

Biomimetic arsenic trioxide nanoplatform induces Caspase-3/GSDME pyroptosis and remodels the glioma immune microenvironment

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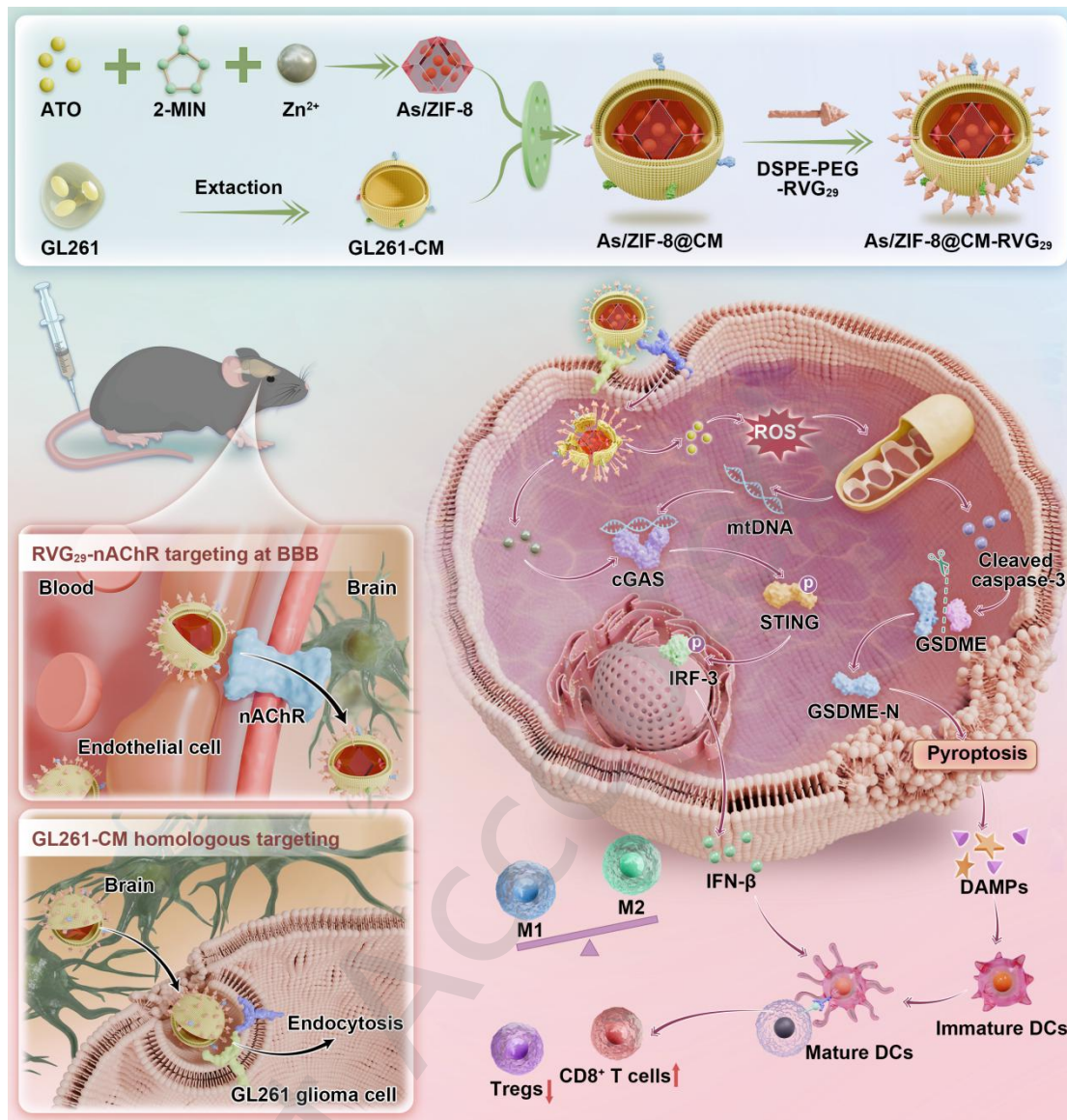
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Scheme 1. Schematic diagram of immunotherapy study using As/ZIF-8@CM-RVG₂₉

for glioma treatment.

Research Article

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Abstract

Glioma (GBM) is characterized by severe invasiveness and grim clinical outcomes. The therapeutic efficacy of conventional treatments is severely constrained by the blood-brain barrier (BBB), while the immunosuppressive tumor microenvironment (TME) further compromises treatment responsiveness. In GBM, abnormal expression of pyroptosis-related genes is closely associated with tumor malignancy and patient prognosis. Core pyroptosis-related genes were identified through bioinformatics screening. The active component arsenic trioxide (ATO) from traditional Chinese medicine was employed as a therapeutic agent, and it possesses unique advantages in inducing pyroptosis in tumor cells. Herein, we developed a

brain-targeted biomimetic nanoplatform, termed As/ZIF-8@CM-RVG₂₉. The engineered nanoplatform exhibited efficient BBB penetration, enhanced tumor accumulation, and pH-responsive drug release. ATO triggered Caspase-3/GSDME-dependent pyroptotic cell death in glioma cells, remodeled the tumor immune microenvironment, and effectively suppressed orthotopic glioma growth. Furthermore, combination treatment with programmed cell death protein-1 (α PD-1) generated a synergistic antitumor response and significantly prolonged median survival in tumor-bearing mice. Collectively, these findings suggest a promising therapeutic strategy and provide evidence for the development of targeted immunotherapy against glioma.

Keywords: glioma; arsenic trioxide; biomimetic nanomedicine; pyroptosis; immune remodeling

1. Introduction

Glioma (GBM) rank among the most common and lethal malignant neoplasms. Its incidence has been rising steadily over recent decades [1-2]. Although the current standard clinical treatment regimen involves maximal safe resection combined with postoperative temozolomide-based concurrent chemoradiotherapy. The median overall survival of affected patients remains a dismal 15 months, and fewer than 10% of patients survive beyond 5 years [3-5]. Blood-brain barrier (BBB) is a physiological protective barrier that hinders the delivery of chemotherapeutic drugs for brain tumors [6-7]. The GBM microenvironment is highly immunosuppressive, characterized by infiltration of immune cells and elevated levels of suppressive cytokines, which

collectively drive immune evasion and disease progression [8-9]. Therefore, penetrating the BBB and reversing immunosuppression are critical for improving GBM outcomes.

In recent years, strategies for inducing immunogenic cell death (ICD) in tumor cells have attracted considerable attention. Pyroptosis is a form of programmed cell death characterized by inflammatory responses. Unlike apoptosis, pyroptosis is morphologically defined by cell swelling, plasma membrane pore formation and rupture, and the release of intracellular contents [10]. Tumor cells release not only pro-inflammatory cytokines such as interleukin-1 β (IL-1 β) and interleukin-18 (IL-18), but also damage-associated molecular patterns (DAMPs) including adenosine triphosphate (ATP), high mobility group box 1 protein (HMGB1), and heat shock proteins (HSPs). These signaling molecules act as danger signals, recruiting innate immune cells and promoting dendritic cell (DC) maturation and antigen presentation [11]. Concurrently, pyroptosis suppresses regulatory T cells (Tregs) and M2 type tumor-associated macrophage (TAMs), converting the “cold” TME into a “hot” one that sustains long-term antitumor immunity and inhibits recurrence and metastasis [12-13].

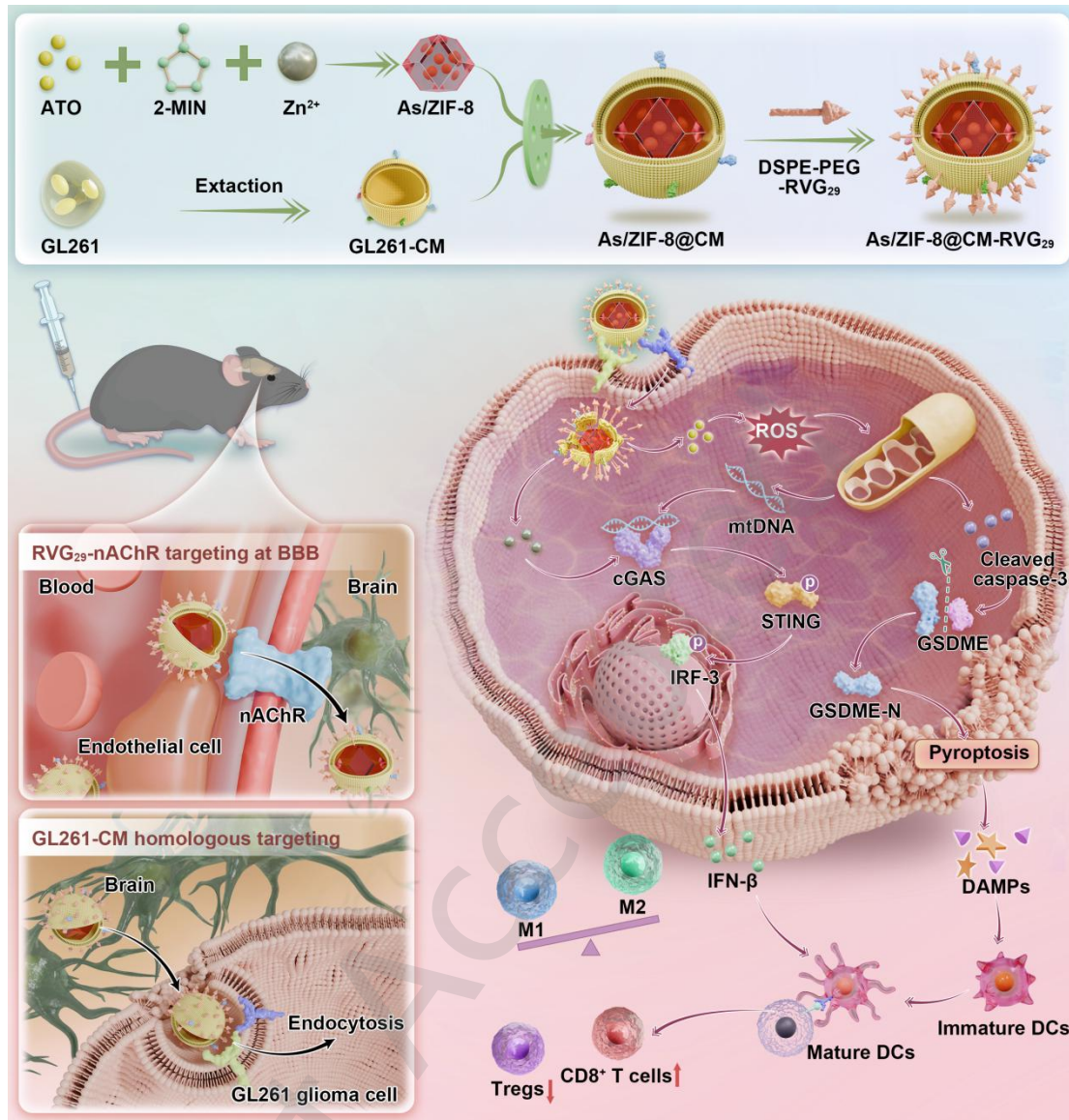
Arsenic trioxide (ATO) has achieved remarkable therapeutic efficacy in the treatment of leukemia, and its characteristic mechanism of "combating poison with poison" is highly consistent with the inflammatory nature of pyroptosis [14]. In terms of antitumor activity, ATO can induce programmed cell death in tumor cells through multiple pathways, including the generation of reactive oxygen species (ROS),

activation of the p53 signaling pathway, and inhibition of the NF- κ B pathway [15-16]. Given the disease specificity of glioma, we analyzed the differential expression patterns between glioma and normal brain tissues using public glioma datasets from the cancer genome atlas (TCGA) and the gene expression omnibus (GEO), and screened for core hub genes. Our bioinformatics analysis revealed that GSDME, a key executioner of pyroptosis, is aberrantly expressed in glioma and correlates with patient prognosis. In addition, our preliminary experiments confirmed that ATO activates the caspase-3/GSDME pyroptotic axis in glioma cells, supporting its use as a pyroptosis inducer. Although ATO demonstrates significant antitumor potential, it faces numerous limitations in the treatment of gliomas because of its poor BBB penetration, unfavorable pharmacokinetic profile, limited tumor selectivity, and systemic toxicities at relevant doses [17].

Nanotechnology offers an ideal solution to drug delivery challenges [18]. The nanocarriers primarily used for the treatment of gliomas include liposomes, polymer nanoparticles, metal-organic frameworks (MOFs), and exosomes [19]. Among these, MOFs can efficiently deliver various chemotherapeutic agents, photosensitizers, and immunomodulators, enabling controlled drug release and demonstrating broad application prospects in combination cancer therapy. To enhance the targeting efficiency and biocompatibility of nanomedicines, biomimetic cell membrane coating strategies have garnered considerable research interest in recent years [20]. Tumor cell membrane coatings preserve the antigenic characteristics and adhesion molecules of their source cells, thereby endowing nanocarriers with homologous targeting

capability, reduced immune recognition, prolonged circulation time, and enhanced tumor accumulation [21]. Furthermore, functionalization with the RVG₂₉ peptide enables receptor-mediated transport across the BBB via specific interactions with brain capillary endothelial cells (BCECs), thereby improving active brain-targeting efficiency [22-23].

Based on these findings, we designed a targeted peptide-modified biomimetic nanodelivery system, As/ZIF-8@CM-RVG₂₉, which achieves BBB penetration via RVG₂₉ peptide-mediated recognition, tumor-specific accumulation through homologous targeting conferred by GL261 glioma cell membrane coating, and precise ATO release in the TME enabled by the pH-responsive degradation of the ZIF-8 core. Mechanistically, ATO induced excessive intracellular ROS production, leading to mitochondrial dysfunction and subsequent mitochondrial DNA (mtDNA) release. ATO activated caspase-3-mediated cleavage of GSDME. DAMPs released during pyroptosis acted in concert with interferon- β (IFN- β) to promote dendritic cell maturation and enhance tumor antigen presentation. When combined with immune checkpoint blockade therapy, this combinatorial regimen induced long-term immune memory and effectively suppressed tumor growth and recurrence at the primary site. Collectively, this integrated therapeutic strategy offers a promising therapeutic platform and provides experimental support for the development of targeted immunotherapy against glioblastoma.



Scheme 1. Schematic diagram of immunotherapy study using As/ZIF-8@CM-RVG₂₉ for glioma treatment.

2. Materials and methods

2.1. Materials

Arsenic trioxide, zinc nitrate hexahydrate, and 2-methylimidazole were purchased from Sinopharm (China). DSPE-PEG2000-Mal and RVG₂₉ peptide were obtained from Xi'an Ruixi Biotechnology (China). Cell culture consumables were supplied by Corning (USA). Paraformaldehyde, Triton X-100, potassium bromide,

and indocyanine green were purchased from Sigma-Aldrich (USA). Fluorophore-conjugated antibodies were purchased from BioLegend (USA), and ELISA kits were obtained from Hangzhou Lianke Biotechnology (China).

2.2. Preparation and Characterization of As/ZIF-8@CM-RVG₂₉

2.2.1. Preparation of As/ZIF-8@CM-RVG₂₉

The brain-targeting conjugate DSPE-PEG-RVG₂₉ was synthesized via thiol-maleimide click reaction [24]. Briefly, 100 mg of DSPE-PEG2000-Mal was dissolved in 3 mL of dry N,N-dimethylformamide (DMF), and then RVG₂₉ peptide was added at a molar ratio of 1:2. The mixture was stirred at room temperature for 12 h in the dark to avoid peptide degradation. The crude product was transferred to a dialysis bag. The dialysate was gathered and freeze-dried to acquire white flocculent DSPE-PEG-RVG₂₉, which was stored at -20 °C until use.

Arsenic trioxide-loaded zeolitic imidazolate framework-8 (As/ZIF-8) was prepared by an optimized one-pot method [25]. Briefly, Zn(NO₃)₂ · 6H₂O and 2-methylimidazole were dissolved separately in 5 mL and 50 mL deionized water, respectively. The two solutions were mixed rapidly at 25 °C and reacted for 15 min, then ATO was added at a mass ratio of 1:5 and stirred overnight at room temperature. The nanoparticles were collected by centrifugation at 12000 rpm for 10 min, washed three times with deionized water, and lyophilized. GL261 cell membranes were extracted by differential centrifugation. Cells grown to 90% confluence were collected, rinsed twice with ice-cold PBS, and lysed on ice for 30 minutes in RIPA buffer. The lysate was sequentially centrifuged at 2000 g for 10 min, 15000 g for 20 min, and

100000 g for 60 min at 4 °C. The membrane precipitate was resuspended in PBS [26].

To construct As/ZIF-8@CM-RVG₂₉, As/ZIF-8 (1 mg/mL) was mixed with cell membranes at a mass ratio of 1:2. The suspension was extruded in sequence through polycarbonate membranes with pore sizes of 800 nm, 400 nm and 200 nm 11 times each in an ice bath [27]. DSPE-PEG-RVG₂₉ was then added at an optimized mass ratio of 1:10 and incubated at 37 °C for 60 min with gentle shaking [28].

2.2.2. Nanoparticle Characterization

Particle size, PDI, and zeta potential were measured by DLS. Morphological characteristics were examined using transmission electron microscopy (TEM) and scanning electron microscopy (SEM) after sample deposition on carbon-coated grids or gold sputter-coating, respectively [29]. Energy-dispersive X-ray spectroscopy (EDS) and X-ray photoelectron spectroscopy (XPS) were used to test elemental composition and distribution [30]. Fourier transform infrared (FTIR) spectra were acquired from 4000 to 400 cm⁻¹ via the KBr pellet method for chemical structure analysis. N₂ adsorption-desorption isotherms were measured at 77 K, and the Brunauer-Emmett-Teller (BET) method was used to calculate surface area and pore size [31].

2.3.3. Fluorescence colocalization and SDS-PAGE protein analysis

Fluorescein Isothiocyanate (FITC)-labeled ZIF-8 nanoparticles and DiR-labeled GL261 cell membranes were used to verify membrane coating [32]. The colocalization of fluorescent signals was examined by CLSM and quantified using Pearson's correlation coefficient. For sodium dodecyl sulfate-polyacrylamide gel

electrophoresis (SDS-PAGE) protein analysis, equal amounts (1 mg protein) of pure GL261 cell membranes, As/ZIF-8, As/ZIF-8@CM and As/ZIF-8@CM-RVG₂₉ were resuspended in 100 μ L RIPA lysis buffer, incubated on a constant temperature shaker at 4 °C for 30 min with vortexing every 10 min, and centrifuged at 12000 rpm for 20 min at 4 °C. The supernatants were mixed with 5 $\times$ loading buffer and boiled for 5 min to prepare electrophoresis samples. Proteins were separated by SDS-PAGE with a mini vertical electrophoresis system, then the gel was stained for 30 min using Coomassie Brilliant Blue dye and destained with deionized water overnight on a shaker with fresh water replacement every 4 h. After the blue background was completely removed, the gel was imaged and analyzed using a gel imaging system [33].

2.2.4. Drug Release

The drug release behavior of As/ZIF-8, As/ZIF-8@CM and As/ZIF-8@CM-RVG₂₉ was investigated by the dialysis method [34]. Briefly, 2 mL aliquots of each nanoparticle suspension, containing an equivalent ATO dose of 1 mg, were loaded into a dialysis bag with a molecular weight cutoff (MWCO) of 3500 Da. The bag was hermetically sealed and submerged in 50 mL of fresh release medium. The sample was maintained at 37 °C and shaken continuously at 120 rpm. At predetermined time points (0, 1, 2, 4, 6, 8, 12, 24, 36, and 48 h), the entire release medium was withdrawn and replaced with an equal volume of pre-warmed fresh medium. The solution was filtered through a 0.22 μ m microporous membrane, and the arsenic concentration was measured by inductively coupled plasma optical emission

spectroscopy (ICP-OES).

2.3. In vitro studies

2.3.1. In vitro BBB model and transcytosis assay

The BBB model was established by seeding bEnd.3 cells at 6×10^4 cells/well on gelatin-precoated Transwell inserts [35]. When transendothelial electrical resistance (TEER) exceeded $200 \Omega \cdot \text{cm}^2$, the inserts were transferred to 24-well plates containing GL261 cells in the lower chambers. DiI-labeled nanoparticles were added to the upper chambers. After incubation for 4 h, the fluorescence intensity in the lower chambers was measured by a microplate reader, and cellular uptake by GL261 cells was observed by laser confocal scanning microscope (LCSM). Three independent Transwell inserts were used for each group.

2.3.2. Cellular uptake and lysosomal escape

GL261 cells were seeded onto glass-bottom confocal dishes at a density of 5×10^5 cells per dish and allowed to adhere overnight before incubation with FITC-labeled As/ZIF-8@CM-RVG₂₉ for 2 h. The cells were incubated with 4% paraformaldehyde for fixation, followed by staining with Hoechst 33342 and imaged by LCSM. For lysosomal escape assay, cells were incubated with FITC-labeled nanoparticles for 0.5 h and 4 h. LysoTracker Red was added 30 min before the end of incubation to label lysosomes. The Pearson correlation coefficient was calculated using ImageJ to quantify colocalization.

2.3.3. 3D tumor sphere penetration

A 1.5% agarose solution was dissolved at 80 °C and sterilized at 115 °C. Each

well of a 96-well plate was coated with 60 μ L of the solution. After solidification, GL261 cells (2×10^3 well) were seeded. The plate was sealed and centrifuged at 4 °C and 1000 $\times$ g for 10 min to promote cell aggregation. Spheroids were cultured for 7 days to reach approximately 300 μ m in diameter, and then treated with DiI/ZIF-8, DiI/ZIF-8@CM-RVG₂₉ in serum-free DMEM for 4 h. Some wells were irradiated with a 640 nm laser for 5 min and further incubated for 2 h, non-irradiated wells were directly incubated for 2 h. Spheroids were collected, washed three times with PBS, and imaged using a confocal microscope. Optical sectioning was performed from the top to the middle at 10 μ m intervals to assess nanoparticle penetration [36].

2.3.4. Mitochondrial damage

Intracellular ROS production was evaluated using the DCFH-DA probe [37]. Following treatment with the indicated formulations, GL261 cells were incubated with 12 μ M DCFH-DA and analyzed by CLSM and flow cytometry. Mitochondrial membrane potential was assessed using JC-1 staining after PBS washing, followed by CLSM imaging.

2.3.5. Western blot analysis

Total proteins were isolated using RIPA buffer supplemented with protease and phosphatase inhibitors. Equal quantities of proteins were resolved by SDS-PAGE and electrotransferred onto PVDF membranes. Membranes were blocked with 5% skim milk for 1 h at room temperature and then incubated overnight at 4°C with primary antibodies against GSDME (1:1000), cleaved GSDME (1:1000), caspase-3 (1:1000), cleaved caspase-3 (1:1000). After three consecutive washes with TBST

for 5 min each, the membranes were incubated with HRP-conjugated secondary antibodies (1:5000) diluted in blocking buffer for 1 h at room temperature. Protein bands were subsequently visualized using an enhanced chemiluminescence (ECL) reagent and imaged with a digital chemiluminescence detection system [38]. Each Western blot experiment was performed with three independent biological replicates.

2.3.6. Dendritic cell maturation assay

Bone marrow-derived dendritic cells (BMDCs) were isolated from the bone marrow of C57BL/6 mice and cultured in RPMI 1640 medium containing 20 ng/mL GM-CSF and 10 ng/mL IL-4 to induce differentiation over 6-7 days. A small number of cells were washed with PBS and then identified by flow cytometric staining. The proportion of CD11c⁺ cells is 40%-60%, and the proportion of CD80⁺ CD86⁺ cells within the CD11c⁺ cell population is 10%-20%, indicating appropriate differentiation and maturation. GL261 cells were seeded in the upper chambers of Transwell inserts and treated with different formulations for 24 h. BMDCs were seeded in the lower chambers at 1×10^5 cells/well and co-cultured for another 24 h. Collected cells were incubated with FITC-conjugated anti-CD80, PE-conjugated anti-CD86, and APC-conjugated anti-CD11c antibodies. Flow cytometry was performed to quantify the percentage of mature DCs [39]. Three independent biological replicates were performed.

2.4. In vivo studies

2.4.1. Establishment of orthotopic glioma model

Mice were anesthetized with 0.8% pentobarbital sodium (20 mg/kg,

intraperitoneal injection) and fixed in a stereotaxic apparatus. A 1 mm burr hole was made at coordinates 1 mm anterior to the bregma and 2.5 mm lateral to the sagittal suture. GL261-Luc cells (5×10^4 cells in 5 μ L PBS) were injected into the right striatum at a depth of 3.5 mm at a rate of 0.5 μ L/min. To prevent cell reflux, the needle was maintained in situ for 2 min prior to slow retraction. The drilled hole was sealed with bone wax, and the scalp incision was closed with sutures. Tumor growth was confirmed by bioluminescence imaging 10-14 days post-inoculation [40].

2.4.2. Targeting and biodeistribution

Tumor-bearing mice were intravenously injected with ICG-labeled formulations and subjected to in vivo fluorescence imaging using an IVIS Spectrum system at predetermined time points. After euthanasia, major organs were collected for ex vivo fluorescence imaging and quantitative analysis using Living Image software. For the biodistribution study, mice were intravenously administered arsenic at a dose of 2.0 mg/kg and euthanized at 1, 6, and 24 h post-injection. After the organs were weighed, tissue homogenates were prepared [41].

2.4.3. Pharmacokinetic study

SD rats were intravenously administered arsenic-containing formulations [42]. Blood samples were collected at predetermined time points, and plasma was separated by centrifugation. After acid digestion, plasma arsenic concentrations were determined by inductively coupled plasma mass spectrometry (ICP-MS) for pharmacokinetic analysis.

2.4.4. Antitumor efficacy in orthotopic glioma model

Tumor-bearing mice were randomly divided into five groups. Different formulations were injected into the jugular vein. Tumor progression was monitored by bioluminescence imaging, and body weight and survival time were recorded throughout the experiment. At the end of the experiment, brain tissue was fixed, embedded, sectioned, and stained with Hematoxylin and Eosin (H&E) for histopathological examination [43].

2.4.5. Antitumor efficacy in recurrent glioma model

On day 14 post-inoculation, 80%-90% of the visible tumor mass was surgically resected under a stereomicroscope. Mice with residual tumor fluorescence were randomly divided into 4 groups: PBS control, α PD-1, As/ZIF-8@CM-RVG₂₉, and As/ZIF-8@CM-RVG₂₉ + α PD-1. α PD-1 was administered every 2 days for a total of five treatments.

2.4.6. In vivo immune mechanism studies

After the final treatment, all mice were euthanized. Tumor and spleen tissues were excised and mechanically minced to obtain single-cell suspensions. Cell surface staining was performed using fluorochrome-conjugated antibodies against CD3, CD4, CD8, CD11c, CD80, CD86, F4/80, and CD206. After incubation for 20 min at 4 °C in the dark, cells were analyzed by flow cytometry. In addition, serum samples were collected to detect the concentrations of TNF- α , IFN- γ , IL-6, IL-10 and IL-12 using ELISA kits.

2.5. Statistical analysis

Statistical analysis was performed using GraphPad Prism 9.0 software.

Measurement data were expressed as mean $\pm$ standard deviation (SD). Single-factor ANOVA was employed for comparisons among multiple groups, while the t-test was used for comparisons between two groups. A p-value <0.05 was considered statistically significant.

3. Results

3.1 Bioinformatics Analysis and Preliminary Drug Validation

A total of 6,507 differentially expressed genes (DEGs) were identified between glioma and normal tissues, including 3,420 upregulated and 3,087 downregulated genes (Fig. 1A). Intersection with 118 known PRGs yielded 63 DE-PRGs, of which 29 were up-regulated and 34 down-regulated in glioma (Fig. 1B). Violin plots showed a clear separation of DE-PRG expression between normal and tumor tissues. Univariate Cox regression analysis identified GSDME as a significant protective prognostic factor (Fig. 1C), which was selected as core hub genes for subsequent modeling. Consensus clustering based on the core genes stratified patients into two distinct subtypes: low-pyroptosis (C1) and high-pyroptosis (C2). Kaplan-Meier analysis demonstrated that C2 patients had significantly longer OS and PFS than C1 patients (Fig. 1D). In the GBM subgroup, median OS was 16.7 months for C2 versus 9.3 months for C1, indicating that higher pyroptosis activity was associated with better prognosis. Gene ontology (GO) and kyoto encyclopedia of genes and genomes (KEGG) enrichment revealed that DEGs in the C2 subtype were predominantly enriched in T-cell activation, NK-mediated cytotoxicity, antigen processing and presentation, and antitumor immune pathways (Fig. 1E-F). Immune- cell infiltration

clustering analysis (Fig. 1G) further displayed distinct immune landscapes between two pyroptosis-related subtypes. The high-pyroptosis C2 subtype exhibited higher infiltration of antitumor immune cells, whereas the low-pyroptosis C1 subtype was characterized by enrichment of immunosuppressive cell populations, further supporting the correlation between pyroptosis status and the glioma immune microenvironment.

Light microscopy revealed that ATO treatment caused dose-dependent morphological alterations, including cytoplasmic granulation, membrane blebbing, and cell rupture (Fig. 1H). TEM further confirmed pyroptotic ultrastructural features: chromatin condensation, cytoplasmic vacuolation, mitochondrial swelling, and membrane pore formation with content release (Fig. 1I). The LDH release assay showed a significant concentration-dependent increase in LDH leakage after ATO exposure (Fig. 1J), confirming loss of membrane integrity. Western blot analysis demonstrated that ATO upregulated cleaved Caspase-3 and cleaved GSDME accompanied by decreased full-length GSDME and increased IL-1 β expression (Fig. 1K-L). qRT-PCR confirmed that ATO significantly elevated the mRNA levels of Casp3, GSDME-N, and IL-1 β (Fig. 1M). Collectively, these findings demonstrate that ATO triggers pyroptosis through the Caspase-3/GSDME axis in GL261 cells.

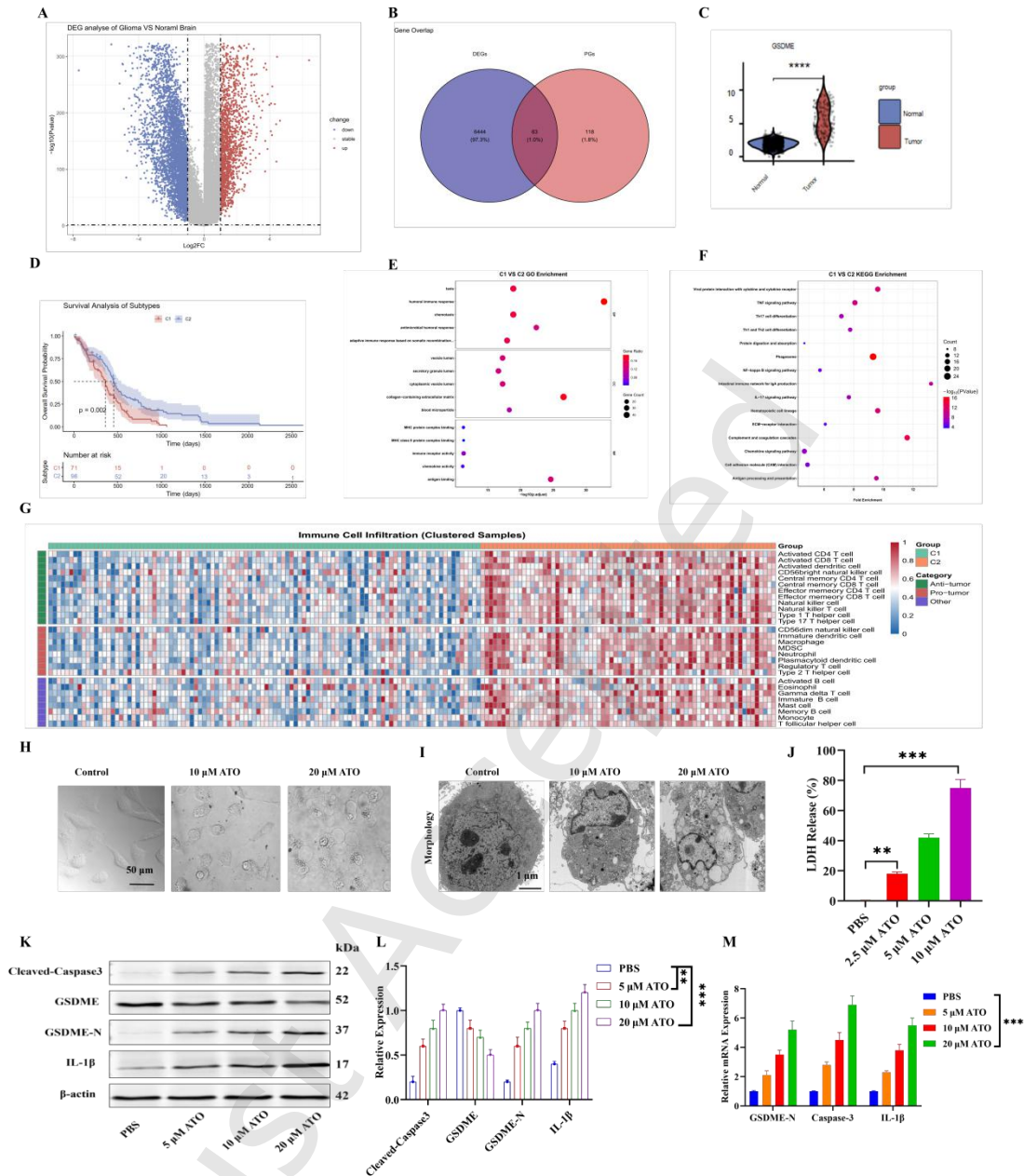


Fig. 1. Analysis of public glioma datasets and in-vitro experimental validation in GL261 cells. (A) Volcano plot of differential genes. (B) Venn diagram of differentially expressed pyroptosis-related genes. (C) Violin plot of pyroptosis gene expression in normal versus tumor tissues. (D) Survival analysis curves for different molecular subtypes. (E-F) Functional enrichment of different pyroptosis subtypes. (G) Cluster analysis of immune-cell infiltration profiles. (H) Microscopic observation of cellular morphological alterations. (I) Transmission electron microscopy images of GL261

cells treated with different concentrations of ATO. (J) Quantitative analysis of LDH release rate. (K-L) Effects of different ATO concentrations on pyroptosis-related protein expression in GL261 cells. (M) Relative mRNA levels of pyroptosis-related genes.

3.2. Physicochemical characterization of As/ZIF-8@CM-RVG₂₉ nanoplatform

As shown in (Fig. 2A), As/ZIF-8 nanoparticles exhibited a hydrodynamic diameter of 165.3 ± 2.5 nm. Following cell membrane coating, the particle size increased to 178.7 ± 3.2 nm, accompanied by a surface charge reversal to -12.3 ± 0.9 mV. Subsequent RVG₂₉ modification further increased the particle size to 182.5 ± 4.1 nm. These changes collectively suggested successful membrane encapsulation and peptide functionalization. The morphology of the nanoparticles was examined by TEM and SEM. As/ZIF-8 nanoparticles displayed a uniform dodecahedral morphology with well-defined edges and minimal aggregation. As/ZIF-8@CM exhibits a core-shell structure, indicating that the cell membrane has been successfully encapsulated onto the ZIF-8 surface (Fig. 2B). Notably, the crystalline morphology of the inner core remained intact. SEM images further confirmed that ATO was successfully loaded into ZIF-8. For validation of RVG₂₉ insertion, the RVG₂₉ peptide was probed using specific antibodies for immunofluorescent staining. As shown in (Fig. S2), confocal microscopy revealed green fluorescence signals on the cell membrane surface, indicating successful insertion.

Elemental further verified successful drug incorporation. EDS spectra detected characteristic As signals in addition to Zn, C, and N elements originating from the

ZIF-8 matrix. Consistently, XPS analysis revealed the presence of Zn 2p, As 3d, C 1s, N 1s, and O 1s peaks without noticeable shifts in Zn 2p binding energy, indicating preservation of the ZIF-8 coordination environment (Fig. 2D-E). Elemental mapping confirmed the homogeneous distribution of Zn, C, N, O, and As throughout the nanoparticles (Fig. 2C). Compared with blank ZIF-8, As/ZIF-8 exhibited a new absorption peak at approximately 800 cm^{-1} , which was assigned to the As-O stretching vibration of ATO. This peak was also observed in the free ATO spectrum but was absent in blank ZIF-8. Meanwhile, the characteristic absorption bands of ZIF-8 were retained without obvious shifts. As/ZIF-8 retained the characteristic absorption bands of ZIF-8 while exhibiting additional peaks attributed to As-O vibrations (Fig. 2F). XRD analysis showed that blank ZIF-8 exhibited sharp and characteristic peaks, which were consistent with the standard simulated spectrum (Fig. 2G), indicating that ATO incorporation did not disrupt the crystalline framework and was likely present in an amorphous or highly dispersed state within the pores. The porous structure of As/ZIF-8 was further evaluated by N_2 adsorption-desorption measurements at 77 K (Fig. 2H). The isotherm exhibited a steep increase at low relative pressure and a hysteresis loop at higher relative pressure, indicating the coexistence of micropores and mesopores.

The construction of biomimetic nanoplateforms is critical for investigating the biocompatibility and active targeted delivery of therapeutic drugs [44]. As/ZIF-8@CM and As/ZIF-8@CM-RVG₂₉ exhibited almost identical protein band profiles to the native CM, with all characteristic membrane protein bands well

retained, indicating that the extrusion coating process and subsequent RVG₂₉ modification did not cause significant degradation or loss of membrane proteins (Fig. 2J). As shown in (Fig. 2I), the Pearson correlation coefficient between the green and red fluorescence signals reached 0.91, indicating a high spatial colocalization between the nanoparticle core and cell membrane signals. The cumulative release rate of ATO from As/ZIF-8@CM-RVG₂₉ was only (18.43±1.23)% within 48 h under physiological pH conditions (Fig. 2K). The release rate of ATO in each group followed the order of As/ZIF-8 > As/ZIF-8@CM-RVG₂₉ under acidic conditions, indicating that the CM coating and RVG₂₉ modification further enhanced the controlled release performance of the nanoplatform.

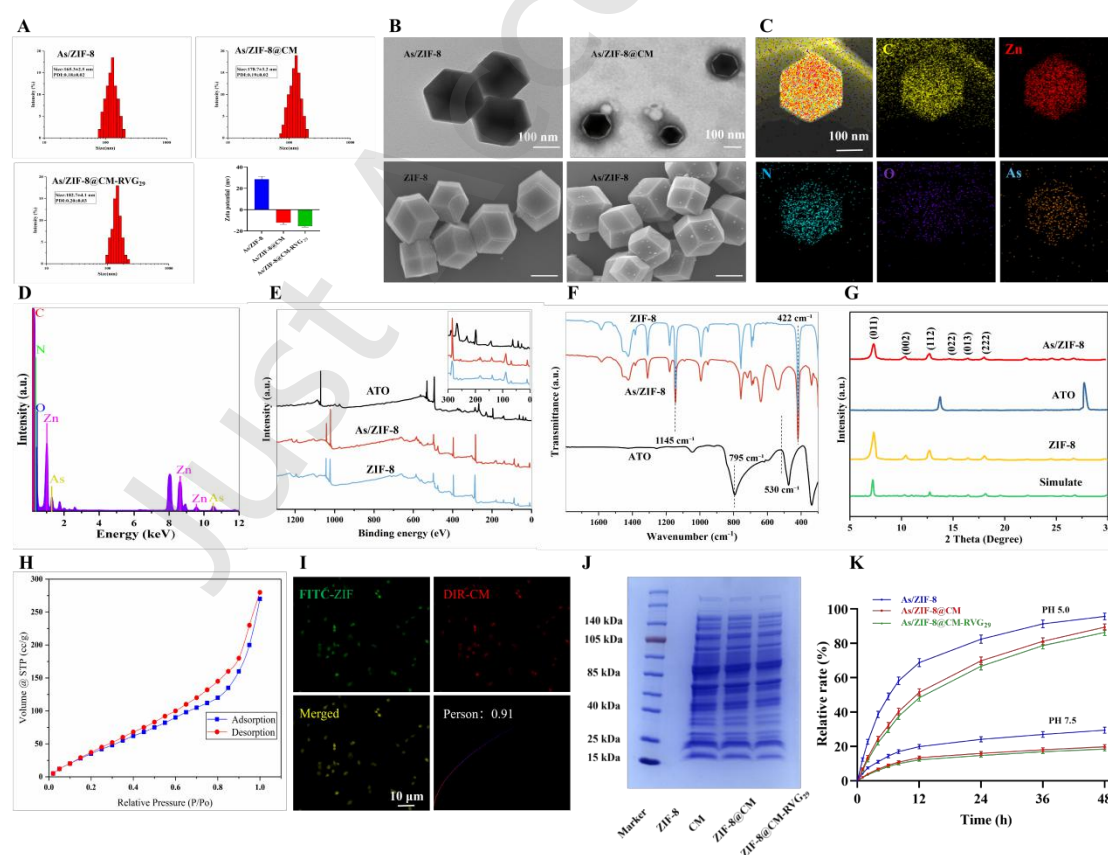


Fig. 2. Construction and characterization of As/ZIF-8@CM-RVG₂₉. (A) Particle size and zeta potential measurement. (B) TEM and SEM images, the scale is 200 nm. (C)

Elemental mapping images in As/ZIF-8. (D) EDS analysis of As/ZIF-8. (E) XPS analysis. (F) FTIR analysis. (G) XRD analysis. (H) N₂ Adsorption-desorption isotherms of As/ZIF-8. (I) Fluorescence co-localization analysis of As/ZIF-8@CM-RVG₂₉. (J) SDS-PAGE analysis of membrane protein retention. (K) In vitro release profiles of different formulation groups at pH 5.0 and 7.5 (n=3, means±SD).

3.3. In vitro targeting validation of As/ZIF-8@CM-RVG₂₉

BBB penetration is essential for brain-targeted delivery (Fig. 3A). The trans-BBB ability of different formulations was evaluated by fluorescence imaging (Fig. 3B). For the free ICG and unmodified ZIF-8 groups, the majority of the fluorescence signal was retained in the bEnd.3 cell monolayer. In contrast, the DiI-labeled As/ZIF-8@CM-RVG₂₉ group showed strong fluorescence signal in the lower chamber GL261 cells, with significantly higher intracellular fluorescence intensity than the other groups.

To investigate intracellular trafficking, GL261 cells were incubated with Cou6-labeled As/ZIF-8@CM-RVG₂₉ for 6 h. Abundant green fluorescence originating was widely distributed throughout the cytoplasm, indicating efficient cellular internalization (Fig. 3C). Intracellular lysosomal trafficking was subsequently assessed using lysotracker red staining. At the early stage of incubation, substantial overlap between nanoparticle-derived green fluorescence and lysosomal red fluorescence was observed (Fig. 3D). However, after prolonged incubation, the coefficient decreased to 0.21, accompanied by widespread cytoplasmic diffusion of

the nanoparticle signal (Fig. 3E). These findings suggest that As/ZIF-8@CM-RVG₂₉ initially accumulated within lysosomes and subsequently escaped into the cytoplasm.

3D GL261 tumor spheroids with a diameter of approximately 200 μm were constructed to evaluate the deep penetration ability of the nanopatform (Fig. 3F). CLSM Z-stack scanning showed that free DiI and unmodified ZIF-8 were merely attached to the surface layer of the tumor spheroids, with almost no fluorescence signal detected in the core area. In contrast, DiI-labeled ZIF-8@CM-RVG₂₉ penetrated the outer dense cell layer of the tumor spheroids and diffused continuously to the core area, achieving uniform fluorescence distribution throughout the whole spheroid after 4 h of incubation (Fig. 3G-H). The PBS control group showed intact tumor spheroid structure with predominantly green fluorescence from live cells fluorescence and negligible red fluorescence from dead cells. As/ZIF-8@CM-RVG₂₉ group showed a large area of strong red dead cell fluorescence throughout the tumor spheroid, with a significantly reduced live cell ratio, and the spheroid structure was loose and damaged (Fig. 3I-J). These results confirmed that As/ZIF-8@CM-RVG₂₉ exhibited excellent deep tumor penetration capability and potent cytotoxicity on 3D glioma spheroids.

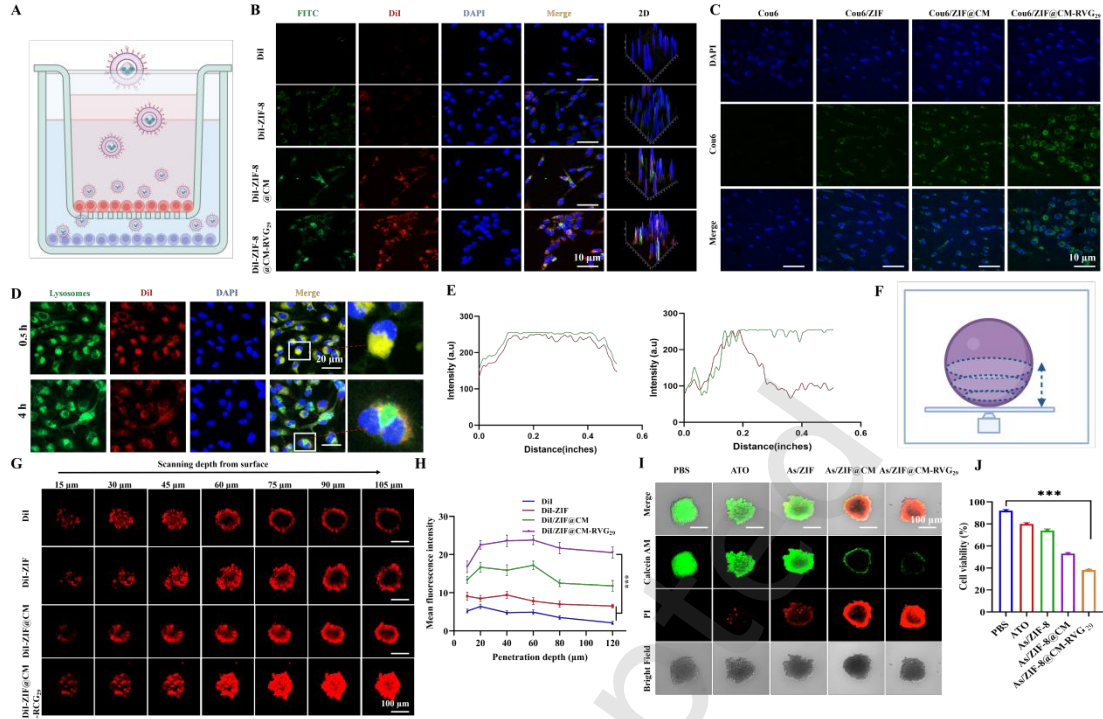


Fig. 3. In vitro targeting validation. (A) Construction of the BBB model. (B) In vitro blood-brain barrier crossing capability. (C) Cell uptake assay. (D) Lysosomal escape fluorescence imaging, scale bar, 10 μm. (E) Co-localization ratio. (F) Construction of 3D tumor spheroid model. (G-H) Tumor spheroid permeation assay images and MFI analysis. (I-J) Live/dead fluorescence imaging of tumor spheroids and cytotoxicity evaluation (** $P < 0.01$, *** $P < 0.001$, $n=3$).

3.4. In vitro antitumor activity of As/ZIF-8@CM-RVG₂₉

All formulations showed dose-dependent cytotoxicity against GL261 cells after 48 h of incubation (Fig. 4A). The IC₅₀ value of As/ZIF-8 was (4.85±0.41) μM (Fig. S4), indicating that CM coating and RVG₂₉ modification synergistically enhanced the tumor cell killing effect of ATO by improving the targeting and cellular uptake efficiency of the nanocarrier. ROS is a key upstream trigger of ATO-induced pyroptosis [45]. Cells in the PBS group exhibited only weak background fluorescence,

with intracellular ROS levels remaining stable. Following treatment with As/ZIF-8@CM-RVG₂₉, the intracellular green fluorescence signal significantly intensified (Fig. 4B). Semiquantitative analysis confirmed this finding (Fig. S5). Mitochondrial membrane potential imbalance is an important hallmark of pyroptosis initiation. In the As/ZIF-8@CM-RVG₂₉ group, the red fluorescence decreased significantly, with the red/green fluorescence ratio was reduced by 82.3% compared with the control group (Fig. 4C), indicating severe mitochondrial membrane potential (MMP) depolarization and mitochondrial damage. Meanwhile, immunofluorescence staining showed that As/ZIF-8@CM-RVG₂₉ group induced markedly release of mtDNA (Fig. 4D). This result suggests a potential link between pyroptosis and innate immune activation.

To further evaluate drug-induced ICD substance release and the release of intracellular contents [46], the expression levels of calreticulin (CRT) and HMGB1 leakage were measured in GL261 glioma cells treated with As/ZIF-8@CM-RVG₂₉. Immunofluorescence staining showed that As/ZIF-8@CM-RVG₂₉ treatment significantly increased CRT exposure on the cell surface (Fig. 4E), accompanied by a substantial increase in HMGB1 release into the extracellular space (Fig. 4F). These results demonstrate that ATO effectively induces ICD in tumor cells and activates local immune responses in the host.

mtDNA released from damaged mitochondria acts as a typical DAMP and can be recognized by the cytosolic sensor cyclic GMP-AMP synthase (cGAS) to initiate downstream immune signaling [47]. Compared with the PBS group,

As/ZIF-8@CM-RVG₂₉ nanoparticles remarkably enhanced the abundance of these activated pyroptotic markers (Fig. 4H). Quantitative analysis was shown in (Fig. S7). These results indicate that As/ZIF-8@CM-RVG₂₉ treatment is associated with activation of the caspase-3/GSDME pyroptotic axis in glioma cells, which may contribute to its tumor cell killing effect. Western blot analysis showed that As/ZIF-8@CM-RVG₂₉ treatment significantly increased the expression and phosphorylation of cGAS-STING pathway-associated proteins (Fig. 4I), indicating enhanced activation of cGAS-STING-associated signaling. However, these data alone do not establish direct causal dependence. DAMPs generated during ICD are known to facilitate DC maturation [48]. Accordingly, a GL261-BMDC Transwell co-culture model was established (Fig. 4J). Flow cytometry analysis showed that As/ZIF-8@CM-RVG₂₉ treatment significantly increased the proportion of mature DCs compared with the control groups (Fig. 4K), and quantitative analysis confirmed this increase (Fig. 4L). These findings suggest that As/ZIF-8@CM-RVG₂₉ effectively promotes DC maturation and enhances tumor-associated immune activation.

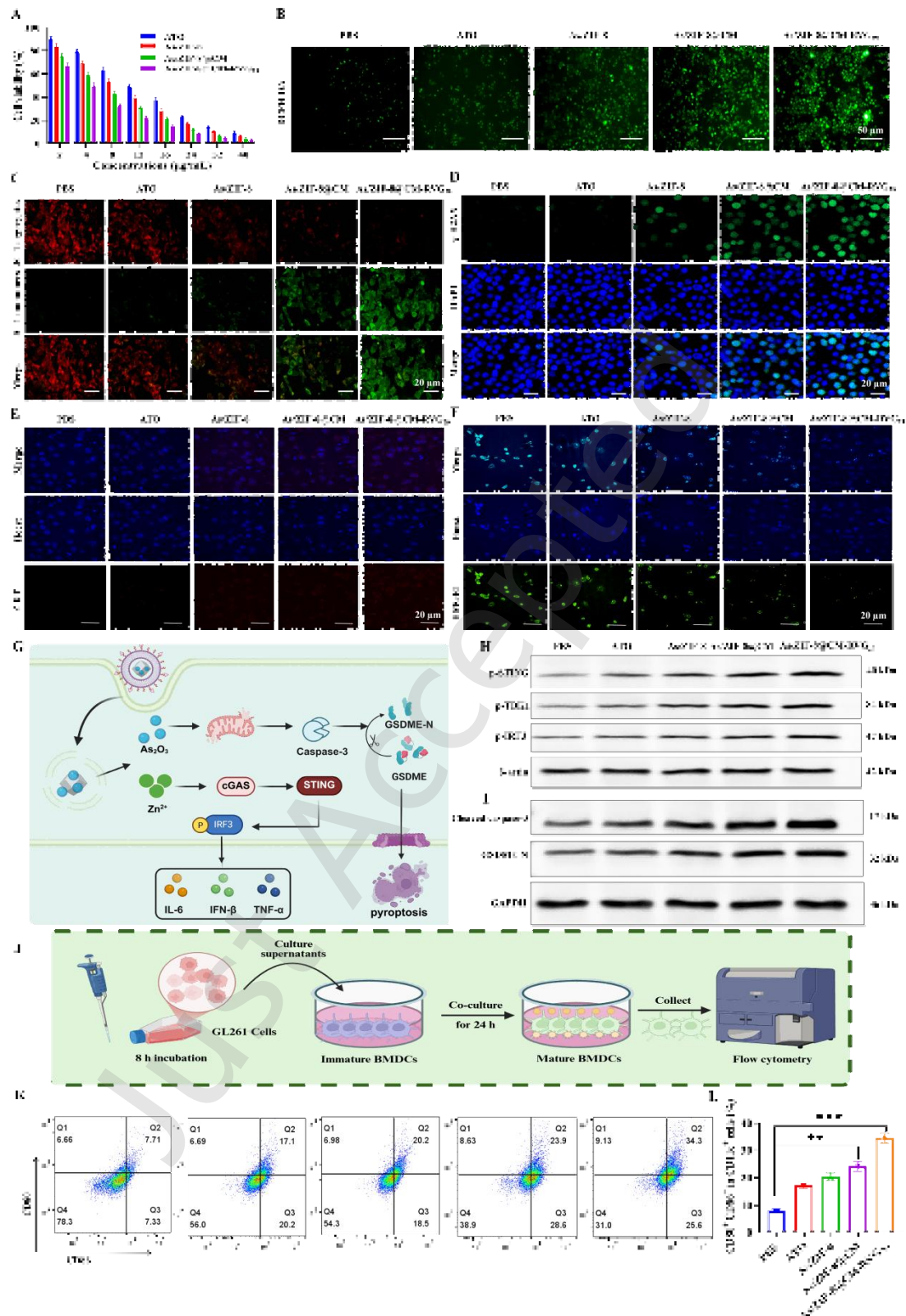


Fig. 4. In vitro antitumor effects and mechanism studies of nanoparticle formulations.

(A) Effects of different concentrations on GL261 cell viability. (B) Generation of ROS. (C) CLSM observation of mitochondrial membrane potential changes in GL261

cells. (D) mtDNA release. (E) CRT exposure on the cell surface. (F) Intracellular expression of HMGB1. (G) Intracellular pyroptosis and STING pathway activation. (H) Expression of pyroptosis-related proteins. (I) Expression of STING pathway-associated proteins. (J) Construction of tumor cell-dendritic cell co-culture systems. (K) Flow cytometry analysis of mature DCs. (L) Quantitative flow cytometry analysis (** $P < 0.01$, *** $P < 0.001$, $n=3$).

3.5. In vivo brain-targeting property and pharmacokinetic behavior

As shown in (Fig. 5A), free ICG rapidly distributed throughout the body and was subsequently cleared, resulting in negligible fluorescence accumulation within the brain tumor region. Similarly, ICG/ZIF-8 primarily accumulated in the liver, indicating limited brain delivery. In contrast, ICG/ZIF-8@CM-RVG₂₉ exhibited progressive fluorescence enrichment at the tumor site from 2 to 24 h post-injection, with maximal signal intensity observed at 24 h. To further assess intracranial distribution, mice were sacrificed at 24 h, and brains were collected for imaging and histological analysis. Ex vivo fluorescence imaging demonstrated substantially higher fluorescence retention in the brains of mice treated with ICG/ZIF-8@CM-RVG₂₉ compared with the free ICG group (Fig. 5B). Brain sections containing the orthotopic GL261 glioma and adjacent normal brain tissue were imaged to evaluate the intracranial distribution of the ICG-labeled formulations. The results showed that free ICG and ICG/ZIF-8 exhibited only weak fluorescence in the tumor region, whereas ICG/ZIF-8@CM-RVG₂₉ showed markedly stronger fluorescence predominantly localized within the tumor tissue, with only weak signals in the adjacent normal brain

region (Fig. 5C-D).

To elucidate the *in vivo* metabolic characteristics, circulation half-life, and bioavailability of As/ZIF-8@CM-RVG₂₉, a systematic pharmacokinetic evaluation was conducted. As shown in (Fig. 5E). The plasma drug concentration in the free ATO group increased rapidly and then declined sharply, indicating fast *in vivo* elimination, a small apparent volume of distribution, and poor long-circulating properties. The plasma drug elimination rate was slower in the As/ZIF-8 group, but rapid sequestration by the reticuloendothelial system still existed. In contrast, the plasma drug concentration in the As/ZIF-8@CM-RVG₂₉ group decreased gently, with a significantly prolonged *in vivo* retention time (Table. S1). At 24 h after injection, the ATO concentration in the brain tissue of the As/ZIF-8@CM-RVG₂₉ group was evidently higher than the free ATO group. Free ATO was mainly distributed in the kidney, with rapid renal excretion, while As/ZIF-8@CM-RVG₂₉ significantly reduced the ATO accumulation in the kidney and heart, and only showed moderate accumulation in the liver (Fig. 5F).

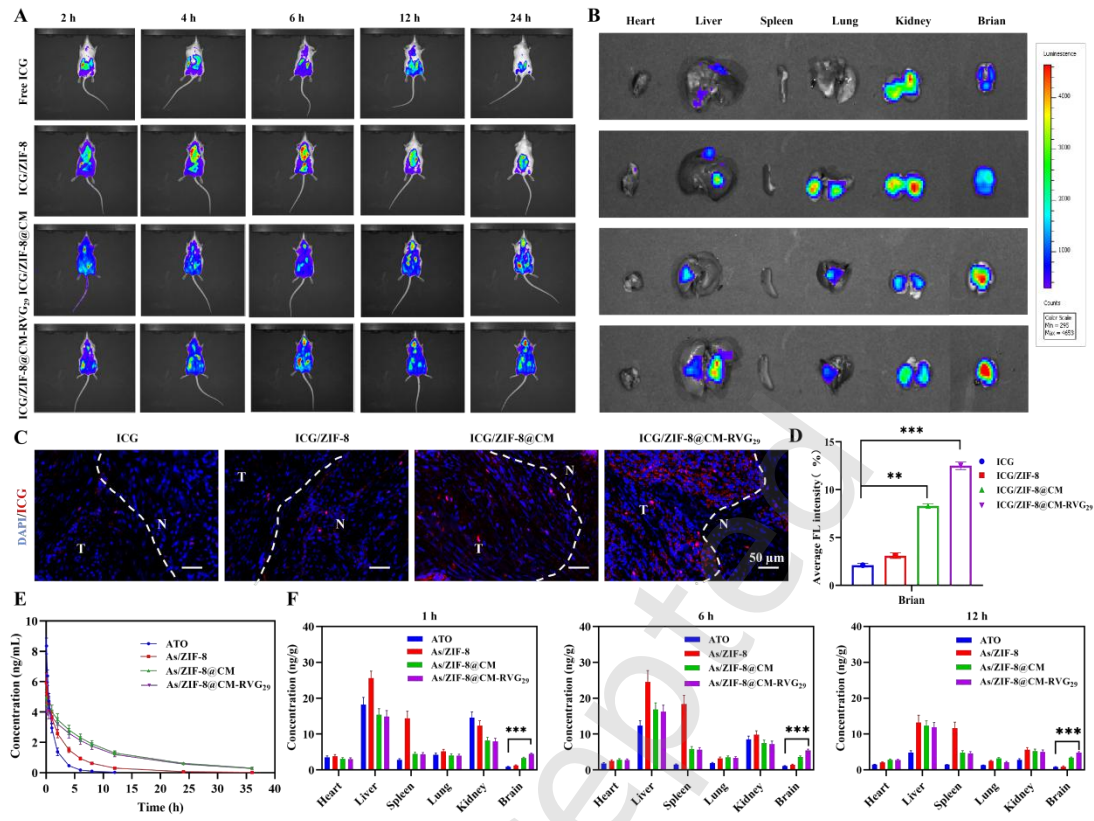


Fig. 5. In vivo biodistribution and pharmacokinetic analysis. (A) In vivo fluorescence imaging. (B) Ex vivo fluorescence imaging of major organs at 24 h post-injection. (C) CLSM images of brain sections (D) Brain targeting index. (E) Plasma pharmacokinetic parameters (mean $\pm$ SD, n=3). (F) ATO distribution in major organs at 1, 6, and 12 h post-injection (** $p < 0.01$, *** $p < 0.001$, n=3).

3.6. In vivo antiglioma efficacy and immune microenvironment remodeling of As/ZIF-8@CM-RVG₂₉

The PBS control group exhibited the most rapid tumor growth, with the strongest bioluminescence signal and obvious tumor mass occupying the right brain hemisphere at the end of treatment, confirming successful model establishment and unrestricted tumor progression (Fig. 6B). The free ATO group showed only slight tumor growth inhibition, which was attributed to the poor BBB penetration ability of free ATO,

resulting in extremely low drug accumulation in brain tissues. The As/ZIF-8@CM group exhibited further improved antitumor efficacy, as evidenced by markedly decreased tumor volume and fluorescence intensity. Notably, the As/ZIF-8@CM-RVG₂₉ group achieved the most antitumor effect, weakest fluorescence signal, and obvious tumor regression in some mice.

Mice in the PBS group exhibited progressive weight loss beginning on day 12, reaching a maximum reduction of 11.2% by day 21 (Fig. 6D). In contrast, mice in the As/ZIF-8@CM group maintained relatively stable body weight and even showed a slight increase during the treatment period. Survival analysis further demonstrated the therapeutic advantage of the targeted nanoplateform (Fig. 6E). The median survival time of the free ATO group was 28.3 days. Notably, treatment with As/ZIF-8@CM-RVG₂₉ extended the median survival time to 42.8 days, representing an 82.1% increase compared with the PBS group. Histopathological examination was subsequently performed to evaluate tumor pathology (Fig. 6F). Extensive tumor infiltration, high cellular density, marked nuclear atypia, necrosis, and microvascular proliferation were observed in the PBS group. Although tumor size was reduced in the free ATO group, active proliferative regions remained evident. In contrast, only minimal residual lesions were observed in mice treated with As/ZIF-8@CM-RVG₂₉, along with sparse tumor cell distribution and inflammatory cell infiltration.

To investigate the occurrence of pyroptosis *in vivo*, tumor sections were stained. As shown in (Fig. 6I-J), extensive positive staining was detected in tumors treated with As/ZIF-8@CM-RVG₂₉, whereas substantially weaker signals were observed in

the other groups. These findings indicate robust activation of the caspase-3/GSDME-mediated pyroptotic pathway within tumor tissues. Notably stronger red fluorescence signals were visualized in tissue slices from the As/ZIF-8@CM-RVG₂₉ cohort relative to other experimental groups (Fig. 6K-L). These results demonstrate that As/ZIF-8@CM-RVG₂₉ effectively induces pyroptosis in glioma cells in vivo, likely through a synergistic effect combining RVG₂₉-mediated BBB penetration with CM-mediated homologous targeting. These findings are consistent with the possibility that dsDNA generated during pyroptosis may be recognized by cGAS and contribute to downstream STING signaling activation.

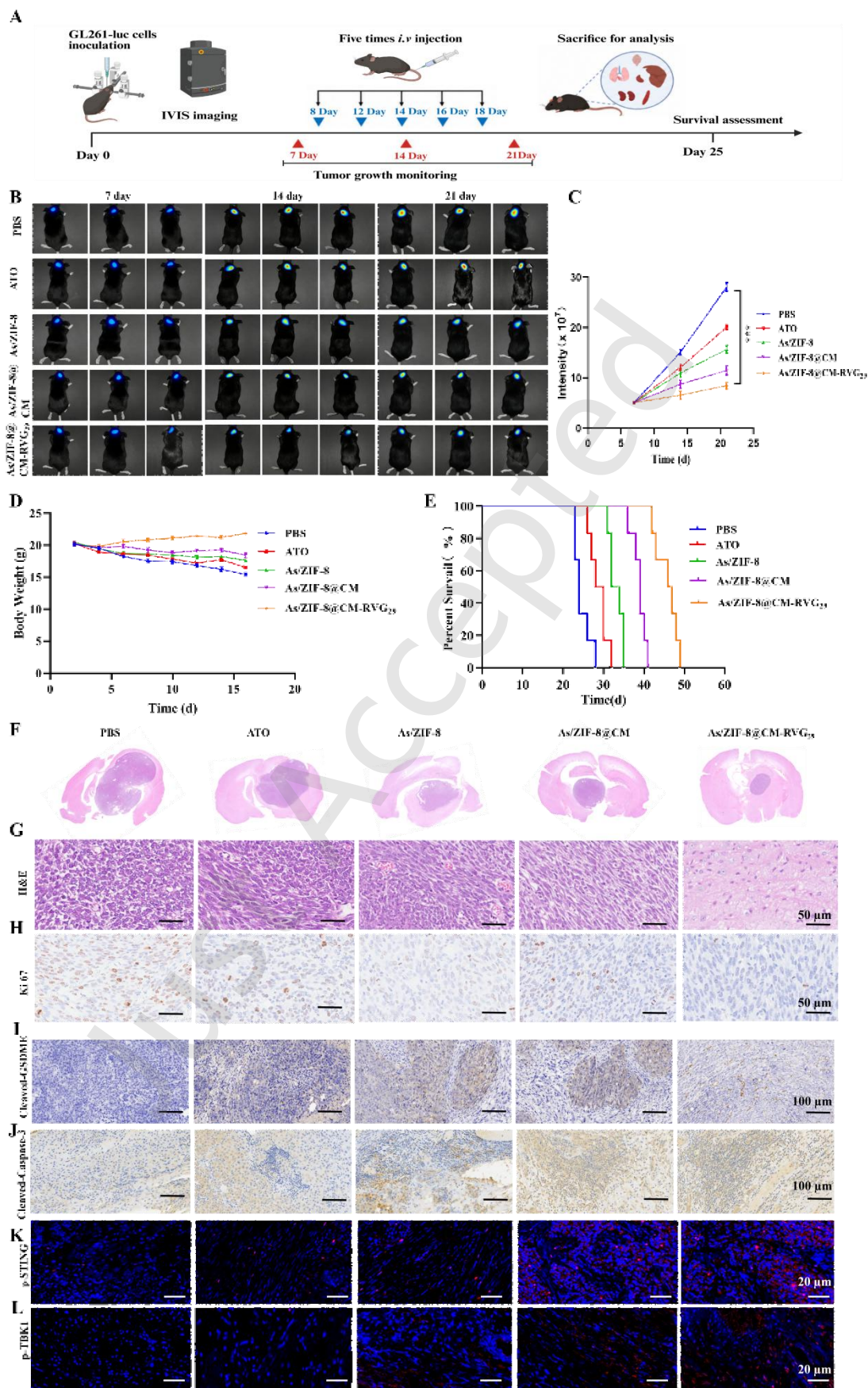


Fig. 6. Pharmacodynamic evaluation. (A) Modeling and administration process of

glioma model mice. (B) Real-time bioluminescence images and semi-quantitative analysis (n=3). (C) Semi-quantitative analysis. (D) Body weight changes. (E) Survival curves (n=6). (F) Whole-brain tissue sections. (G) Panoramic H&E staining of brain tissue. (H) Ki67 staining. (I-J) Immunohistochemical staining. (K-L) Fluorescence staining of STING markers in tumor tissues (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n=6).

3.7. Remodeling of the tumor immune microenvironment

To investigate immune modulation within glioma tissues, multicolor immunofluorescence staining was performed. As shown in (Fig. 7A), As/ZIF-8@CM-RVG₂₉ group markedly increased the fluorescence intensity of CD86 while reducing CD206 expression relative to the other groups, suggesting a shift in macrophage phenotype toward a pro-inflammatory state. Analysis of T-cell subsets revealed a similar trend (Fig. 7B). Compared with the control groups, As/ZIF-8@CM-RVG₂₉ treatment increased CD4⁺ T-cell infiltration while reducing the abundance of Foxp3⁺ Tregs. These observations indicate a more immunologically active TME following treatment.

TAMs represent a critical population of immune cells within the TME, primarily classified into M1-type cells with antitumor effects and M2-type cells that promote tumor progression. In the PBS group, M2-like macrophages constituted the dominant population (25.2%), whereas M1 macrophages accounted for only 8.25% (Fig. 7E-F). In contrast, As/ZIF-8@CM-RVG₂₉ treatment increased M1 macrophages to 15.5%. These findings indicate a pronounced shift toward an immunostimulatory macrophage phenotype. Additionally, Systemic immune responses were further assessed by

measuring serum cytokine levels. As/ZIF-8@CM-RVG₂₉ group exhibited elevated the pro-inflammatory cytokines compared with the other groups (Fig. 7G).

RNA sequencing was performed on tumor tissues to elucidate the regulatory mechanisms of targeted nanomedicines on the in situ glioma microenvironment at the global transcriptional level. Principal component analysis (PCA) revealed clear separation between the PBS and As/ZIF-8@CM-RVG₂₉ groups, with strong clustering among biological replicates, indicating distinct transcriptional profiles after treatment (Fig. 7H). A total of 816 DEGs were identified, including 632 upregulated genes and 184 downregulated genes (Fig. 7I-J). GO enrichment analysis showed that these DEGs were primarily associated with immune-cell chemotaxis, immune activation, antigen processing and presentation, type I interferon signaling, and pyroptosis-related biological processes (Fig. 7K). (KEGG pathway analysis further revealed significant enrichment of pathways related to cytosolic DNA sensing, pyroptosis, and antitumor immunity (Fig. 7L). Consistently, gene set enrichment analysis (GSEA) demonstrated enhanced enrichment of pyroptosis-associated and T-cell activation gene signatures in the As/ZIF-8@CM-RVG₂₉ group (Fig. 7M). Collectively, these transcriptomic findings support the immunomodulatory effects of As/ZIF-8@CM-RVG₂₉ and its ability to promote pyroptosis-associated immune activation within the glioma microenvironment.

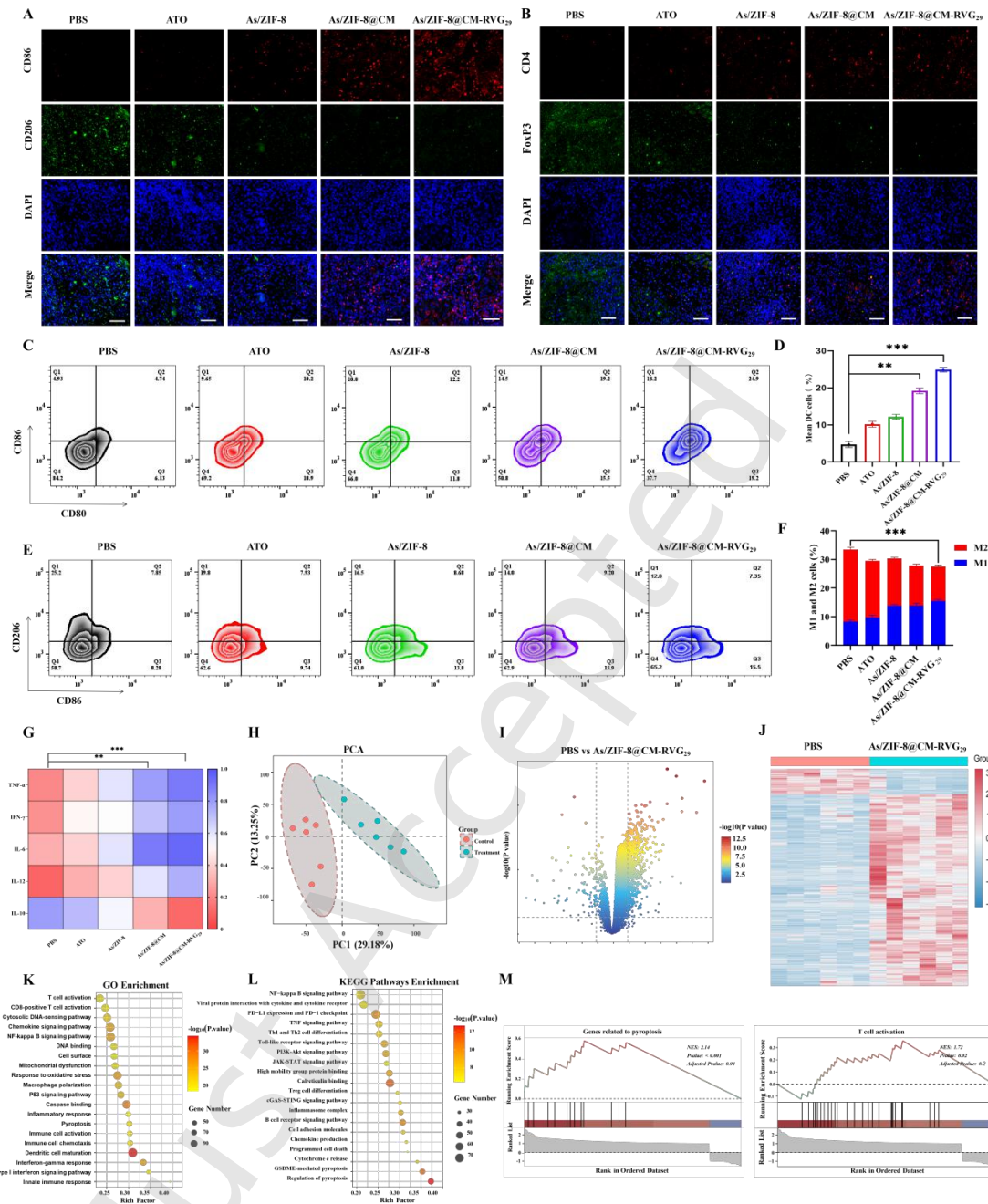


Fig. 7. Immunofluorescence analysis and transcriptomic profiling. (A-B) Analysis of multi-marker immunofluorescence staining in tumor tissues, the scale bar is 10 μ m. (C-D) DC cells in tumor tissues and their proportion. (E-F) Flow cytometry analysis and proportions (B) CD206⁺ M2-like macrophages and CD86⁺ M1-like macrophages in tumor tissue. (G) Concentrations of TNF- α , IFN- γ , IL-6, IL-12, and IL-10 in mouse serum. (H) PCA diagram. (I) Volcano plot of differentially expressed genes. (J) heat

map clustering analysis.(K) GO function analysis.(L) KEGG enrichment analysis.(M) GSEA pathway ($*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $n=3$).

3.8. Synergistic antitumor effect of As/ZIF-8@CM-RVG₂₉ in combination with anti-PD-1 immunotherapy in postoperative recurrence models

To investigate whether As/ZIF-8@CM-RVG₂₉ could enhance immune checkpoint blockade therapy, a postoperative glioma recurrence model was established and treated (Fig. 8A). The PBS group exhibited rapid tumor recurrence, accompanied by progressively increasing fluorescence signals throughout the observation period (Fig. 8B). Treatment with α PD-1 alone produced only modest suppression of tumor regrowth, whereas As/ZIF-8@CM-RVG₂₉ monotherapy delayed tumor progression to a greater extent. Notably, the combination treatment achieved the strongest therapeutic effect, with minimal increases in bioluminescence intensity and visible tumor regression in several animals. Quantitative analysis of fluorescence signals further supported these observations (Fig. 8C). To assess tumor proliferation and apoptosis in the recurrent glioma model, Ki67 and TUNEL staining was performed (Fig. 8D). The combination group exhibited the lowest Ki67 positivity and the highest TUNEL positivity, consistent with its superior antitumor efficacy and prolonged survival.

Body weight changes were monitored throughout the treatment period (Fig. 8E). Compared to the PBS group, The treated with As/ZIF-8@CM-RVG₂₉ maintained relatively stable body weight. The median survival time of the PBS group was 18.5 days (Fig. 8F). Treatment with α PD-1 or As/ZIF-8@CM-RVG₂₉ alone extended

median survival to 25.6 and 38.5 days, respectively. The combination regimen further prolonged median survival to 58.5 days, corresponding to a 216.2% increase relative to the control group. Importantly, several mice in the combination group remained alive at the 80-day study endpoint.

As shown in (Fig. 8G-H), CD8⁺ T cells accounted for only 4.9 % of immune cells in the PBS group. This proportion increased to 8.7 % following α PD-1 treatment and to 13.6% after administration of As/ZIF-8@CM-RVG₂₉ alone. In the combination group, CD8⁺ T cells accounted for 21.6% of immune cells (Fig. 8I-J). These findings suggest enhanced antitumor immune activation following combination treatment. Finally, a systematic biosafety evaluation was performed (Fig. S10-S12). The formulation exhibited no significant hemolysis, and serum biochemical markers of liver and kidney function remained within normal ranges after administration, with no evident pathological changes.

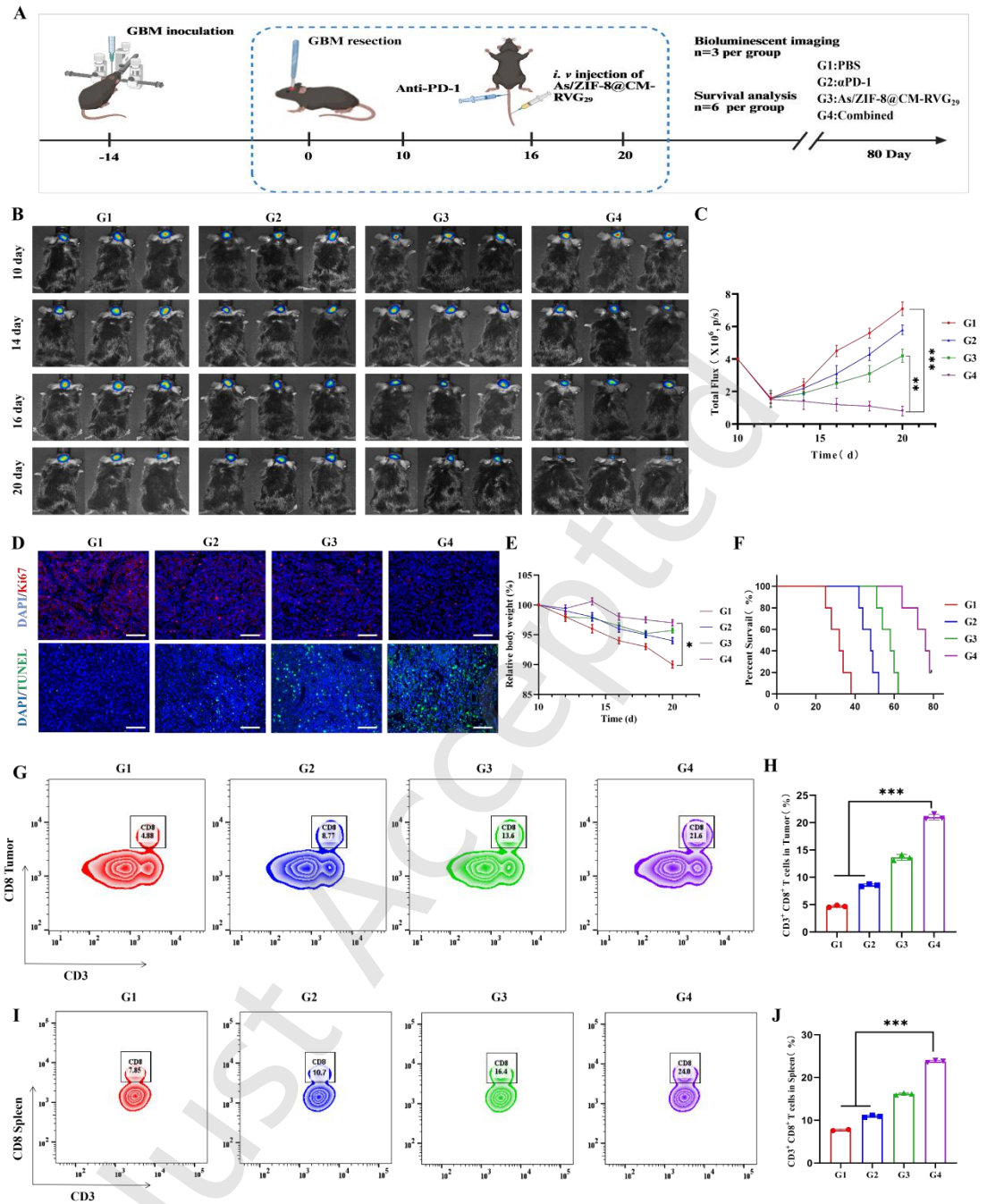


Fig. 8. Recurrent model therapy. (A) Schematic of the mouse glioma recurrence model. (B) Representative in vivo bioluminescence images of mice in each treatment group (n=3 mice per group). (C) Quantitative analysis of intracranial fluorescence values. (D) Ki67 and TUNEL immunofluorescence staining of tumor tissues, the scale is 20 μm . (E) Weight changes. (F) Survival curves (n=6). (G) Flow cytometry analysis of CD8⁺ T cells in tumor tissue. (H) Quantification of CD8⁺ T cells in tumor tissues. (I)

Flow cytometry analysis of CD8⁺ T cells in the spleen. (J) Quantification of CD8⁺ T cells in the spleen. (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, n=6).

4. Discussion

Unlike conventional nanocarriers that primarily function as passive drug reservoirs, the engineered platform actively participates in the amplification of therapeutic signaling through the coordinated actions of its individual components. RVG₂₉ facilitates transport across the BBB, and the glioma cell membrane coating promotes homologous tumor recognition and immune evasion [49]. This hierarchical design addresses multiple biological barriers that traditionally limit the effectiveness of brain tumor nanomedicine.

Among the 63 differentially expressed PRGs, the prominence of GSDME is particularly compelling. Unlike GSDMD, which is broadly expressed in myeloid cells, GSDME is frequently epigenetically silenced (via promoter hypermethylation) in numerous malignancies, serving as a classical gatekeeper against metastasis [50]. Our data revealing that higher GSDME expression correlates with improved survival in glioma challenges the conventional view of GSDME merely as a bystander. Instead, it suggests that tumors retaining GSDME expression harbor an intrinsic vulnerability—they possess the "executioner machinery" ready to convert apoptotic signals into highly immunogenic pyroptotic cell death.

The classical view of ATO's anticancer action has revolved around apoptosis induction [51]. Our study provides compelling evidence that at the concentrations delivered by our nanocarrier, ATO predominantly induces a different programmed cell

death pathway. This is a crucial distinction, apoptotic cells are typically immunologically silent or even tolerogenic, whereas pyroptotic cells actively alert the immune system. The transition from apoptosis to pyroptosis can depend on the intensity and duration of the insult.

The nanotherapy primes the TME by converting it from immunosuppressive to immunoresponsive, while α PD-1 “releases the brakes” on newly infiltrated yet potentially exhausted cytotoxic T lymphocytes (CTLs). The immune activation was not limited to the primary tumor site, as evidenced by increased CD8⁺ T cells in the spleen. This is a critical prerequisite for combating distant micro-metastases and preventing long-term recurrence, which remains the ultimate goal of GBM immunotherapy. The generation of immunological memory is a likely outcome, although formal proof using rechallenge experiments is warranted.

The precise molecular interactions between pyroptosis and the cGAS-STING signaling complex require further investigation. Although the present data show increased expression and phosphorylation of cGAS-STING pathway components after treatment, these findings indicate an association rather than definitive causality. Genetic knockdown or pharmacological inhibition studies will be necessary to establish whether cGAS-STING activation is directly required for the observed immune remodeling and antitumor efficacy. Furthermore, the long-term immunological memory induced by this therapeutic strategy and its efficacy in patient-derived or spontaneous glioma models remain to be explored.

5. Conclusions

In summary, this study employed bioinformatics analysis to elucidate the prognostic significance and immunomodulatory role of pyroptosis-related genes in gliomas, demonstrating that ATO induces pyroptosis by activating the Caspase-3/GSDME pathway. The successful development of the As/ZIF-8@CM-RVG₂₉ biomimetic nanosystem enables penetration across the BBB, targeted delivery to gliomas, pH-responsive drug release, enhanced bioavailability of ATO, and reduced systemic toxicity. In vivo experiments showed that this system was associated with pyroptosis induction and remodeling of the immune microenvironment, and it exhibited anti-glioma efficacy. The involvement of cGAS-STING signaling is proposed based on pathway marker activation, and further causal validation is required. When combined with α PD-1 inhibitors, it significantly improves treatment outcomes for recurrent tumors. This study establishes a novel delivery strategy for ATO-based glioma therapy and lays theoretical and experimental foundations for precision immunotherapy and combination therapies. However, there are still limitations, future efforts should focus on optimizing preparation methods, enhancing drug-targeting accuracy, and advancing molecular mechanisms and preclinical translational research to advance its clinical application.

Declarations

Ethical Approval and Informed Consent

All animal experiments were conducted in compliance with the Guidelines for Care and Use of Laboratory Animals issued by Zhejiang University of Traditional Chinese Medicine, and were approved by the Institutional Animal Care and Use

Committee of Zhejiang University of Traditional Chinese Medicine (Approval No.: 20240413-05).

Consent for publication

Not applicable

Availability of data and materials

All data are included in the manuscript and its supporting information files.

Competing interest

The 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.

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CRedit authorship contribution statement

Hao Wang: Writing-original draft, Methodology, Investigation, Conceptualization. SenSen Zheng: Software, Conceptualization. Dingfeng Chen: Formal analysis, Data curation. Jia Li: Methodology, Data curation. TianZe Tao: Validation, Investigation. Xiaoying Zhao: Writing-review & editing Fanzhu Li: Writing-review & editing, Supervision, Project administration, Funding acquisition.

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Electronic Supplementary Material

Biomimetic arsenic trioxide nanoplatform induces Caspase-3/GSDME pyroptosis and remodels the glioma immune microenvironment

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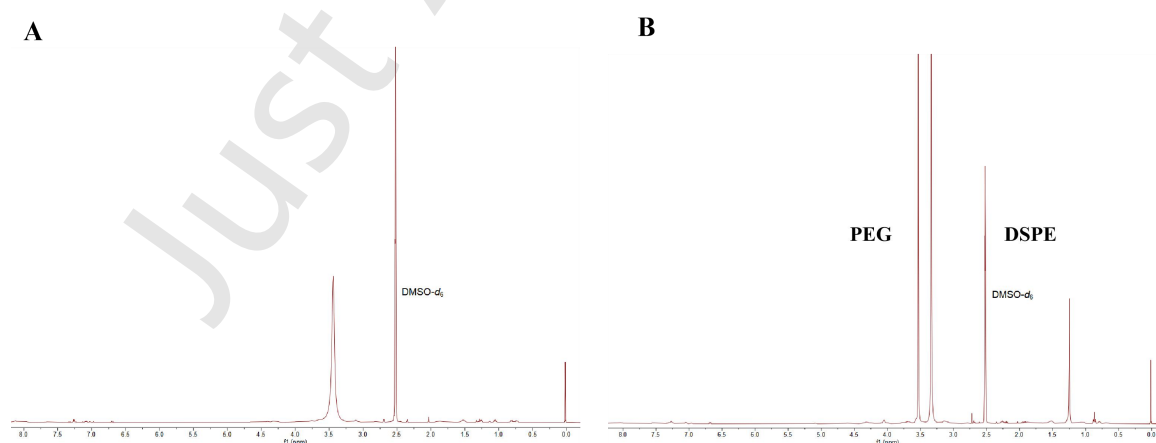


Fig. S1 ¹H NMR spectra of RVG₂₉ (A) and DSPE-PEG-RVG₂₉ (B).

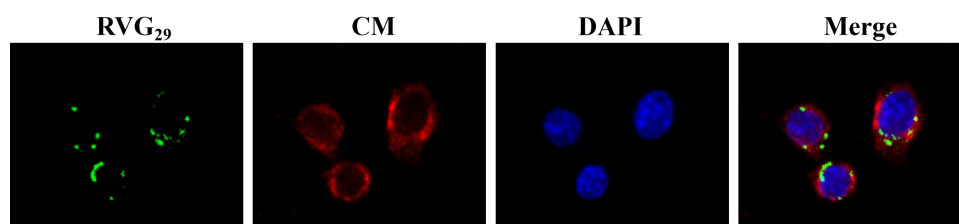


Fig. S2 Immunofluorescence staining.

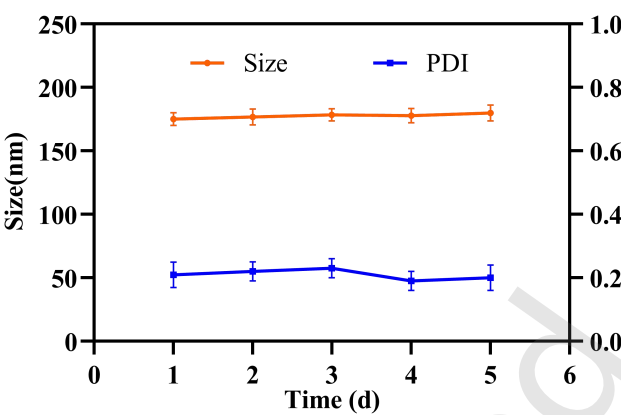


Fig. S3 Stability study.

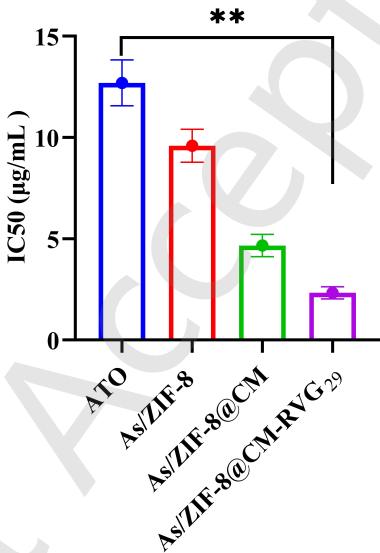


Fig. S4 IC₅₀ values of different formulations on GL261 cells.

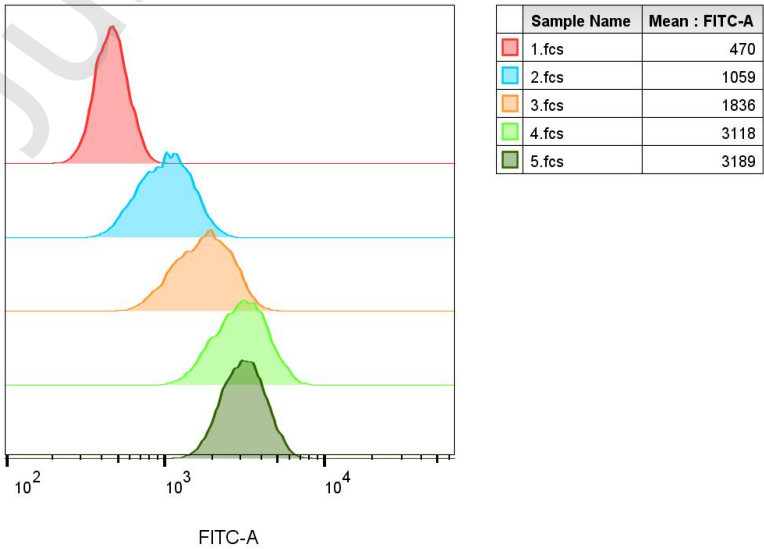


Fig. S5 Quantitative analysis of intracellular reactive oxygen species generation.

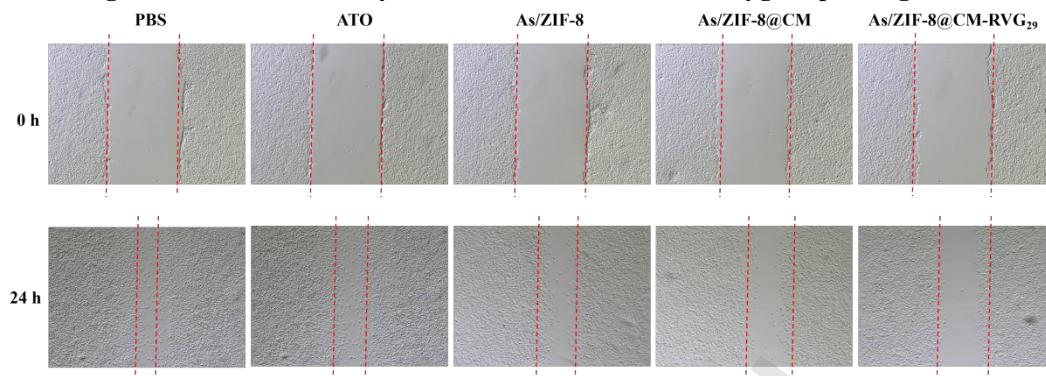


Fig. S6 Cell scratch assay of GL261 cells after treatment with different formulations.

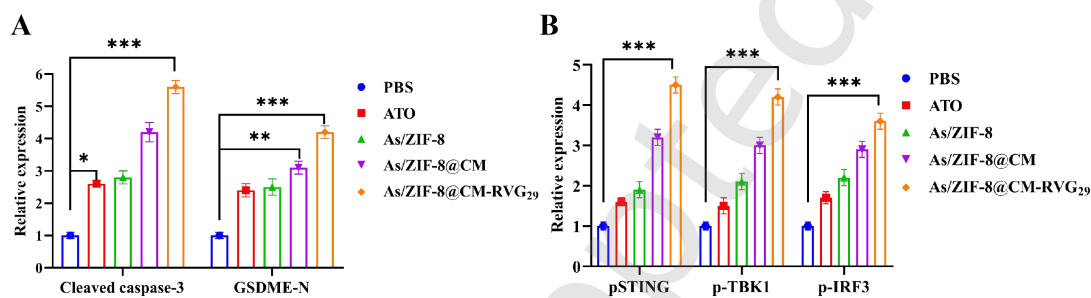


Fig. S7 Quantitative analysis of pyroptosis-related proteins (A) and STING pathway-associated proteins (B) after treatment with different formulations.

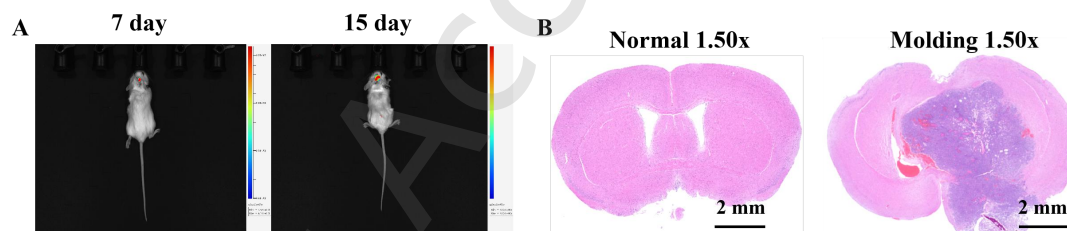


Fig. S8 In vivo imaging (A) and H&E staining (B) validation of modeling results.

Table S1 Plasma pharmacokinetic parameters in rats after tail vein injection of different groups (Mean±SD, n=3).

药代动力学参数	ATO	As/ZIF-8	As/ZIF-8@CM	As/ZIF-8@CM-RVG ₂₉
C _{max} (mg/L)	8.35±0.92	5.93±0.65* * *	4.95±0.54* * *	4.52±0.57 * * * #
t _{1/2α} (h)	0.21±0.04	0.48±0.05	1.08±0.11	1.05±0.13
t _{1/2β} (h)	2.92±0.38	5.81±0.64* *	14.86±1.63* * * ##	13.52±1.49* * * ##
AUC _{0→∞} (mg·h/L)	6.38±0.79	17.42±1.98* * *	56.38±6.20* * * ##	50.15±5.52* * * ##
MRT (h)	2.01±0.22	4.56±0.50	15.92±1.75	14.56±1.66

CL (L/h/kg)	0.47±0.05	0.17±0.02* *	0.053±0.006* * * ##	0.060±0.007* * * ##
V _d (L/kg)	1.98±0.25	1.42±0.16* *	1.13±0.12* * *	1.17±0.13* * *

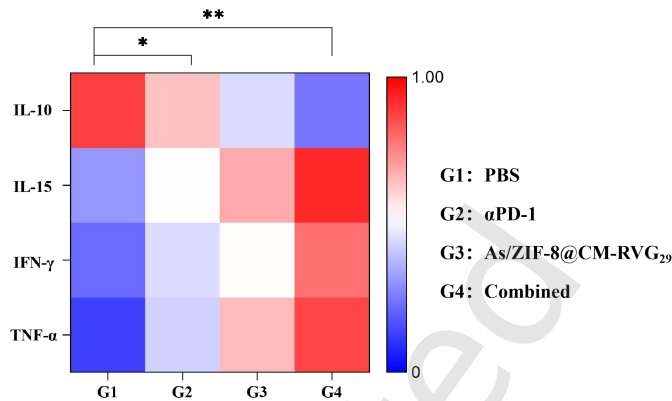


Fig. S9 Serum factor analysis in mice with relapse model after drug administration.

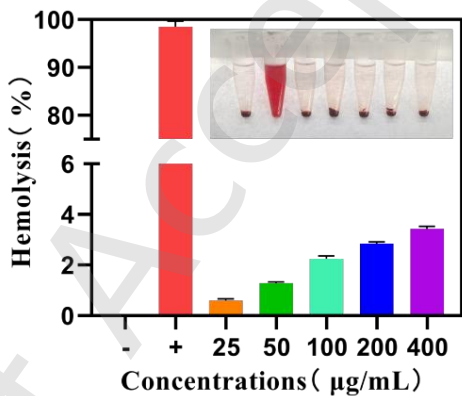


Fig. S10 Hemolytic rate of blank ZIF-8@CM-RVG₂₉ vector at different concentrations (n=3).

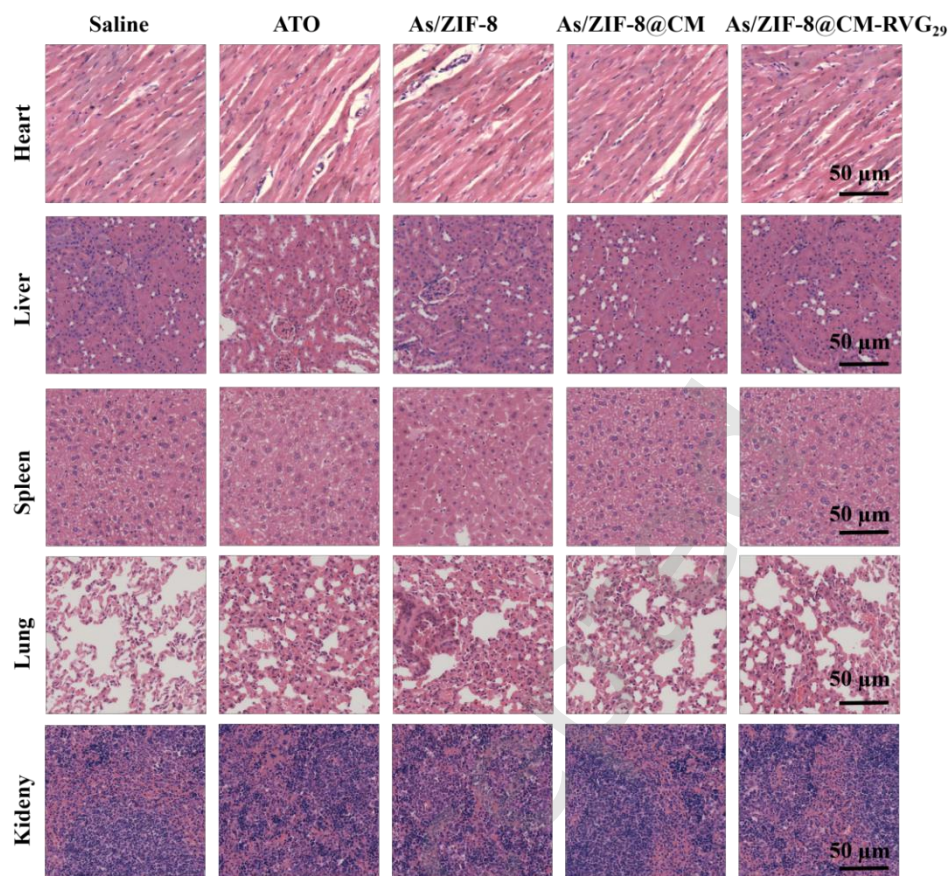


Fig. S11 H&E staining of major tissues and organs in mice from each group, the scale bar is 50 μm .

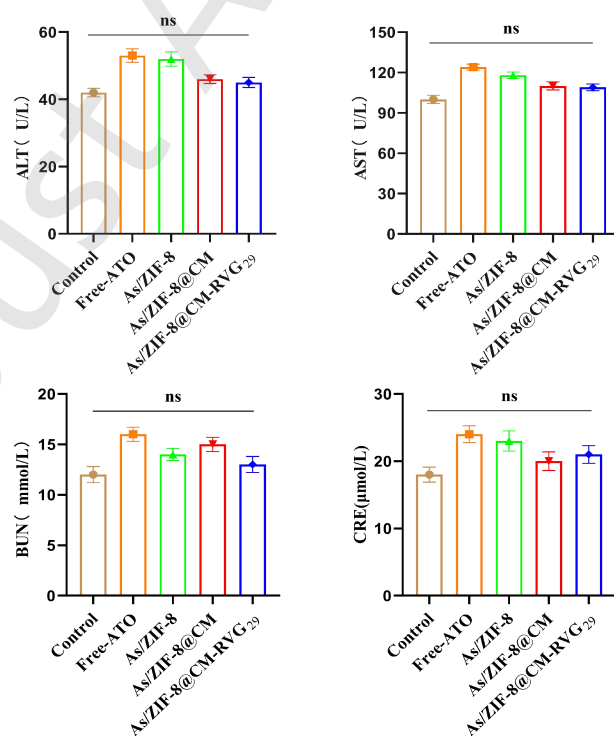


Fig. S12 Serum biochemical tests in mice after different formulation treatments (n=3).