ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

ESMO OPEN SCIENCE FOR OPTIMAL CANCER CARE

ORIGINAL ARTICLE

First-in-human phase I study of hypoxia-targeting ^{64}Cu-ATSM radioligand therapy in patients with malignant brain tumors (STAR-64)

H. Kurihara[1*], Y. Narita[2], K. Ito[3], H. Sato[4], Y. Miyakita[2], M. Takahashi[2†], M. Ohno[2], S. Yanagisawa[2], N. T. Okita[5], R. Sadachi[5], R. Machida[5], K. Tateishi[6], Y. Yoshii[7*], A. Waki[8], H. Hashimoto[8], H. Suzuki[8], M.-R. Zhang[8], K. Kawamura[8], N. Honda[9], M. Yoshimoto[10] & H. Matsumoto[11]

[1]Diagnostic and Interventional Radiology, Kanagawa Cancer Center Hospital, Yokohama; [2]Neurosurgery and Neuro-Oncology, National Cancer Center Hospital, Tokyo; [3]Diagnostic Radiology, National Cancer Center Hospital, Tokyo; [4]Neurosurgery, Kanagawa Cancer Center Hospital, Yokohama; [5]Clinical Research Support Office, National Cancer Center Hospital, Tokyo; [6]Neurosurgery, Yokohama City University, Yokohama; [7]LinqMed Inc., Tokyo; [8]National Institutes for Quantum Science and Technology, Chiba; [9]Pharmacy, National Cancer Center Hospital, Tokyo; [10]Exploratory Oncology Research & Clinical Trial Center, National Cancer Center East, Chiba; [11]Isotope Science Center, The University of Tokyo, Tokyo, Japan

Available online xxx

Background: Hypoxic microenvironments throughout tumors contribute to proliferation and resistance to chemoradiotherapy. Copper-64 diacetyl-bis(N^4-methylthiosemicarbazone) (^{64}Cu-ATSM) is a first-in-class drug enabling hypoxia-targeting radioligand therapy with β^- particles and high-linear energy transfer Auger electrons, originally employed for positron emission tomography imaging. We report the first-in-human study of ^{64}Cu-ATSM therapy in patients with recurrent brain tumors.

Patients and methods: This phase I dose-escalation trial (STAR-64, jRCT2091220362) evaluated intravenous ^{64}Cu-ATSM administered four times at 30, 60, 99, or 150 MBq/kg weekly in patients with recurrent malignant brain tumors, using the 3 + 3 design. Primary objectives were to assess dose-limiting toxicities (DLTs) and determine the maximum tolerated dose (MTD). Secondary objectives included efficacy, pharmacokinetics, and organ dosimetry.

Results: Eighteen patients received ^{64}Cu-ATSM: grade 3 glioma (anaplastic astrocytoma and anaplastic oligodendroglioma) 27.8%, grade 4 glioma (glioblastoma) 50%, malignant meningioma 11.1%, and metastatic brain tumors 11.1%. DLTs, consisting only of grade 3 lymphopenia, were observed in four patients: one at 60 MBq/kg, one at 99 MBq/kg, and two at 150 MBq/kg. The MTD was established as 99 MBq/kg. Two patients had a radiographic complete response by RECIST v1.1 with long-term survival: $\geq$45 months for a patient with anaplastic oligodendroglioma (60 MBq/kg) and $\geq$23 months for a patient with glioblastoma (99 MBq/kg). The 1-year and median overall survival (OS) were 76.6% and 29.4 months, respectively, in all 18 patients; 12 patients (66.7%) were long-term survivors of $\geq$12 months. Among nine patients with glioblastoma, the 1-year and median OS were 64.8% and 17.7 months, respectively, suggesting favorable preliminary outcomes compared with historical references. Pharmacokinetic and dosimetry analyses revealed a clinically favorable distribution and organ radiation doses of ^{64}Cu-ATSM, supporting the selected dosing regimen and schedule.

Conclusions: ^{64}Cu-ATSM therapy was well tolerated and demonstrated preliminary efficacy in patients with recurrent malignant brain tumors, particularly glioblastoma, supporting further evaluation in an ongoing phase III study (STEP-64, jRCT2031240090).

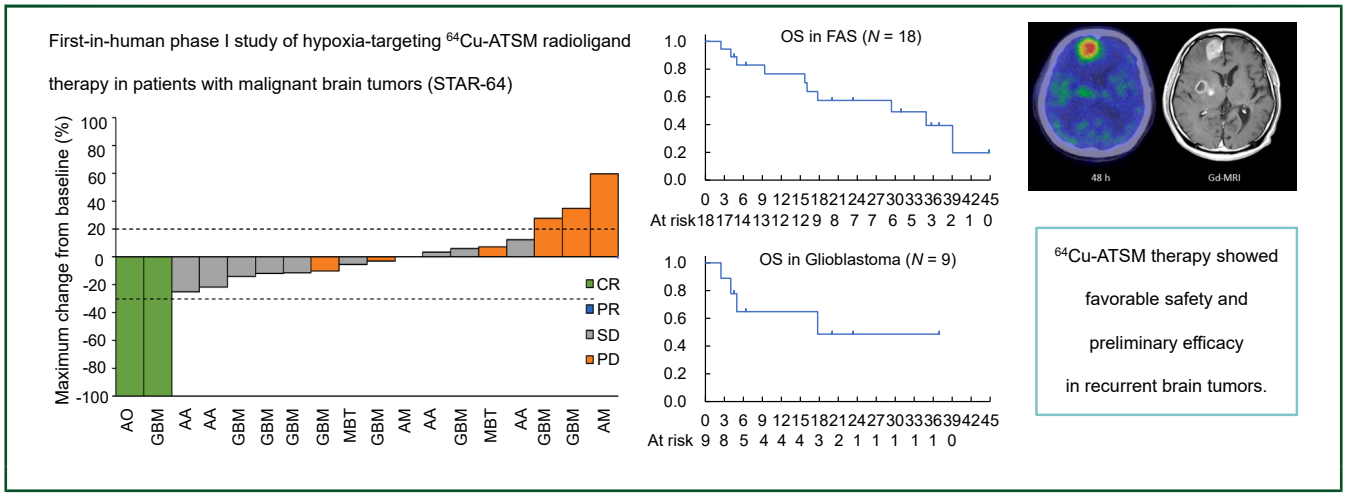
Key words: ^{64}Cu-ATSM, hypoxia, malignant brain tumor, phase I study, radioligand therapy

INTRODUCTION

Malignant brain tumors, including glioblastoma, remain highly lethal despite multimodal therapy comprising surgery, radiotherapy, and chemotherapy, with approximately 23 800 new primary brain tumors diagnosed each year in the United States.[1] Prognosis remains particularly poor; for example, glioblastoma is associated with a 5-year overall survival (OS) of only approximately 10% and a median survival of approximately 15 months.[2] Relapse is common, and

*Correspondence to: Dr Hiroaki Kurihara, Diagnostic and Interventional Radiology, Kanagawa Cancer Center Hospital, 2-3-2 Nakao, Asahi-ku, Yokohama 241-8515, Japan. Tel: +81-45-520-2222
E-mail: kurihara.0420j@kanagawa-pho.jp (H. Kurihara).
Dr Yukie Yoshii, LinqMed Inc., 3-11-5 Nihonbashi Honcho, Chuo-ku, Tokyo 103-0023, Japan. Tel: +81-3-6661-7661
E-mail: yoshii.yukie@linqmed.co.jp (Y. Yoshii).
†Present address: Neurosurgery, Tokai University School of Medicine, Isehara, Japan.

GRAPHICAL ABSTRACT



effective therapies for recurrent malignant gliomas remain limited, representing a significant clinical challenge.[3,4] Hypoxic microenvironments throughout tumors have been reported to play critical roles in tumor proliferation, aggressiveness, and resistance to chemoradiotherapy, leading to poor outcomes in malignant brain tumors.[5,6]

Copper-64 diacetyl-bis(N^4-methylthiosemicarbazone) (^{64}Cu-ATSM) is a first-in-class hypoxia-targeting radiotherapeutic agent for malignant tumors.[7-11] Cu-ATSM labeled with $^{60/61/62/64}$Cu radioisotopes was originally developed for positron emission tomography (PET) imaging agents that can highly accumulate in tumor hypoxia characterized by an overreducing status;[7-9,12] Cu-ATSM rapidly diffuses into cells and tissues due to its small molecular size and hydrophobic properties. Under the overreducing tumor hypoxia, Cu (II) in Cu-ATSM is reduced to Cu (I), which dissociates from ATSM and becomes trapped within cancer cells. Our previous clinical PET study demonstrated that ^{62}Cu-ATSM showed high accumulation in glioma,[13,14] with uptake significantly greater in higher-grade gliomas than in lower-grade gliomas and positively correlated with expression of hypoxic marker HIF-1α.[13] ^{64}Cu with a physical half-life of 12.7 h is a unique radionuclide suitable for internal radiotherapy and PET imaging, as it emits β^- (0.574 MeV, 40%) and Auger electron (42.6%) for therapy, and β^+ (0.653 MeV, 17.4%) for imaging.[7,15,16] Previous studies have demonstrated that tumor cells incorporated ^{64}Cu into their nucleus following ^{64}Cu-ATSM administration under hypoxia and that high-linear energy transfer (LET) Auger electrons together with β^- particles, emitted from ^{64}Cu, induce DNA double-strand breaks and cluster damage in ^{64}Cu-ATSM treatment.[8,9,15-17] This is similar to high-LET carbon ion irradiation that can overcome the low oxygen enhancement ratio.[16] A preclinical study using a human glioblastoma U87MG xenograft mouse model demonstrated that administering 37 MBq of ^{64}Cu-ATSM weekly for 4 weeks effectively inhibited tumor growth and significantly prolonged survival in the absence of serious toxicity.[18] In recent neuro-oncological research, targeted radioligand therapy has been increasingly explored; adequate delivery across the blood—brain barrier is a key determinant of successful clinical development.[4,19,20] Cu-ATSM has been reported to demonstrate the ability to cross the blood—brain barrier.[21] While β^- emitters such as ^{177}Lu and ^{90}Y and α-emitters such as ^{225}Ac have been widely investigated in radiotherapeutics, no radioligand therapy has yet reached the approval or late stages of clinical development for malignant brain tumors.[19] The hypoxia-selective retention mechanism of Cu-ATSM is dependent on copper redox chemistry within the ATSM complex; therefore, the radiopharmaceutical properties of Cu-ATSM cannot be readily reproduced by simple substitution with other radionuclides.[8,9,12] Based on these findings, this first-in-human, open-label, phase I dose-escalation trial (STAR-64, jRCT2091220362) was conducted to evaluate the safety, preliminary therapeutic efficacy, pharmacokinetics, and organ radiation doses of ^{64}Cu-ATSM therapy in patients with malignant brain tumors.

PATIENTS AND METHODS

Study design and eligibility criteria

This was a phase I, multicenter, investigator-initiated, nonrandomized, open-label, single-arm, 3 + 3 dose-escalation study evaluating ^{64}Cu-ATSM monotherapy in patients with recurrent malignant brain tumors, conducted at the National Cancer Center Hospital (Japan) and Kanagawa Cancer Center (Japan). The study was approved by institutional review boards at each site and was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice guidelines. Written informed consent was obtained from all patients.

Patients were eligible if they had histologically diagnosed glioma grade 3 or higher, primary central nervous system malignant lymphoma (PCNSL), atypical or malignant meningioma (grade 2 or 3) based on the most recent

pathological diagnosis with the World Health Organization (WHO) 2016 classification,[22] or metastatic brain tumor based on their clinical course or imaging studies. Patients were required to have one of the following: (i) recurrence of glioblastoma or grade 3 glioma after completion of standard therapy; (ii) PCNSL recurrence after completion of standard therapy consisting of at least one course of high-dose methotrexate therapy and whole-brain irradiation; (iii) in patients with metastatic brain tumors having completed standard treatment for the primary lesion, with confirmation of recurrence or aggravation after external irradiation for brain metastases; or (iv) atypical and malignant meningioma (grade 2 or 3) with confirmed recurrence or exacerbation after one or more surgeries. Patients were required to be aged between 20 and 75 years at the time of registration with a Karnofsky performance status (KPS) score of at least 60. Patients were required to have a measurable lesion with a maximum diameter of 10 mm or more on contrast-enhanced head MRI before registration. Patients were required to have all the following on their latest examination within 14 days before registration: (i) neutrophil count $\geq$1500 /mm^3, (ii) platelet count $\geq 10 \times 10^4$/mm^3, (iii) hemoglobin $\geq$9.0 g/dL, (iv) alanine aminotransferase $\leq$100 U/L, (v) aspartate aminotransferase $\leq$100 U/L, (vi) prothrombin time international normalized ratio $<$1.5, (vii) creatinine $\leq$1.5 mg/dL, and (viii) urinary protein qualitative ($-$) to (1$+$). To exclude pseudoprogression at study initiation, patients with prior radiotherapy were required to be registered at least 90 days after the final irradiation day, and eligibility required the presence of recurrent/progressive disease after completion of standard therapy.

Treatment with ^{64}Cu-ATSM and study endpoints

^{64}Cu-ATSM was synthesized at the National Institute for Quantum Science and Technology and the National Cancer Center Hospital.[23] ^{64}Cu-ATSM was administered intravenously weekly four times with four dose cohorts (level 1, 30 MBq/kg; level 2, 60 MBq/kg; level 3, 99 MBq/kg; and level 4, 150 MBq/kg). One week constituted one course, and treatment with ^{64}Cu-ATSM was continued for up to four courses or until DLT occurred.

The primary endpoints of this phase 1 study were dose-limiting toxicity (DLT) and the recommended dose for the next trial. The secondary endpoints included the objective response rate (ORR), progression-free survival (PFS), incidence of adverse events (AEs), pharmacokinetics, and dosimetry.

Statistical analysis

The primary endpoint was the proportion of DLT in the full analysis set (FAS). The secondary endpoints included ORR, PFS, OS, and organ radiation doses in FAS, and AE in the safety analysis set (SAS). The 95% confidence intervals (CIs) for DLT and ORR were estimated using the Clopper—Pearson method. OS and PFS were estimated using the Kaplan—Meier method. The 95% CIs for median OS and PFS were estimated using the Brookmeyer and Crowley method, and those for 1-year OS and 6-month PFS were estimated using Greenwood's formula. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC).

Table 1. Patient characteristics

Characteristic	Registered	FAS
No. of patients	20	18
Age, years		
Median	49.5	49.0
Interquartile range	42.5-56.5	41.0-56.0
Range	30-71	30-71
Mean	50.0	48.9
SD	11.9	11.7
Sex		
Male	11	10 (55.6)
Female	9	8 (44.4)
KPS, %		
100	1 (5.0)	1 (5.6)
90	9 (45.0)	9 (50.0)
80	6 (30.0)	6 (33.3)
70	4 (20.0)	2 (11.1)
60	0 (0.0)	0 (0.0)
Histological classification		
Anaplastic astrocytoma	4 (20.0)	4 (22.2)
Anaplastic oligodendroglioma	2 (10.0)	1 (5.6)
Glioblastoma	9 (45.0)	9 (50.0)
PCNSL	0 (0.0)	0 (0.0)
Atypical meningioma (WHO grade 2)	2 (10.0)	2 (11.1)
Malignant meningioma (WHO grade 3)	0 (0.0)	0 (0.0)
Metastatic brain tumor	3 (15.0)	2 (11.1)

Data in the FAS are presented as *n* (%).
FAS, full analysis set; KPS, Karnofsky performance status; PCNSL, primary central nervous system lymphoma; SD, standard deviation; WHO, World Health Organization.

Supplementary materials and methods

Detailed methods on the synthesis of ^{64}Cu-ATSM, organ radiation doses, and pharmacokinetics analysis, as well as the protocol including study design, eligibility criteria, and DLT criteria, are provided in Supplementary Materials S1 and S2, available at https://doi.org/10.1016/j.esmoop.2026.108249, respectively.

RESULTS

Patients

Twenty patients were enrolled between November 2018 and January 2023. One patient withdrew before treatment due to neurological deterioration. Of the remaining 19 patients in the SAS, 1 was deemed ineligible, resulting in 18 patients (FAS) who received ^{64}Cu-ATSM in dose exploration [level 1, 30 MBq/kg ($n = 3$); level 2, 60 MBq/kg ($n = 6$); level 3, 99 MBq/kg ($n = 6$); and level 4, 150 MBq/kg ($n = 3$)]. Baseline patient characteristics are shown in Table 1 and Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2026.108249. Overall, 55.6% of patients were male; the median age was 49.0 years (range 30-71 years), and 11.1% of patients had a KPS of $\leq$70. The most common tumor types were glioblastoma (9; 50.0%), anaplastic astrocytoma (4; 22.2%), atypical meningioma

Table 2. Adverse events in the SAS ($N = 19$)

CTCAE toxicity	Grades 1–2	Grade 3	Grade 4	Total
Blood and lymphatic system disorders	4 (21.1)			4 (21.1)
Anemia	4 (21.1)			4 (21.1)
Gastrointestinal disorders	4 (21.1)			4 (21.1)
Epigastric pain	1 (5.3)			1 (5.3)
Constipation	1 (5.3)			1 (5.3)
Nausea	2 (10.5)			2 (10.5)
General disorders and administration site conditions	1 (5.3)			1 (5.3)
Fever	1 (5.3)			1 (5.3)
Infections and infestations	1 (5.3)			1 (5.3)
Sinusitis	1 (5.3)			1 (5.3)
Investigations	8 (42.1)	8 (42.1)	3 (15.8)	19 (100)
Alanine aminotransferase increased	2 (10.5)			2 (10.5)
Aspartate aminotransferase increased	1 (5.3)			1 (5.3)
Blood albumin decreased	1 (5.3)			1 (5.3)
Creatine phosphokinase increased	2 (10.5)			2 (10.5)
Blood pressure increased	1 (5.3)			1 (5.3)
Fibrin D-dimer increased	1 (5.3)			1 (5.3)
Lymphocyte count decreased	6 (31.6)	7 (36.8)	3 (15.8)	16 (84.2)
Neutrophil count decreased	9 (47.4)	1 (5.3)		10 (52.6)
Platelet count decreased	11 (57.9)			11 (57.9)
Weight loss	1 (5.3)			1 (5.3)
Weight gain	1 (5.3)			1 (5.3)
White blood cells decreased	6 (31.6)			6 (31.6)
Metabolism and nutritional disorders	13 (68.4)	1 (5.3)		14 (73.7)
Hypercalcemia	2 (10.5)			2 (10.5)
Hyperglycemia	6 (31.6)			6 (31.6)
Hyperkalemia	3 (15.8)			3 (15.8)
Hypernatremia	2 (10.5)			2 (10.5)
Hypoalbuminemia	7 (36.8)			7 (36.8)
Hypocalcemia	1 (5.3)			1 (5.3)
Hypoglycemia	1 (5.3)			1 (5.3)
Hypokalemia	1 (5.3)			1 (5.3)
Hyponatremia	3 (15.8)	1 (5.3)		4 (21.1)
Hypoproteinemia	2 (10.5)			2 (10.5)
Loss of appetite	1 (5.3)			1 (5.3)
Musculoskeletal and connective tissue disorders	2 (10.5)			2 (10.5)
Back pain	1 (5.3)			1 (5.3)
Musculoskeletal pain	1 (5.3)			1 (5.3)
Nervous system disorders	4 (21.1)	2 (10.5)		6 (31.6)
Aphasia	1 (5.3)			1 (5.3)
Dysgeusia	1 (5.3)			1 (5.3)
Headache	1 (5.3)			1 (5.3)
Hemiplegia	2 (10.5)			2 (10.5)
Memory impairment	1 (5.3)			1 (5.3)
Seizures	1 (5.3)	2 (10.5)		3 (15.8)
Psychiatric disorders	1 (5.3)			1 (5.3)
Delirium	1 (5.3)			1 (5.3)
Renal and urinary disorders	2 (10.5)			2 (10.5)
Hematuria	1 (5.3)			1 (5.3)
Proteinuria	1 (5.3)			1 (5.3)
Respiratory, thoracic, and mediastinal disorders	1 (5.3)			1 (5.3)
Hiccups	1 (5.3)			1 (5.3)
Skin and subcutaneous tissue disorders	2 (10.5)			2 (10.5)
Papules	1 (5.3)			1 (5.3)
Pruritus	1 (5.3)			1 (5.3)

All data are presented as *n* (%).
CTCAE, Common Terminology Criteria for Adverse Events; SAS, safety analysis set.

(2; 11.1%), metastatic brain tumor (2; 11.1%), and anaplastic oligodendroglioma (1; 5.6%) in the FAS.

Safety

Eighteen patients in the FAS were evaluated for DLTs (Supplementary Table S2, available at https://doi.org/10.1016/j.esmoop.2026.108249), and four patients (22.2%; 95% CI% 6.4%-47.6%) experienced grade 3 lymphopenia as DLT; 1 at 60 MBq/kg, 1 at 99 MBq/kg, and 2 at 150 MBq/kg. The MTD and recommended dose for the next trial was determined to be 99 MBq/kg. The incidence of AEs in the SAS was 100% (19/19 patients; Table 2). No treatment-related deaths occurred. Grade $\geq$3 lymphopenia was observed in 52.6% (10/19), seizures in 10.5% (2/19), and neutropenia and hyponatremia in 5.3% (1/19 each); the seizures were unrelated to ^{64}Cu-ATSM.

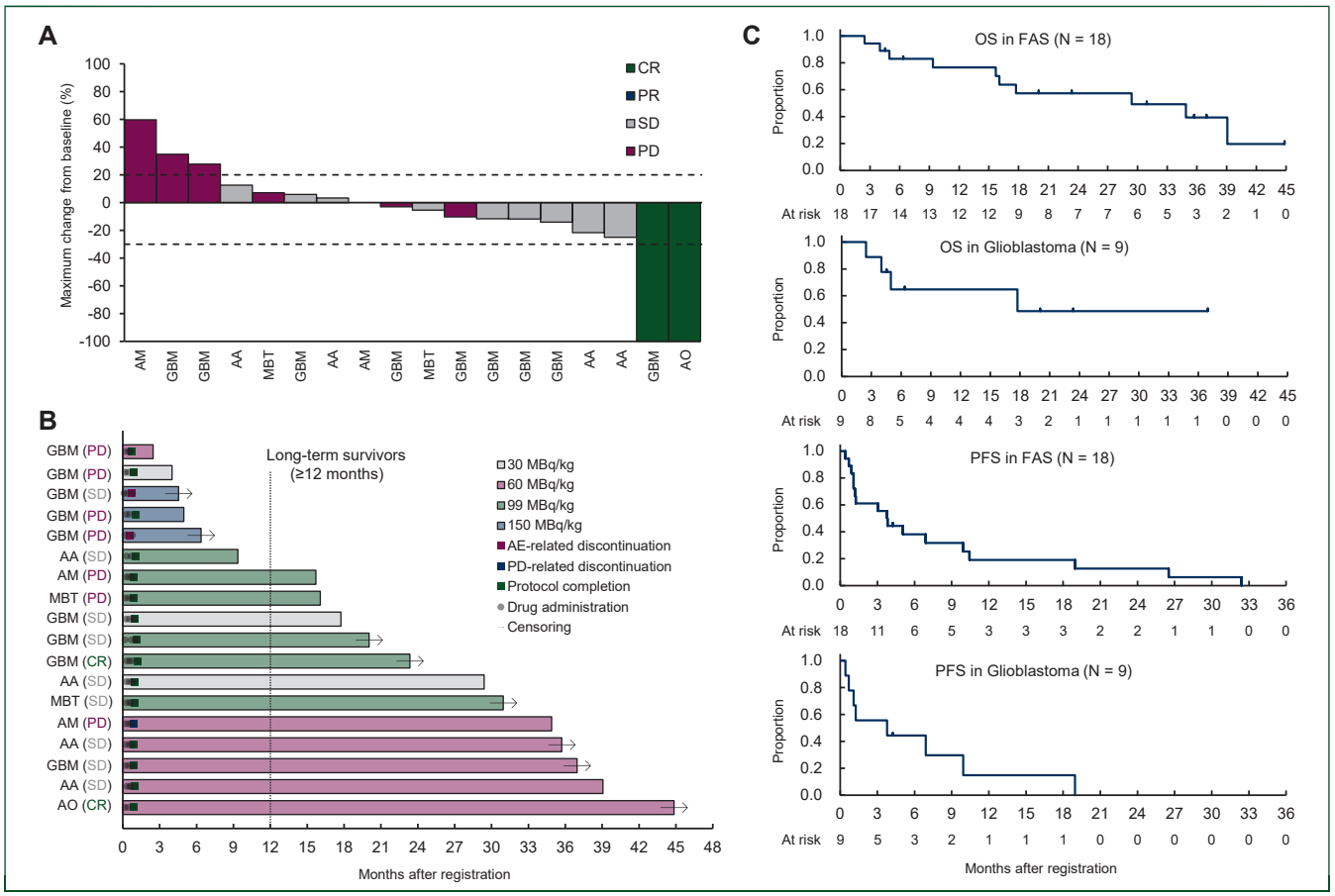


Figure 1. Efficacy outcomes of ^{64}Cu-ATSM therapy in the phase 1 study (STAR-64). (A) Waterfall plot depicting percentage change in target lesions (RECIST) in 18 patients. (B) Swimmer plot depicting 18 study participants. (C) Kaplan—Meier estimates of OS in the FAS and patients with glioblastoma; PFS in the FAS and patients with glioblastoma (from top to bottom).

AA, anaplastic astrocytoma; AE, adverse event; AM, atypical meningioma; AO, anaplastic oligodendroglioma; CR, complete response; FAS, full analysis set; GBM, glioblastoma; MBT, metastatic brain tumor; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease.

Antitumor effects and survival

All 18 patients in the FAS had measurable disease at baseline, were assessed according to RECISTv1.1[24] for response, and were included in the waterfall plot depicted in Figure 1A. Among the 18 patients, the best overall response was CR in 2 patients, stable disease (SD) in 9 patients, and PD in 7 patients; the ORR was 11.1% (95% CI 1.4%-34.7%). The swimmer plot in Figure 1B illustrates the duration of study participation for each patient. Among the 18 patients, 12 patients (66.7%) were long-term survivors ≥12 months, and 14 (77.8%) survived ≥6 months. For patients with CR, one patient received 60 MBq/kg. This

Table 3. PFS and OS in FAS and in glioblastoma with historical comparisons

	1-year OS (95% CI)	6-month PFS (95% CI)	Median OS (95% CI)	Median PFS (95% CI)
This study (STAR-64)				
FAS	76.60%	38.10%	29.4 months	3.8 months
(*N* = 18)	(48.8%-90.6%)	(16.6%-59.5%)	(9.4-NE)	(1.1-9.9)
No. of events	-	-	10	17
Glioblastoma	64.80%	44.40%	17.7 months	3.8 months
(*N* = 9)	(25.3%-87.2%)	(13.6%-71.9%)	(2.5-NE)	(0.4-9.9)
No. of events	-	-	4	8
Historical references				
Glioblastoma (EORTC-26101)[25] Lomustine + bevacizumab	31.50%	-	9.1 months	4.2 months
Glioblastoma (JO22506) Japan[26] Bevacizumab	34.50%	33.90%	10.5 months	3.3 months

1-year OS, overall survival rate at 1 year; 6-month PFS, progression-free survival rate at 6 months; CI, confidence interval; FAS, full analysis set; N/E, not evaluated; NE, not estimable; OS, overall survival; PFS, progression-free survival.

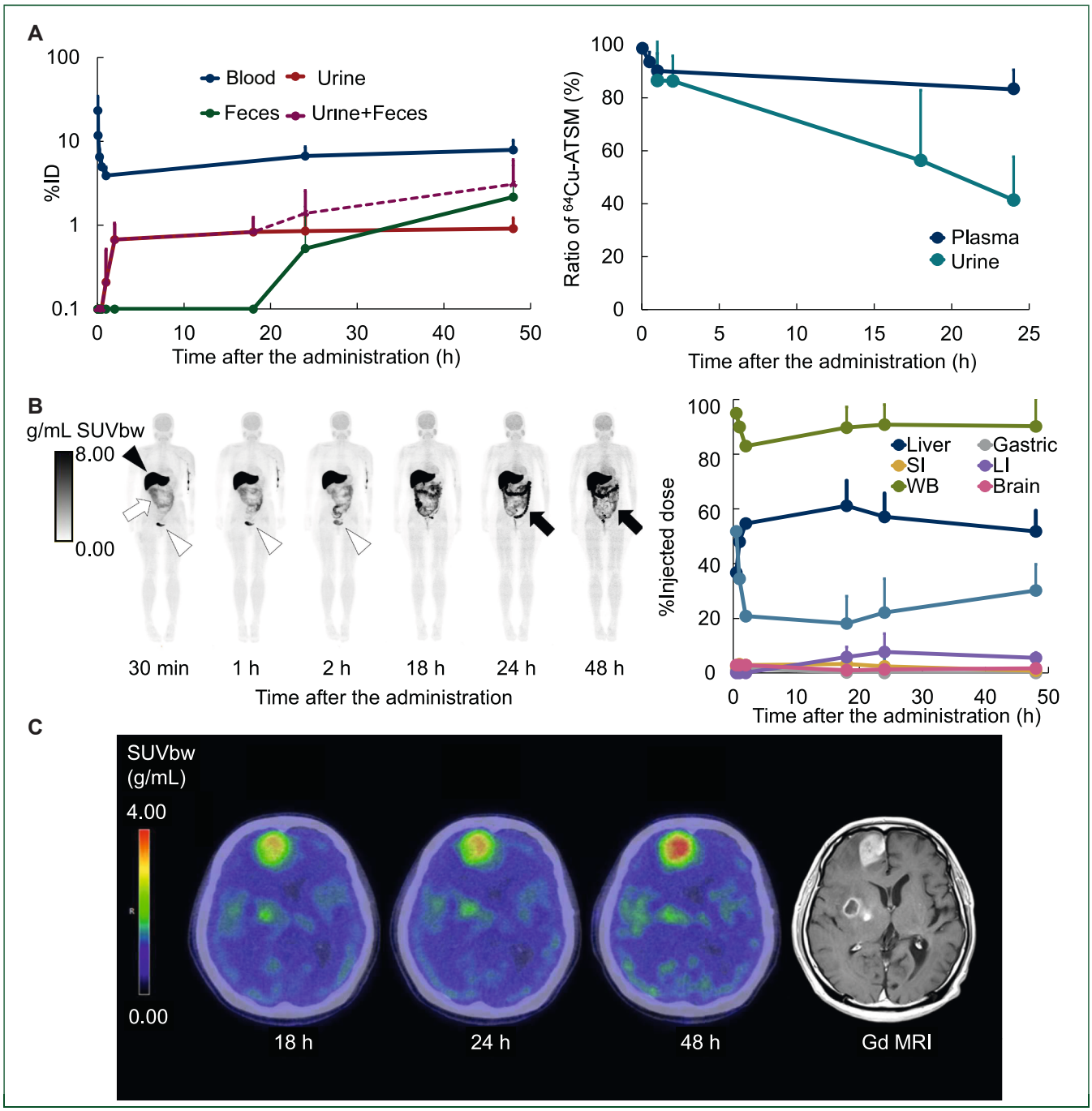


Figure 2. Pharmacokinetics and PET/CT imaging. (A) The rate for cumulative excretion and blood distribution of ^{64}Cu activity after ^{64}Cu-ATSM administration in patients with brain tumors (left). Data were obtained at 1, 2, 18, 24, and 48 h after ^{64}Cu-ATSM administration. Values are expressed as the % injected dose. Radiometabolite analysis of ^{64}Cu-ATSM (right). Ratios of ^{64}Cu-ATSM in the radioactivity in plasma and urine are shown. Values are shown as means and SDs of nine patients. (B) Representative WB PET/CT maximum intensity projection images obtained at 30 min, 1 h, 2 h, 18 h, 24 h, and 48 h after [^{64}Cu] Cu-ATSM administration (left). Closed arrowhead indicates liver, open arrow indicates small intestine, closed arrow indicates large intestine, and open arrowhead indicates urinary bladder, respectively. Time course for [^{64}Cu] Cu-ATSM distribution in the observed organs (right). Values are expressed as the % injected dose accumulated in the WB, liver, gastric, SI, LI, brain, and remWB. The remWB was calculated by subtracting the sum of organs (liver, gastric, SI, LI, and brain) from WB. Values are shown as means of the following: $n = 1$ for 30 min, 1 h, 2 h, $n = 4$ for 18 h, $n = 6$ for 24 h, and $n = 5$ for 48 h. (C) Representative PET/CT fusion images of a patient with glioblastoma obtained at 18, 24, and 48 h after [^{64}Cu] Cu-ATSM administration and gadolinium-enhanced MRI. Standard uptake value of [^{64}Cu] Cu-ATSM within the tumor was 3.27, 3.61, and 4.02 at 18, 24, and 48 h, respectively.

CT, computed tomography; LI, large intestine; PET, positron emission tomography; remWB, remainder of the body; SI, small intestine; SUVbw, standardized uptake value body weight; WB, whole body.

patient was a 41-year-old man (KPS 90) with anaplastic oligodendroglioma (IDH, mutant; 1p/19q, codeleted; pTERT, not available; pMGMT, positive), previously treated with gross total resection and temozolomide-based therapy. Serial MRI showed a CR, with survival of ≥45 months (and censored). Another patient received 99 MBq/kg; this patient was a 49-year-old woman (KPS 90) with glioblastoma (IDH, wild-type; 1p/19q, non-codeleted, pTERT, mutant; pMGMT, positive) previously treated with gross total resection, whole-brain radiotherapy, stereotactic

radiotherapy, and temozolomide. This patient had a CR, showing a survival of ≥23 months (and censored). All nine patients with SD showed survival ≥5 months, and seven patients showed survival ≥12 months. Kaplan–Meier curves are shown in Figure 1C, and OS and PFS are summarized in Table 3 for the FAS and for patients with glioblastoma. Among the 18 FAS patients, the 1-year OS was 76.60% (95% CI 48.8%-90.6%), and the median OS was 29.4 months (95% CI 9.4 months to NE); the 6-month PFS was 38.10% (95% CI16.6%-59.5%), and median PFS was 3.8 months (95% CI 1.1-9.9 months). Among the nine patients with glioblastoma, the 1-year OS was 64.80% (95% CI 48.8%-90.6%), and the median OS was 17.7 months (95% CI 9.4 months to NE); the 6-month PFS was 44.40% (95% CI13.6%-71.9%), and median PFS was 3.8 months (95% CI 0.4-9.9 months), indicating that the OS and PFS.

Pharmacokinetics and PET imaging

Nine patients were included in the pharmacokinetic analysis. Blood radioactivity concentrations following intravenous administration of ^{64}Cu-ATSM (Supplementary Figure S1, available at https://doi.org/10.1016/j.esmoop.2026.108249) were fitted by the bi-exponential curve and shown in Figure 2A as %ID. Supplementary Table S3 (available at https://doi.org/10.1016/j.esmoop.2026.108249) summarizes the pharmacokinetic parameters of ^{64}Cu-ATSM. The half-life of ^{64}Cu-ATSM in the distribution phase ($t_{1/2(\alpha)}$) was 2.75 ± 1.66 min, and that in the elimination phase ($t_{1/2(\beta)}$) was 15.42 ± 4.71 h. No dose-dependent changes of pharmacokinetic parameters were observed. Urinary and fecal excretion accounted for less than 5% (Figure 2A). Most of the radioactivity in plasma remained as intact ^{64}Cu-ATSM, exceeding 90% up to 1 h and more than 80% up to 24 h after the intravenous administration (Figure 2A).

Sixteen patients underwent a ^{64}Cu-ATSM PET/computed tomography (CT) study. Representative whole-body PET/CT images at 30 min, 1 h, 2 h, 18 h, 24 h, and 48 h after ^{64}Cu-ATSM administration, and organ time activity curves are shown in Figure 2B. The liver had the highest standardized uptake value (SUV; 31.7 ± 13.7) of all the organs in the body at each time point. The large intestines had high accumulation at 24 h and 48 h after injection (SUV: 13.2 ± 10.2 and 10.2 ± 4.1, respectively), suggesting that radioactivity was excreted into the bile and feces. The radiation dosimetry of ^{64}Cu-ATSM is estimated by the MIRD method[27] and summarized in Supplementary Table S3, available at https://doi.org/10.1016/j.esmoop.2026.108249. The estimated organ doses of the liver and lower large intestines were 0.23 and 0.14 mSv/MBq, respectively. As a preliminary observation beyond the study endpoints, PET/CT scans were performed in patients with glioblastoma at 18, 24, and 48-h post-administration. Representative images are shown in Figure 2C. Notably, high accumulation of ^{64}Cu-ATSM was observed within the brain tumors; the mean tumor SUV (3.2 ± 1.1) was higher than that of normal brain tissue (1.4 ± 0.1) at 48 h after administration across 19 patients.

DISCUSSION

This study represents the first-in-human phase I evaluation of ^{64}Cu-ATSM for therapeutic purposes as a hypoxia-targeting radioligand therapy. DLTs were limited to grade 3 lymphopenia; the MTD was established at 99 MBq/kg administered weekly for four times. Radiographic CRs were observed in two patients. Among patients with glioblastoma, the 1-year OS rate was 64.8% and the median OS was 17.7 months, suggesting favorable preliminary efficacy.

These findings highlight the potential of ^{64}Cu-ATSM as a first-in-class hypoxia-targeting radioligand therapy for recurrent malignant brain tumors. Primary brain tumors remain a significant clinical challenge worldwide, with an estimated 321 624 new cases annually,[28] including high-grade gliomas (grade 3 or 4) 22.9%, PCNSL 1.8%, and grade 2/3 meningiomas 0.54%.[29] Despite recent therapeutic advances, approximately 90% of glioblastomas relapse within 2 years of diagnosis.[30] Tumor hypoxia is a key driver of disease progression and therapeutic resistance. Hypoxic markers such as HIF-1α are frequently overexpressed, with reported positivity rates of approximately 72% in glioblastoma[31] and 69.7% in metastatic brain tumors.[32] Given these observations, hypoxia-targeting ^{64}Cu-ATSM therapy offers a rational approach to overcome therapy resistance in malignant brain tumors.

Although various chemotherapeutic approaches have been attempted worldwide for recurrent high-grade gliomas, no treatment has demonstrated sufficient efficacy to be established as a standard of care. In the randomized phase III EORTC 26101 trial in patients with progressive glioblastoma, lomustine plus bevacizumab improved PFS than lomustine alone (median PFS, 4.2 versus 1.5 months) but did not improve OS (median, OS 9.1 versus 8.6 months).[25] In a Japanese phase II trial (JO22506), 29 patients with recurrent glioblastoma showed a 6-month PFS of 33.9% (median PFS 3.3 months) and a median OS of 10.5 months (1-year OS 34.5%).[26] These results indicate that bevacizumab provides a limited survival benefit in recurrent glioblastoma. In the present study, ^{64}Cu-ATSM therapy achieved a 6-month PFS of 44.4% (median PFS, 3.8 months) and a median OS of 17.7 months (1-year OS, 64.8%) in patients with recurrent glioblastoma. These efficacy findings are of interest in the context of recurrent glioblastoma, for which limited efficacy has been reported with bevacizumab monotherapy or lomustine plus bevacizumab; however, the current results remain exploratory and should be interpreted with caution. Larger, controlled studies are warranted to confirm these preliminary observations.

Intravenous administration of ^{64}Cu-ATSM was safe and effective, with only minor toxicity limited to grade 3 lymphopenia. Pharmacokinetic analysis revealed a distribution half-life of $t_{1/2(\alpha)}$ 2.75 ± 1.66 min and an elimination half-life of $t_{1/2(\beta)}$ 15.42 ± 4.71 h across all dose cohorts. Metabolite analysis demonstrated that most circulating radioactivity remained as intact ^{64}Cu-ATSM during the elimination phase in the plasma, supporting the feasibility

of repeated weekly dosing. Given the 12.7-h half-life of ^{64}Cu and that less than 10% of administered radioactivity was excreted, clearance appears to be driven by physical decay. Dosimetry analysis identified the liver and lower large intestine as the most affected organs for radiation. However, no severe hepatic or intestinal AEs were observed in this phase I study. Collectively, these data provide a solid rationale for dose selection and scheduling in ongoing and future clinical trials. This phase 1 safety study selected fractionated weekly dosing, which would be beneficial for patients with recurrent brain tumors who have very poor prognosis. From a clinical feasibility perspective, the weekly administration schedule used in this study may appear intensive; however, in the present study, the treatment was feasible without major logistical difficulties, leading to the potential future applicability of this treatment in clinical practice. For the clinical role of ^{64}Cu-ATSM, it may be most appropriate in the recurrent setting after standard treatment for malignant brain tumors, while its potential use in combination with other therapies should be explored in future studies.

Given that ^{64}Cu-ATSM can penetrate the blood—brain barrier,[13,14] this therapy represents a novel, minimally invasive, and less toxic form of internal radiotherapy for patients with recurrent malignant brain tumors. Previously, Cu-ATSM labeled with $^{60/61/62}$Cu or ^{64}Cu had been used exclusively for PET imaging in clinical studies, demonstrating safety at diagnostic doses (up to 925 MBq/body[33]). This study provides the first clinical evidence of safety and establishes the MTD (99 MBq/kg) for therapeutic administration, along with preliminary signals of efficacy for ^{64}Cu-ATSM therapy. Given marked resistance of recurrent malignant brain tumors to standard chemoradiotherapy, the cytocidal radiation emitted by ^{64}Cu—comprising both β^- particles and high-LET Auger electrons—may help overcome therapeutic resistance by directly damaging DNA in hypoxic tumor cells. This mechanism underpins the potential role of ^{64}Cu-ATSM as a rational last-line treatment option for refractory malignant brain tumors.

This study has several limitations, including its small sample size, the phase I design without a control arm, and the heterogeneous tumor population. Therefore, the efficacy outcomes should be regarded as exploratory and require confirmation in larger prospective studies. Moreover, eligibility and histological categorization were based on the 2016 WHO classification, which was the prevailing standard when this trial began. Since then, the WHO classification of central nervous system tumors has been updated (WHO CNS5, 2021),[34] incorporating integrated molecular features (e.g. IDH mutation and 1p/19q codeletion). The available molecular features have been described to Supplementary Table S1, available at https://doi.org/10.1016/j.esmoop.2026.108249. Future studies using molecularly integrated classification are expected to further evaluate the relationship between these molecular features and treatment outcomes. Furthermore, in this first-in-human phase I study, dosimetry focused on normal organs to ensure safety and determine DLT, in line with regulatory requirements. Tumor dosimetry was not included as a prespecified endpoint and was challenging to estimate reliably owing to the small cohort and heterogeneous tumor types. In addition, PET imaging was performed after therapeutic administration, and at variable time points depending on patient discharge from radiation-controlled rooms and clinical conditions. Therefore, tumor dosimetry was not considered to be sufficiently robust for meaningful analysis in the present study. Future studies with pretherapeutic low-dose administration for diagnostic imaging would be necessary to enable more reliable tumor dosimetry and the evaluation of dose—response relationships.

Conclusion

Collectively, these results demonstrate that ^{64}Cu-ATSM is well tolerated and demonstrates encouraging efficacy in recurrent malignant brain tumors, particularly glioblastoma. These findings support further clinical evaluation in the ongoing phase III study[35] (STEP-64, jRCT2031240090) and highlights its potential as a first-in-class hypoxia-targeting radioligand therapy, offering a novel therapeutic option for patients with these devastating and otherwise treatment-refractory diseases.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the patients and their families for their participation in this study. The authors acknowledge the efforts of Dr Kenichi Nakamura in contributing to this study. They also thank Mr Kenta Anjo, Mr Satoshi Azuma, Mrs Yukari Nagasaka, and Mrs Keiko Ohata for their assistance with this study.

FUNDING

This work was supported by funds from the Japan Agency for Medical Research and Development (AMED) [grant numbers JP17ck0106297 and JP20ck0106567].

DISCLOSURE

HK reports a relationship with LinqMed Inc. that includes: equity or stocks. YY reports a relationship with LinqMed Inc. that includes: board membership, employment, and equity or stocks. AW reports a relationship with LinqMed Inc. that includes: equity or stocks. HM reports a relationship with LinqMed Inc. that includes: equity or stocks. The other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019;69:7-34.
2. Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med*. 2005;352:987-996.
3. Weller M, Cloughesy T, Perry JR, Wick W. Standards of care for treatment of recurrent glioblastoma—are we there yet? *Neuro Oncol*. 2013;15:4-27.

4. Wen PY, Weller M, Lee EQ, et al. Glioblastoma in adults: a Society for Neuro-Oncology (SNO) and European Society of Neuro-Oncology (EANO) consensus review on current management and future directions. *Neuro Oncol*. 2025;27:2751-2788.
5. Xiong Z, Liu H, He C, Li X. Hypoxia contributes to poor prognosis in primary IDH-wt GBM by inducing tumor cells MES-like transformation trend and inhibiting immune cells activity. *Front Oncol*. 2021;11:782043.
6. Singleton DC, Macann A, Wilson WR. Therapeutic targeting of the hypoxic tumour microenvironment. *Nat Rev Clin Oncol*. 2021;18:751-772.
7. Lewis J, Laforest R, Buettner T, et al. Copper-64-diacetyl-bis(N^4-methylthiosemicarbazone): an agent for radiotherapy. *Proc Natl Acad Sci U S A*. 2001;98:1206-1211.
8. Obata A, Yoshimi E, Waki A, et al. Retention mechanism of hypoxia selective nuclear imaging/radiotherapeutic agent cu-diacetyl-bis(N^4-methylthiosemicarbazone) (Cu-ATSM) in tumor cells. *Ann Nucl Med*. 2001;15:499-504.
9. Obata A, Kasamatsu S, Lewis JS, et al. Basic characterization of ^{64}Cu-ATSM as a radiotherapy agent. *Nucl Med Biol*. 2005;32:21-28.
10. Holland JP, Giansiracusa JH, Bell SG, Wong LL, Dilworth JR. In vitro kinetic studies on the mechanism of oxygen-dependent cellular uptake of copper radiopharmaceuticals. *Phys Med Biol*. 2009;54:2103-2119.
11. Yoshii Y, Yoneda M, Ikawa M, et al. Radiolabeled Cu-ATSM as a novel indicator of overreduced intracellular state due to mitochondrial dysfunction: studies with mitochondrial DNA-less ρ0 cells and cybrids carrying MELAS mitochondrial DNA mutation. *Nucl Med Biol*. 2012;39:177-185.
12. Fujibayashi Y, Taniuchi H, Yonekura Y, Ohtani H, Konishi J, Yokoyama A. Copper-62-ATSM: a new hypoxia imaging agent with high membrane permeability and low redox potential. *J Nucl Med*. 1997;38:1155-1160.
13. Tateishi K, Tateishi U, Sato M, et al. Application of ^{62}Cu-diacetyl-bis (N^4-methylthiosemicarbazone) PET imaging to predict highly malignant tumor grades and hypoxia-inducible factor-1alpha expression in patients with glioma. *AJNR Am J Neuroradiol*. 2013;34:92-99.
14. Toriihara A, Ohtake M, Tateishi K, et al. Prognostic implications of ^{62}Cu-diacetyl-bis (N^4-methylthiosemicarbazone) PET/CT in patients with glioma. *Ann Nucl Med*. 2018;32:264-271.
15. Yoshii Y, Furukawa T, Kiyono Y, et al. Internal radiotherapy with copper-64-diacetyl-bis (N^4-methylthiosemicarbazone) reduces CD133$^+$ highly tumorigenic cells and metastatic ability of mouse colon carcinoma. *Nucl Med Biol*. 2011;38:151-157.
16. McMillan DD, Maeda J, Bell JJ, et al. Validation of ^{64}Cu-ATSM damaging DNA via high-LET Auger electron emission. *J Radiat Res*. 2015;56:784-791.
17. Tinganelli W, Durante M. Carbon ion radiobiology. *Cancers (Basel)*. 2020;12:3022.
18. Yoshii Y, Matsumoto H, Yoshimoto M, et al. Multiple administrations of ^{64}Cu-ATSM as a novel therapeutic option for glioblastoma: a translational study using mice with xenografts. *Transl Oncol*. 2018;11:24-30.
19. Albert NL, Le Rhun E, Minniti G, et al. Translating the theranostic concept to neuro-oncology: disrupting barriers. *Lancet Oncol*. 2024;25:e441-e451.
20. Weller M, Albert NL, Galldiks N, et al. Targeted radionuclide therapy for gliomas: emerging clinical trial landscape. *Neuro Oncol*. 2024;26:S208-S214.
21. Huuskonen MT, Tuo QZ, Loppi S, et al. The copper bis(thiosemicarbazone) complex Cu^{II}(atsm) is protective against cerebral ischemia through modulation of the inflammatory milieu. *Neurotherapeutics*. 2017;14:519-532.
22. WHO classification of tumours of the central nervous system. In: WHO Classification of Tumours. *rev 4th* ed. Vol 1. 1 IARC Publications; 2016.
23. Matsumoto H, Kaneko E, Igarashi C, et al. Process development of [^{64}Cu]Cu-ATSM: efficient stabilization and sterilization for therapeutic applications. *J Radioanal Nucl Chem*. 2019;322:467-475.
24. Eisenhauer EA, Therasse P, Bogaerts J, et al. New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). *Eur J Cancer.* 2009;45:228-247.
25. Wick W, Gorlia T, Bendszus M, et al. Lomustine and bevacizumab in progressive glioblastoma. *N Engl J Med*. 2017;377:1954-1963.
26. Nagane M, Nishikawa R, Narita Y, et al. Phase II study of single-agent bevacizumab in Japanese patients with recurrent malignant glioma. *Jpn J Clin Oncol*. 2012;42:887-895.
27. Stabin MG, Sparks RB, Crowe E. OLINDA/EXM: the second-generation personal computer software for internal dose assessment in nuclear medicine. *J Nucl Med*. 2005;46:1023-1027.
28. Ferlay J, Em LF, Laversanne M, et al. Global Cancer Observatory: Cancer Today. version 1.1. International Agency for Research on Cancer. Available at https://gco.iarc.who.int/today. Accessed October 14, 2025.
29. Price M, Ballard C, Benedetti J, et al. CBTRUS statistical report: primary brain and other central nervous system tumors diagnosed in the United States in 2017-2021. *Neuro Oncol*. 2024;26:vi1-vi85.
30. Rogers S, Gross M, Ermis E, et al. Re-irradiation for recurrent glioblastoma: a pattern of care analysis. *BMC Neurol*. 2024;24:462.
31. Li H, Niu X, Cheng R. Prevalence, prognostic and clinicopathological value of HIF-1α in glioblastoma patients: a systematic review and meta-analysis. *Neurosurg Rev*. 2024;47:860.
32. Ebright RY, Zachariah MA, Micalizzi DS, et al. HIF1A signaling selectively supports proliferation of breast cancer in the brain. *Nat Commun*. 2020;11:6311.
33. Lewis JS, Laforest R, Dehdashti F, Grigsby PW, Welch MJ, Siegel BA. An imaging comparison of ^{64}Cu-ATSM and ^{60}Cu-ATSM in cancer of the uterine cervix. *J Nucl Med*. 2008;49:1177-1182.
34. Central nervous system tumours. In: *WHO Classification of Tumours*. 5th ed. Vol 6. IARC Publications; 2021.
35. Ando Y, Yanagisawa S, Ohno M, et al. Efficacy of radioactive hypoxia-targeting therapeutic agent ^{64}Cu-ATSM on recurrent malignant glioma: a study protocol for a phase-III, investigator-sponsored, randomized controlled trial. *Jpn J Clin Oncol*. 2025;55:543-546.