Nanoparticle-mediated convection-enhanced delivery of a DNA intercalator to gliomas circumvents temozolomide resistance

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In patients with glioblastoma, resistance to the chemotherapeutic temozolomide (TMZ) limits any survival benefits conferred by the drug. Here we show that the convection-enhanced delivery of nanoparticles containing disulfide bonds (which are cleaved in the reductive environment of the tumour) and encapsulating an oxaliplatin prodrg and a cationic DNA intercalator inhibit the growth of TMZ-resistant cells from patient-derived xenografts, and hinder the progression of TMZ-resistant human glioblastoma tumours in mice without causing any detectable toxicity. Genome-wide RNA profiling and metabolomic analyses of a glioma cell line treated with the cationic intercalator or with TMZ showed substantial differences in the signalling and metabolic pathways altered by each drug. Our findings suggest that the combination of anticancer drugs with distinct mechanisms of action with selective drug release and convection-enhanced delivery may represent a translational strategy for the treatment of TMZ-resistant gliomas.

Despite rapid advances in cancer research during the past decades, glioblastoma (GBM) remains the most aggressive brain tumour in adults1, with a rate of 15,000 deaths every year in the United States alone, and a 5-year survival rate of less than 10%. While TMZ increases the survival rate of GBM patients by methylating DNA and inducing toxicity in tumour cells, its therapeutic benefits are limited by resistance, which arises via numerous mechanisms, including the acquisition of mismatch repair defects and re-expression of O6-methylguanine-DNA-methyltransferase (MGMT)4–8. Anticancer agents with alternative mechanisms of action are needed to treat TMZ-resistant GBM patients.

Platinum-based compounds—such as the third-generation platinum anticancer drug oxaliplatin and a cationic platinum DNA intercalator (5,6-dimethyl-1,10-phenanthroline (1S,2S)-diaminocyclohexane) platinum(II) (56MESS)—have been widely used for the delivery of chemotherapeutics21. Despite their remarkable antitumour efficacy, the therapeutic applications of oxaliplatin and 56MESS are hindered by toxicity15,17. Here we propose to address this problem by encapsulating these agents in reduction-responsive nanoparticles (NPs)15,16,18–20, an approach widely used for the delivery of chemotherapeutics21.

As an emerging class of nanocarriers, reduction-responsive polymers possess great potential for tumour-specific delivery of bioactive molecules22–25. Reduction-responsive polymers usually incorporate disulfide bonds that are sufficiently stable in the extracellular space, but are rapidly cleaved in the reductive tumour environment23,27. The glutathione (GSH) concentration in tumour tissue is fourfold higher than in non-neoplastic tissue25. Moreover, TMZ-resistant glioma cell lines show even higher levels of GSH than TMZ-sensitive cell lines22. Such differences make reduction-responsive NPs especially attractive for GBM chemotherapy21.

The blood–brain barrier, which is impermeable to most drugs21–31, is another obstacle for GBM therapy. Recent clinical trials have shown that convection-enhanced delivery (CED) safely bypasses the blood–brain barrier and directly delivers drugs to target brain regions32. Using CED, the drugs can diffuse to a
wider region compared with bolus injection or implants, where the diffusion is driven solely by the concentration gradient\textsuperscript{33,34}. Combining the advantages of these technologies, we demonstrate here that CED of reduction-responsive NPs containing highly potent platinum agents serves as a promising therapeutic strategy for TMZ-resistant GBM.

Fig. 1 | Synthesis of the reduction-responsive polymer and formation of NPs. a, Synthesis of poly (CHTA-co-HD)-PEG. b, Structures of OxaPt(iv) and 56MESS. c.d, Formation of NPs by nanoprecipitation. c, Formation of NP-OxaPt(iv). d, Preparation of NP-56MESS. e, A schematic illustrating that oxaliplatin and 56MESS induce cell death after GSH-mediated drug release. ER, endoplasmic reticulum; rRNA, ribosomal RNA.
The surface charge of NPs influence intracellular uptake. The hydrodynamic diameters in aCSF, PDI in aCSF, surface charges in aCSF, and PDI in aCSF were 0.19 and 0.15, respectively (Fig. 2d); both NP types were negatively charged, with zeta-potentials of −26 mV and −22 mV, respectively (Fig. 2e); these parameters were within the range of optimal internalization identified previously. In addition, we observed that the encapsulation efficiency of OxaPt(iv) (36.2%) was lower than that of 56MESS (63.4%) (Fig. 2f).

To test whether our polymer was reduction-sensitive, we designed a thiol–disulfide exchange reaction using thioglycolic acid and found that disulfide bonds broke quickly in the presence of a reducing agent (Fig. 2g,h). The consumption rate of thioglycolic acid is displayed in Fig. 2i. It has been reported that the GSH concentration in tumour tissue is fourfold higher than in normal tissue and that TMZ-resistant glioma cell lines possess higher levels of GSH than TMZ-sensitive cell lines. To investigate the triggered release of platinum drugs, we incubated the NPs in aCSF and GSH–aCSF solutions with different GSH concentrations—0.5 mM, 5 mM, and 20 mM—and observed that approximately 83% of platinum was released from the NP-OxaPt(iv) with continuous incubation in 5 mM GSH solution over 3 d, whereas only 11.3% OxaPt(iv) was released during incubation in aCSF over the same time period. Similarly, approximately 72% of platinum was released from NP-56MESS in 5 mM GSH solution, whereas only 9.1% was released in aCSF. Both types of NPs dissociated faster in 20 mM GSH and −22 mV, respectively (Fig. 2e); these parameters were within the range of optimal internalization identified previously. In addition, we observed that the encapsulation efficiency of OxaPt(iv) (36.2%) was lower than that of 56MESS (63.4%) (Fig. 2f).
solution and more slowly in 0.5 mM GSH solution. These data suggest that NPs are responsive to reductive conditions.

To evaluate intracellular uptake of NPs, we formulated NPs loaded with a fluorescent tracer, Dil (NP-Dil). NP-Dil physical characteristics were similar to NP-OxaPt(iv) and NP-56MESS (hydrodynamic diameter 90 ± 2 nm, PDI 0.13 and zeta potential −22.7 mV) (Fig. 3b–d). Two human GBM cell lines, TMZ-resistant LN229 (LN229-TR) and cells from a patient-derived xenograft PDX101, were used to study intracellular uptake of NP-Dil by flow cytometry. The mean fluorescence intensity of the cells increases over time (Fig. 3e–f).

**Fig. 3** | *Intracellular uptake of dye-loaded NPs.*

**a**. Schematic of experimental procedure. **b–d**, Characterization of NPs containing Dil (NP-Dil), including the hydrodynamic diameter (**b**; mean = 90 nm), PDI (**c**; mean = 0.14) and zeta potential (**d**; mean = −23 mV). *n* = 3, data are mean ± s.d. **e**, Intracellular uptake of NP-Dil by LN229-TR (**e**) and PDX (**f**) cells, measured by flow cytometry. The mean fluorescence intensity of the cells increases over time. *n* = 3, data are mean ± s.d. **g**, Quantification of mean fluorescence intensity over time. **h**, NP-Dil NPs exhibit perinuclear localization in both LN229-TR cells and PDX cells 12 h after incubation. F-actin is labelled with a phalloidin antibody (green). Scale bars, 5 µm. **i–m**, Subcellular localization of NP-Dil. LN229-T5 cells are stained with early endosome antigen 1 (EEA1) antibodies. The spatial signal in the dashed square in **i** is quantified and presented in **j** (EEA1) and **k** (NP-Dil). The signal along the dotted arrow in **i** is measured and plotted in **l** (EEA1) and **m** (NP-Dil). L1, L2 and L3 refer to image layers 1, 2 and 3 from confocal imaging.
Antitumour efficacy of NPs in vitro

To test the anticancer activities of OxaPt(iv) and 56MESS, we performed growth-delay assays using human GBM cell lines including TMZ-sensitive LN229 (LN229-TS), TMZ-resistant LN229 (LN229-TR), PDX and U87 (Fig. 4b–c). The half-inhibitory concentration (IC50) of TMZ in LN229-TS was 2.0 µM (Fig. 4b), whereas the IC50 of TMZ in LN229-TR was 162.6 µM (Fig. 4c), confirming an approximately 81-fold resistance to TMZ in the LN229-TR cell line. Both OxaPt(iv) and 56MESS showed higher potencies than TMZ in all cell lines tested, especially in TMZ-resistant cells (Table 1). After loading into NPs, the IC50 of both drugs decreased in most cell lines, except in the LN229-TS cell line, where the IC50 values were similar for both free drugs and their NP-loaded forms (Fig. 4f–i and Table 1).

Antitumour efficacy of NPs in an animal model

In previous studies, platinum-based drugs have typically been administered intraperitoneally in doses ranging from 5 mg kg−1 to 60 mg kg−1 to achieve a therapeutic effect67–69. To compare the safety profiles of CED versus intraperitoneal injection of platinum-based drugs, we performed whole blood cell counts (Fig. 5a–c) and examined tissue histology after drug administration (Supplementary Figs. 8 and 9). We found that at therapeutic doses, CED was safer than intraperitoneal injection: intraperitoneal injection of drug-loaded NPs reduced the number of white blood cells, platelets and red blood cells. By contrast, the white blood cell, platelets, and red blood cell counts of the CED-treated groups were within the normal ranges (Fig. 5a–c). Furthermore, no toxicity was detected in the organs from mice treated with CED, whereas intraperitoneal injection of the NPs caused splenic abnormalities (Supplementary Figs. 8 and 9) such as cells with brown–black pigment in the spleen. This pigment could be the result of macrophage engulfment of effete or damaged red blood cells during drug-induced haemolytic anaemia60–64.

Table 1 | IC50 values (in µM) of TMZ, OxaPt(iv) and 56MESS in different cell lines

| Drug          | LN229-TS | LN229-TR | U87 | PDX |
|---------------|----------|----------|-----|-----|
| TMZ           | 2.0      | 162.6    | 39.9| 189.6|
| OxaPt(iv)     | 0.7 (2.9)| 0.6 (2.71)| 0.2 (199.5)| 0.3 (632)|
| 56MESS        | 1.1 (1.8)| 0.5 (325.2)| 1.1 (36.3)| 1.7 (111.5)|
| NP-OxaPt(iv)  | 1.0 (2.0)| 0.2 (813)| 0.1 (399)| 0.2 (948)|
| NP-56MESS     | 0.6 (3.3)| 0.2 (813)| 0.7 (57)| 0.7 (270.9)|

The numbers within parentheses represent the ratios of the IC50 values of TMZ to those of each drug.
To test the antitumour efficacy of the NPs in vivo, we first established an animal model by transducing LN229-TR cells with lentivirus to express luciferase (LN229-TR-LUC, Supplementary Fig. 10a) and implanting these cells into mice. Two weeks after the implantation, we investigated luciferin kinetics using an in vivo imaging system (IVIS) and found that the bioluminescent signal from the tumours peaked approximately 17 min after injection of luciferin (Supplementary Fig. 10b,c). We next tested the antitumour efficacy of our NP formulations in mice bearing LN229-TR-LUC tumours. We observed that the TMZ-treated mice died around day 27, which was not significantly different to the survival of mice in the PBS group (around 23 d). Conversely, NP-OxaPt substantially inhibited tumour growth and tripled the survival time of mice bearing LN229-TR-LUC tumours. Most notably, 80% of NP-56MESS-treated
**Fig. 6** | Transcriptional analysis of LN229 cells treated with TMZ, 56MESS and NP-56MESS. **a**, Heat map depicting transcriptional alterations. **b**, KEGG pathway enrichment analysis of differentially expressed genes between the TMZ group and the 56MESS group. Sixty pathways are arranged from top to bottom according to q values. ‘Cell cycle’ has the lowest q value. ‘Nucleotide excision repair’ has the highest q value in this chart. n = 3 biological replicates. “Resis” stands for resistance.
of cancer cells. In addition, CED, a delivery approach being widely tested in clinical trials, was implemented to carry the drugs into the region of interest, bypassing the blood–brain barrier and enhancing drug distribution. Genome-wide RNA profiling and metabolome analysis uncovered the transcriptional and metabolic changes resulting from 56MESS treatment, confirming that its mechanism of action was distinct from that of TMZ. Together, CED of disulfide NPs with a cationic DNA intercalator substantially prolonged the survival of mice bearing TMZ-resistant GBM tumours without detectable systemic toxicity. Future research will include validation of the therapeutic efficacies of NP-56MESS with PDX models\(^{55,175}\), identification of its molecular target\(^5\), potential improvement of the polymer with a targeting component for GBM\(^5\) and assessment of neurotoxicity by behavioural assays\(^5\). We envision that the integrated approach presented in this proof-of-concept study could lead to promising avenues for the treatment of refractory GBM.

**Methods**

**Materials.** Dimethylformamide, dimethyl sulfoxide, oxaliplatin, hydrogen peroxide, dodecyl isocyanate, potassium tetrachloroplatinate(II), 1,25-diaminocyclohexane and 2-hydroxyethyl disulfide were purchased from Sigma-Aldrich. GSH (catalogue no. 78259) and DiI (cat. no. D282) were purchased from Thermo Fisher Scientific. aCSF (cat. no. 59-7316) was procured from Sigma-Aldrich. Dimethylformamide, dimethyl sulfoxide, oxaliplatin, hydrogen peroxide, dodecyl isocyanate, potassium tetrachloroplatinate(II), 1,25-diaminocyclohexane and 2-hydroxyethyl disulfide were purchased from Sigma-Aldrich. GSH (catalogue no. 78259) and DiI (cat. no. D282) were purchased from Thermo Fisher Scientific. aCSF (cat. no. 59-7316) was procured from Sigma-Aldrich. Dimethylformamide, dimethyl sulfoxide, oxaliplatin, hydrogen peroxide, dodecyl isocyanate, potassium tetrachloroplatinate(II), 1,25-diaminocyclohexane and 2-hydroxyethyl disulfide were purchased from Sigma-Aldrich. GSH (catalogue no. 78259) and DiI (cat. no. D282) were purchased from Thermo Fisher Scientific. aCSF (cat. no. 59-7316) was procured from Sigma-Aldrich. Dimethylformamide, dimethyl sulfoxide, oxaliplatin, hydrogen peroxide, dodecyl isocyanate, potassium tetrachloroplatinate(II), 1,25-diaminocyclohexane and 2-hydroxyethyl disulfide were purchased from Sigma-Aldrich. GSH (catalogue no. 78259) and DiI (cat. no. D282) were purchased from Thermo Fisher Scientific. aCSF (cat. no. 59-7316) was procured from Sigma-Aldrich. Dimethylformamide, dimethyl sulfoxide, oxaliplatin, hydrogen peroxide, dodecyl isocyanate, potassium tetrachloroplatinate(II), 1,25-diaminocyclohexane and 2-hydroxyethyl disulfide were purchased from Sigma-Aldrich. GSH (catalogue no. 78259) and DiI (cat. no. D282) were purchased from Thermo Fisher Scientific. aCSF (cat. no. 59-7316) was procured from Sigma-Aldrich. Dimethylformamide, dimethyl sulfoxide, oxaliplatin, hydrogen peroxide, dodecyl isocyanate, potassium tetrachloroplatinate(II), 1,25-diaminocyclohexane and 2-hydroxyethyl disulfide were purchased from Sigma-Aldrich. GSH (catalogue no. 78259) and DiI (cat. no. D282) were purchased from Thermo Fisher Scientific. aCSF (cat. no. 59-7316) was procured from Sigma-Aldrich.

**Products.** Luciferin (122799) was purchased from Perkin Elmer. Puralube vet ointment (17033-211-38) was procured from Dechra Veterinary Products. Luciferin (122799) was purchased from Perkin Elmer.

**Bone wax (W31G) was obtained from Ethicon. Reflex 9 mm wound clips were from CellPoint Scientific. Triple antibiotic ointment (cat. no. 9004788) was obtained from Henry Schein. Polyimide microbore tubing (TPI-34X-12) was bought from Professional Plastics. Epoxy 907 adhesive system was acquired from Miller-Stephenson. Betadine solution swabs were obtained from Betadine. Puralube vet ointment (17033-211-38) was procured from Dechra Veterinary Products. Luciferin (122799) was purchased from Perkin Elmer.

**Instruments.** Proton nuclear magnetic resonance (1H NMR) and 13C NMR spectra were completed using a 300 MHz NMR. Matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF–MS) was conducted with an Autoflex III (Bruker). The hydrodynamic diameter of each NP formulation was measured by dynamic light scattering (Malvern Panalytical). Flow cytometry experiments were performed using the Attune NxT. An Olympus confocal microscope was used for fluorescence imaging. An IVIS system (Perkin Elmer) was used to monitor tumour growth in vivo. A stereoscopic frame with UMP3 system (TAXIC-600), a mouse adapter (cat. no. 502063) and a micro drill (503598) were obtained from World Precision Instruments. A reflex skin closure system (72-6060 to 72-6064) were purchased from Harvard Apparatus. A vacuum centrifuge concentrator (SPD120) was obtained from Thermo Fisher Scientific. The MALDI-TOF–MS instrument ( Autoflex III) was acquired from Bruker.

**Synthesis of OxaPt(IV).** To prepare OxaPt(IV)-OH, 0.5 g of oxaliplatin was suspended in 20 ml of H₂O₂ (30%, v/v). The resulting solution was stirred at 50 °C until it was clear. After the solution was cooled to room temperature, needle-like crystals precipitated. The crystals were washed with acetone and dried in a...
desiccator. Afterwards, oxaloplatin(v)-OH was isolated. To synthesize OxaPt(iv), 400 mg of Oxap(t)-OH was dissolved in 5 ml of anhydrous dimethylformamide (DMF), followed by adding 0.744 ml of dodecyl isocyanate to the mixture. The solution was stirred at 50°C until it was clear. The solution was then added into ice water to precipitate the reaction product. The product was washed with acetonitrile, acetonitrile ether and dried under the vacuum to obtain Oxap(t)(iv) (45%). ‘H NMR (300 MHz, DMSO) δ 9.70 (s, 2H), 8.43 (s, 2H), 6.74 (s, 2H), 2.89 (tt, J = 13.2, 6.7 Hz, 4H), 2.58 (d, J = 1.4 Hz, 2H), 2.17 (d, J = 11.8 Hz, 2H), 1.52 (d, J = 4.8 Hz, 2H), 1.27 (d, J = 2.6 Hz, 4H), 0.85 (s, J = 6.2 Hz, 6H). High-resolution mass spectrometry for C31H24N2O12Pt calculated: 584.0422; observed: 584.1291.

Synthesis of 56MESS. The synthesis and characterization of 56MESS were performed as described45.

Synthesis of poly (CHTA-co-HD)-PEG. Five millilitres of DMF was used to dissolve 89 mg of 2-hydroxyethyl disulphide and 96 mg of 1,4,5-cyclo-hexanetetraacarboxyl disulphide. The reaction proceeded for 24 h under nitrogen protection. Afterwards, 200 mg of MPEG-ZK-OH (0.02 mmol) was added to the system. The reaction proceeded at 50°C overnight. Five to ten millilitres of diH2O was added to the system, after which the product was dialysed for 48 h and lyophilized.

Preparation and characterization of NPs. Poly (CHTA-co-HD)-PEG (PEG) (100 mg) was dissolved in 1 ml of DMF. One millilitre of DMSO was used to dissolve DiI-Poly (CHTA-co-HD)-PEG (100 mg) and DMSO into a glass vial. Oxap(t)(iv) (66.7 g) polymer (400 µl), DMF (400 µl) and DMSO (133.3 µl) were added to a glass vial. 56MESS (600 µl) and polymer (400 µl) were mixed. The mixture was added dropwise to 3 ml of deionized water in a glass vial with stirring at 1,000 rpm at room temperature, followed by stirring for 2 h in a fume hood to remove organic solvent. Afterwards, the mixture and 3 ml of deionized water were transferred to a dialysis tube, cat. no. 025-0160, 3,000 g MWCO, E1500, and incubated on ice for 10 min. The NPs were respended in 5 ml of deionized water and centrifuged at 2,500 g for 30 min. This step was repeated twice to remove organic solvent. Finally, the NPs were resuspended in 1 ml of deionized water or aCSF depending on the experiment. The hydrodynamic diameter, polydispersity index and surface charge of the NPs were measured by dynamic light scattering (Malvern Panalytical). NPs were incubated at 37°C. Ten microlitres of solution was collected at various time points to assess stability of NPs.

Release of 56MESS and Oxap(t)(iv) from NPs. A filter (Thermo Fisher Scientific, Slide-A-Lyzer mini dialysis device, 0.5 ml, 10 K MWCO) was placed in a well of a 24-well plate. One and a half ml of 0.5 mM GSH, 5 mM GSH, 20 mM GSH solution or aCSF was added to each well, followed by addition of 200 µl NP-56MESS or NP-Oxap(t)(iv) to each filter. The 24-well plate was incubated at 37°C. The release of 56MESS and Oxap(t)(iv) was evaluated using the same protocol. Using 56MESS as an example, 10 µl of aCSF solution was collected from the wells at various time points (0 h, 1 h, 2 h, 3 h, 6 h, 12 h, 24 h, 48 h and 72 h) to measure 56MESS concentration (C) in a 96-well plate by an in vitro assay described below.

Cell culture. LN229 and PDX (G22) cells were acquired from R. Bindura (Yale University). LN229-TR (MGMT+) cells, engineered by transfecting LN229 cells with MGMT in the pSv2GMGT vector and selecting with 1.5 µg ml−1 G418, were obtained from B. Kamen. U87 and P89 cells were purchased from ATCC. U87-RFP cells were from H. Xiao (Chinese Academy of Sciences). Cells were cultured in DMEM medium supplemented with 10% FBS and 1% penicillin-streptomycin at 37°C with 5% (v/v) CO2 in a humidified atmosphere.

Characterization of the subcellular localization of NP-DiI. Ten-thousand LN229-TS cells were seeded into one well of a 24-well plate, followed by addition of NP-DiI (2 µl) 12 h later. Cells were rinsed with PBS twice (500 µl each) followed by fixation with 4% paraformaldehyde at 30 min, 2 h and 24 h after addition of NP-DiI. Afterwards, the cells were rinsed with PBS three times, incubated in blocking buffer (1×PBS, 5% BSA, 0.3% Triton X-100) at room temperature for 1 h followed by three washes with PBS and staining with an E1A1 antibody (CST, cat. no. 3288S, 1:200 in antibody dilution buffer (1×PBS, 1% BSA, 0.3% Triton X-100) at 4°C for 24 h. Afterwards, the cells were washed with PBS three times (5 min each), incubated with secondary antibody at room temperature for 1.5 h and washed with PBS three times (5 min each). Gold Polyclonal Reagent (DAP, cat. no. 4961, 5 µl) was used to decorate the cells. Images of cells were collected using a confocal microscope (Olympus, ×100 objective).

Animal survival experiment. All procedures were approved by the Yale University Institutional Animal Care and Use Committee and performed in accordance with the guidelines and policies of the Yale Animal Resource Center. Female mice from Charles River (Fox chase CD1, strain code 250, 4 weeks old) were used for the survival experiments. The procedures for CED were detailed in previous publications30,31. In brief, mice were anaesthetized with an intraperitoneal injection of a ketamine/xylazine mixture (100 mg kg−1 ketamine, 10 mg kg−1 xylazine), followed by a pre-empive dose (15 µm) of buprenorphine (0.06 mg kg−1) and meloxicam (0.3 mg kg−1). Mice were then restrained using a stereotaxic frame and injected intracranially over 3 min on day 0. Three more doses of buprenorphine (0.06 mg kg−1, every 12 h) and one more dose of meloxicam (0.3 mg kg−1) were intraperitoneally administrated for post-operative care. Tumours grew for 4 d before being subjected to different therapeutic treatments. Specifically, for the mice in the TMZ group, TMZ was administrated by intraperitoneal injection weekly. For the mice in other groups, a single dose of PBS or drugs (4µl) was given through CED at 0.5 µl min−1.

In vivo biodistribution of NPs. CED of NP-56MESS-FITC was performed 4 d after intracranial implantation of U87-RFP (125,000 cells per mouse). Mice brains were collected, flash-frozen, and cryo-sectioned (30 µm per slide) 4 h after
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Kitange, G. J. et al. Induction of MGMT expression is associated with

56MESS (1.1 M) or NP-56MESS (0.6 mM) for 36h. Three distinct samples, 1 million cells per sample, from each treatment group were collected to purify RNA. The RNA quality was confirmed using a NanoDrop 2000c Spectrophotometer. The sequencing data was submitted to the National Center for Biotechnology Information (NCBI) Sequence Read Archive (SRA) database (Bioproject ID PRJNA668937), which will be released upon publication. BGISEQ-500 was employed for sequencing. RSEM was used to quantify the transcription levels of

The authors declare that the main data supporting the findings of this study generated during the study is too large (2.3 GB) to be publicly shared, but the data are available for research purposes from the corresponding authors on reasonable request.

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Author contributions

Y.W., R.S.B., H.X. and W.M.S. discussed and designed the study. D.W., Y.Y. and L.Z. prepared and characterized the polymer and drugs. P.S. and T.L. helped conduct the cell viability assays. H.K.M. assisted in analysing the flow cytometry data. A.S.P.-D. helped perform toxicity experiments. A.H. analysed the haematoxylin and eosin images from the brain. X.L. and Z.Z. aided in the metabolome analysis. A.J., Y.C., Y.Z., P.S. and F.W. performed toxicity experiments. A.H. analysed the haematoxylin and eosin images from the brain. X.L. and Z.Z. aided in the metabolome analysis. A.J., Y.C., Y.Z., P.S. and F.W. contributed to characterization of NPs. X.C. and F.L. helped with the statistical analysis. Y.W. and Y.J. conducted all other experiments in this manuscript. All authors discussed the data and reviewed the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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*Our web collection on statistics for biologists contains articles on many of the points above.*

Software and code

**Policy information about availability of computer code**

**Data collection** Detailed descriptions are included in Methods. Briefly, Malvern Panalytical was used to collect the sizes, polydispersity indexes and zeta potentials of nanoparticles. Fluorescence images were collected using an Olympus confocal microscope (100x). MALDI-TOF-MS data were collected using an Autoflex III (Bruker). An IVIS (Perkin Elmer) was used to monitor tumour growth. A SpectraMax microplate reader was used to determine cell viability. An Attune NxT flow cytometer was used to assess nanoparticle uptake.

**Data analysis** The data were analysed using CellProfiler and GraphPad Prism 6. RSEM was used to quantify the transcription levels of genes. R packages ggplot2 and gghthemes were used to generate volcano graphs. The heat map was plotted by pheatmap. R phyper was used for KEGG enrichment analysis. Cytoscape were utilized to generate protein–protein interaction networks. ImageJ was used to analyse haematoxylin and eosin images. Olympus confocal images were analysed using FV3000 software. FlowJo was used to analyse flow-cytometry data.

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The authors declare that the main data supporting the findings of this study are available within the paper and its Supplementary Information. The raw data generated for the RNA-seq analysis is available from the NCBI Sequence Read Archive database under the accession code PRJNA668337. The metabolomic dataset
generated during the study is too large (2.3 GB) to be publicly shared, yet the data are available for research purposes from the corresponding authors on reasonable request.

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

| Sample size | We selected sample sizes to make the statistical power greater than 0.8. |
| Data exclusions | No data were excluded from the experiments. |
| Replication | All experimental findings, including material characterization and animal experiments, were reliably reproduced. |
| Randomization | Experimental groups were formed on the basis of what was being tested, with random selections. Materials, cells and animals were randomly divided into multiple groups. |
| Blinding | The investigators were blinded to group allocation during data collection. |

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| Animals and other organisms    | MRI-based neuroimaging |
| Human research participants    |         |
| Clinical data                  |         |
| Dual use research of concern   |         |

Antibodies

Antibodies used

EEA1 antibody (CST, Cat. 3288S) was used to characterize the intracellular uptake of the nanoparticles.

Validation

Validation of each antibody was done under standard information offered by the supplier.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

LN229 and PDX (G22) cells were acquired from Dr. Ranjit Bindra at Yale University. LN229-TR (MGMT+) cells were obtained from Dr. Bernd Kaina. U87 and F98 cells were purchased from ATCC. U87-RFP cells were from Dr. Haihua Xiao.

Authentication

Cell line authentication was initially performed by ATCC.

Mycoplasma contamination

Cell lines were tested for mycoplasma contamination in the Clinical Virology Laboratory at Yale University.

Commonly misidentified lines

No commonly misidentified cell lines were used.
Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research.

| Laboratory animals | Female mice from Charles River (Fox chase SCID beige, strain code 250, 4 weeks old) were used for the survival experiments. |
|--------------------|------------------------------------------------------------------------------------------------------------------------|
| Wild animals       | The study did not involve wild animals.                                                                                   |
| Field-collected samples | The study did not involve samples collected from the field.                                                                 |
| Ethics oversight   | All procedures were approved by the Yale University Institutional Animal Care and Use Committee (IACUC) and performed in accordance with the guidelines and policies of the Yale Animal Resource Center (YARC). |

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

- The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).
- The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).
- All plots are contour plots with outliers or pseudocolor plots.
- A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

| Sample preparation | One hundred thousand cells were seeded in each well of a 6-well plate. Twelve hours later, cells were incubated with nanoparticles. Afterwards, the cells were washed with PBS, digested with Trypsin-EDTA, centrifuged and resuspended with PBS before evaluation with flow cytometry. |
|--------------------|-----------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| Instrument         | Attune NxT flow cytometer from Thermo Fisher Scientific.                                                                                                                                          |
| Software           | FlowJo                                                                                                                                                                                                                                           |
| Cell population abundance | Live cells were more than 98% of the population in the all groups.                                                                                                                         |
| Gating strategy    | Gating was performed using forward scatter channel and side scatter channel to identify cells of interest and singlets. BL3 (excitation=549 nm, emission=565 nm) was used to detect fluorescent nanoparticles. |

- Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.