-RGD) oncolytic adenovirus: replication and immunotherapeutic effects in recurrent malignant glioma. *J. Clin. Oncol.* **36**, 1419–1427 (2018).
18. Nassiri, F. et al. Oncolytic DNX-2401 virotherapy plus pembrolizumab in recurrent glioblastoma: a phase 1/2 trial. *Nat. Med.* **29**, 1370–1378 (2023).
19. Liu, T. C. et al. An E1B-19 kDa gene deletion mutant adenovirus demonstrates tumor necrosis factor-enhanced cancer selectivity and enhanced oncolytic potency. *Mol. Ther.* **9**, 786–803 (2004).
20. Del Vecchio, M. et al. Interleukin-12: biological properties and clinical application. *Clin. Cancer Res.* **13**, 4677–4685 (2007).
21. Motzer, R. J. et al. Phase I trial of subcutaneous recombinant human interleukin-12 in patients with advanced renal cell carcinoma. *Clin. Cancer Res.: J. Am. Assoc. Cancer Res.* **4**, 1183–1191 (1998).
22. Cohen, J. IL-12 deaths: explanation and a puzzle. *Science* **270**, 908 (1995).
23. Leonard, J. P. et al. Effects of single-dose interleukin-12 exposure on interleukin-12-associated toxicity and interferon-gamma production. *Blood* **90**, 2541–2548 (1997).
24. Wang, P. et al. Re-designing Interleukin-12 to enhance its safety and potential as an anti-tumor immunotherapeutic agent. *Nat. Commun.* **8**, 1395 (2017).
25. Zhang, Z. et al. A tumor-targeted replicating oncolytic adenovirus Ad-TD-nsIL12 as a promising therapeutic agent for human esophageal squamous cell carcinoma. *Cells* **9**, 2438 (2020).
26. Hönikl, L. S. et al. Postoperative communicating hydrocephalus following glioblastoma resection: incidence, timing and risk factors. *Front. Oncol.* **12**, 953784 (2022).
27. Li, Y. et al. Choroid plexus mast cells drive tumor-associated hydrocephalus. *Cell* **186**, 5719–5738.e28 (2023).
28. Parker, J. N., Bauer, D. F., Cody, J. J. & Markert, J. M. Oncolytic viral therapy of malignant glioma. *Neurotherapeutics* **6**, 558–569 (2009).
29. Mirlekar, B. & Pylayeva-Gupta, Y. IL-12 family cytokines in cancer and immunotherapy. *Cancers (Basel)* **13**, 167 (2021).
30. Chiocca, E. A. et al. Regulatable interleukin-12 gene therapy in patients with recurrent high-grade glioma: Results of a phase 1 trial. *Sci. Transl. Med.* **11**, eaaw5680 (2019).
31. Chiocca, E. A. et al. Combined immunotherapy with controlled interleukin-12 gene therapy and immune checkpoint blockade in recurrent glioblastoma: An open-label, multi-institutional phase I trial. *Neuro. Oncol.* **24**, 951–963 (2022).
32. Orringer, D. et al. Extent of resection in patients with glioblastoma: limiting factors, perception of resectability, and effect on survival. *J. Neurosurg.* **117**, 851–859 (2012).
33. Hauer, L. et al. Cerebrovascular manifestations of herpes simplex virus infection of the central nervous system: a systematic review. *J. Neuroinflammation* **16**, 19 (2019).
34. Wang, S. et al. Targeting M2-like tumor-associated macrophages is a potential therapeutic approach to overcome antitumor drug resistance. *npj Precis. Oncol.* **8**, 31 (2024).
35. Molenaar, R. J. et al. The combination of IDH1 mutations and MGMT methylation status predicts survival in glioblastoma better than either IDH1 or MGMT alone. *Neuro Oncol.* **16**, 1263–1273 (2014).
36. Carrillo, J. A. et al. Relationship between tumor enhancement, edema, IDH1 mutational status, MGMT promoter methylation, and survival in glioblastoma. *AJNR Am. J. Neuroradiol.* **33**, 1349–1355 (2012).
37. Wen, P. Y. et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *J. Clin. Oncol.* **28**, 1963–1972 (2010).

Acknowledgements

We would like to express our deepest gratitude to all the patients and their families who graciously participated in this study. Their invaluable contributions have made this research possible. We are also profoundly thankful to the dedicated clinical staff who played a crucial role in this trial. Their expertise and commitment are greatly appreciated. This research was supported by the National Key R&D Programme of China, (2019YFC1316104, FNL).

Author contributions

Concept and design: Y.W., S.W., F.L., and H.Z. Acquisition, analysis, or interpretation of data: X.Q. Draughting of the manuscript: X.Q., L.C.D., and Y.W. Viral intervention: X.Q., W.N., and Y.G. Clinical treatment: L.M., Y.Qu., H.W., C.G., and M.Z. Sample tests: S.Li., D.S., and D.L. Follow-ups: X.Q. and L.M. Statistical analysis: X.Q. Administrative, technical, or material support: all authors. Supervision: Y.W., S.W. and H.Z. Materials and correspondence should be addressed to Yaohe Wang, Shengdian Wang, and Hongwei, Zhang.

Competing interests

Y.W is an inventor of the patent of Ad-TD-nsIL12 (Modified interleukin 12 and its use in manufacture of a medicament for treatment of tumours, PCT/CN2016/098527). All other authors declare no conflict of interest.

Additional information

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41467-024-53041-7.

Correspondence and requests for materials should be addressed to Yaohe Wang, Shengdian Wang or Hongwei Zhang.

Peer review information *Nature Communications* thanks Rimas Lukas, Minoru Tanaka and the other, anonymous, reviewer(s) for their contribution to the peer review of this work. A peer review file is available.