Clinical safety, tolerability and preliminary efficacy of injection and liposomal honokiol treatment in patients with recurrent high-grade glioma

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Submitted to: JMIR Cancer
on: October 13, 2024

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Abstract

Background: OBJECTIVE: To observe the clinical safety, tolerability, pharmacokinetics and preliminary efficacy of lipo-HK for injection as a monotherapy in patients with recurrent high-grade glioma and to provide the basis for drug dose and method of administration.

Methods: Patients with recurrent high-grade glioma were screened according to inclusion and exclusion criteria. A total of 5 experimental dose groups were included. The initial dose was 80 mg, and the dose was escalated according to the modified Fisher method, and the highest dose was 420 mg. Each patient received only one dose of administration. This clinical trial consists of two phases - a dose escalation phase and a dose expansion phase. The dose escalation phase includes a single-dose period (1 day of single dose, followed by three days of observation), multiple-dose period (5 days of continuous dosing per week, two days off, three weeks, one week off) and multiple-dose extension periods (3 weeks of multiple doses, one week of rest, then three weeks of additional doses, each week for five consecutive days and two days off). The dose-expansion phase consists of single-dose period and multiple-dose period. If the patient tolerates during the single-dose period, they could enter the multiple-dose period. If the patient gets clinical benefits from HK under clinical evaluation after the multiple dosing period, the patient could enter the multiple extended dosing period. Clinical safety was assessed using NCI CTCAE 4.03 criteria, and RANO criteria were used to evaluate the efficacy.

Results: A total of 24 patients were enrolled in this clinical study. The clinical symptoms of many subjects improved after the drug was administered, and the clinical effects of 10 subjects were evaluated as "Stable disease" after multiple administration periods.Long-term use is well tolerated by patients.

Conclusion: lipo-HK for injection is clinically safe and well tolerated in the treatment of patients with recurrent high-grade glioma. The recommended dose for subsequent studies is 420 mg.

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(JMIR Preprints 13/10/2024:67502)
DOI: https://doi.org/10.2196/preprints.67502

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Clinical safety, tolerability and preliminary efficacy of injection and liposomal honokiol treatment in patients with recurrent high-grade glioma

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Innovative introduction: Honokiol has a variety of pharmacological activities, its anti-tumor activity makes it a potential natural anti-tumor drug. It is an intravenous drug formed by honokiol, which overcomes the disadvantages of poor solubility and low oral bioavailability. There is a lack of clinical studies of this drug in recurrent high-grade glioma. In this study, the protocol for injection liposomal honokiol(Lip-HNK) to treat patients with recurrent grade glioma.

Abstract

OBJECTIVE: To observe the clinical safety, tolerability, pharmacokinetics and preliminary efficacy of lipo-HK for injection as a monotherapy in patients with recurrent high-grade glioma and to provide the basis for drug dose and method of administration.
Methods: Patients with recurrent high-grade glioma were screened according to inclusion and exclusion criteria. A total of 5 experimental dose groups were included. The initial dose was 80 mg, and the dose was escalated according to the modified Fisher method, and the highest dose was 420 mg. Each patient received only one dose of administration. This clinical trial consists of two phases - a dose escalation phase and a dose expansion phase. The dose escalation phase includes a single-dose period (1 day of single dose, followed by three days of observation), multiple-dose period (5 days of continuous dosing per week, two days off, three weeks, one week off) and multiple-dose extension periods (3 weeks of multiple doses, one week of rest, then three weeks of additional doses, each week for five consecutive days and two days off). The dose-expansion phase consists of single-dose period and multiple-dose period. If the patient tolerates during the single-dose period, they could enter the multiple-dose period. If the patient gets clinical benefits from HK under clinical evaluation after the multiple dosing period, the patient could enter the multiple extended dosing period. Clinical safety was assessed using NCI CTCAE 4.03 criteria, and RANO criteria were used to evaluate the efficacy.
Results: A total of 24 patients were enrolled in this clinical study. The clinical symptoms of many subjects improved after the drug was administered, and the clinical effects of 10 subjects were evaluated as "Stable disease" after multiple administration periods.Long-term use is well tolerated by patients.
Conclusion: lipo-HK for injection is clinically safe and well tolerated in the treatment of patients with recurrent high-grade glioma. The recommended dose for subsequent studies is 420 mg.
Keyword: Lip-HNK, Recurrent high-grade glioma, Clinical trail

Introduction

Gliomas are the most common primary intracranial malignancy, accounting for 35.2%~61.0% of malignant brain tumors, with an annual incidence of about 3-8/100,000 population (1, 2). Among them, glioblastoma is the most common glioma (about 45% of all gliomas) and the subtype with the worst prognosis, with a 5-year survival rate of only 4.7% (3, 4). Although the incidence of glioma is not the highest in the population, its extremely poor prognosis and high mortality make new and effective drugs and treatment options urgent (5). However, the presence of the blood-brain barrier (Blood Brain Barrier, BBB) and the blood-cerebrospinal fluid barrier (Blood-Cerebrospinal Fluid Barrier, BCSFB) greatly reduces the efficacy of most conventional and targeted therapies because most drugs can not pass BBB and BCSFB or achieve effective intracranial concentrations due to the presence of both barriers (6).

Lip-HNK is made from hydroponol from the plant (Magnolia officinalis Rehd. et Wils.) (7). It was extracted, purified, and sterile prepared by high purity, honokiol and liposome packaging. Lyophilized powder for injection and lipo-HK were administered by intravenous infusion, overcoming the disadvantages of free and poor solubility and low oral bioavailability, and greatly improving the bioavailability of honokiol. Many studies at home and abroad have illustrated that honokiol plays an active anti-tumor effect through various mechanisms: (1) inducing cell cycle arrest of tumor cell, thus inhibiting tumor cell proliferation; (2) inducing tumor cell apoptosis through the mitochondrial pathway and apoptosis receptor pathway; and (3) reducing tumor cell metastasis by inhibiting angiogenesis and lymphangiogenesis. And the molecular mechanism of the anti-tumor effect of honokiol induced apoptosis by acting on GRP 78 and then apoptosis. It also inhibited the binding of HSP 90 and its client protein and promoted the degradation of HSP 90 client protein. EGF-α/Akt, c-RAF and Akt all belong to HSP 90 client protein, thus inhibiting EGF-α/Akt and EGF-α/ Erk signaling pathway and playing an anti-tumor role (8, 9). HIF-α is also a client protein of HSP 90, and honokiol affects the neovascularization by affecting the autocrine regulation of HIF- α on VEGF-α(10).

Materials and methods

Inclusion and exclusion criteria

Subjects meeting the following criteria may be included in the trial: 1.18 to 80 years, Gender is not limited; 2. If confirmed by histology or cytology, patients with recurrent high-grade glioma who have failed the previous standard treatment regimen and who have failed to tolerate or reject existing therapies; 3. The presence of evaluable tumor lesions according to the RANO criteria; 4. No severe hematopoietic abnormalities (absolute neutrophil value 1.5x109 / L, Platets 80x109 / L, Hemoglobin 100g / L); 5. No organic severe lesions in the heart, lung, liver and kidney (LVEF (left ventricular ejection fraction) 50%; Total bilirubin: 1.5 ULN; Gluamic aminotransferase (ALT) 2.5 ULN (5 ULN if liver metastasis); Grain grass aminotransferase (AST) 2.5 ULN (5 ULN for liver metastasis); Serum creatinine 1.5 ULN or CCr> 40 mL/min); 6. No severe coagulation function abnormalities (PT 1.5 ULN, APTT 1.5 ULN, TT 1.5 ULN); 7. Before enrollment, at least four weeks after the last antitumor therapy (chemotherapy, radiation therapy, biological therapy, hormonal therapy and targeted therapy); 8. Expected survival time of 12 weeks; 9. The KPS score was> 50 points; 10. Those who agreed to participate in this study signed the informed consent form.

Subjects with any of the following were not included in the trial: 1. The toxicity of previous anticancer therapy did not return to grade I or below, or had not fully recovered from the previous surgery; 2. Patients with previous or concurrent patients of other malignant tumors, except for skin basal cell carcinoma or cervical carcinoma in situ that has been cured for more than five years; 3. Pregnant or lactating female patients; 4. Refuse of contraception for fertile women / men during the trial; 5. Serious complications and underlying diseases, such as gastrointestinal bleeding, intestinal obstruction, intestinal paralysis, interstitial pneumonia, pulmonary fibrosis, renal failure,

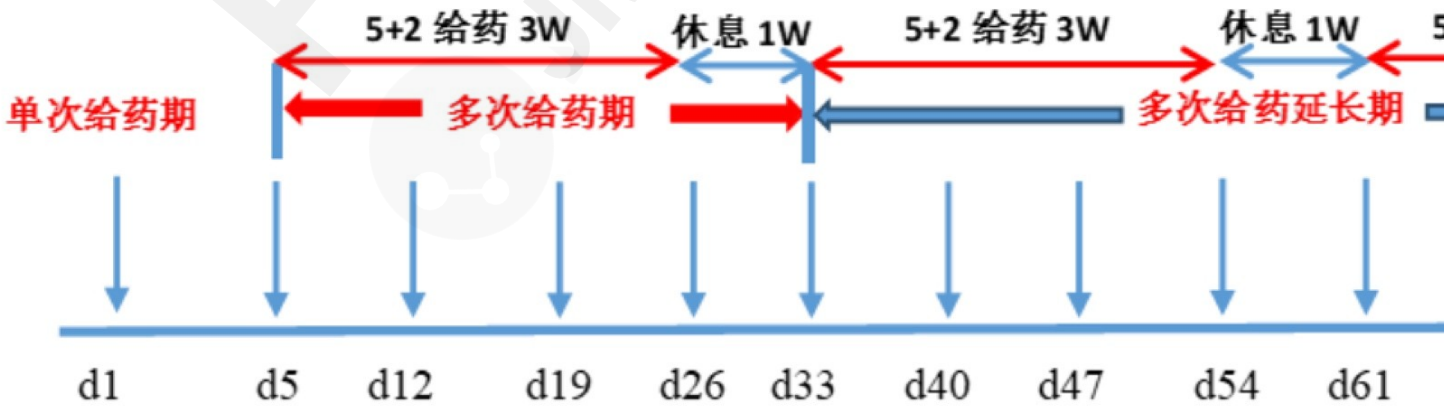
uncontrollable diabetes, etc.; 6. History of acute myocardial infarction, unstable angina, or stroke within the six months prior to inclusion, Congestive heart failure with the New York Society of Cardiology (NYHA) grade 2 or above (including grade 2); 7. Mental disorders; 8. With a history of immunodeficiency, including: HIV positive, having other acquired or congenital immunodeficiency diseases, or having a history of organ transplantation; 9. HBsAg or HBcAb positive individuals, peripheral blood HBV DNA titer detection of 1103 IU / mL, and the HCV-positive patients; 10. There are contraindications to PICC catheterization (e. g., no suitable puncture vessel for catheter catheterization; Infection or injury at the puncture site; History of trauma, vascular surgery, radiotherapy, and venous thrombosis; Superior vena cava compression syndrome, etc.), the patient fails to complete the PICC catheterization, or the failure of the PICC catheterization; 11. Alcohol, hormone dependence, or drug abuse; 12. Allergic to this product or related ingredients; 13. Those who have participated in other drug clinical trials in the past month; 14. Other factors considered by the investigator to participate in the trial.

Subjects may withdraw from this clinical study at any time without any reason. The subject may withdraw from the trial if (1) proven tumor progression at any time, (2) intolerable toxicity, and (3) the subject unprescribed other treatments; the subject is considered unsuitable for continuation. If the decision is due to serious adverse events or clinically apparent abnormal laboratory values, the study treatment should be discontinued and appropriate treatment measures should be taken.

Research design

The subjects of this clinical study were patients with recurrent high-grade glioma. The study was conducted in two phases, including the dose escalation and expansion phase. The dose escalation phase includes a single dose period (1 day of single dose, Then observe it for three days, To ensure that the patient is not allergic to the drug), multiple doses (5 consecutive days per week, followed by two days off the medication, for a duration of three weeks, with one week off before resuming.) and multiple administrations (3 weeks for multiple administration, followed by one week off. Subsequently, another cycle of three weeks of weekly administrations was carried out, with each weekly administration lasting five days, followed by two days off.); Each subject received only one dose; The dose extension phase consisted of multiple dose periods and multiple dose extension, in order to evaluate the clinical safety, tolerability, and preliminary efficacy of Lipo-HK.(The dosing stage is in Table 1.)

Table 1: Schematic diagram of the dosing stage



This clinical study underwent dose escalation according to the modified Fibonacci method, initially

preset 7 test dose groups. Doses of 20mg and 40mg have been proven safe and tolerable in other advanced solid tumors. Considering the presence of the blood-brain barrier to achieve effective drug concentrations in the skull, the study started at 80mg, with 3-6 subjects in each group. For each dose group, after the dose-limiting toxicity (Dose Limited Toxicity, DLT) in the first subject, two subjects were enrolled; 3 subjects had all DLT observed in the next dose group or new subjects in this dose group. If none of the three subjects in a dose group had DLT, process to the next dose group. If one out of the three subjects in a dose group had DLT, 3 subjects in this dose group. If the increased 3 subjects had no DLT, escalated to the next dose group. If the increased 3 subjects had 1 DLT (i. e. 2 total number of DLT patients), the previous dose was determined as the maximum tolerated dose (Maximal Tolerance Dose, MTD). If two out of the three subjects in a dose group developed a DLT, the previous dose of this dose was determined to be the MTD.

This product is white or white lipid-like lyophilized powder containing 20mg of honokiol, developed by the State Key Laboratory of Biological Therapy of Sichuan University, Chengdu Jinrui Foundation Biotechnology Co., Ltd., which provides drug and drug test certificates. Each bottle of Lipo-HK lyophilized powder was dissolved with about 10 mL of sterilized injection water at room temperature, fully shaken until completely dissolved, and dilute the liquid with 5% glucose solution to the administered volume. Infusion should be completed within 6h after the preparation. The drug was administered by peripheral central venous puncture, and the dose volume was 250 ml. The infusion time was about 2h. The actual infusion time was recorded, and the administration concentration, administration speed, administration time and administration volume were adjusted according to the safety and tolerability of the subjects.

Subject information and medical history were collected

During the screening period, all subjects required to sign informed consent. Additionally, pathological histological examinations are conducted, and demographic data, medical history, and treatment history are collected. The following data should be collected and recorded during the screening period, single dose period, multiple administration period, multiple dose extension period, trial end or withdrawal: blood sample collection, physical examination, vital signs, KPS score, laboratory examination (blood routine, urine routine, blood biochemistry, coagulation function), cerebrospinal fluid examination, 12-lead electrocardiogram, myocardial enzyme spectrum examination, cardiac abnormalities such as pain, palpitations or echocardiography as required by the investigator.

Safety evaluation

The NCI CTCAE 4.03 criteria were used in this clinical study.

Main safety evaluation indicators: the incidence and composition of post-treatment adverse events, adverse reactions and serious adverse events. Safety assessments include vital signs, physical examination, KPS physical strength scores, laboratory tests (blood routine, urine routine, blood biochemistry, coagulation function), electrocardiogram, myocardial enzyme spectrum, echocardiography, and observation and assessment of early withdrawal for safety or tolerability reasons. All patients treated with the study drug have been evaluated for tolerability and safety assessment. Safety information was collected until 28 days after the last dose.

Effectiveness evaluation

The effect of HK in subjects with recurrent high-grade glioma was assessed by RANO criteria. The evaluation time points were before subject administration, after multiple doses and after multiple administrations, and when the subject had a tendency or manifestation of disease progression. Efficacy was assessed by enhanced MR imaging of the head. Efficacy confirmation should be performed four weeks after efficacy assessment in subjects with first disease remission (CR / PR). The efficacy evaluation measures were progression-free survival (Progression-Free-Survival, PFS) and disease control rate (Disease Control Rate, DCR). In this clinical study, PFS was defined as the time from the start of medication to tumor progression or death; DCR was the proportion of patients whose tumor shrank or stabilized for a certain period of time, including cases with complete response

(CR), partial response (PR), and stable (SD). (See Table 2 below for the RANO criteria.)

Table 2. The RANO criteria

	Complete Response (CR)	Partial Response (PR)	Disease stability (SD)
Focal MRI IT 1 enhanced image	not have	Reduce oneself 50%	Reduce by <50% increase by <25%
Focal MRI IT 2 enhanced images or FLAIR images	Stabilize or reduce	Stabilize or reduce	Stabilize or reduce
New onset lesions	not have	not have	not have
hormone	not have	Stabilize or reduce	Stabilize or reduce
clinlcal symptom	Stabilize or improve	Stabilize or improve	Stabilize or improve

Note: a the imaging change of the lesion refers to the product of the vertical diameter at the maximum cross-sectional area, and the sum of the product of the multilesion; b is the disease progression, but the increase in hormone dosage cannot be used solely as the basis of disease progression (RANO is the evaluation of the nerve sac).

Quality of life assessment

Quality of life was assessed using the KPS physical status scoring criteria (see Table 3 below). KPS score is the Karnofsky (Ka, KPS, percentage) functional state score criteria. The higher the score, the better the health, and the more the body can tolerate the side effects of treatment, and thus it is possible to receive thorough treatment. It is generally considered that Karnofsky 80 score above is a non-dependent level (independent), that is, self-care level. 50~70 is divided into semi-dependent level (semi-independent), that is, semi-self-care. Below 50 is the dependency level (dependent), where life needs help from others. Those with more than 80 had better postoperative status and longer survival. This trial intends to include subjects with KPS score above 70, indicating the ability to perform basic activities independently.

Table 3 Functional status scores of the KPS

grade	Physical condition
100 Points	Ability to move normally without symptoms of discomfort or disease
90 Points	Ability to perform normal activities, with mild symptoms
80 Points	Can arely for normal activities. There are some medical symptoms or physic
70 Points	Life can take care of themselves, but can not maintain normal activities or a
60 Points	Occasionally need the help of others in life, but most of the time can take ca
50 Points	Life is often need the help of others, or frequent medical care
40 Points	They cannot take care of themselves and need special help and care
30 Points	Life is completely can not take care of themselves, should be hospitalized death
20 Points	Must be hospitalized, become seriously ill, and require active supportive car
10 Points	Critical condition, near death
0 Points	die

Related ethics

Clinical studies for injection and park lipo-HK strictly complied with the Declaration of Helsinki (the Declaration of Helsinki) and the ethical guidelines for medical research in human subjects, and the trial protocol was implemented after approval by the Medical Ethics Committee of the Clinical Trial Unit.

Results

Basic subject characteristics

As of November 2021, 24 subjects were included in this clinical study, and the basic information is shown in Table 4 below.

Of the 24 enrolled subjects, 15 were male (62.5% of the total) and 9 were female (37.5% of the total). The age is 26-59 years, and the average age is about 43.54 years, which is basically consistent with the age of glioma onset. Among the included subjects with recurrent high-grade gliomas, 50% each had pathological grades of WHO III and WHO V, and all WHO grade IV subjects had recurrent glioblastoma pathology. In the early stage of dose escalation, the patients were mainly other advanced malignant tumors, and the safety of the drug was confirmed. This clinical study mainly started with the injection of lipo-HK 80mg (1 case), 140mg(2 cases), 210mg (3 cases), 300mg (6 cases), 420mg (6 cases). Almost all subjects (95.83%, 23/24) successfully completed the multiple administration period, and some subjects (41.67%, 10/24) entered the multiple administration extension period as "clinically stable" after the completion of the multiple administration period.

Adverse event

Adverse event (Adverse Event, AE) is an adverse medical event after a patient or a clinical trial subject, but is not necessarily causally related to treatment. An adverse event can be any adverse and non-intended sign (including abnormal laboratory findings), symptoms or illness related to drug use (study), regardless of whether the drug is related. It includes, but is not limited to: (1) exacerbation of pre-existing disease prior to the use of the study drug; (2) increased frequency or severity of pre-existing episodes prior to the use of the study drug; (3) abnormal changes identified or diagnosed after the use of the study drug, although such abnormal changes may have existed before treatment; (4) exacerbation of disease or symptoms persisting prior to the start of the study.

10 of 24 subjects in the five dose groups had adverse events (Table 5), for a total of 27 episodes and an incidence of 41.67%. Among all the adverse events, combined with the study protocol, after the investigator comprehensively analyzed the specific conditions of the adverse events and the subjects, the previous medical history, incidence and concomitant medication of the subjects, only five subjects had 19 adverse events in the treatment period, which may be related to the experimental drug, with an incidence of 30.83%. In conclusion, the incidence of adverse reactions to intravenous administration of lipo-HK for injection is low.

Table 5 Summary table of adverse events

	80mg N=1			140mg N=2			210mg N=3			300mg N=6			420mg N=12			amount to N=24		
project	example count	example Times	incidence %	example count	example Times	incidence %	example count	example Times	incidence %	example count	example Times	incidence %	example count	example Times	incidence %	example count	example Times	incidence %
TEAE	1	1	100.0	1	1	50.0	1	2	33.3	4	13	66.7	3	10	25.0	10	27	41.67
Adverse reactions during the treatment period	1	1	25.0	0	0	0	0	0	0	1	8	16.7	3	10	25.0	5	19	30.83

A TEAE of grade 3	0	0	0	0	0	0	0	0	0	1	1	16.7	1	1	8.3	2	2	8.3
Grade 3 adverse effects	0	0	0	0	0	0	0	0	0	1	1	16.7	1	1	8.3	2	2	8.3
stage of therapy	0	0	0	0	0	0	0	0	0	0	0	0	1	1	8.3	1	1	4.17
SAE																		
Serious adverse reactions during the treatment period	0	0	0	0	0	0	0	0	0	0	0	0	1	1	8.3	1	1	4.17
Leading to a reduced dose of TEAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
TEAEs leading to pause	0	0	0	0	0	0	0	0	0	1	5	16.7	0	0	0	1	5	4.17
TEAEs leading to a permanent discontinuation	0	0	0	0	0	0	0	0	0	0	0	0	1	1	8.3	1	1	4.17
TEAEs that led to withdrawal from the trial	0	0	0	0	0	0	0	0	0	0	0	0	1	1	8.3	1	1	4.17
A TEAE leading to the death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

All adverse events (Table 2) include: 1. physical and sensory discomfort: facial numbness, hip pain, pain in upper abdomen, dyspnea, etc.; 2. abnormal physiological function: diarrhea, constipation, etc. 3. abnormal blood routine (decreased white blood cells, red blood cells, hemoglobin, platelets, etc.), biochemical abnormalities (increased alanine aminotransferase, aspartate aminotransferase, γ -glutamyltransferase), abnormal coagulation function (blood fibrinogen reduction), weight gain, etc. Treatment period adverse reactions mainly involve all kinds of adverse reactions, including abnormal blood routine (mainly for bone marrow transplantation: white blood cells, red blood cells, hemoglobin, platelets, etc.), abnormal liver function (including alanine aminotransferase, aspartate aminotransferase, γ -glutamyltransferase), weight gain, difficulty breathing. The adverse reactions in the treatment period of each dose group were mostly grades 1 to 2, all of which were mild. There was no need to stop or change the treatment dose. After the relevant symptomatic treatment, the subjects could generally achieve complete remission. The more serious adverse reaction was an elevation of alanine aminotransferase and a DLT (grade CTCAE 3). After giving the relevant symptomatic treatment after withdrawal, the relevant indicators returned to normal. The most serious adverse effect was that another subject presented with dyspnea during the medication, and the subject was completely relieved after the withdrawal and, therefore, withdrew from the clinical study. There were no suspected or unexpected serious adverse reactions. In conclusion, the types of adverse reactions for injection and lipo-HK are few and mild, and they can be completely relieved with relevant symptomatic treatment.

This clinical study proveds that the treatment of patients with recurrent high-grade glioma using lipo-HK has a low incidence of adverse events and few types of adverse events, mainly focusing on hematology and liver function abnormalities. The elevation of alanine aminotransferase was a DLT for injection and lipo-HKs. All possible adverse reactions associated with medication were mild and resolved with relevant symptomatic treatment without withdrawal or dose change. These findings provide evidence that injection lipo-HK were safe and well tolerated by subjects.

Anti-tumor effect

As of 2023.07.02, of the 24 subjects included in the study, 10 subjects had stable disease after multiple dosing period (Stable Disease, SD), 41.67% of the total number of subjects, and had multiple extended dosing periods (see Schedule 3). In terms of clinical symptoms, 1 of 24 subjects withdrew from clinical experiments, and the remaining 23 subjects, 3 (12.5%) improved, 17 (70.83%) were stable and 3 (12.5%) were aggravated. Initially, the efficacy of patients with injection and lipo-HK for recurrent high-grade glioma was observed.

From the perspective of each dose group, with the increase of dose, the proportion of SD evaluated in relapsed high-grade glioma subjects increased significantly, and showed a certain dose-dependent trend. In the 300mg and 420mg dose groups, the percentage of patients with stable disease (SD) was 33% and 50%, respectively, and the percentage of patients with stable disease (SD) was 33% and 40%, respectively (Table 4), which showed a significant volume-effect relationship, further confirming the efficacy of Lipo-HK for recurrent high-grade gliomas, especially recurrent glioblastomas, as the drug dose increased, patient benefit may be higher.

Table 4. Basic Information of the subjects

Basic information table		
sex	man	15
	woman	9
Tumor grade	III	12
	IV	12
Drug dose grouping	80mg	1
	140mg	2
	210mg	3

	300mg	6
	420mg	12
age	average age	43.54
	age-bracket	26-59

For subjects enrolled before April 2020 (subsequent subjects have short enrollment time and survival data to further mature), the median survival has reached more than 24 months and the longest survival is 80 months. In subjects with recurrent glioblastoma, the median survival had reached 18 months, and the longest survival was 27.9 months. In contrast, historical data indicate that the median survival of patients with recurrent glioblastoma is 7-9 months [1]. In contrast to previous data, injectable and lipo-HK greatly prolonged patient survival in this clinical study.

Some of the enrolled subjects will significantly benefit after entering the dosing period. The following are some typical subjects before and after medication:

304 Subject: male, 55 years old. May 2018 Head magnetic resonance: right temporoparietal space occupying lesion. In August 2005, the subject underwent intracranial space resection with postoperative pathology: anaplastic astrocytoma (grade WHO III) and local glioblastoma change (grade WHO IV). Postoperatively, subjects received concurrent chemoradiotherapy and six cycles of standard temozolomide oral chemotherapy. In June 2019, the subject showed tumor progression. Subjects were enrolled in this clinical study at the 80mg dose group in July 2019. The patient was assessed as having stable disease (SD) following both single administration and multiple doses. Subsequently, they successfully completed multiple extended administrations with the best efficacy evaluation for SD. Currently OS 24 months +. Figure 1 shows the magnetic resonance imaging of the head during the enrolled medication. Note in the magnetic resonance imaging of the head at baseline, 30 days of treatment, 78 days, SD.

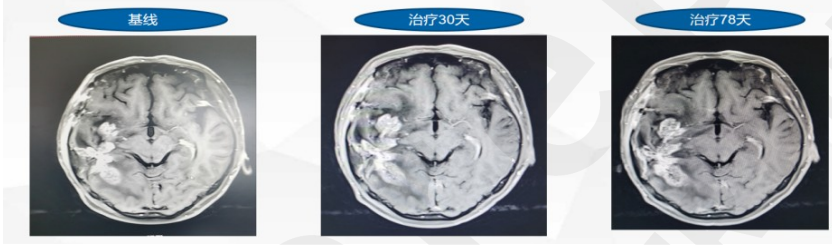


Figure 1 304 Head MR findings during medication

402 Subject: female, 45 years old. June 2016 head magnetic resonance showed: right parietal space occupying lesion. On June 29, 2016, postoperative pathology: metaobcell tumor (grade WHO III). Postoperatively, the subjects received concurrent chemoradiotherapy and 6 cycles of oral chemotherapy with temozolomide. In April 2018, the subject's head magnetic resonance review indicated tumor progression and underwent tumor recurrence surgery. On April 24, 2018, postoperative pathology: high-grade glioma, grade III-IV, anaplastic astrocytoma, and gliosis. Postoperatively, the subjects received 12 cycles of oral chemotherapy with temozolomide. On Aug 16, 2019, the head MRI indicated tumor progression. On August 30, 2019, the subject was enrolled in the 140mg dose group of this clinical study and successfully completed multiple extended doses with the best efficacy evaluation SD. Currently OS 23 months +. Figure 2 shows the head MR imaging of the subjects at baseline, 35 days, 79 days, and 139 days, without significant lesion enlargement or new lesions.

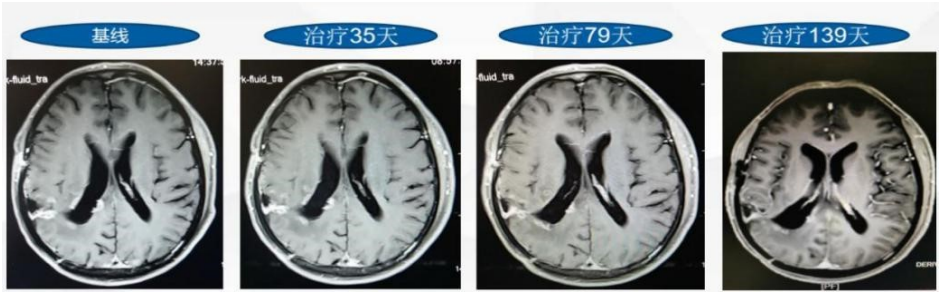


Figure 2 402 Head MR findings during medication

605 Subject: female, 44 years old, underwent craniotomy for intracranial space-occupying lesion on March 10, 2020, postoperative pathology: glioblastoma (grade WHO IV). The subject started concurrent chemoradiotherapy on 09, 2020 with oral temozolomide (75mg / m2 / day) on June 4, 2020 and ended treatment on June 5, 2020. Later subjects started temozolomide chemotherapy combined with allotinib plus piperacillin, which ended on July 14, 2020. On August 16, 2020, the subject reviewed the head MRI to consider tumor recurrence. On August 20, 2020, the clinical study received 300mg dose, successfully completed the evaluation SD after single dose and multiple doses, and successfully completed multiple extended doses without new clinical symptoms, and the best efficacy evaluation SD. Current subject has OS for eight months. Figure 3 shows the magnetic resonance imaging of the head at baseline, 33, 69 and 124 days of treatment, with no significant enlargement or new lesions.

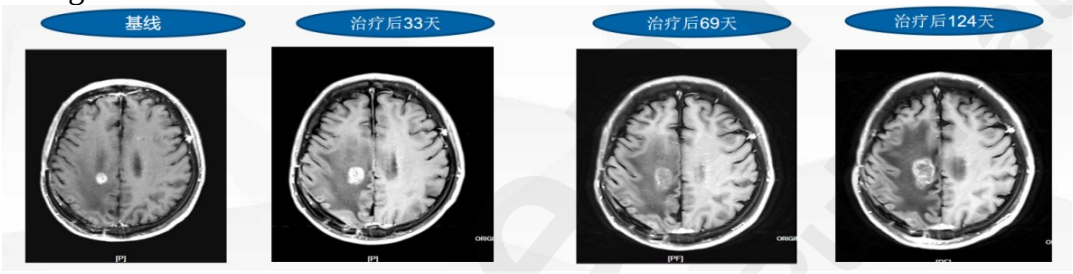


Figure 3 605 Head MR findings during medication

606 Subject: Male, 59 years old, left parietal craniotomy resection on July 13, 2018, postoperative pathology: anaplastic oligodendroglioma (grade WHO I II). The Subjects underwent concurrent chemoradiotherapy from 30 July 2018 to 4 September 2018,4100 CGY / 24 times / 32 days and temozolomide 100mg / day / 32 days. 6 cycles of adjuvant chemotherapy with temozolomide 5 / 28 regimen between October 2018 and March 2019. Since then, it has been reviewed regularly, and the head MRI review on August 7, 2020 showed new lesions compared with the left ventricle in April 23, 2019. For further treatment, the patient was admitted to the clinical study at 420mg dose on September 1, 2020, successfully completed single dose and multiple doses, and successfully completed multiple extended doses without new clinical symptoms, and the best efficacy evaluation SD. Current subject OS 29.9 months. Figure 4 shows the magnetic resonance imaging of the head at baseline, 33 days and 69 days of treatment, and there was no significant enlargement or new lesions.

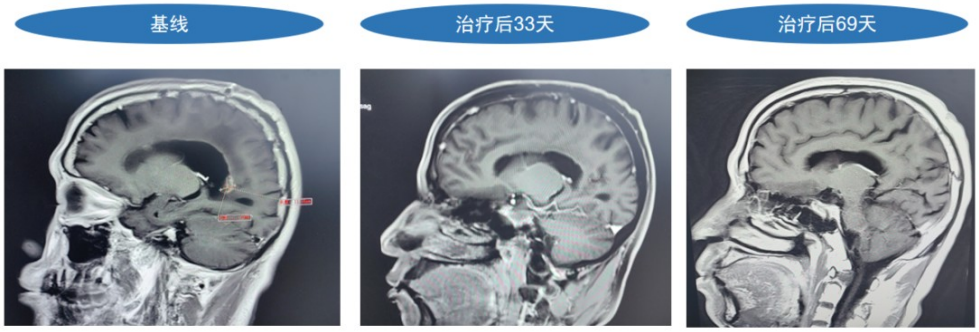


Figure 4 606 Head MR findings during medication

703 Subject: male, 55 years old, received antiepileptic treatment in March 2011 due to no right upper limb jitter and seizure speech. In July 2013, symptoms were progressive and frontopetal tumor resection was performed in July 2013. Postoperative pathology: oligodendroglioma (grade WHO II). After tumor recurred in August 2018, resection of left frontal tumor was performed on September 14, 2018, postoperative pathology: oligodendroglioma (grade WHO III), the subject underwent whole-brain simultaneous radiotherapy and chemotherapy from October 24, 2018 to December 04, 2018, 30 times, DT: 6000 cGy, temozolomide 140mg * 38. From January 2019 to October 2020, adjuvant chemotherapy in 5 / 28 on November 28, 2020, October 2020 entered the clinical study at 420mg dose on December 4, 2020, successfully completed single dose and multiple dose evaluation SD, and successfully completed multiple extended dose without new clinical symptoms, and the best efficacy evaluation SD. Current subject OS 26.7 months. Figure 5 shows the magnetic resonance imaging of the head at baseline, 33 days and 69 days of treatment, and there was no significant enlargement or new lesions.

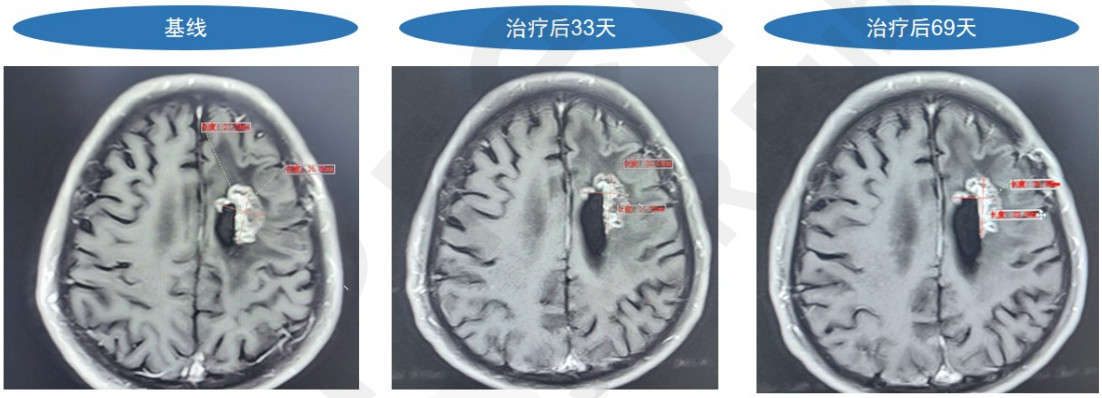


Figure 5 703 Head MR findings during medication

704 Subject: male, 57 years old, underwent the resection of the left occipital lobe lesion on December 31, 2019, postoperative pathology: glioblastoma (grade WHO IV). The subject underwent concurrent chemoradiotherapy ten times from 10-02-2020-20-25 / 03 / 2020 with temozolomide unknown at 30 doses. Six cycles of 5 / 28 days of temozolomide were followed by adjuvant chemotherapy at a dose of 150 – 200mg / m2/ sky. Post-regular review. On October 20, 2020, MRI of the brain showed a significant increase in the range of annular enhancement in the left temporal-occipital area, with obvious peripheral edema. Tumor resection + artificial dural repair was performed on November 6, 2020. One cycle of adjuvant chemotherapy with cisplatin plus temozolomide was given on 04 December 2020 (ended on December 11, 2020). In order for further treatment, the 420mg dose on January 14, 2021 successfully completed single dose and multiple dose evaluation SD, and successfully completed multiple extended doses without new clinical symptoms and efficacy evaluation SD. Current subject OS 29.5 months. Figure 6 shows the magnetic resonance

images of the head at baseline, 33 and 69 days of treatment, with no significant enlargement or new lesions of the original lesions.

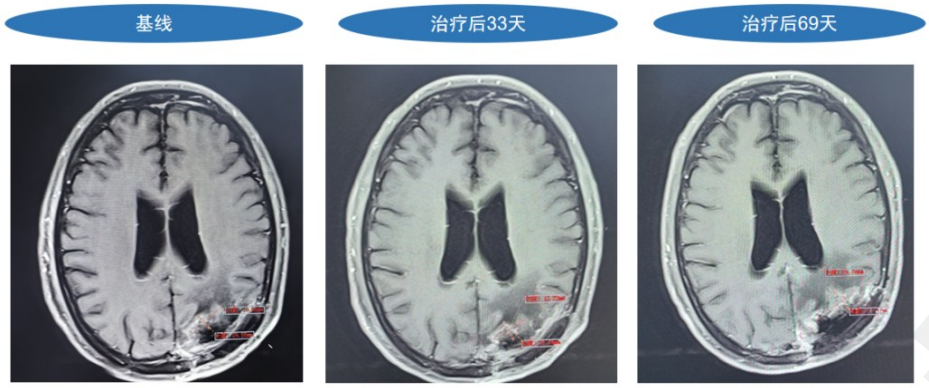


Figure 6 704 Head MR findings during medication

707 Subject: Male, 36 years old, underwent left frontal lobe tumor resection on September 13, 2020, with residual postoperative disease, postoperative pathology: glioblastoma (Grade WHO IV). The subject underwent head chemoradiation (total dose 60 Gy) from October 9, 2020 to November 19, 2020, Tmozolomide 100mg * 42 days, Postoperative two cycles of adjuvant chemotherapy with temozolomide 5 / 28 regimen, End of the last dose on January 18, 2021, Head MRI review on January 6, 2021: after left frontal glioma, Abnormal enhancement, Considering the recurrence, For further treatment, The 420mg dose was enrolled in this clinical study on March 4, 2021, SD after single dose and multiple doses, And successfully completed multiple extended doses, No new-onset clinical symptoms, therapeutic evaluation SD. Current subject has OS 12.6 months. Figure 7 shows the magnetic resonance images of the head at baseline, 33,69 and 124 days of treatment, with no significant enlargement or new lesions of the original lesions.

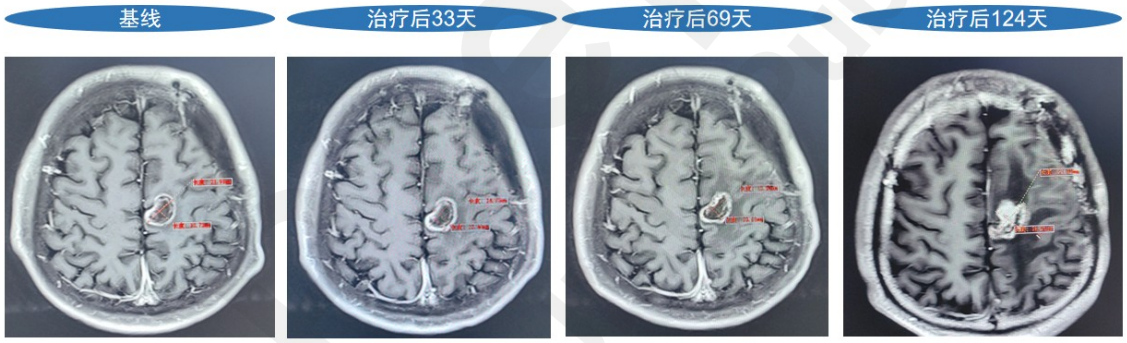


Figure 7 707 Head MR findings during medication

709 Subject: female, 45 years old, performed on January 23, 2018, right frontal craniotomy tumor resection, residual, postoperative pathology: anaplastic oligodendroglioma (grade WHO III). The subject received postoperative oral temozolomide concurrent radiotherapy on March 26, 2018, 42 times, dose unknown, concurrent oral temozolomide chemotherapy at a dose of 120mg / day, followed by 4 cycles of temozolomide chemotherapy at 340mg in June 2018 and ended at the end of September 2018. He was followed up and reviewed on March 4,2020, considering tumor recurrence. On April 15, 2020, bevacizumab injection 300mg with oral chemotherapy with temozolomide. On September 27, 2020, varizumab injection 300mg was combined with oral chemotherapy with temozolomide. On March 8,2021, the clinical study received 420mg dose, successfully completed the evaluation SD after single dose and multiple administration, and successfully completed multiple extended doses without new clinical symptoms, and efficacy evaluation SD. Current subject OS 23.6 months. Figure 8 shows the magnetic resonance images of the head at baseline, 33 and 69 days of treatment, with no significant enlargement or new lesions.

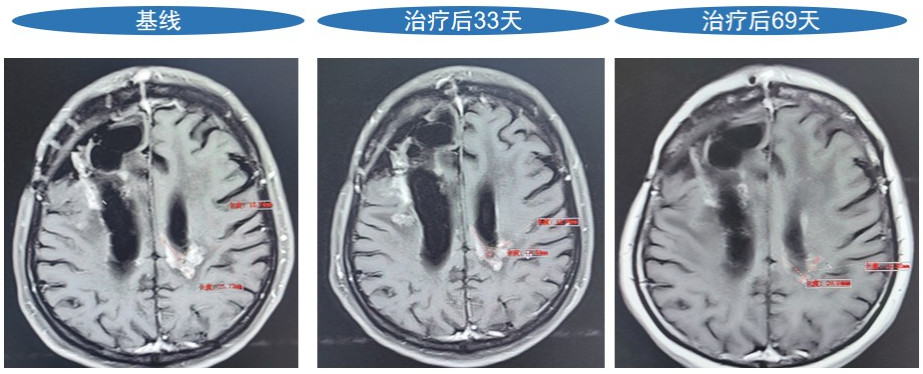


Figure 8 709 Head MR findings during medication

711 Subject: 39-year-old female, treated on 11 March 2019, left temporoparietal tumor resection, residual, postoperative pathology: diffuse astrocytoma (grade WHO II). The subject underwent whole brain concurrent chemoradiotherapy from April 3, 2019 to 11 May 2019, 30 times, DT: 6000 cGy, temozolomide 100mg * 38 to August 2020, adjuvant chemotherapy, tumor recurrence on November 25, 2019 and September 3, 2020, September 2020, adjuvant chemotherapy with cisplatin plus temozolomide for one cycle, temozolomide chemotherapy on October 16, 2020. On November 24, 2020, December 30, 2020, February 2, 2021, with increased symptoms after two weeks. For further treatment, the 420mg dose on April 19, 2021 successfully completed evaluation SD after single dose and multiple doses, and successfully completed multiple extended doses without new clinical symptoms and efficacy evaluation SD. Current subject has OS 22.3 months. Figure 9 shows the magnetic resonance imaging of the head at baseline, 33 days and 124 days of treatment, with no significant enlargement or new lesions

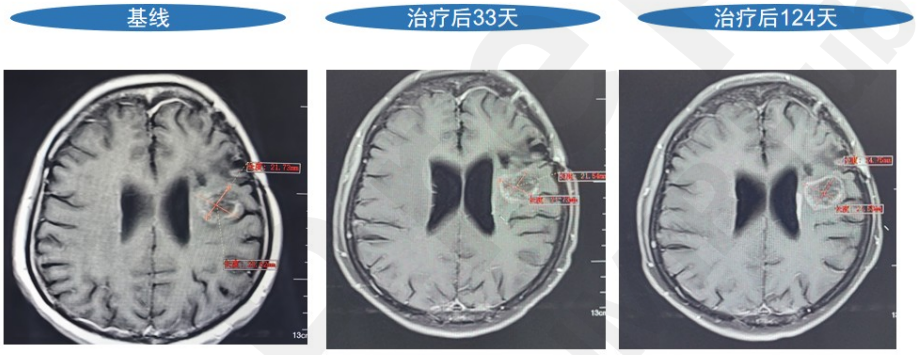


Figure 9 711 Head MR findings during medication

714 Subject: male, 34 years old, developed vomiting in August 2017 without systematic treatment, the above symptoms appeared again on October 8, 2017, right temporal occipital craniotomy tumor resection was performed on November 7, 2017, postoperative pathology: anaplastic oligodendroastrocytoma (grade WHO I-II), without systematic examination and chemotherapy thereafter. On May 8, 2021, for further treatment, the clinical study was enrolled on May 24, 2021, 420mg dose, single administration and multiple administration evaluation SD, and successfully completed multiple extended administration without new clinical symptoms, efficacy evaluation SD. Current subject has OS 15.4 months. Figure X shows the head MR images of the subjects at baseline and 30 days of treatment, with no significant enlargement or new lesions

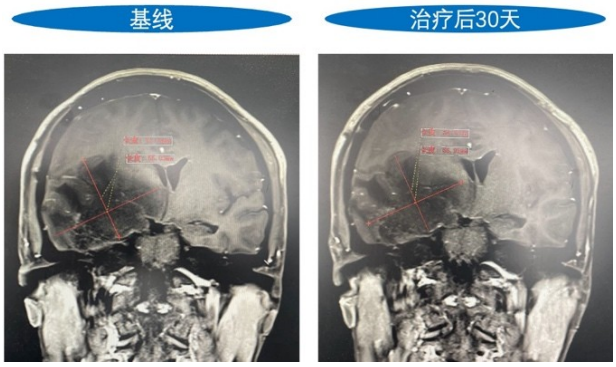


Figure 10 714 Head MR findings during medication

Quality of life score

To ensure that subjects had essentially tolerable body traits, all subjects had a KPS score greater than 80, indicating their ability to function independently.. After the completion of this clinical study, the reevaluation of the subjects showed that all their KPS scores were greater than 60, and their life was not significantly affected, and they were basically self-care. This further indicates good safety of injection lipo-HK.

The results of other studies

Of the enrolled subjects, two subjects (one each from the 140mg and 300mg dose groups) exhibited likely radiation-induced damage to the scalp and temporal hair follicles. After the administration, two subjects observed hair growth in the cranial roof and temporal areas and continued to grow in the later follow-up, with no hair drop. This phenomenon suggests that the lipo-HK may promote the regeneration of damaged hair follicles and promote hair growth after radiotherapy.

Discussion

Of all primary CNS malignancies, brain gliomas have the highest prevalence, accounting for about half of all intracranial malignancies. Among them, glioblastoma is the most common malignancy and the worst prognosis subtype, and the median survival of such patients is approximately 7-9 months. For primary glioma, the standard treatment plan includes surgery, radiotherapy, chemotherapy and a series of means. Among them, radiotherapy and chemotherapy can be adjusted in the use period and dosage according to the situation of patients, including neoadjuvant therapy (preoperative chemoradiotherapy), concurrent chemoradiotherapy, etc., so as to achieve the best therapeutic effect. For patients with brain glioma, the recurrence of the disease is often found in the regular review after the initial treatment. Epidemiology has found that glioma recurrence is very common, and the recurrence rate is even as high as 75% -90%, which means that almost all patients with high-grade glioma will face tumor recurrence (11). Some patients with glioma undergo secondary resection after recurrence, and the postoperative pathological hint is higher pathological grade than the first detection, usually high-grade glioma, namely WHO III to IV, which also means that glioma will have a worse prognosis after recurrence.

No effective method has yet been found for the treatment of recurrent glioma. Surgery, radiotherapy, and chemotherapy as standard treatments are difficult to apply to patients with recurrent glioma. First, it is limited by non-resectable recurrence sites. Secondly, for some patients with recurrent glioma, the disease advances rapidly and their own condition is poor, so the patients cannot tolerate complete radiotherapy and chemotherapy. Therefore, for patients with recurrent high-grade glioma, the national comprehensive cancer network (National Comprehensive Cancer Network, NCCN) guidelines first recommended in clinical trials, which can not only provide patients with a full range of treatment care, may also through clinical trials for current common treatment benefits, the most ideal is to extend the patients with PFS and OS.

Many clinical trials of recurrent high-grade gliomas are ongoing or will be conducted. Studies have

demonstrated that temozolomide therapy is effective despite initial treatment with temozolomide at initial treatment (5). A European randomized phase II clinical trial demonstrated a significantly higher proportion of 6-month progression-free survival in patients treated with temozolomide compared with those with methylazine, but only 1.5 months (12). In recent years, targeted therapy in multiple clinical trials has demonstrated that anti-angiogenic targeted therapy does not significantly affect progression-free survival and overall survival in either newly diagnosed glioma or recurrent glioma (13). It can be seen that most of the clinical trials for recurrent high-grade glioma, whether new attempts of chemotherapy drugs, or targeted therapy including immunotherapy, the outcomes have been suboptimal. This highlights the urgent need for new therapeutic agents and approaches in clinical practice.

Honokiol (3,5-di- (2-propenyl) -1,1-biphenyl-2,2-diol) is a naturally active drug derived from the deciduous or concave leaf trees in the Magnoliaceae. Previous basic experiments have proved that honokiol has anti-tumor activity, and its inhibitory effects are mainly by inhibiting cell proliferation, inducing apoptosis, antiangiogenesis, inhibiting tumor cell invasion, causing tumor autophagy, and regulating immune cells in the tumor microenvironment of glioma. This scope of action involves many tumors, including lymphoma, head and neck tumors, lung cancer, liver cancer, thyroid cancer, glioma, chondrosarcoma, colorectal cancer, ovarian cancer, breast cancer, gastric cancer and so on. Previous in vitro studies proved that the liposome had significant inhibition of the growth of human glioma U251 cells in vitro, and the IC50 of half inhibition rate (48h) was between 20 μ M and 80 μ M.

Further research has discovered that it is possible to effectively inhibit the growth of brain tumors in both rat 9L brain glial sarcoma models and human U251 xenograft glioma models. This inhibition occurs through the blood-brain barrier (BBB) and blood cerebrospinal fluid barrier (BCSFB), which provides its into the intracranial inhibition of intracranial malignancy (14). However, animal experiments showed that oral and honokiol monomers were poorly absorbed in the gastrointestinal tract, and the oral bioavailability was low, while the drug curves after intravenous injection and honokiol monomers complied with the three-chamber open model, which was characterized by rapid distribution and rapid decrease of blood concentration (15). However, the lipo-HK prepared by liposome wrapping cleverly makes up for the deficiencies such as low oral bioavailability, poor solubility and rapid decrease of blood concentration of honokiol monomer, and improves the body tolerance. In vivo pharmacodynamic test, human U251 glioma cells were used for subcutaneous tumor model, indicated the tumor volume decreased (50.21%), accompanied by a improvement in survival outcomes. At the same time, previous toxicological studies found that there was no significant toxicity in the heart and central nervous system. In addition, no adverse reactions of the drug were found by genotoxicity or reproductive toxicity. These findings indicates a good safety profile for injection and lipo-HKs.

Based on the above research basis, we carried out this clinical study. In order to obtain more accurate test results, all the KPS scores of the subjects included in the study were above 80, indicating the subjects could basically take care of themselves. In the course of all 24 subjects, most patients completed all dosing periods. In all subjects, only one DLT occurred with elevated ALT, but the index normalized after withdrawal and symptomatic treatment. Only one subject experienced dyspnea during medication, achieved complete remission after withdrawal, and was withdrawn from the clinical study. All remaining adverse events were mild and could recover after the dosing period. There were no suspected or unexpected serious adverse reactions. In conclusion, the adverse effects in the treatment of patients with recurrent high-grade glioma are safe and mild.

Based on the specificity of CNS tumors, this trial used the RANO criteria to evaluate the efficacy of lesion MRI T 1 augmentation, lesion MRI T 2 augmentation or FLAIR imaging, the presence of new lesions, and the hormones and symptoms. In this trial, 10 of all 24 subjects (41.67%) were rated as stable disease according to the RANO criteria. In terms of clinical symptoms, 20 subjects (83.3%) were above stable, three subjects were improved, and three subjects had worse clinical symptoms.

Although not all patients with stable clinical symptoms achieved the expected stable results in the final overall evaluation, evaluation of the subjects with stable clinical symptoms also benefited from the characteristics of glioma patients with rapid progression and poor quality of life.

It should be noted that the proportion of the subjects of rated SD increased with the dose increase, with 33% and 50% in the 300mg and 420mg dose groups, respectively. This phenomenon was more pronounced in subjects with recurrent glioblastoma, where the percentage of SD cases was 33% and 40% in the 300mg and 420mg dose groups, respectively. In the case of many current gliomas, especially recurrent glioblastoma, this is an encouraging outcome.

The subjects included in this clinical study all had a KPS score above 80 points, which enabled the subjects to tolerate the relevant treatment after enrollment better. However, after completing the relevant dosing stage, the subjects' KPS physical status score was above 60, and they were basically able to take care of themselves. As is well known, chemotherapy drugs will not only have a certain inhibitory effect on tumor drugs, but also have a certain effect on normal cells, which is often manifested as a toxic effect on normal physiological cells. Therefore, after the completion of anti-tumor treatment, most patients' normal physiological activities will be affected to a certain extent, and they cannot even take care of themselves in serious cases. In this clinical study, there was no significant decrease in KPS score before and after the treatment, which indicates that injection and polol liposome did not affect the physical condition of the subjects and had good safety. However, this also needs to be confirmed with more sample sizes.

In addition, as a clinical study of anti-tumor drugs, it is hoped to explore the effective concentration of Lipo-HK in addition to evaluating the safety, tolerability and efficacy of Lipo-HK. With the increasing dose of Lipo-HK, the anti-tumor effect was continuously strengthened. However, to obtain the best effective action concentration of Lipo-HK, it needs to be proved by expanding the patient sample size in the subsequent clinical trials.

In addition to the above main observations, subjects underwent CSF collection before and within 10min after the end of the single dose period. This was done to explore the drug concentration of the drug in the CSF and evaluate the ability of Lipo-HK to pass through BBB in order to guide clinical medication. The results proved that a certain concentration of honokiol could be detected in CSF, but no certain relationship of concentration. This suggests that the Lipo-HK have the ability to pass through the BBB. However, considering the possible multiple injuries brought by multiple lumbar punctures, the collection of CSF is limited by the collection time and frequency, and the real and effective penetration effect of HK cannot be obtained, which requires more specimen collection.

In this study, there were unexpected findings besides the above results. In the group of 140mg and 300mg, respectively, two patients regained hair in the area of the roof and temporal region, and continued to grow in the later follow-up. This suggests that Lipo-HK may promote hair follicle regeneration injured by radiotherapy. Based on this observation, we induced damage to hairless skin post-radiotherapy, and observed the regrowth of mice after intravenous Lipo-HK, indicating that Lipo-HK can promote the regeneration of injured hair follicles after radiotherapy. The specific mechanism of action needs further discovery in subsequent basic experiments.

Conclusion

Lipo-HK treatment in patients with recurrent high-grade glioma had few and mild adverse effects, with elevation of alanine transferase identified as the dose-limiting toxicity, which could be completely relieved by drug withdrawal and related symptomatic therapy. Overall, the treatment was clinically safe and well tolerated. total of 24 patients with recurrent high-grade glioma were enrolled, and ten subjects had stable disease efficacy; in clinical evaluation, 20 subjects had stable clinical evaluation or above, and three subjects had improved clinical evaluation. Among all the subjects, there were 12 subjects with recurrent glioblastoma. Of these, four subjects had stable efficacy evaluation disease, 11 subjects had stable clinical evaluation or above, and one subject showed

improved clinical evaluation and showed a certain quantitative-effect relationship. Injection and magnopolol liposomes have certain efficacy on recurrent high-grade gliomas, especially recurrent glioblastoma. Specific drug effects will need to be demonstrated in additional clinical trials. The recommended dose for subsequent studies is 420mg. Simultaneous injection lipo-HK may be able to promote the regeneration of injured hair follicles after radiotherapy, which still needs further experimental demonstration.

Acknowledgements

The authors thank all the patients and the personnel involved in this study.

Conflict of Interest

The authors declare no conflicts of interest.

Author Contributions

author's name	sex	education background	professional title	Contribution in this study
Wang Ce	famale	Master	Physician-in-charge	Observation of cases, data collection, sorting and statistics, data sorting and analysis results, writing and revising the paper
Wen-bin Li	male	Doctor	Professor	Guided the study, reviewed the collation and analysis results of the data, and guided the writing and revision of the paper

Data availability

All data in this study are included in the main or supplementary materials. The raw data are available from the corresponding author upon request.

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Table 1: Schematic diagram of the dosing stage

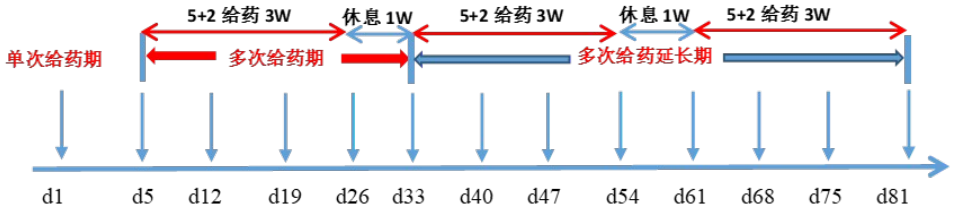

Table 2. The RANO criteria

	Complete Response (CR)	Partial Response (PR)	Disease stability (SD)
Focal MRI IT 1 enhanced image	not have	Reduce oneself 50%	Reduce by <50% increase by <25%
Focal MRI IT 2 enhanced images or FLAIR images	Stabilize or reduce	Stabilize or reduce	Stabilize or reduce
New onset lesions	not have	not have	not have
hormone	not have	Stabilize or reduce	Stabilize or reduce
clinlcal symptom	Stabilize or improve	Stabilize or improve	Stabilize or improve

Note: a the imaging change of the lesion refers to the product of the vertical diameter at the maximum cross-sectional area, and the sum of the product of the multilesion; b is the disease progression, but the increase in hormone dosage cannot be used solely as the basis of disease progression (RANO is the evaluation of the nerve sac).

Table 3 Functional status scores of the KPS

grade	Physical condition
100 Points	Ability to move normally without symptoms of discomfort or disease
90 Points	Ability to perform normal activities, with mild symptoms
80 Points	Can arely for normal activities. There are some medical symptoms or physic
70 Points	Life can take care of themselves, but can not maintain normal activities or a
60 Points	Occasionally need the help of others in life, but most of the time can take ca
50 Points	Life is often need the help of others, or frequent medical care
40 Points	They cannot take care of themselves and need special help and care
30 Points	Life is completely can not take care of themselves, should be hospitalized death
20 Points	Must be hospitalized, become seriously ill, and require active supportive car
10 Points	Critical condition, near death
0 Points	die

Table 4. Basic Information of the subjects

Basic information table		
sex	man	15
	woman	9
Tumor grade	III	12
	IV	12
Drug dose grouping	80mg	1
	140mg	2
	210mg	3
	300mg	6
	420mg	12

age	average age	43.54
	age-bracket	26-59

Table 5 Summary table of adverse events

	80mg N=1			140mg N=2			210mg N=3			300mg N=6			420mg N=12			amount to N=24		
project	example count	example Times	incidence %	example count	example Times	incidence %	example count	example Times	incidence %	example count	example Times	incidence %	example count	example Times	incidence %	example count	example Times	incidence %
TEAE	1	1	100.0	1	1	50.00	1	2	33.30	4	13	66.7	3	10	25.0	10	27	41.67
Adverse reactions during the treatment period	1	1	25.0	0	0	0	0	0	0	1	8	16.7	3	10	25.0	5	19	30.83
A TEAE of grade 3	0	0	0	0	0	0	0	0	0	1	1	16.7	1	1	8.3	2	2	8.3
Grade 3 adverse effects	0	0	0	0	0	0	0	0	0	1	1	16.7	1	1	8.3	2	2	8.3
stage of therapy	0	0	0	0	0	0	0	0	0	0	0	0	1	1	8.3	1	1	4.17
SAE																		
Serious adverse reactions during the treatment period	0	0	0	0	0	0	0	0	0	0	0	0	1	1	8.3	1	1	4.17
Leading to a reduced dose of TEAE	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

	80mg N=1			140mg N=2			210mg N=3			300mg N=6			420mg N=12			amount to N=24		
project	example count	example Times	incidence %	example count	example Times	incidence %	example count	example Times	incidence %	example count	example Times	incidence %	example count	example Times	incidence %	example count	example Times	incidence %
TEAEs leading to pause	0	0	0	0	0	0	0	0	0	1	5	16.7	0	0	0	1	5	4.17
TEAEs leading to a permanent discontinuation	0	0	0	0	0	0	0	0	0	0	0	0	1	1	8.3	1	1	4.17
TEAEs that led to withdrawal from the trial	0	0	0	0	0	0	0	0	0	0	0	0	1	1	8.3	1	1	4.17
A TEAE leading to the death	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Appendix

Schedule 1

Dose-limiting toxicity definition One of the first dose occurred related to the study drug from the end of Day 15 (D 1 to D 19): Grade 4 neutropenia; Granulocytopenic fever (defined as absolute neutrophil value [ANC] <1000 / mm3With fever body temperature greater than 38.3℃ or body temperature continuously higher than more than 38℃ 1 h); Grade 3 neutropenia with evidence of infection; Thrombocytopenia of grade 3 or above; Any grade 3 or above non-hematologic toxicity. Except for Nausea, vomiting, and hair loss; The patient had normal bilirubin, liver transaminase or alkaline phosphatase at baseline and grade 3 during DLT observation; patients with liver metastases had liver transaminase 6 ULN with bilirubin grade 2 or above (including grade 2) during DLT observation.

Schedule 2 List of adverse events

Subjects number	Research Center No	Dose group	Adverse event name	In the stage	PT	SOC	date commenced	order of severity / CTC	Whether the rapeutic intervention	Measures related to the study drug	Association with the study drug	whether or not SAE	Transfer type	Date of return (if death is recorded as date of death)	Exit from the trial due to an adverse event
304	03	80mg	Falling fibrinogen	Extension period with multiple administration	Blood fibrinogen was decreased	All kinds of inspection	2019-09-30	Level 1	deny	The dose is unchanged	Probably about	deny	complete remission	2019-10-07	deny
403	03	140mg	anemia	Multiple dosing periods	anemia	Blood and lymphatic system disorders	2019-10-25	Level 1	deny	The dose is unchanged	Certainly irrelevant	deny	unknown		deny
501	03	210mg	Facial numbness	Screening period	hypaesthesia	Various kinds of neurological diseases	2019-11-14		deny	The dose is unchanged	Certainly irrelevant	deny	complete remission	2019-11-14	deny
501	03	210mg	Rump pain	Screening period	Bone muscle pain	Various musculoskeletal and connective tissue disorders	2019-11-14		deny	The dose is unchanged	Certainly irrelevant	deny	complete remission	2019-11-14	deny
601	03	300mg	stomachache	A single dosing period	epigastric pain	Gastrointestinal system diseases	2020-04-23		deny	The dose is unchanged		deny	complete remission	2020-04-23	deny

Subjects number	Research Center No	Dose group	Adverse event name	In the stage	PT	SOC	date commenced	order of severity / CTC	Whether the rapeutic intervention	Measures related to the study drug	Association with the study drug	whether or not SAE	Transfer type	Date of return (if death is recorded as date of death)	Exit from the trial due to an adverse event
603	03	300 mg	ALT up	go	Multiple dosing periods	Alanine aminotransferase was elevated	All kinds of inspection	2020-06-29	Level 1	deny	The dose is unchanged	Probably about	deny	make or become heavier		deny
603	03	300 mg	ALT up	go	Multiple dosing periods	Alanine aminotransferase was elevated	All kinds of inspection	2020-07-02	Level 3	yes	Suspension of medication	Probably about	deny	partial remission	2020-07-06	deny
603	03	300 mg	ALT up	go	Multiple dosing periods Extension period with multiple administration	Alanine aminotransferase was elevated	All kinds of inspection	2020-07-06	Level 2	yes	Suspension of medication	Probably about	deny	partial remission	2020-07-13	deny
603	03	300 mg	ALT up	go	Extension period with multiple administration	Alanine aminotransferase was elevated	All kinds of inspection	2020-07-10	Level 2	yes	Suspension of medication	Probably about	deny	partial remission	2020-07-13	deny
603	03	300 mg	ALT up	go	Extension period with multiple administration	Alanine aminotransferase was elevated	All kinds of inspection	2020-07-13	Level 1	yes	Suspension of medication	Probably about	deny	partial remission	2020-07-16	deny

Subjects number	Research Center No	Dose group	Adverse event name	In the stage	PT	SOC	date commenced	order of severity / CTC	Whether the rapeutic intervention	Measures related to the study drug	Association with the study drug	whether or not SAE	Transfer type	Date of return (if death is recorded as date of death)	Exit from the trial due to an adverse event
603	03	300 mg	ALT up	go The drug period was administered multiple times	Alanine aminotransferase was elevated	All kinds of inspection	2020-07-01	Level 2	deny	The dose is unchanged	Probably about	deny	make or become heavier		deny
603	03	300 mg	AST up	go Multiple dosing periods	Raised in the aspartate aminotransferase	All kinds of inspection	2020-06-29	Level 1	deny	The dose is unchanged	Probably about	deny	non-remission		deny
603	03	300 mg	GGT up	go Multiple dosing periods	Elevated γ-glutamyltransferase	All kinds of inspection	2020-07-06	Level 1	deny	Suspension of medication	Probably about	deny	non-remission		deny
605	03	300 mg	astriction	Multiple dosing periods	astriction	Gastrointestinal system diseases	2020-08-27	Level 1	yes	The dose is unchanged	It may not matter	deny	complete remission	2020-08-27	deny

Subjects number	Research Center No	Dose group	Adverse event name	In the stage	PT	SOC	date commenced	order of severity / CTC	Whether the rapeutic intervention	Measures related to the study drug	Association with the study drug	whether or not SAE	Transfer type	Date of return (if death is recorded as date of death)	Exit from the trial due to an adverse event
605	03	300 mg	astriction	Extension period with multiple administration	astriction	Gastrointestinal system diseases	2020-10-20	Level 1	yes	The dose is unchanged	It may not matter	deny	complete remission	2020-10-20	deny
605	03	300 mg	diarrhoea	Multiple dosing periods	diarrhoea	Gastrointestinal system diseases	2020-09-02	Level 1	yes	The dose is unchanged	It may not matter	deny	complete remission	2020-09-02	deny
606	03	300 mg	diarrhoea	Extension period with multiple administration	diarrhoea	Gastrointestinal system diseases	2020-10-27	Level 1	yes	The dose is unchanged	Certainly irrelevant	deny	complete remission	2020-10-28	deny
701	03	420 mg	expiratory dyspnea	A single dosing period	expiratory dyspnea	Respiratory system, thoracic, and mediastinal diseases	2020-10-15	Level 4	yes	Permanent withdrawal of drugs	Probably about	yes	complete remission	2020-10-15	yes

Subjects number	Research Center No	Dose group	Adverse event name	In the stage	PT	SOC	date commenced	order of severity / CTC	Whether the rapeutic intervention	Measures related to the study drug	Association with the study drug	whether or not SAE	Transfer type	Date of return (if death is recorded as date of death)	Exit from the trial due to an adverse event
703	03	420 mg	gain weight	Extension period with multiple administration	gain weight	All kinds of inspection	2021-02-22	Level 1	deny	The dose is unchanged	Probably about	deny	non-remission		deny
704	03	420 mg	White blood cell reduction	Multiple dosing periods	White blood cell count was decreased	All kinds of inspection	2021-02-08	Level 1	deny	The dose is unchanged	Probably about	deny	non-remission		deny
704	03	420 mg	Red blood cells were reduced	Multiple dosing periods	The red blood cell count was decreased	All kinds of inspection	2021-02-08	Level 1		The dose is unchanged	Probably about	deny			deny
704	03	420 mg	The hematocrit was decreased	Multiple dosing periods	The hematocrit was decreased	All kinds of inspection	2021-02-08	Level 1		The dose is unchanged	Probably about	deny			deny
704	03	420 mg	Lymphocytes were decreased	Multiple dosing periods	The lymphocyte count was decreased	All kinds of inspection	2021-02-08	Level 1		The dose is unchanged	Probably about	deny			deny

Subjects number	Research Center No	Dose group	Adverse event name	In the stage	PT	SOC	date commenced	order of severity / CTC	Whether the rapeutic intervention	Measures related to the study drug	Association with the study drug	whether or not SAE	Transfer type	Date of return (if death is recorded as date of death)	Exit from the trial due to an adverse event
704	03	420 mg	Mean hemoglobin concentration per liter of red blood cells	Multiple dosing periods	Mean cellular hemoglobin concentration	All kinds of inspection	2021-02-08	Level 1		The dose is unchanged	Probably about	deny			deny
704	03	420 mg	The hemoglobin was reduced	Multiple dosing periods	The hemoglobin was reduced	All kinds of inspection	2021-02-08	Level 1	deny	The dose is unchanged	Probably about	deny	complete remission	2021-02-10	deny
704	03	420 mg	Platelet reduction	Multiple dosing periods	Platelet count was decreased	All kinds of inspection	2021-02-08	Level 1		The dose is unchanged	Probably about	deny			deny
704	03	420 mg	Lower neutrophils	Multiple dosing periods	Neutrophil count was decreased	All kinds of inspection	2021-02-08	Level 1		The dose is unchanged	Probably about	deny			deny

Schedule 3 efficacy data in recurrent high-grade glioma

	Dose group	ss number	age	sex	single-dose	Tumor type	WHO classify	Multiple dosing periods Effectiveness evaluation	Extension period with multiple administration Effectiveness evaluation	clinlcal symptom appraise	OS (By the end of July)	The proportion of SD cases
1	80mg	304	55	man	On July 25,2019	Glioma brain	4	SD	SD	take a turn for the better	27.6	100%（1/1）
2	140mg	402	45	woman	On September 5,2019	Glioma brain	3	SD	PD	take a turn for the better	26.2	50%（1/2）
3	140mg	403	45	man	On October 24,2019	Glioma brain	4	PD	—	make or become heavier	10.6	
4	210mg	501	54	man	On November 14,2019	Glioma brain	4	PD	—	stabilize	23.9	
5	210mg	504	36	man	On April 16,2020	Glioma brain	4	PD	—	take a turn for the better	3.0	0（0/3）
6	210mg	505	29	man	On May 7,2020	Glioma brain	3	PD	—	stabilize	6.3	
7	300mg	601	26	woman	On April 23,2020	Glioma brain	4	PD	—	stabilize	5.5	
8	300mg	602	43	man	On May 14,2020	Glioma brain	4	PD	—	stabilize	17.9	
9	300mg	603	54	man	On June 18,2020	Glioma brain	3	PD	—	make or become heavier	16.8	33%（2/6）
10	1300mg	604	49	man	On July 30,2020	Glioma brain	3	PD	—	make or become heavier	6.1	
11	1300mg	605	44	woman	On August 20,2020	Glioma brain	4	SD	SD	stabilize	8.4	
12	1300mg	606	59	man	On September 3,2020	Glioma brain	3	SD	SD	stabilize	14.3	
13	1420mg	701	45	woman	On October 15,2020	Glioma brain	3	—	—	—	12.9	
14	1420mg	703	55	man	On December 10,2020	Glioma brain	3	SD	SD	stabilize	11.1	
15	1420mg	704	57	man	On January 14,2021	Glioma brain	4	SD	Not to the evaluation time	stabilize	9.9	
16	1420mg	705	39	woman	On January 14,2021	Glioma brain	4	PD	—	stabilize	9.6	
17	1420	70	5	ma	On March	Glio	4	PD		stabilize	8.2	

Schedule 4 Efficacy data for subjects with recurrent glioblastoma

	Dose group	ss number	age	sex	single-dose	Tumor type	WHO classify	Multiple dosing periods Effectiveness evaluation	Extension period with multiple administration Effectiveness evaluation	clinlcal symptom apprais e	OS	The proportion of SD cases
1	80mg	304	55	man	On July 25,2019	glioblastoma	4	SD	SD	take a turn for the better	27.6	100%（1/1）
2	140mg	403	45	man	On October 24,2019	glioblastoma	4	PD	—	make or become heavier	10.6	0（0/1）
3	210mg	501	54	man	On November 14,2019	glioblastoma	4	PD	—	stabiliz e	23.9	
4	210mg	504	36	man	On April 16,2020	glioblastoma	4	PD	—	take a turn for the better	3.0	0（0/2）
5	210mg	601	26	woman	On April 23,2020	glioblastoma	4	PD	—	stabiliz e	5.5	
6	300mg	602	43	man	On May 14,2020	glioblastoma	4	PD	—	stabiliz e	17.9	33%（1/3）
7	300mg	605	44	woman	On August 20,2020	glioblastoma	4	SD	SD	stabiliz e	8.4	
8	420mg	704	57	man	On January 14,2021	glioblastoma	4	SD	SD	stabiliz e	9.9	
9	420mg	705	39	woman	On January 14,2021	glioblastoma	4	PD	—	stabiliz e	9.6	
10	420mg	706	56	man	On March 4,2021	glioblastoma	4	PD	—	stabiliz e	8.3	40%（2/5）
11	420mg	707	36	man	On March 4,2021	glioblastoma	4	SD	SD	stabiliz e	8.3	
12	420mg	713	33	man	On May 13,2021	glioblastoma	4	PD	—	stabiliz e	5.0	

Supplementary Files