Targeting the GBM Recurrence Program: ErbB4 as a Therapeutic Node in Chemotherapy-Resistant Glioblastoma

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BIOS 4500 / BIOL 8803: Drug Discovery

Selected Drug Target and Treatment
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Revisiting Glioblastoma

What We Established :

- WHO Grade IV IDH-wildtype glioma — most lethal brain tumor
- Median survival: 15 months with standard of care (Stupp et al., 2005)
- Standard of care: Surgery → Radiation (60 Gy) → Temozolomide (TMZ)
- 5-year survival: <10% (Ostrom et al., 2023)

What Remains Unsolved:

- Over 90% recurrence rate — nearly every single patient relapses
- Recurrent GBM: no effective therapy
- Median survival after recurrence: 6–9 months
- No drug has meaningfully extended post-recurrence survival in 20 years

We can treat the primary tumor. We cannot treat the tumor that comes back. That is the problem I set out to solve.

The Central Question

- *What cells survive temozolomide chemotherapy and drive tumor recurrence?*

Primary GBM Tumor → TMZ Chemotherapy → 69% Cell Death → 31% Survivors → RECURRENCE

Three Sub-Questions :

- What is the molecular identity of chemotherapy-resistant cells?
- Is resistance pre-programmed or acquired under treatment?
- Can we target the resistance mechanism therapeutically?

Dataset — 128,670 Single Cells Across 76 GBM Patients

Dataset Overview:

- 128,670 single cells from 76 GBM patients
- Primary tumors: 44 patients
- Matched recurrent tumors: 32 patients
- Technology: 10x Genomics Chromium snRNA-seq
- Data source: GSE174554 (Garofano et al., 2021)
- Technology: 10x Genomics Chromium snRNA-seq
- Cell types identified: 7 tumor states (Neftel et al., 2019)

Computational Approach:

1. Differential gene expression: primary vs recurrent tumors
2. Identified 28-gene recurrence signature
3. Scored all 128,670 cells for signature expression
4. Analyzed cell type composition shifts

Experimental Validation:

- T5224 GBM cell line treated with TMZ (100 µM, 72 hours)
- snRNA-seq before and after treatment

A 28-Gene Recurrence Program Shared Across All Tumor States

Key Findings:

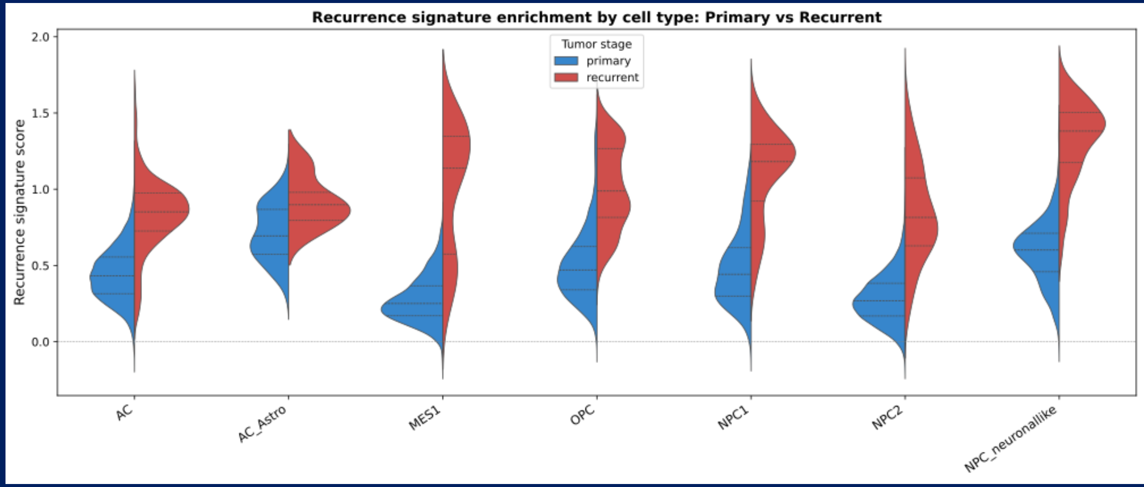
• All 7 tumor cell types show significantly higher recurrence signature at relapse (all $p < 10^{-50}$)

• NPC_neuronallike: largest shift ($\Delta = +0.73$, $p = 4\times10^{-159}$)

• NPC1 and NPC2: also massively enriched ($\Delta = +0.63$, $\Delta = +0.56$)

• Astrocytic (AC) and mesenchymal (MES) cells: smaller but significant increases

• Neural progenitor populations are most therapy-resistant (Neftel et al., 2019)



Violin plots showing recurrence signature scores across seven tumor cell states in primary versus recurrent GBM samples.

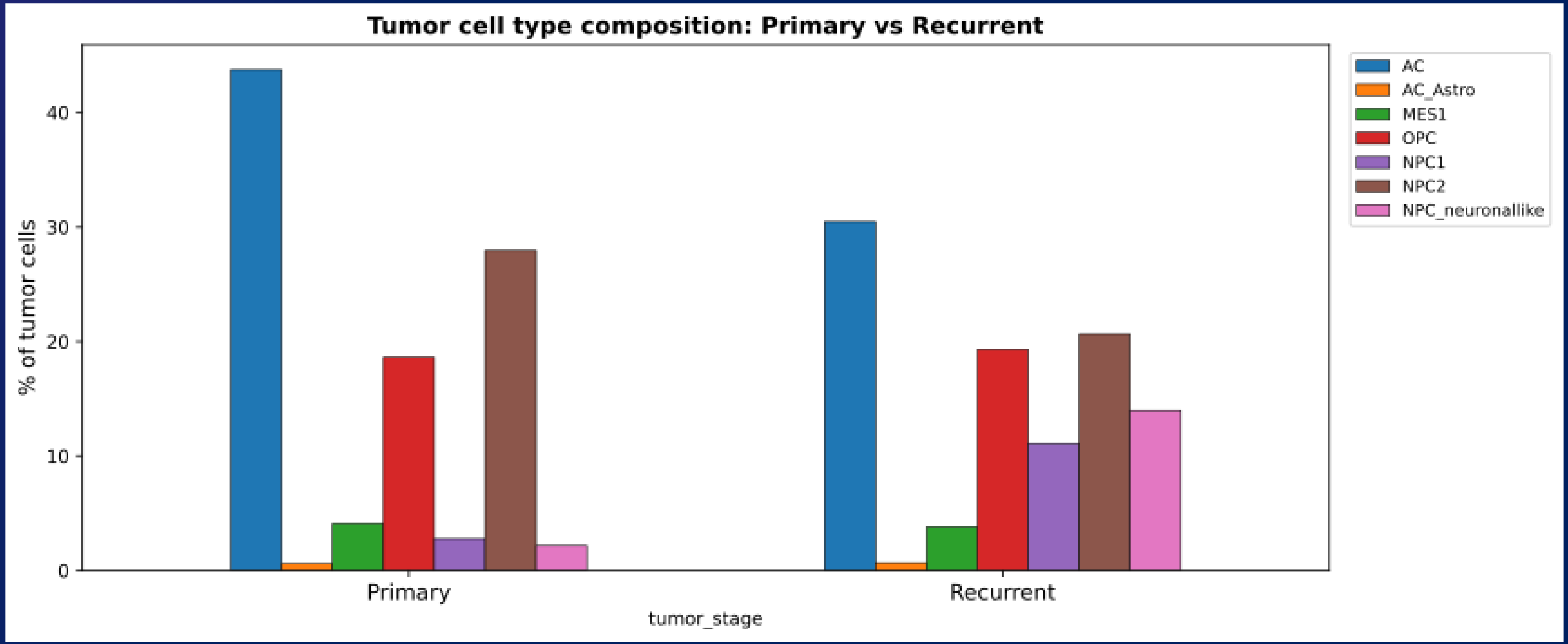
	cell_type	n_primary	n_recurrent	primary_mean	recurrent_mean	delta	p_value	sig
1	MES1	2736	89	0.2756	1.0056	0.73	1.83e-45	✅
2	NPC2	18504	482	0.2821	0.8466	0.5644	1.87e-243	✅
3	OPC	12340	451	0.5067	1.0121	0.5054	2.49e-201	✅
4	AC	28964	711	0.441	0.8343	0.3933	3.17e-301	✅
5	NPC_neuronallike	1436	326	0.5902	1.3208	0.7306	4.05e-159	✅
6	AC_Astro	396	16	0.7115	0.9195	0.208	4.97e-05	✅
7	NPC1	1841	259	0.4771	1.1044	0.6273	5.54e-115	✅

Detailed view of the magnitude of recurrence signature elevation at relapse across tumor states.

NPC_neuronallike Cells Expand 6.4-Fold at Recurrence

Key Findings:

- NPC_neuronallike: 2.2% → 14.0% (+11.8 percentage points, 6.4× fold change)
- NPC1: 2.8% → 11.1% (+8.3 percentage points, 4.0× fold change)
- Tumor_AC: 43.7% → 30.5% (−13.2 percentage points, 30% reduction)



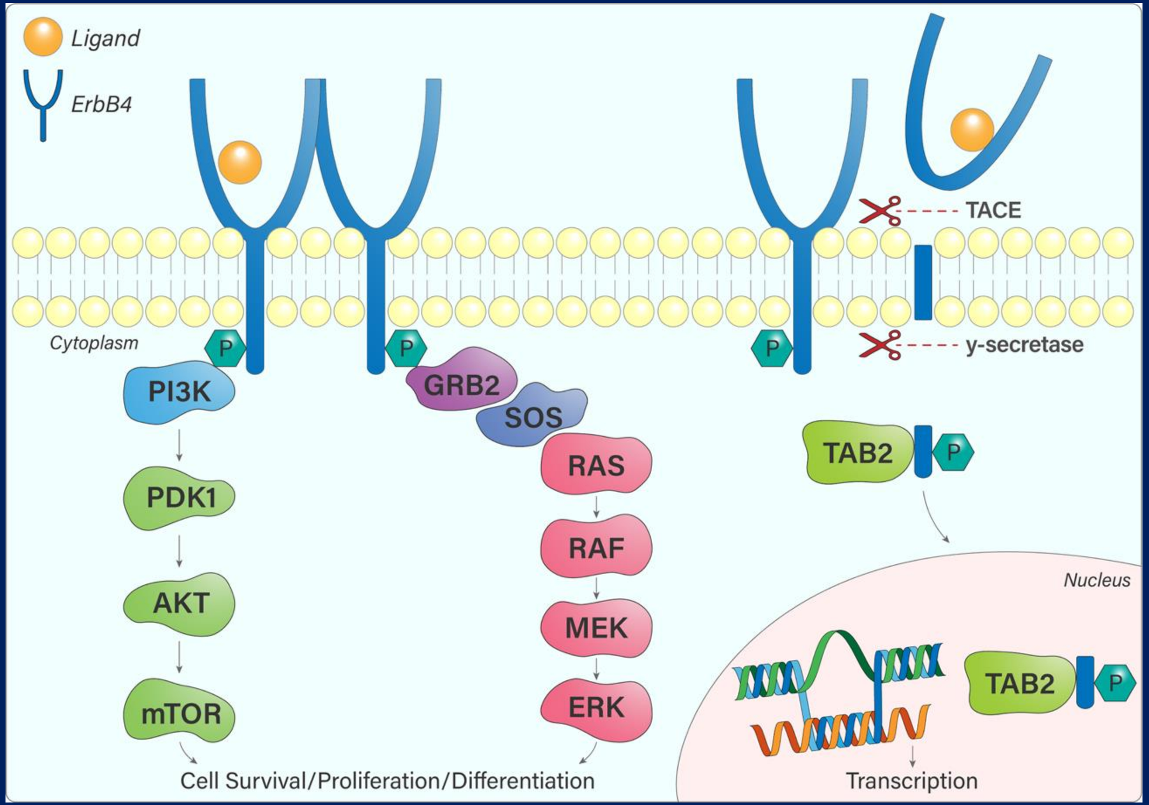
Cellular composition changes between primary and recurrent GBM tumors.

Interpretation: Recurrent tumors are rebuilt by neural progenitor cell expansion (Garofano et al., 2021), not by AC/MES cell survival. GBM cellular plasticity enables state transitions under therapy (Suvà & Tirosh, 2020).

The Molecular Target — ERBB4-NRG Autocrine Survival Loop

1. ERBB4/NRG Autocrine Signaling

- ERBB4 — neuregulin receptor (tyrosine kinase)
- NRG1, NRG3 — neuregulin ligands
- Self-sustaining proliferation signal independent of EGF/EGFR
- High p-ERBB4 → 12.0 months survival vs. 22.5 months (Pearce et al., 2018)
- ErbB4 is primary NRG receptor in CNS; high expression = poor prognosis



ERBB4 activation triggers PI3K/AKT (survival) and MAPK (proliferation) pathways. High p-ERBB4 expression → 47% shorter survival in GBM (Pearce et al., 2018).

The Molecular Target — ERBB4-NRG Autocrine Survival Loop

2. Synaptic & Axon Guidance Machinery

- SYT1 — synaptotagmin-1 (synaptic vesicle fusion)
- NRXN1 — neurexin-1 (synapse formation with neurons)
- DCC — deleted in colorectal cancer (axon guidance receptor)
- Enables tumor-neuron synaptic communication (Venkatesh et al., 2019; Venkataramani et al., 2019)

Electrical and synaptic integration of glioma into neural circuits

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Affiliations + expand

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The Molecular Target — ERBB4-NRG Autocrine Survival Loop

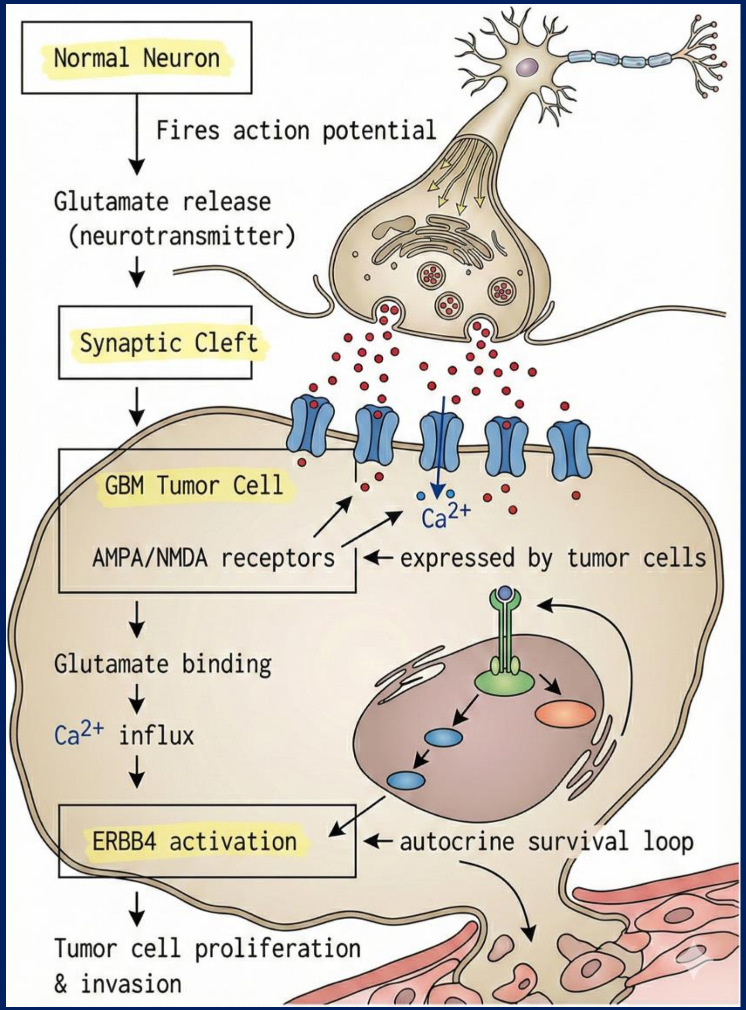
3. Neural Transcription Factors -

- RBFOX1 — RNA-binding neuronal splicing factor
- ZFPM2 — FOG2 transcription factor
- Locks cells into neuronal identity state

Mechanism Summary:
These cells wire themselves into the brain like neurons, form functional synapses with normal neurons, and use glutamate signals to drive ERBB4-dependent survival

Neuronal Hijacking — GBM Cells Integrate into Neural Circuits

- **Discovery (Monje Lab, 2019):**
  - GBM cells express postsynaptic proteins: **NRXN1, SYT1, DCC**
  - Form bona fide glutamatergic synapses with normal neurons
  - Electron microscopy confirms synaptic vesicles and membrane specializations
- **Functional Consequences:**
  - Neuronal activity → glutamate release → GBM cell depolarization
  - Ca^{2+} signaling activates **PI3K/AKT** and **MAPK** proliferation pathways
  - Blocking synapses (with tetrodotoxin or glutamate receptor antagonists) reduces tumor growth by 50%
- **Clinical Relevance:**
  - Tumors in high-activity brain regions (e.g., motor cortex) grow faster
  - Seizures correlate with accelerated progression
  - **ERBB4 links synaptic input to survival signaling** — connecting neuronal hijacking to the autocrine loop



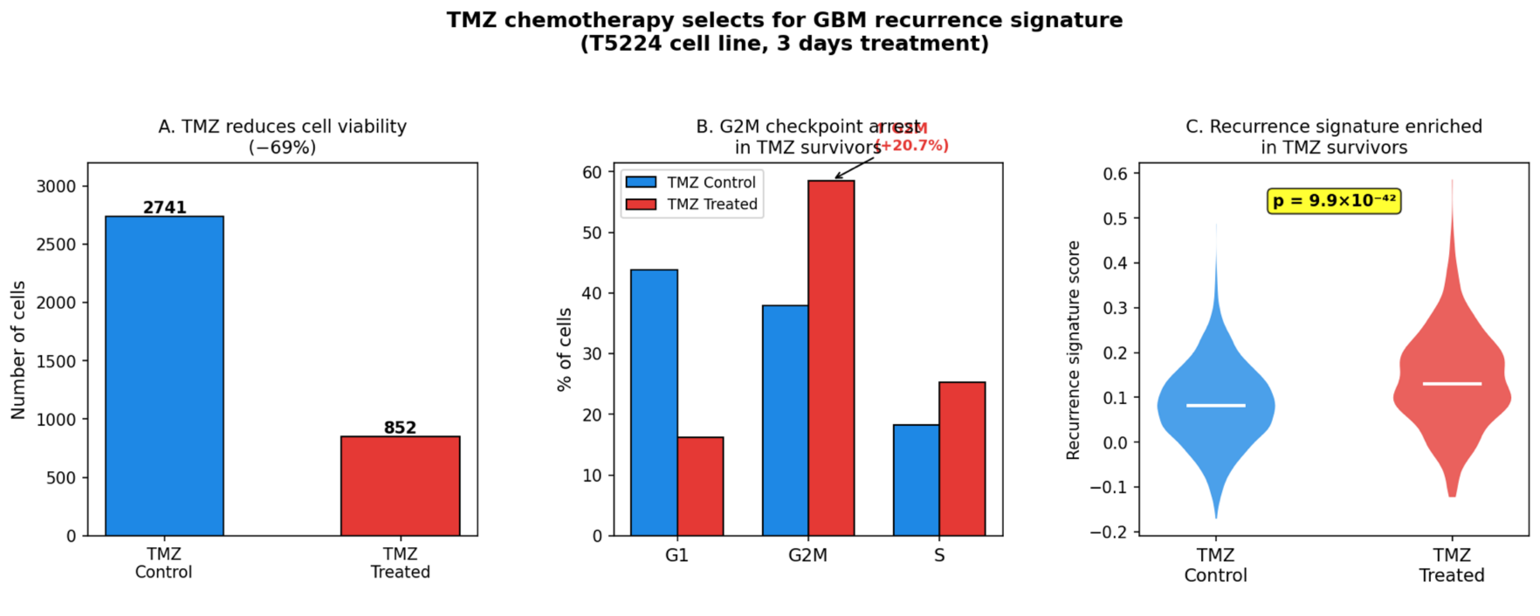
GBM cells don't just invade the brain — they wire themselves into it and use brain activity as fuel.

GBM cells form glutamatergic synapses with normal neurons, hijacking neuronal activity to drive ERBB4-dependent tumor proliferation and invasion.

Experimental Proof — TMZ Chemotherapy Directly Selects for the Recurrence Signature

Key Results:

(A) 69% cell death, 31% survival — survivors cluster separately (distinct transcriptional state)
(B) G2M checkpoint arrest in survivors (20.7% vs. 16.1% in control) — active DNA damage response
(C) Recurrence signature elevated in survivors: $p = 9.9\times10^{-42}$ — TMZ directly selects for this program



TMZ chemotherapy directly selects for the GBM recurrence signature. *(A) 69% cell death after 72h treatment. (B) Survivors show G2/M checkpoint arrest (+20.7%). (C) Recurrence signature dramatically elevated in survivors ($p = 9.9\times10^{-42}$).*

TMZ Treatment Experiment:

- T5224 GBM cell line, 100 µM TMZ, 72 hours
- snRNA-seq before (2,741 cells) and after (852 cells) treatment
- 69% cell death, 31% survival

Proposed Treatment — ERBB4 Inhibition + CHK1/2 Checkpoint Blockade

Proposed Combination Therapy:

Drug 1: ERBB4 Inhibitor

Afatinib (FDA-approved pan-ERBB inhibitor)

- Irreversibly binds EGFR, ERBB2, ERBB4
- CNS penetration: moderate BBB crossing
- FDA-approved for NSCLC (2013)

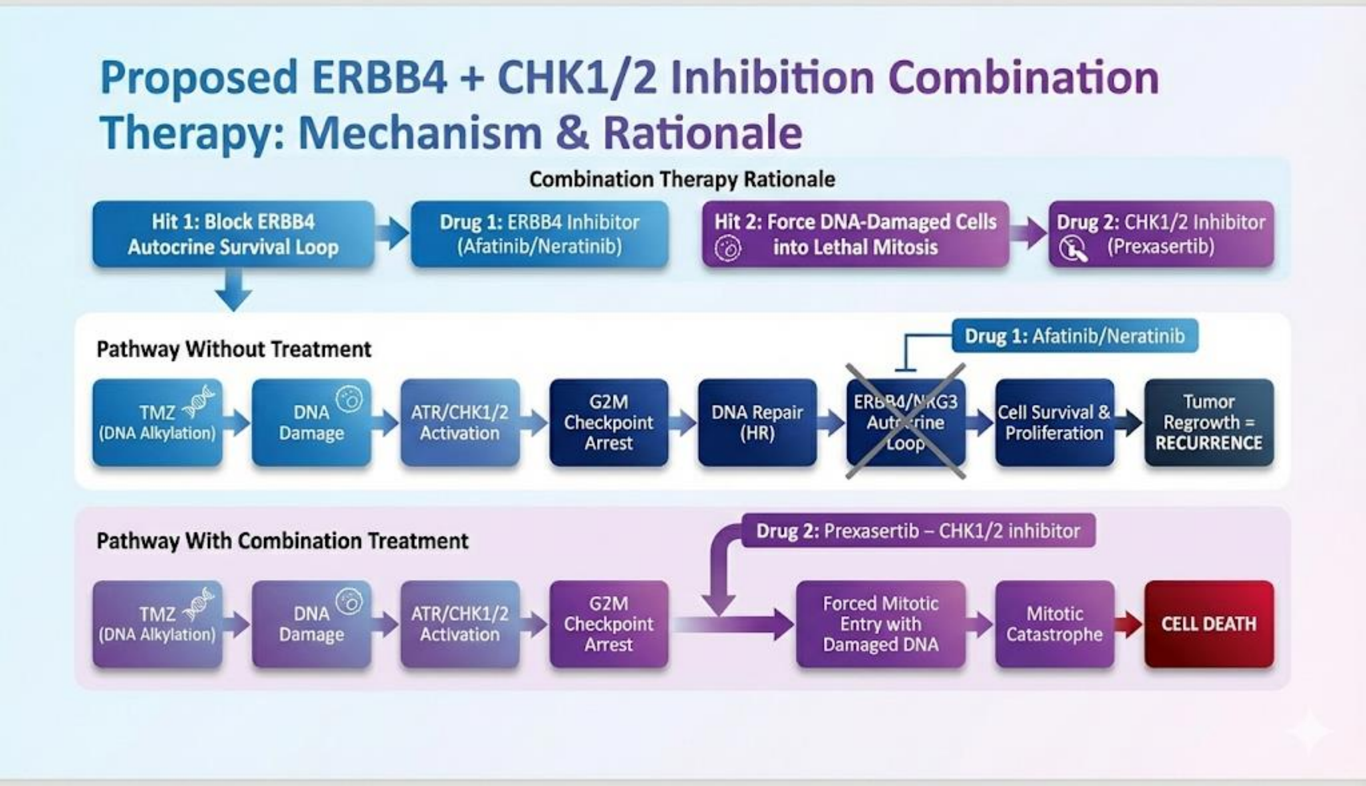
Neratinib (alternative)

- Irreversible pan-ERBB inhibitor
- Higher ERBB4 selectivity
- Phase II trials in brain metastases

Drug 2: CHK1/2 Inhibitor

Prexasertib (LY2606368)

- Potent CHK1/2 inhibitor
- Phase II trials in solid tumors
- Mechanism: forces mitotic catastrophe in checkpoint-arrested cells



Proposed combination therapy targeting chemotherapy-resistant GBM survivors.

Proposed Treatment — ERBB4 Inhibition + CHK1/2 Checkpoint Blockade

Administration Protocol

Afatinib (ERBB4 Inhibitor)

- Recurrent GBM: 280 mg orally every 7 days (pulsatile dosing for CNS penetration)
- Newly diagnosed: 40 mg daily during RT, then maintenance
- Taken on empty stomach (1 hr before or 3 hrs after food)

Prexasertib (CHK1/2 Inhibitor)

- 105 mg/m^2 IV infusion over 60 minutes
- Day 1 of every 14-day cycle
- Requires mandatory G-CSF prophylaxis 24-72 hrs post-infusion

Sequencing Strategy

- Days 1-3: Afatinib primes replication stress and checkpoint arrest
- Day 3-4: Prexasertib forces mitotic catastrophe in arrested cells
- Days 5-14: Recovery period for neutrophil reconstitution

Rationale:

Hit 1: Block ERBB4 autocrine survival loop

Hit 2: Force DNA-damaged cells into lethal mitosis before repair completes

Synergistic mechanism targets the exact resistance pathway identified

Key Requirements

- Confirm ERBB4 amplification/expression before treatment
- Weekly CBC monitoring (Cycles 1-2), then every 2 weeks
- Adjust afatinib dose if on dexamethasone (CYP3A4 interaction)

Summary

Five Key Findings:

1. **GBM recurrence is driven by NPC_neuronallike cell expansion**
    - 6.4-fold increase at relapse; primary tumor shifts from AC-dominant to NPC-dominant
2. **A 28-gene neuronal survival program defines resistant cells**
    - ERBB4/NRG3 autocrine loop, synaptic machinery (SYT1, NRXN1), neural TFs (RBFOX1)
3. **TMZ chemotherapy directly selects for this program**
    - Survivors show elevated recurrence signature ($p = 9.9\times10^{-42}$)
    - Not quiescence — active G2M checkpoint-mediated survival
4. **ERBB4 is the master regulator and therapeutic target**
    - Druggable with FDA-approved pan-ERBB inhibitors (Afatinib, Neratinib)
5. **Proposed treatment: TMZ + Afatinib (ERBB4i) + Prexasertib (CHK1/2i)**
    - Two-hit strategy: block survival signal + force mitotic catastrophe

References

- Louis DN, Perry A, Wesseling P, et al. The 2021 WHO Classification of Tumors of the Central Nervous System: a summary. *Neuro Oncol.* 2021;23(8):1231-1251.
- Stupp R, Mason WP, van den Bent MJ, et al. Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma. *N Engl J Med.* 2005;352(10):987-996.
- Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma. *Lancet Oncol.* 2009;10(5):459-466.
- Ostrom QT, Cioffi G, Gittleman H, et al. CBTRUS Statistical Report: Primary Brain and Other Central Nervous System Tumors Diagnosed in the United States in 2016-2020. *Neuro Oncol.* 2023;25(Suppl 4):iv1-iv99.
- Weller M, van den Bent M, Preusser M, et al. EANO guidelines on the diagnosis and treatment of diffuse gliomas of adulthood. *Nat Rev Clin Oncol.* 2021;18(3):170-186.
- Tykocki T, Eltayeb M. Ten-year survival in glioblastoma: a systematic review. *J Clin Neurosci.* 2018;54:7-13.
- Garofano L, Migliozzi S, Oh YT, et al. Pathway-based classification of glioblastoma uncovers a mitochondrial subtype with therapeutic vulnerabilities. *Nat Cancer.* 2021;2(2):141-156.
- Voldborg BR, Damstrup L, Spang-Thomsen M, Poulsen HS. Epidermal growth factor receptor (EGFR) and EGFR mutations, function and possible role in clinical trials. *Ann Oncol.* 1997;8(12):1197-1206.
- Sundvall M, Iljin K, Kilpinen S, et al. Role of ErbB4 in breast cancer. *J Mammary Gland Biol Neoplasia.* 2008;13(2):259-268.
- Venkatesh HS, Morishita W, Geraghty AC, et al. Electrical and synaptic integration of glioma into neural circuits. *Nature.* 2019;573(7775):539-545.
- Venkataramani V, Tanev DI, Strahle C, et al. Glutamatergic synaptic input to glioma cells drives brain tumour progression. *Nature.* 2019;573(7775):532-538.
- Neftel C, Laffy J, Filbin MG, et al. An integrative model of cellular states, plasticity, and genetics for glioblastoma. *Cell.* 2019;178(4):835-849.
- Monje M. Synaptic Communication in Brain Cancer. *Cancer Res.* 2020;80(14):2979-2982.
- Dungo RT, Keating GM. Afatinib: first global approval. *Drugs.* 2013;73(13):1503-1515.
- Rabindran SK, Discafani CM, Rosfjord EC, et al. Antitumor activity of HKI-272, an orally active, irreversible inhibitor of the HER-2 tyrosine kinase. *Cancer Res.* 2004;64(11):3958-3965.
- Hong D, Infante J, Janku F, et al. Phase I Study of LY2606368, a Checkpoint Kinase 1 Inhibitor, in Patients With Advanced Cancer. *J Clin Oncol.* 2016;34(15):1764-1771.
- Hoffknecht P, Tufman A, Wehler T, et al. Efficacy of the irreversible ErbB family blocker afatinib in epidermal growth factor receptor (EGFR) tyrosine kinase inhibitor (TKI)-pretreated non-small-cell lung cancer patients with brain metastases or leptomeningeal disease. *J Thorac Oncol.* 2015;10(1):156-163.
- King C, Diaz HB, McNeely S, et al. LY2606368 Causes Replication Catastrophe and Antitumor Effects through CHK1-Dependent Mechanisms. *Mol Cancer Ther.* 2015;14(9):2004-2013.
- Brennan CW, Verhaak RG, McKenna A, et al. The somatic genomic landscape of glioblastoma. *Cell.* 2013;155(2):462-477.