

Comprehensive Multimodal Therapy for Recurrent Glioblastoma: A Patient Trial Combining Advanced Surgical Techniques, Crispr Enhanced Immunotherapy and Metabolic Modulation

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Abstract—Glioblastoma GBM remains one of oncology's most formidable challenges with median survival stagnating at 12 to 15 months despite aggressive treatment. This prospective phase I and II trial evaluated a novel four pronged therapeutic approach in 23 patients with recurrent IDH wild type GBM.

1. Real time intraoperative mass spectrometry iknife for precision tumor resection
2. CRISPR engineered NKG2D CAR NK cells with PD 1 knock out
3. Personalized oncolytic adenovirus Delta 24 RGD IL12
4. Metabolic preconditioning using a ketogenic diet with dichloroacetate DC and metformin

Results demonstrated a median progression-free survival of 9.8 months 95% CI 7.4 12.1 versus 4.2 months historically $p < 0.001$ with 62% 12 month overall survival. Three patients (13%) achieved complete radiographic responses while cognitive function was preserved mean MMSE 28.1 post-treatment. This first in human protocol represents a significant advancement in GBM treatment warranting phase III evaluation.

I. INTRODUCTION

Glioblastoma's therapeutic resistance stems from three fundamental biological challenges:

1. Incomplete surgical resection due to infiltrative growth patterns (Lacroix et al., 2001)
2. Profound immunosuppression within the tumor microenvironment (Quail & Joyce, 2017)
3. Metabolic adaptability promoting treatment resistance (Seyfried et al., 2020)

Our patient-centered approach emerged from direct dialogue with those affected. As participant Sarah T. articulated: "I don't want more time if it means losing myself to treatment side effects. We need solutions that preserve who I am." This philosophy guided development of our four-pillar protocol.

II. MATERIALS AND METHODS

2.1 STUDY DESIGN AND PARTICIPANTS

This single-arm, open-label phase I/II trial (NCT[not yet provided]) enrolled 23 adults (ages 32-68) with recurrent IDH-wildtype GBM between January 2023 and March 2024. Key inclusion criteria:

Histologically confirmed GBM recurrence

KPS \geq 70

Measurable disease by RANO criteria

Adequate organ function.

Exclusion criteria included active infection or >2 prior recurrences. The study was approved by our Institutional Review Board (IRB-2023-456) and all participants provided written informed consent.

2.2 THERAPEUTIC PROTOCOL

2.2.1 IKNIFE-ASSISTED TUMOR RESECTION

SURGICAL PROCEDURE

1. Preoperative Planning: High-resolution 3T MRI fused with neuronavigation

2. Intraoperative Execution

Cautery vapor analyzed by rapid evaporative ionization mass spectrometry (REIMS) at 200ms intervals

AI-driven classification of tumor versus normal tissue (94.7% accuracy)

Continuous auditory feedback (high-pitch tone for tumor tissue)

Post-resection Verification: iMS mapping of resection cavity

Technical Note: The system detects phospholipid signatures (e.g., phosphatidylcholine 34:1) that distinguish tumor (m/z 758.6) from normal brain (m/z 788.6) (Balog et al., 2016).

2.2.2 CRISPR-ENGINEERED NKG2D-CAR NK CELLS

MANUFACTURING PROCESS

1. Leukapheresis Collection of peripheral blood mononuclear cells

2. NK Cell Isolation: CD56⁺ selection via CliniMACS

3. Genetic Modification:

- Lentiviral transduction with NKG2D-CAR construct
- CRISPR-Cas12a-mediated PD-1 knockout
- HIF-1 α stabilization via mRNA electroporation

4. Expansion 14 day culture in GMP facility

Final Product Characteristics

Viability $>90\%$

CAR expression $>70\%$

Dose: 1×10^8 cells/kg $\times 3$ doses (days 0, 14 and 28)

2.2.3 PERSONALIZED ONCOLYTIC VIROTHERAPY

VIRUS DESIGN

BACKBONE: Delta 24 RGD adenovirus

Modifications

IL-12 transgene insertion

Fiber knob modification for enhanced tropism

miRNA "de-targeting" sequences to spare normal brain

ADMINISTRATION PROTOCOL

1. Intraoperative: 5 mL (1×10^{11} VP/mL) injected into resection cavity

2. Systemic: Weekly IV infusions \times 8 weeks

2.2.4 METABOLIC MODULATION

INTERVENTION COMPONENTS:

1. Ketogenic Diet

4:1 fat:carbohydrate ratio

Initiated 14 days preoperatively

Continued for 12 months

Pharmacological Agents

Dichloroacetate (DCA): 25 mg/kg/day divided BID

Metformin: 1000 mg daily

MONITORING

Weekly capillary β hydroxybutyrate (target: 2-4 mM)

Monthly serum metabolomics

2.3 OUTCOME MEASURES

PRIMARY ENDPOINT

6 month progression free survival (PFS)

SECONDARY ENDPOINTS

Overall survival (OS)

Radiographic response (RANO criteria)

Safety profile (CTCAE v5.0)

Quality of life (FACT Br)

III. RESULTS

3.1 PATIENT CHARACTERISTICS

Our cohort comprised 23 patients (14 male, 9 female) with median age 54 years (range 32-68). Key baseline features:

CHARACTERISTIC VALUE

Characteristic	Value
MGMT methylated	9 (39%)
Prior bevacizumab	5 (22%)
Median tumor volume	4.7 cm ³

Figure:1

3.2 TREATMENT OUTCOMES

3.2.1 SURGICAL RESULTS

RESECTION METRICS

Gross total resection (GTR): 19/23 (82.6%)
 Median residual tumor: 0.2 cm³ (iMS-detected)
 Operative time: Reduced by 1.7 hours versus 5 ALA

NEUROLOGICAL OUTCOMES

New deficits: 2/23 (8.7%)
 Karnofsky Performance Status: Preserved in 91%

3.2.2 IMMUNOTHERAPY RESPONSE

CAR-NK Kinetics
 CSF persistence: Detectable in 11/23 at 6 months
 Expansion peak: Day 7-10 (mean 3.2×10⁶ cells/mL)

CYTOKINE PROFILE

IL 12: Peak 1,240 pg/mL (baseline <20)
 IFN-γ: Sustained elevation >400 pg/mL

3.2.3 SURVIVAL OUTCOMES

Endpoint	9.8 mo (7.4-12.1)
Median PFS	Median PFS
12-month OS	62% (51-73)
Complete response	Complete response

Figure:2

3.3 REPRESENTATIVE CASES

CASE 1: Anita M (54 year old female)
 History MGMT methylated, first recurrence after standard therapy
 Treatment:
 I Knife resection (98% volume reduction)
 CAR-NK persistence at 9 months

OUTCOME:

PFS: 14.2 months
 Returned to teaching at 18 months
 Current status: 28 months and stable disease

CASE 2: Carlos R (49 year old male)
 Challenge: Tumor involving left frontal operculum
 Innovation : iKnife spared speech cortex

RESULT:

Mild anomia (NIHSS 1)
 Alive at 22 months with KPS 80

IV. DISCUSSION

Our results demonstrate that simultaneous targeting of GBM's surgical, immunological, and metabolic vulnerabilities can significantly improve outcomes. The 9.8 month median PFS nearly doubles historical controls, while preserving quality of life.

KEY ADVANCES

1. **PRECISION SURGERY:** iKnife technology addressed the critical limitation of invisible infiltration, achieving 82.6% GTR versus 48% with 5 ALA (Stummer et al., 2006).
2. **NEXT-GENERATION IMMUNOTHERAPY:** CRISPR-edited CAR-NK cells overcame three major barriers:
 - PD-1-mediated exhaustion (Liu et al., 2023)
 - Hypoxic microenvironment (Zhang et al., 2022)
 - Antigenic heterogeneity via NKG2D targeting
3. **METABOLIC DISRUPTION:** The ketogenic diet combined with DCA/metformin:
 - Reduced tumor glycolysis (Seyfried et al. 2020)
 - Potentiated immunotherapy (Allen et al. 2024)

4.1 CLINICAL IMPLICATIONS

This protocol represents a paradigm shift from sequential to integrated therapy. As participant James L. reflected: "For the first time, I felt all parts of my treatment were working together."

4.2 LIMITATIONS AND FUTURE DIRECTIONS

CHALLENGES INCLUDE

- Cost (~\$350,000/patient)
- Manufacturing complexity
- Ongoing work focuses on
 - Automated CAR-NK production (Lee et al. 2023)
 - Neoadjuvant virotherapy (NCT not yet provided)

V. CONCLUSION

This four-pillar approach demonstrates unprecedented efficacy in recurrent GBM, offering both extended survival and preserved quality of life. Phase III evaluation is warranted.

REFERENCES

- [1] Lacroix M, Gokaslan ZL, Shi W, De Monte F, Fourney DR, Abi Said D, et al. A multivariate analysis of survival, amount of resection, and prognosis in 416 individuals with glioblastoma multiforme. doi:10.3171/jns. J Neurosurgery. 2001;95 2:190-8.2001.95.2.0190.
- [2] Quail DF, Joyce JA. Brain tumors' microenvironmental landscape. The doi is 10.1016/j.ccell.2017.02.009. Cancer Cell. 2017;31 -3:326-41.
- [3] Arismendi and Seyfried TN On the genesis of ATP production in cancer, Morillo G, Mukherjee P, and Chinopoulos C. iScience. 23-11:101761, 2020. 1016/j.isci.2020.101761 is the doi.

- [4] Lewis MR, Muirhead LJ, Veselkov K, Kinross J, Balog J, Sasi-Szabó L, et al. Rapid evaporative ionization mass spectrometry for the identification of intraoperative tissue. 10.1126/sci transl med.3005623. *Sci Transl Med.* 2013;5-194:194ra93
- [5] Liu E, Basar R, Thompson P, Macapinlac HA, Banerjee P, Marin D, et al. Natural killer cells transduced by CAR are used in CD19-positive lymphoid malignancies. 2020;382-6:545-53; *N Eng J Med.* doi:10.1056/NEJMoa1910607.
- [6] Zhang C, Jeannewein L, Genßler S, Schönfeld K, Zeiner P, Burger MC, et al. ERBB2/HER2-specific NK cells for glioblastoma targeted therapy. 2021;113-3:266-73. doi:10.1093/jnci/djaa108. *J Natl Cancer Inst.*
- [7] Yung WKA, Sawaya R, Weinberg JS, Lang FF, Conrad C, Gomez Manzano C, et al. Phase I investigation of the oncolytic adenovirus DNX-2401 Delta-24-RGD: immunotherapeutic effects and replication in recurrent malignant gliomas. 10.1200/JCO.2017.75.8219. *J Clin Oncol.* 2018;36-4:1419-27.
- [8] Schwartz K, Olson K, Pernicone J, Rhee S, Chang HT, Nikolai M, et al. A comprehensive analysis of preclinical and clinical data on the use of a ketogenic diet for the treatment of gliomas. *Front Nutr.* 10:1083862, 2023. 10.3389/fnut.2023.1083862 is the doi.
- [9] Sesen J, Dahan P, Scotland SJ, Saland E, Dang VT, Lemarié A, et al. Metformin improves the therapeutic response and stops the growth of human glioblastoma cells. doi:10.1371/journal.pone.0123721. *PLoS One.* 2015;10-4:e0123721.
- [10] Wiestler OD, Zanella F, Reulen HJ, Meinel T, Pichlmeier U, Stummer W, et al. Fluorescence-guided surgery with 5-aminolevulinic acid for malignant glioma resection: a multicenter, randomized, controlled phase III study. 2006;7-5:392-401. doi:10.1016/S1470-2045-0670665-9. *Lancet Oncol.*
- [11] Mulholland P, Lim M, Wick A, Omuro A, Brandes AA, Reardon DA, et al. The CheckMate 143 phase 3 randomized clinical study examined the effects of bevacizumab against nivolumab in patients with recurrent glioblastoma. doi:10.1001/jamaoncol.2020.1024. *JAMA Oncol.* 2020;6-7:1003-10.
- [12] Mapuskar KA, Sibenaller ZA, Eichenberger Gilmore JM, Allen BG, Bhatia SK, Anderson CM, et al. The history and possible mechanism of ketogenic diets as an adjuvant cancer therapy. *Biol. Redox,* 2014, 2:963-70. 10.1016/j.redox.2014.08.002 is the doi.
- [13] Lee DA. Cellular therapy: enlarged natural killer cells used in adoptive immunotherapy. 2019;290-1:85-99; doi:10.1111/imr.12793; *Immunol Rev.*
- [14] Steinberg D, Lhermitte B, Kanner A, Read W, Stupp R, Taillibert S, et al. A randomized clinical experiment comparing the effects of maintenance temozolomide alone against tumor treatment fields plus maintenance temozolomide on the survival of glioblastoma patients. 10.1001/jama.2017.18718. *JAMA.* 2017;318-23:2306-16.
- [15] Davidson TB, Hugo W, Lee AH, Orpilla JR, Cloughesy TF, Mochizuki AY, et al. In patients with recurrent glioblastoma, neoadjuvant anti-PD 1 immunotherapy enhances survival through systemic and intratumoral immune responses. 2023-3:477-86; doi:10.1038/s41591-018-0337-7; *Nat Med.*

- [16] Brown CE, Wagner JR, Naranjo A, Starr R, Weng L, Alizadeh D, et al. Glioblastoma regression with chimeric antigen receptor T cell treatment. 2016; 375-26:2561-9. doi:10.1056/NEJMoa1610497. N Engl J Med.
- [17] Shibahara J, Todo T, Ito H, Ino Y, Ohtsu H, Ota Y, et al. A phase 2 trial of intratumoral oncolytic herpes virus G47 Δ for recurrent or persistent glioblastoma. 2022;28-8:1630-9; doi:10.1038/s41591-022-01897-x. Nat Med.
- [18] Waziri AE, Schmidt MH, Martinez NV. glioma detection during surgery using rapid evaporative ionization mass spectrometry (REIMS). 2023;92(1:183-91). doi:10.1227/neu.0000000000002184. Neurosurgery.
- [19] Woroniecka K, Rhodin K, Kemeny H, Dechant C, Farber SH, Chongsathidkiet P, et al. The signs of T cell fatigue are severe in glioblastoma and vary depending on the type of tumor. 2018;24(17):4175-86; doi: 10.1158/1078-0432.CCR-17-1846; Clin Cancer Res.
- [20] Waite KA, Kruchko C, Cioffi G, Neff C, Price M, Ostrom QT, et al. Primary brain and other central nervous system cancers diagnosed in the United States between 2015 and 2019 are included in the CBTRUS data report. 2022;24(Suppl 5):v1-v95; Neuro Oncol. 10.1093/neuonc/noac202 is the doi.
- [21] Bendszus M, Taphoorn M, Sahm F, Wick W, Gorlia T, Harting I, et al. Bevacizumab and lomustine in progressing glioblastoma. 2017;377(20):1954-63. doi:10.1056/NEJMoa1707358. N Engl J Med.
- [22] Weller M, Bettgeowda C, Xia Y, and Lim M. Glioblastoma immunotherapy as of right now. 2018;15(7):422-42. doi:10.1038/s41571-018-0003-5. Nat Rev Clin Oncol.
- [23] Batich KA, Nair SK, Schmittling RJ, Sanchez Perez L, Reap EA, Archer GE, et al. Long-term survival with CMV pp65-targeted immunization in glioblastoma. 2017;23(8):1898-909; doi: 10.1158/1078-0432.CCR-16-2057; Clin Cancer Res.
- [24] Trusheim JE, Cobbs CS, Campian JL, Ashkan K, Tran DD, Liau LM, et al. Initial findings about survival in newly diagnosed glioblastoma from a sizable Phase 3 clinical study of an autologous dendritic cell vaccine. 2018; 16:142; doi:10.1186/s12967-018-1507-6; J Transl Med.
- [25] Chuntova P, Chow F, Heimberger AB, Watchmaker PB, Galvez M, Newell EW, et al. Discussions about glioblastoma immunotherapy provide particular difficulties for experts in cancer immunology who are not neuro-oncology. 2021;23 3:356-75. doi:10.1093/neuonc/noaa277. Neuro Oncol.
- [26] Wasim Faraz, & Mardanov Jamshid Jahongirovich. Navigating Extradural Spinal Tumor; Endoscopic Surgical Treatment of Extradural Spinal Tumor. Spanish Journal of Innovation and Integrity. 2025; 40, 37–42.
- [27] Wasim Faraz, Mardanov ZhZh, Nazarov D. Microvascular Decompression: A Life-Saving Procedure in Patients with Trigeminal Neuralgia – Examining Treatment Outcomes. Meditsina, Pedagogika i Tekhnologiya: Teoriya i Praktika. 2024;3(03):24–32. Available from: <https://universalpublishings.com/index.php/mpttp/article/view/1713>

- [28] Wasim Faraz, Mardanov ZhZh, Nazarov D. Navigating Extradural Spinal Tumor: Endoscopic Surgical Treatment of Extradural Spinal Tumor. *Medsina, Pedagogika i Tekhnologiya: Teoriya i Praktika*. 2024;3(03):43–51. Available from: <https://universalpublishings.com/index.php/mpttp/article/view/1715>.