

Volume 3, Issue 3

Research Article

Date of Submission: 04 August, 2026

Date of Acceptance: 10 September, 2026

Date of Publication: 30 September, 2026

BBB-Gated h-Foci Map State-Resolved Anchors for Cell Therapy and Targeted Protein Degradation in Glioblastoma

Fred Y. Ye^{1,2,*}

¹DaKES Institute, Boston, MA 02171, USA

²International Joint Informatics Lab, Nanjing University, Nanjing 210008, China

***Corresponding Author:**

Fred Y. Ye, DaKES Institute, Boston, MA 02171, USA and International Joint Informatics Lab, Nanjing University, Nanjing 210008, China.

Citation: Ye, F. Y. (2026). BBB-Gated h-Foci Map State-Resolved Anchors for Cell Therapy and Targeted Protein Degradation in Glioblastoma. *J Med Sci Health Care Res*, 3(3), 01-11.

Highlights

- Three control-aware cohorts define 4,762 concordant GBM genes
- Continuous h selection resolves three foci and 54 second-order hubs
- No active Drugs@FDA compound reverses both represented GBM lines
- The null redirects discovery to state-aware CAR and degradation anchors

Abstract

Computational drug repurposing in glioblastoma is vulnerable to cell-composition confounding, lineage-mismatched perturbation models and unmeasured blood-brain barrier exposure. We integrated three case-control cohorts (247 tissues) to define 4,762 directionally concordant genes and applied continuous h selection to extract a 293-node differential network with three h-foci and 54 second-order hubs. Donor-aware analysis of 21,943 cells localized these programs across Neftel states without treating tumour-only data as a case-control comparison. Among 53 perturbagens with active Drugs@FDA products scored in U251MG and LN229—the only represented GBM lines—none showed reproducible reversal in both lines, and every relaxed signal failed a conservative CNS descriptor screen. Afatinib and temozolomide served as complementary controls for sufficiency and necessity. This null result redirected the network into a spatial modality atlas: CD276 and IL13RA2 prioritized cell-therapy tests, whereas ROCK1, DBF4, ODC1 and CCNA2 prioritized targeted protein degradation. These are falsifiable experimental anchors, not efficacy claims.

Introduction

Glioblastoma is a lethal primary brain tumor whose treatment remains anchored by maximal safe resection, radiotherapy and temozolomide [1]. Therapeutic failure reflects more than a single driver: regional blood-brain and blood-tumor barriers, infiltrative growth, myeloid-rich immunosuppression, and plastic AC-like, MES-like, NPC-like and OPC-like malignant states coexist within individual tumors [2]. Bulk tissue therefore mixes disease programs with cell composition, while any systemic therapy must also achieve adequate unbound exposure in brain and infiltrative margins.

Expression-signature reversal offers a target-agnostic bridge from disease transcriptomes to chemical perturbations [3,4]. Yet a convincing heat map can be generated in an irrelevant lineage, at one dose or time, and then reinforced by physicochemically permissive docking. Independent evaluations have found limited reproducibility and incomplete clinical calibration of such predictions [5,6]. In GBM, the problem is sharper: the originating cell state and genotype must be represented, and molecular weight, polarity, ionization, transporters, metabolism and binding all influence brain exposure [7,8]. Descriptor rules can falsify an implausible candidate, but they cannot measure human BBB penetration.

A null small-molecule result can still define a productive next experiment. Surface antigens can be evaluated as targets for locoregional CAR-T cells, antibody-drug conjugates (ADCs) or bispecific engagers; intracellular dependencies can be tested for proteolysis-targeting chimeras (PROTACs) or molecular glues [9,10]. The modalities are not interchangeable. Surface RNA abundance requires confirmation of extracellular-epitope density and normal-neural or vascular safety; targeted degradation requires causal dependency, a ligandable site, productive E3 engagement and a delivery strategy. Disease-downregulated or tumor-suppressive proteins must be excluded from destructive targeting.

We therefore implemented a prospectively specified analysis plan while allowing documented feasibility audits to revise unworkable assumptions. Three public cohorts with explicit human non-tumor brain controls defined the primary contrast; tumor-dominant TCGA and CGGA collections were not misrepresented as case-control discovery sets. A continuous h rule selected network cardinality without a prespecified K, and adult single-cell data localized the resulting foci using the published Neftel modules. LINCS scoring was restricted to GBM cell lines represented in the current collection and intersected with compounds in active US products before conservative CNS and clinical gates. When no compound survived, the same network was routed to explicitly testable cell-therapy and targeted protein degradation anchors.

Results

A Control-Aware Audit Defines a 4,762-Genes GBM Signature

The prospective plan considered TCGA-GBM, CGGA and GEO as potential sources, but the first two are predominantly tumor collections and cannot independently estimate a tumor-normal effect. We restricted primary discovery to cohorts with explicit human non-tumor brain controls (Figure. 1). GSE4290 contributed 77 samples with an explicit glioblastoma diagnosis and 23 non-tumor epilepsy brain samples; four additional entries carried a series-level GBM label but lacked confirmation in the frozen sample-level diagnostic field and were excluded. GSE50161 contributed 34 GBM and 13 normal brain samples, and GSE147352 contributed 85 adult GBM and 15 normal brain samples (NCBI GEO, GSE4290; NCBI GEO, GSE50161; NCBI GEO, GSE147352). The final evidence base contained 196 GBM and 51 controls (247 tissues).

Figure 1 | A control-aware, BBB-gated and falsification-calibrated GBM study

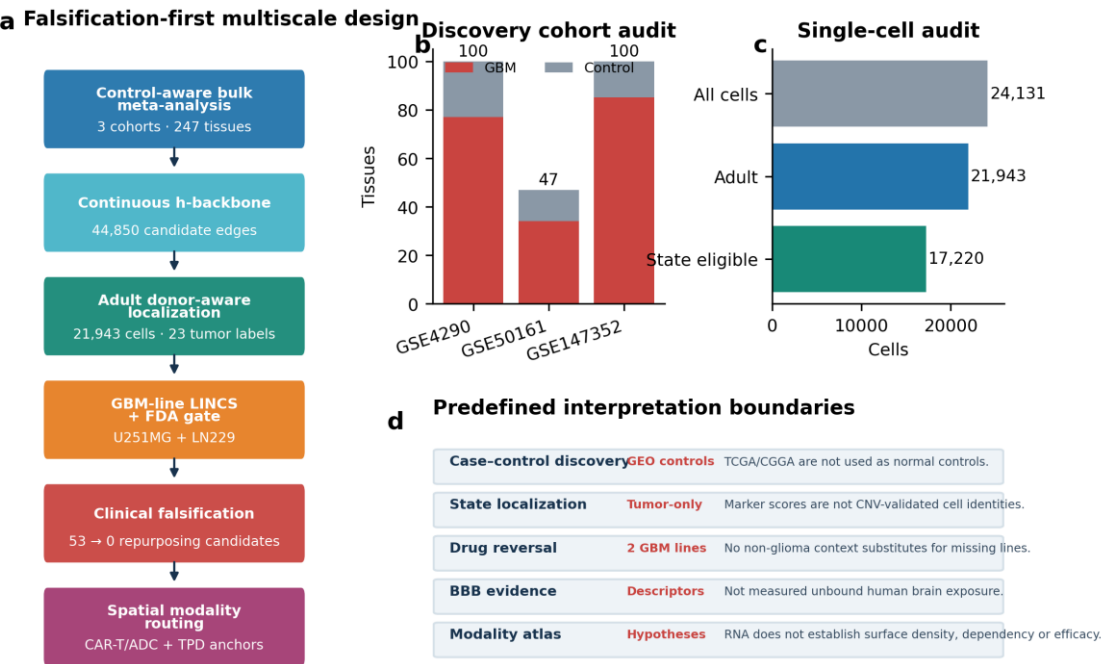


Figure 1: Study Design and Audited Evidence Base. a) Falsification-first Workflow: a Case-Control Bulk Signature and Continuous h-Backbone are Localized in Adult GBM Single Cells, Tested Against Lineage-Matched Perturbations, and Independently Gated by Active Product Status, Conservative CNS Descriptors and Clinical Evidence. b) GSE4290 Contributed 77 Explicitly Annotated GBM and 23 Non-Tumor Samples; GSE50161 Contributed 34 GBM and 13 Normal Samples; GSE147352 Contributed 85 adult GBM and 15 Normal Samples. c) GSE131928 Cell Audit. d) Interpretation Boundaries. TCGA/CGGA were not Misrepresented as Case-Control Cohorts, Marker-Score Compartments are not Copy-Number Identities, and Physicochemical Rules are not Measured Human Brain Exposure

Label-blind probe collapse and protein-coding RNA-seq harmonization yielded 16,225 common genes. Cohort-level Hedges' g effects were directionally correlated (Spearman rho=0.730-0.761). DerSimonian-Laird synthesis followed by meta-FDR<0.01, concordant direction in all three cohorts and I-squared<75% retained 4,762 genes: 2,746 increased and 2,016 decreased (Figure. 2). The resulting effect set spans differences in age and platform; concordance and heterogeneity gates, rather than an unavailable universal covariate model, define its scope.

Figure 2 | Three cohorts define a directionally concordant GBM signature

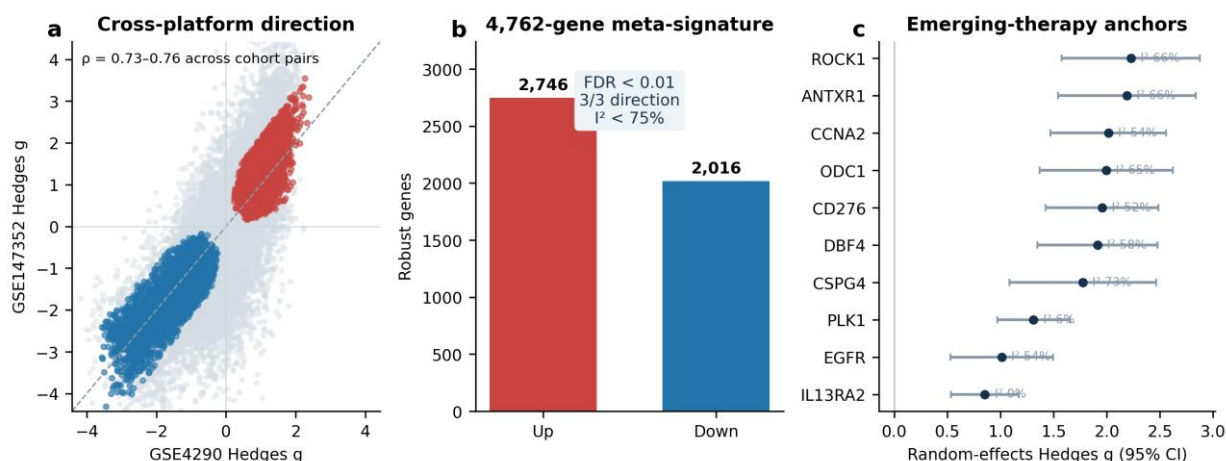


Figure 2: Cross-cohort GBM Meta-Signature. a, Cohort-Level Hedges' g Values are Correlated Despite Platform and Age-Composition Differences. b, Of 16,225 Common Genes, 4,762 Met Meta-FDR<0.01, Three-Cohort Direction Concordance and I-Squared<75%: 2,746 Increased and 2,016 Decreased. c, Random-Effects Estimates with 95% Confidence Intervals for Selected Surface and Intracellular Anchors. These are Tissue-Level Effects; they do not by Themselves Establish Protein Abundance, Cell-Surface Density, Dependency or Therapeutic Efficacy

Continuous h Selection Resolves Three Coupled Disease Foci

The 150 strongest robust increases and 150 strongest robust decreases generated 44,850 gene-pair candidates. Each edge weight combined the magnitude of tumor-normal correlation change with the larger absolute condition-specific correlation. The ordered-weight intersection $w(k) \geq k/M$ selected 5,208 primary edges at 0.1161 without specifying K. A second continuous h pass on inter-community bridge scores retained 1,020 edges, producing a connected 6,228-edge, 293-node backbone (Figure. 3a) [11].

Figure 3 | Continuous h extracts three coupled GBM foci and 54 core hubs

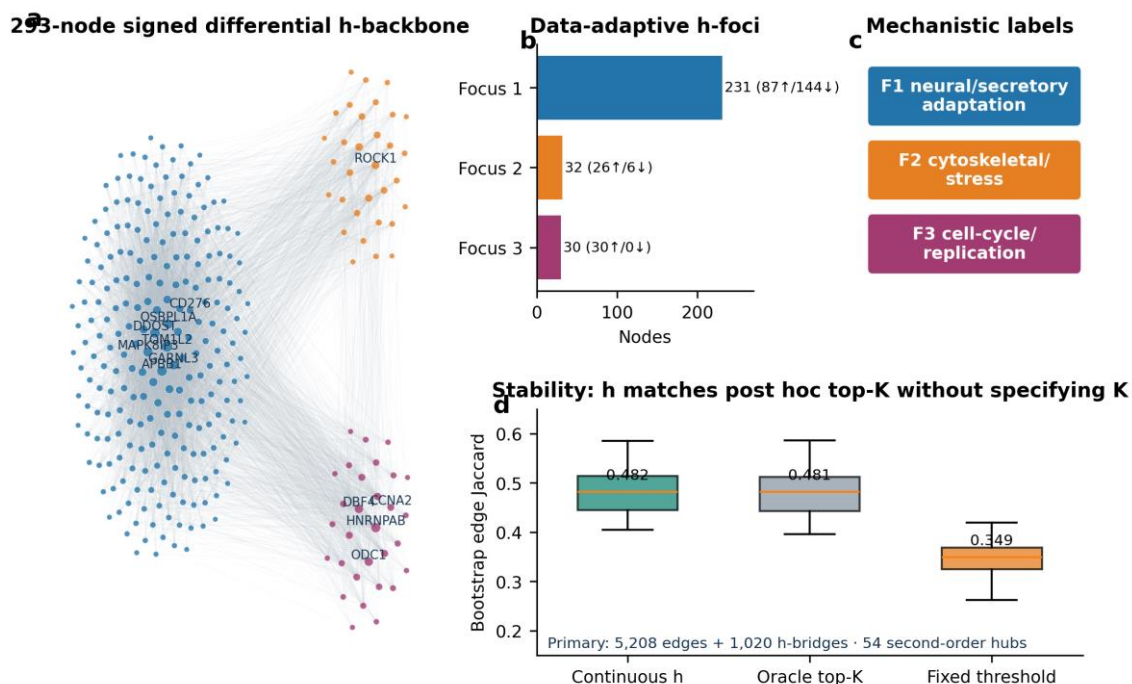


Figure 3: Continuous h-Backbone and h-Foci. a) The Final Signed Differential-Correlation Graph Contains 293 Nodes, 5,208 Primary Edges and 1,020 Recovered Inter-Focus Bridges. Enlarged Labeled Nodes are Second-Order h Hubs. b) Focus Membership and Disease-Direction Counts. c) Mechanistic Labels Derived from Enrichment and Localization. d) Jaccard Similarity to the Full-Data Edge Solution across 25 Stratified Bootstraps. The Oracle top-K Comparator Receives K=5,208 from the Observed h Solution; Continuous h Reaches Essentially the Same Stability without a User-Specified K

An audit-constrained spectral partition selected three h-foci. Focus 1 contained 231 genes (87 increased, 144 decreased) and captured neural or secretory adaptation; focus 2 contained 32 genes (26 increased, 6 decreased) and captured cytoskeletal or stress programs; focus 3 contained 30 uniformly increased genes enriched for cell-cycle and replication pathways (Figure. 3b,c) [12]. A second-order continuous h pass over a composite of strength, degree and inter-focus strength selected 54 hubs; TOM1L2, DDOST, APBB1, DBF4, ODC1, ROCK1 and CCNA2 ranked among the strongest local network cores.

Across 25 stratified bootstraps, median edge-set Jaccard similarity was 0.4816 for continuous h, 0.4814 for an oracle top-K comparator whose K was learned from the full-data h solution, and 0.3490 for a fixed FDR/delta-correlation rule (Figure. 3d). We therefore interpret continuous h as a data-adaptive cardinality rule, not as universally numerically superior to top-K.

Adult Tumor Single Cells Localize State Programs Without Recreating a Control Contrast

GSE131928 contains 24,131 cells from 31 tumor labels profiled by Smart-seq2 and 10X (NCBI GEO, GSE131928) [2]. The primary adult analysis retained 21,943 cells from 23 tumor labels. Because sample-level copy-number assignments were unavailable in the accession metadata, conservative marker scores assigned 17,220 state-eligible glial cells, 2,941 myeloid-like cells, 1,054 pericyte-like cells, 368 endothelial-like cells and 360 T/NK-like cells (Figure. 4). These are localization labels, not validated cell identities.

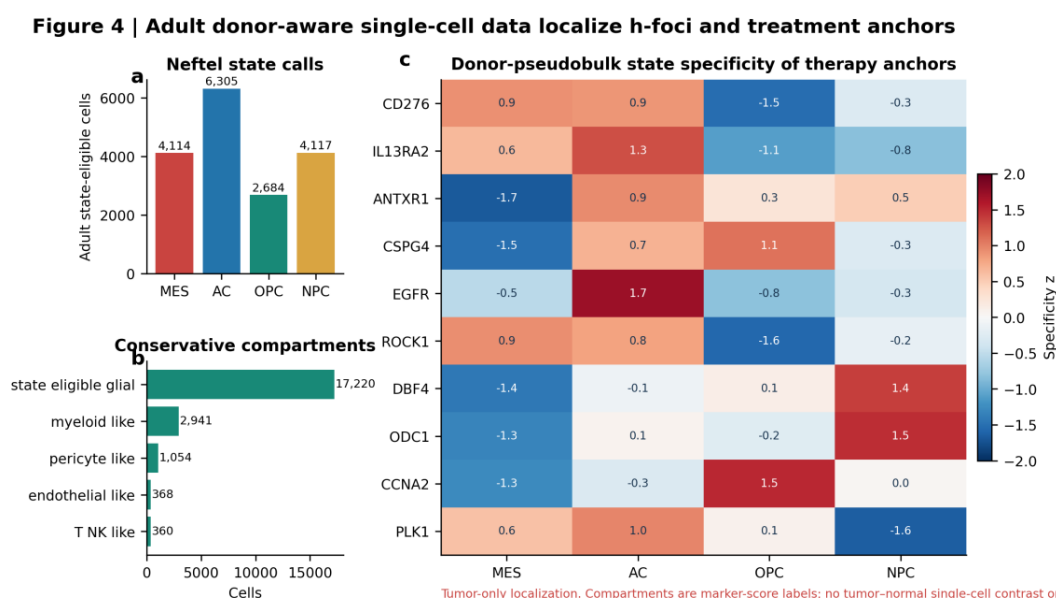


Figure 4: Adult Donor-Aware Single-Cell Localization. a) State Calls among 17,220 State-Eligible Glial Cells. b) Conservative Marker-Score Compartments among 21,943 Adult Cells. c) Donor-Pseudobulk State Specificity z Scores for Therapy Anchors. GSE131928 is a Tumor-only Atlas; these Panels Localize Bulk-Defined Programs but do not Repeat the Tumor-Normal Contrast or Prove Malignant Identity. Neural-Lineage Cells Remain State Eligible because NPC-like and OPC-like Malignant Programs Share Neural Markers

Exact published Neftel modules classified the 17,220 state-eligible cells as 6,305 AC-like, 4,117 NPC-like, 4,114 MES-like and 2,684 OPC-like. Neural-lineage cells remained eligible because malignant NPC-like and OPC-like programs share neural markers. We calculated donor-pseudobulk state and compartment means rather than treating cells as independent replicates. CD276 localized most strongly to the MES-like state, IL13RA2 to AC-like, ROCK1 to MES-like, DBF4 and ODC1 to NPC-like, and CCNA2 to OPC-like (Figure. 4c). These results map bulk-defined nodes within tumors; the tumor-only atlas cannot validate tumor-normal single-cell differential expression.

A Lineage-Coverage Audit Yields no Cross-Line Reverser

The 300-gene query mapped completely in SigCom LINCS [13]. Before scoring, we audited common GBM lines in the current L1000 chemical-perturbation collection. Only U251MG (360 signatures) and LN229 (12 signatures) were represented; A172, T98G and U87MG were absent. We did not substitute non-glioma lines. Intersection with prescription or over-the-counter products in active Drugs@FDA marketing categories dated 24 July 2026 retained 168 signatures for 53 perturbagens [14].

The frozen within-line gate required at least three signatures, at least 60% in the reversal quadrant and median all-signature reversal $z \geq 1.64$. Nine agents passed U251MG only: axitinib, ibrutinib, ixazomib, mirdametinib, moxifloxacin, raloxifene, selumetinib, trametinib and vandetanib. Only afatinib and lapatinib were represented in both lines, but neither passed both line-specific gates (Figure. 5). No cross-line candidate remained before clinical evidence was considered.

Figure 5 | GBM-context replication and clinical controls reduce repurposing to zero

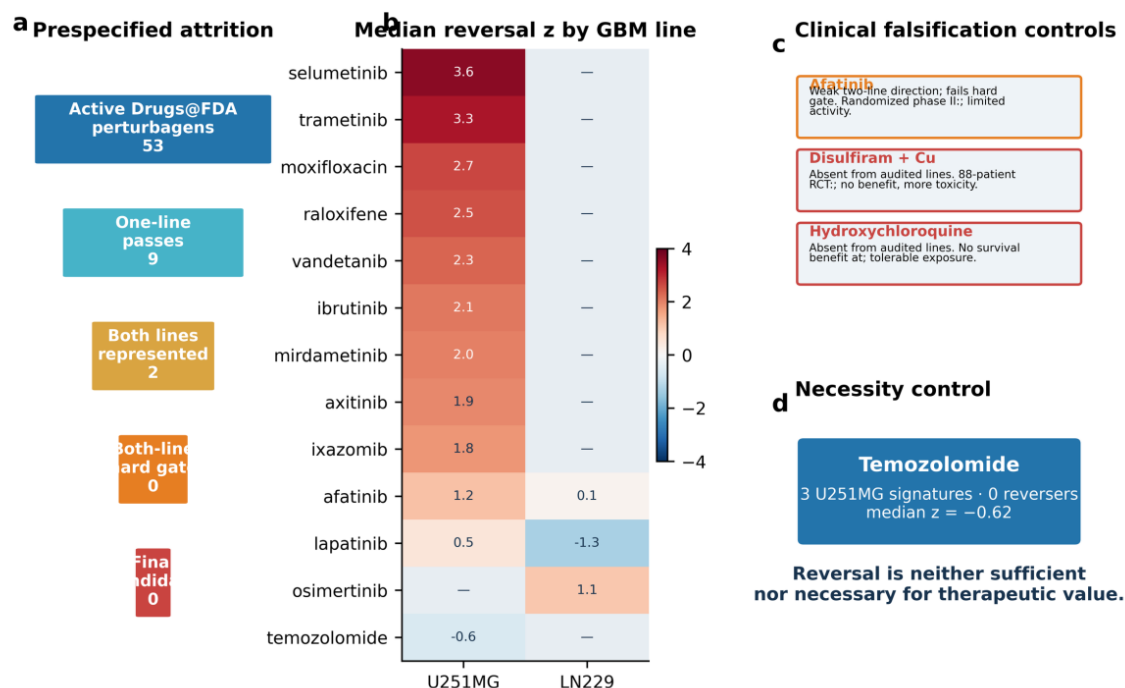


Figure 5: GBM-Context Reversal and Clinical Calibration. a) Attrition from 53 Perturbagens with active Drugs@FDA Products to Zero Two-Line Candidates. b) Median Reversal z by Represented GBM Line. Nine Drugs Pass only U251MG; Afatinib has a Weak Positive Median Direction in both U251MG and LN229 but Fails the Prespecified Within-Line Gate. c) Afatinib is a Computational-Positive, Clinically Limited Calibration; Disulfiram and Hydroxychloroquine are External Clinical-Negative Controls Absent from the Audited Lines. d) Temozolomide is Non-Reversing in Three U251MG Signatures, Showing that Reversal is not Necessary for an Established GBM Therapy

A relaxed exploratory rule identified afatinib as the only agent with a positive median reversal direction in both lines. Each of the nine one-line reversers and afatinib failed a deliberately conservative descriptor screen requiring molecular weight ≤ 450 , topological polar surface area ≤ 90 square angstrom, hydrogen-bond donors ≤ 3 , rotatable bonds ≤ 8 and XlogP between 1 and 4. This screen does not constitute full CNS-MPO, transporter assessment or measured unbound brain exposure; it is a reproducible exclusion layer.

Clinical Controls Show that Reversal is Neither Sufficient nor Necessary

Afatinib provides a direct calibration. It was the only relaxed two-line directional signal, yet it failed the prespecified replication rule. In a phase I/randomized phase II recurrent-GBM study, afatinib had limited single-agent activity and did not improve outcomes when added to temozolomide [15]. Afatinib is therefore computationally positive under a relaxed rule but clinically limited, not a candidate rescued by post hoc thresholding.

Disulfiram and hydroxychloroquine were absent from the two audited GBM perturbation lines and were kept as external clinical-negative controls, not computational hits. An 88-patient randomized disulfiram-plus-copper trial found no survival benefit and higher rates of grade 3 or higher and serious adverse events [16]. Hydroxychloroquine studies did not establish survival benefit or reliable autophagy inhibition at tolerable exposure [17,18]. Conversely, temozolomide produced no reversing signatures among three U251MG profiles (median reversal $z = -0.620$), despite being established therapy. Together these controls make reversal neither a sufficient nor a necessary surrogate for clinical value (Figure. 5).

The Null Redirects Discovery to Surface and Intracellular Therapeutic Anchors

The 54 second-order hubs were routed using canonical UniProt localization [19]. Four were cell-surface, four extracellular or secreted, 12 internal-membrane or organelle and 34 cytosolic or nuclear. Disease direction then vetoed destructive targeting of 24 downregulated hubs. Among surface hubs, CD276 was the only upregulated member (meta-Hedges' $g = 1.956$, $FDR = 7.58 \times 10^{-12}$, $I\text{-squared} = 52.0\%$) and localized to MES-like cells. We therefore designated CD276 as a B7-H3 CAR-T/ADC anchor requiring protein-density, normal-brain and cytokine counter-screens, not an efficacy claim [20].

IL13RA2 was a robust meta-signature extension rather than one of the 54 hubs ($g = 0.853$, $FDR = 1.06 \times 10^{-6}$, $I\text{-squared} = 0\%$) and localized to AC-like cells. Its completed locoregional phase I experience makes it a clinically grounded reference CAR anchor, while the early EGFR-IL13RA2 bivalent trial illustrates both multi-antigen feasibility and the remaining challenge of durable control [21,22]. We therefore prioritize single-antigen CD276 and IL13RA2

constructs, together with CD276-IL13RA2 OR-gated and other logic-gated designs in mixed-state organoids. ANTXR1 and CSPG4 remain conditional because endothelial-like and pericyte-like localization flags narrow their safety windows. Gene-level EGFR is retained only with a separate EGFRvIII-specific external control; bulk RNA does not resolve the mutant epitope [23].

The intracellular branch prioritizes ROCK1 (MES-like; $g=2.228$), DBF4 (NPC-like; $g=1.913$), ODC1 (NPC-like; $g=1.996$) and CCNA2 (OPC-like; $g=2.015$) for degradation discovery (Figure. 6). ROCK1 begins with warhead/linker screening against kinase-inhibition and CRISPR controls; DBF4 requires ligand or glue discovery with CDC7-complex rescue; ODC1 requires degradation versus catalytic-inhibition and polyamine rescue; CCNA2 first requires endogenous-tag proof-of-degradation because no direct warhead is assumed. PLK1 is a robust extension and platform comparator. Existing CDK6 PROTAC chemistry demonstrates modality feasibility but does not make CDK6 a GBM anchor because its meta-effect failed the frozen heterogeneity gate [10,24].

Figure 6 | Repurposing failure activates a spatially compatible CAR-T and degradation atlas

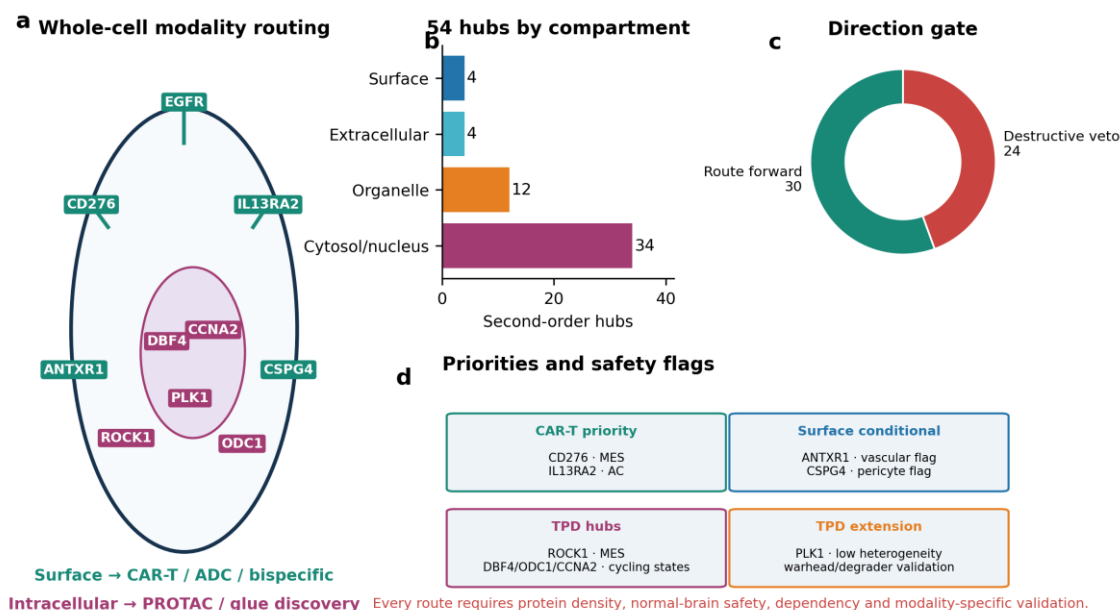


Figure 6: Whole-Cell Modality Atlas Triggered by the Null Repurposing Result. a) Surface and Intracellular Routing of Prioritized Anchors. b) The 54 Second-Order Hubs Comprise 4 Cell-Surface, 4 Extracellular/Secreted, 12 Organelle/Internal-Membrane and 34 Cytosolic/Nuclear Proteins. c) Thirty Hubs are Upregulated and 24 Down Regulated; Destructive Targeting of the Latter is Vetoed. d) Priority and Safety Flags. CD276 is the only Upregulated Surface Member of the 54-Hub Set; IL13RA2 is a Robust Meta-Signature Extension, while ANTXR1 and CSPG4 Require Vascular/Pericyte Counter-Screens. Routing Denotes Experimental Eligibility Rather than Efficacy

Predeclared Validation Turns the Atlas into a Falsifiable Program

The experimental plan begins by quantifying surface molecules per cell across patient-derived AC-, MES-, NPC- and OPC-like cultures, followed by mixed-state organoids under hypoxia and radiation. CAR decisions require killing, cytokine release, state redistribution and antigen-escape measurements against induced-pluripotent-stem-cell neurons, astrocytes and vascular organoids. ANTXR1 and CSPG4 additionally require BBB-organoid integrity and pericyte or endothelial safety tests (Figure. 7).

The degradation arm first tests causal dependency with CRISPRi/knockout and inducible degradation, then advances only targets with rescue-consistent phenotypes to VHL and CRBN chemistry. Go/no-go variables are DC50, Dmax, proteome selectivity, state specificity, normal-neural toxicity and a tractable brain-delivery strategy. Orthotopic testing combines the highest-performing locoregional CAR format with the most tractable systemically or locally delivered degrader. A second docking panel is not an advancement criterion.

Figure 7 | The atlas specifies falsifiable experiments rather than therapeutic claims

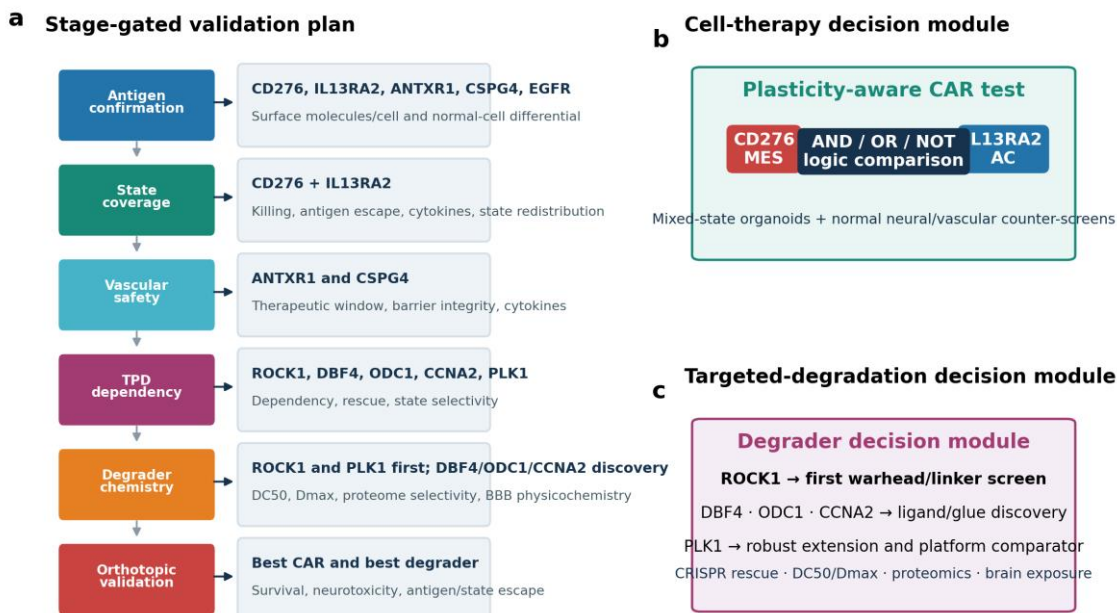


Figure 7: Prospective Validation Blueprint. a) Six Predeclared Stages and go/no-go Outputs. b) Cell-Therapy Module Compares Single-Antigen and CD276-IL13RA2 Logic-Gated Constructs in Mixed-State Organoids with Normal Neural and Vascular Counter-Screens. c) Targeted-Degradation Module Starts with ROCK1 Warhead/Linker Discovery, Retains PLK1 as an Extension Comparator, and Treats DBF4, ODC1 and CCNA2 as Ligand or Glue Discovery Problems. Every Route Requires Orthogonal Genetic Rescue, Proteome Selectivity and Brain-Delivery Assessment

Discussion

This study was built to accept the answer “none.” A reproducible bulk signature, stable data-adaptive network and donor-aware localization did not force a small-molecule nomination: none of the 53 perturbagens with active Drugs@FDA products reproduced reversal across both represented GBM lines, and all relaxed signals failed a conservative CNS descriptor gate. The null is therefore the primary repurposing result, not an inconvenient intermediate hidden by changing thresholds.

Afatinib and temozolomide define complementary boundaries. Afatinib points weakly in the desired direction in both lines but fails the frozen replication rule and has limited activity in a randomized clinical trial. Temozolomide is established therapy yet does not reverse the queried disease signature in the available U251MG profiles. This is the GBM version of a falsification standard: expression reversal is useful for context-specific triage, but it is neither sufficient nor necessary for efficacy. Clinical-negative controls should be designed into computational repurposing studies rather than added only after a favored candidate emerges.

The continuous-h analysis contributes independently of drug nomination. It selects a data-adaptive edge cardinality and exposes a large neural-adaptation focus coupled to stress and cell-cycle foci. Its bootstrap stability is essentially identical to an oracle top-K comparator, which is the honest comparison: the advantage is not a universal gain in Jaccard similarity but removal of a manually chosen K. The 54 second-order hubs likewise arise from a second continuous h intersection rather than a desired fixed count; an anticipated 61-hub count was not imposed on GBM.

The single-cell layer translates tissue effects into state-aware experiments while preserving inferential boundaries. CD276 and ROCK1 align with MES-like cells, IL13RA2 with AC-like cells, DBF4 and ODC1 with NPC-like cells, and CCNA2 with OPC-like cells. These patterns motivate combination and escape experiments, not cell-of-origin claims. The tumor-only atlas lacks normal comparators and sample-level copy-number assignments; marker-score labels and donor-pseudobulks are therefore appropriately conservative.

The emerging-therapy atlas is a modality router, not a clinical ranking. CD276 and IL13RA2 have surface logic and prior CAR evidence, but heterogeneous antigen density, on-target off-tumor effects, neuroinflammation and state switching remain independent gates. ANTXR1 and CSPG4 carry explicit vascular safety liabilities. For the intracellular branch, upregulation is only the first gate: ROCK1, DBF4, ODC1 and CCNA2 require causal dependency, target engagement and rescue before chemistry begins. The physicochemical demands of a bifunctional degrader generally make brain exposure more difficult than for many conventional small molecules; local delivery may be part of the modality, not an afterthought.

Limitations of the Study

Limitations are substantial. GSE50161 is pediatric while the other cohorts are adult, and epilepsy brain is not a pristine matched control; cross-cohort direction and heterogeneity gates reduce but do not erase those differences. The bulk contrast cannot distinguish tumor cells from immune, vascular or reactive glial composition. Current LINCS coverage is markedly imbalanced between U251MG and LN229 and lacks A172, T98G and U87MG, so the null is conditioned on available signatures rather than all possible GBM models. Descriptor rules omit pKa, efflux, plasma protein binding, metabolism and measured unbound brain-to-plasma ratios. Finally, RNA localization does not establish surface protein density, antibody internalization, ligandability, productive ternary complexes or therapeutic window.

The decisive next step is the staged validation panel, not retrospective candidate inflation. It forces surface anchors to survive normal neural and vascular counter-screens, forces multi-antigen constructs to demonstrate coverage under state plasticity, and forces intracellular anchors to show genetic and degradation-rescue concordance. In this framework, computational failure is not the end of discovery: it determines when to stop repurposing and which falsifiable modality-specific experiments should begin.

STAR★METHODS

Key Resources Table

REAGENT OR RESOURCE	SOURCE	IDENTIFIER
Human GBM and control transcriptomes	NCBI GEO	GSE4290; GSE50161; GSE147352
GBM single-cell RNA-seq and state modules	NCBI GEO / Neftel et al.	GSE131928; author repository
Chemical perturbation signatures	SigCom LINCS	l1000_cp; U251MG; LN229
Active US drug products	US FDA	Drugs@FDA; 24 July 2026
Protein localization and chemical descriptors	UniProt / PubChem	Canonical entries; PUG REST
Meta-signature, h-foci and modality atlas	This study	GBM_robust_signature.csv; derived tables
Analysis code	This study	Repository DOI pending

Resource Availability

Lead Contact

Further information and requests should be directed to Fred Y. Ye.

Materials Availability

This study generated no new biological materials.

Data and Code Availability

Public accessions, complete derived tables, source hashes, scripts, figures and the audited workbook accompany this manuscript. Persistent repository identifiers are pending author deposit and must be inserted before submission.

Experimental Model and Study Participant Details

All analyses used de-identified public human tissue or tumor single-cell data. Original studies reported consent and ethics procedures. No new participants were recruited.

Method Details

Study Design and Data Provenance

The prospective analysis plan was treated as a launch protocol, but infeasible assumptions were changed transparently. Public records were accessed through 26 July 2026. Case-control discovery used GSE4290, GSE50161 and GSE147352; TCGA-GBM and CGGA were not treated as normal-controlled discovery cohorts. GSE131928 supplied single-cell localization. Large public raw matrices are not redistributed in the open-science package; accession URLs and SHA-256 values are supplied.

Bulk Preprocessing and Random-Effects Synthesis

Affymetrix series matrices were mapped with GPL570. For each gene, the probe with the highest cohort-wide median signal was selected without disease labels. GSE147352 DESeq-normalized counts were mapped to GENCODE v49 protein-coding symbols, collapsed by median and transformed $\log_2(x+1)$. Each cohort contributed an unpaired Hedges' g and sampling variance. Effects were combined by DerSimonian-Laird random-effects meta-analysis; Benjamini-Hochberg adjustment controlled FDR [25,26]. Robust genes required meta-FDR<0.01, identical effect direction in all cohorts and I-squared<75%.

Differential Co-Expression, Continuous h and Stability

The 150 strongest robust increases and decreases were standardized within cohort before pooling by condition. For each pair, Fisher-transformed tumor and normal correlations were compared. Edge weight was (absolute delta-r/2) times

$\max(\text{absolute } r\text{-tumor}, \text{absolute } r\text{-normal})$. Ordered weights $w(k)$ were retained while $w(k) \geq k/M$. An unsupervised spectral partition was selected by weighted modularity among three to eight communities subject to a minimum focus size of eight. A second continuous h pass over inter-focus bridge scores recovered coupling edges. Hub score was $0.50 \text{ normalized strength} + 0.25 \text{ normalized degree} + 0.25 \text{ normalized inter-focus strength}$, followed by continuous h . Twenty-five stratified sample bootstraps compared continuous h , oracle top- K and a fixed FDR/delta-correlation rule.

Pathway Enrichment and Single-Cell Localization

Enrichr queried MSigDB Hallmark 2020, Reactome 2022 and GO Biological Process 2023 [12]. Exact Neftel meta-modules were retrieved from the authors' public repository [2]. Smart-seq2 and 10X matrices were retrieved from GSE131928 for 663 network, anchor, lineage and state genes. Within each platform, log1p expression was robustly standardized by gene. Marker scores defined conservative immune and vascular-like compartments; remaining adult glial-lineage cells were assigned to the AC, MES, NPC or OPC module with the highest score. Donor-state and donor-compartment pseudobulks required at least ten cells. Specificity z scores were computed across states or compartments for each gene.

LINCS Enumeration and Reversal Scoring

The query comprised the 150 strongest robust increases and 150 strongest robust decreases. SigCom metadata were audited for U251MG, LN229, A172, T98G and U87MG before scoring [13]. Only U251MG and LN229 were represented. All chemical signatures from those lines were retrieved in stable identifier order. Perturbagen salts and aliases were normalized and intersected with ingredients or drug names in active prescription or OTC Drugs@FDA products [14]. LDP3 rank-two-sided characteristic-direction results supplied $z\text{-up}$ and $z\text{-down}$; reversal z was $-(z\text{-up}+z\text{-down})/\sqrt{2}$, and a reversal-quadrant signature required both values to be negative and the returned type to be a reverser.

Frozen Candidate, CNS and Clinical Gates

A line passed when a drug had at least three returned signatures, reversal fraction ≥ 0.60 and median all-signature reversal $z \geq 1.64$. The primary candidate required both U251MG and LN229 to pass. PubChem descriptors were evaluated only after transcriptomic scoring; the exploratory exclusion rule required molecular weight ≤ 450 , TPSA ≤ 90 square angstrom, hydrogen-bond donors ≤ 3 , rotatable bonds ≤ 8 and XlogP 1-4 [27]. The rule is not full CNS-MPO or measured BBB exposure [7,8]. Clinical controls were adjudicated against peer-reviewed human trials only after the computational tables were frozen. A null final set was prespecified as valid.

Spatial Annotation and Modality Routing

Canonical reviewed UniProt entries supplied cell-surface, extracellular/secreted, internal-membrane/organelle or cytosolic/nuclear annotations [19]. Second-order hubs were counted separately from robust meta-signature or backbone extensions. A negative tissue-level effect vetoed destructive targeting. Surface routing triggered CAR-T, ADC or bispecific eligibility pending extracellular-epitope density, internalization and normal-neural or vascular safety. Intracellular routing triggered dependency and degrader-discovery eligibility pending a ligandable site, productive E3 engagement, degradation-rescue concordance, proteome selectivity and a brain-delivery strategy [10]. RNA abundance was never treated as proof of drug ability or efficacy.

Quantification and Statistical Analysis

Analyses used Python 3.12, NumPy, pandas, SciPy, scikit-learn, RDKit, NetworkX and Matplotlib in the versions recorded by the packaged environment report. All inferential tests were two-sided unless stated. The package contains executable scripts, full derived tables, seven main figures and a graphical abstract—including Figures 4 and 6—as SVG and 300/600-dpi PNG files, a formula-audited 20-sheet workbook, source hashes and a file-level SHA-256 manifest. The work generated no new biological materials.

Supplemental Information

The accompanying data and figure package contain the complete derived matrices, all figure formats, the formula-audited workbook, source hashes and executable workflows.

Data Availability

All primary data are public under the GEO accessions and database records listed in the Sources worksheet. Full derived tables, source URLs and exact hashes accompany this manuscript. The persistent public-repository DOI is 10.5281/zenodo.21643514.

Code Availability

Executable analysis, figure and packaging scripts, frozen query outputs and environment records accompany this manuscript. The public-repository URL and software license locate at <https://zenodo.org/records/21643514>.

Acknowledgements

No fund supports this research. The author thanks the contributors and donors who made the GEO, LINCS, Drugs@FDA, and Protein Data Bank resources available.

Author Contributions

F.Y.Y.: Conceptualization; Methodology; Formal analysis; Investigation; Data curation; Validation; Visualization; Writing - original draft; Writing - review and editing; Project administration. F.Y.Y. verified the analyses and approved the submitted manuscript.

Competing Interests

The author declares no competing interests.

Generative-AI Disclosure

ChatGPT 5.6 Sol and Gemini 3.6 Flash assisted code generation, English-language drafting, figure layout and consistency checking. The named author must substantively review, verify and revise every analysis, citation, interpretation and sentence and remains fully accountable for the content.

References

1. Stupp, R., Mason, W. P., Van Den Bent, M. J., Weller, M., Fisher, B., Taphoorn, M. J., ... & Mirimanoff, R. O. (2005). Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *New England journal of medicine*, 352(10), 987-996.
2. Neftel, C., Laffy, J., Filbin, M. G., Hara, T., Shore, M. E., Rahme, G. J., ... & Suvà, M. L. (2019). An integrative model of cellular states, plasticity, and genetics for glioblastoma. *Cell*, 178(4), 835-849.
3. Lamb, J., Crawford, E. D., Peck, D., Modell, J. W., Blat, I. C., Wrobel, M. J., ... & Golub, T. R. (2006). The Connectivity Map: using gene-expression signatures to connect small molecules, genes, and disease. *science*, 313(5795), 1929-1935.
4. Subramanian, A., Narayan, R., Corsello, S. M., Peck, D. D., Natoli, T. E., Lu, X., ... & Golub, T. R. (2017). A next generation connectivity map: L1000 platform and the first 1,000,000 profiles. *Cell*, 171(6), 1437-1452.
5. Lim, N., & Pavlidis, P. (2021). Evaluation of connectivity map shows limited reproducibility in drug repositioning. *Scientific reports*, 11(1), 17624.
6. Koudijs, K. K., Böhringer, S., & Guchelaar, H. J. (2023). Validation of transcriptome signature reversion for drug repurposing in oncology. *Briefings in Bioinformatics*, 24(1), bbac490.
7. Wager, T. T., Hou, X., Verhoest, P. R., & Villalobos, A. (2010). Moving beyond rules: the development of a central nervous system multiparameter optimization (CNS MPO) approach to enable alignment of druglike properties. *ACS chemical neuroscience*, 1(6), 435.
8. Rankovic, Z. (2015). CNS drug design: balancing physicochemical properties for optimal brain exposure. *Journal of medicinal chemistry*, 58(6), 2584-2608.
9. Sakamoto, K. M., Kim, K. B., Kumagai, A., Mercurio, F., Crews, C. M., & Deshaies, R. J. (2001). Protacs: Chimeric molecules that target proteins to the Skp1-Cullin-F box complex for ubiquitination and degradation. *Proceedings of the National Academy of Sciences*, 98(15), 8554-8559.
10. Burslem, G. M., & Crews, C. M. (2020). Proteolysis-targeting chimeras as therapeutics and tools for biological discovery. *Cell*, 181(1), 102-114.
11. Ye FY. Identifying hub genes of Alzheimer's and Parkinson's diseases via h-cutoff: a new methodological approach. *Innovative Medicines & Omics*. 2026.
12. Kuleshov, M. V., Jones, M. R., Rouillard, A. D., Fernandez, N. F., Duan, Q., Wang, Z., ... & Ma'ayan, A. (2016). Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. *Nucleic acids research*, 44(W1), W90-W97.
13. Evangelista, J. E., Clarke, D. J., Xie, Z., Lachmann, A., Jeon, M., Chen, K., ... & Ma'ayan, A. (2022). SigCom LINCS: data and metadata search engine for a million gene expression signatures. *Nucleic acids research*, 50(W1), W697-W709.
14. US Food and Drug Administration. Drugs@FDA data files. Page updated 24 July 2026; archive files stamped 23 July 2026.
15. Reardon, D. A., Nabors, L. B., Mason, W. P., Perry, J. R., Shapiro, W., Kavan, P., ... & Phuphanich, S. (2015). Phase I/ randomized phase II study of afatinib, an irreversible ErbB family blocker, with or without protracted temozolomide in adults with recurrent glioblastoma. *Neuro-oncology*, 17(3), 430-439.
16. Werlenius, K., Kinhult, S., Solheim, T. S., Magelssen, H., Löfgren, D., Mudaisi, M., ... & Jakola, A. S. (2023). Effect of disulfiram and copper plus chemotherapy vs chemotherapy alone on survival in patients with recurrent glioblastoma: a randomized clinical trial. *JAMA network open*, 6(3), e234149.
17. Rosenfeld, M. R., Ye, X., Supko, J. G., Desideri, S., Grossman, S. A., Brem, S., ... & Amaravadi, R. K. (2014). A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme. *Autophagy*, 10(8), 1359-1368.
18. Brazil, L., Swampillai, A. L., Mak, K. M., Edwards, D., Mesiri, P., Clifton-Hadley, L., ... & Short, S. C. (2020). Hydroxychloroquine and short-course radiotherapy in elderly patients with newly diagnosed high-grade glioma: a randomized phase II trial. *Neuro-Oncology Advances*, 2(1), vdaa046.
19. UniProt Consortium. (2025). UniProt: the Universal Protein Knowledgebase in 2025. *Nucleic Acids Res*. 53:D609-D617.
20. Nehama, D., Di Ianni, N., Musio, S., Du, H., Patané, M., Pollo, B., ... & Dotti, G. (2019). B7-H3-redirected chimeric antigen receptor T cells target glioblastoma and neurospheres. *EBioMedicine*, 47, 33-43.
21. Brown, C. E., Hibbard, J. C., Alizadeh, D., Blanchard, M. S., Natri, H. M., Wang, D., ... & Badie, B. (2024). Locoregional delivery of IL-13Ra2-targeting CAR-T cells in recurrent high-grade glioma: a phase 1 trial. *Nature medicine*, 30(4), 1001-1012.

22. Bagley, S. J., Logun, M., Fraietta, J. A., Wang, X., Desai, A. S., Bagley, L. J., ... & O'Rourke, D. M. (2024). Intrathecal bivalent CAR T cells targeting EGFR and IL13R α 2 in recurrent glioblastoma: phase 1 trial interim results. *Nature Medicine*, 30(5), 1320-1329.
23. O'Rourke, D. M., Nasrallah, M. P., Desai, A., Melenhorst, J. J., Mansfield, K., Morrisette, J. J., ... & Maus, M. V. (2017). A single dose of peripherally infused EGFRvIII-directed CAR T cells mediates antigen loss and induces adaptive resistance in patients with recurrent glioblastoma. *Science translational medicine*, 9(399), eaaa0984.
24. Rana, S., Bendjennat, M., Kour, S., King, H. M., Kizhake, S., Zahid, M., & Natarajan, A. (2019). Selective degradation of CDK6 by a palbociclib based PROTAC. *Bioorganic & medicinal chemistry letters*, 29(11), 1375-1379.
25. DerSimonian, R., & Laird, N. (1986). Meta-analysis in clinical trials. *Controlled Clinical Trials*, 7(3), 177-188.
26. Benjamini, Y., & Hochberg, Y. (1995). Controlling the false discovery rate: a practical and powerful approach to multiple testing. *Journal of the Royal statistical society: series B (Methodological)*, 57(1), 289-300.
27. Kim, S., Chen, J., Cheng, T., Gindulyte, A., He, J., He, S., ... & Bolton, E. E. (2023). PubChem 2023 update. *Nucleic acids research*, 51(D1), D1373-D1380.
28. National Center for Biotechnology Information. Gene Expression Omnibus GSE4290: expression data of human glioma and non-tumor brain samples.
29. National Center for Biotechnology Information. Gene Expression Omnibus GSE50161: expression data from human brain tumors and normal brain.
30. National Center for Biotechnology Information. Gene Expression Omnibus GSE147352: coding and non-coding transcriptome of adult glioblastoma and normal brain.
31. National Center for Biotechnology Information. Gene Expression Omnibus GSE131928: single-cell RNA sequencing of adult and pediatric IDH-wild-type glioblastoma.