

Supplementary Materials

Barriers to overcoming immunotherapy resistance in glioblastoma

Julia S. Gillette¹, Elaina J. Wang¹, Richard S. Dowd¹, Steven A. Toms^{1*}

¹Departments of Neurosurgery, The Warren Alpert Medical School of Brown University, Providence, Rhode Island

*** Correspondence:**

Steven A. Toms

steven_toms@brown.edu

1 Supplementary Table 1¹

Mechanisms of Immunotherapy Resistance in Glioblastoma

Mechanisms of Immunotherapy Resistance in Glioblastoma	Example	Reference
<u>Intrinsic (Primary) Resistance</u> Immunosuppression	CNS immune privilege / Blood Brain Barrier	22, 30, 32-41
Inter and intra-tumoral heterogeneity	1) Transcriptional diversity: oncogenic signaling, proliferation, immune signaling, and angiogenesis 2) phenotype changes : histone methylation, transcriptome, RNA methylation, and protein alteration 3) Invasive edge cells that secrete VEGF and IL-8 and over-express HIF-1	42-60
Immune dysfunction	1) T-cell dysfunction 2) tumor-associated macrophages (TAMs), microglia (MG) , and monocyte-derived macrophages (MDMs) populations	1,2,15,18, 61-66
<u>Adaptive (Secondary) Resistance</u> Selection of resistant intratumoral populations and changes in recurrent glioblastoma	1) "tumor microtubes" to accomplish invasion, proliferation, and repopulation after treatment 2) plasticity of glioblastoma stem-like cells (GSCs) 3) treatment-resistant state via IL13R α 2 antigen loss GBM variants, up-regulation of IDO1, PD-L1, and IL-10, plasticity and antigen drift	12, 67-82
Effect of steroids on immune response	High-dose steroid therapy (dexamethasone): CCR7 expression loss in T cells, reduced OS in TTF-treated GBM patients, decreases the efficacy of viral oncolytic immunotherapy, CAN-2409	26, 83-89

2 Supplementary Table 2^{1*}

Ongoing Immunotherapy Clinical Trials for Glioblastoma

Data Extracted from *Home - ClinicalTrials.gov. (n.d.). Retrieved April 3, 2023, from <https://www.clinicaltrials.gov/>

Ongoing Immunotherapy Clinical Trials	Intervention/ Treatment	Trial Title	Phase	Clinical trial Identifier / Reference
Ongoing Checkpoint Inhibitor Clinical Trials	Drug: Pembrolizumab	Pembrolizumab (MK-3475) in Patients With Recurrent Malignant Glioma With a Hypermutator Phenotype	Not Applicable	NCT02658279
	Biological: Ipilimumab Procedure: MRI Biological: Nivolumab	A Study Testing the Effect of Immunotherapy (Ipilimumab and Nivolumab) in Patients With Recurrent Glioma With Elevated Mutational Burden	Phase 2	NCT04145115
	Drug: Epcadostat Drug: Bevacizumab, Radiation: Radiation therapy Procedure: Peripheral blood draw Drug: Retifanlimab	Retifanlimab and Epcadostat in Combination With Radiation and Bevacizumab in Patients With Recurrent Gliomas	Phase 2	NCT03532295
	Drug: Pembrolizumab Drug: Bevacizumab, Radiation: Re-irradiation	Pembrolizumab and Reirradiation in Bevacizumab Naïve and Bevacizumab Resistant Recurrent Glioblastoma	Phase 2	NCT03661723
	Biological: MK-3475 Device: MRI-guided laser ablation Procedure: Surgical resection/debulking, biopsy, blood draw for research purposes, cervical lymph node fine needle aspiration	MK-3475 in Combination With MRI-guided Laser Ablation in Recurrent Malignant Gliomas	Phase 1/ Phase 2	NCT02311582
	Drug: Pembrolizumab at 7 days prior Drug: Pembrolizumab at 14 days post Drug: Pembrolizumab at 35 days post Procedure: Laser Interstitial Thermotherapy	Laser Interstitial Thermotherapy (LITT) Combined With Checkpoint Inhibitor for Recurrent GBM (RGBM)	Phase 1/ Phase 2	NCT03277638
Ongoing (CAR) T-Cell Therapy Clinical Trials	Drug: B7-H3 CAR-T Drug: Temozolomide Molecular Target: B7-H3	Pilot Study of B7-H3 CAR-T in Treating Patients With Recurrent and Refractory Glioblastoma	Phase 1	NCT04385173
	Drug: Temozolomide, Biological: B7-H3 CAR-T Molecular Target: B7-H3	B7-H3 CAR-T for Recurrent or Refractory Glioblastoma	Phase 1/ Phase 2	NCT04077866
	Biological: CD147-CART Molecular Target: CD147	CD147-CART Cells in Patients With Recurrent Malignant Glioma.	Early Phase 1	NCT04045847

	Genetic: (C7R)-GD2.CAR T cells Molecular Target: GD2	C7R-GD2.CAR T Cells for Patients With GD2-expressing Brain Tumors (GAIL-B)	Phase 1	NCT04099797
	Biological: CART-EGFRvIII T cells Biological: Pembrolizumab Molecular Target: EGFRvIII	CART-EGFRvIII + Pembrolizumab in GBM	Phase 1	NCT03726515
	Biological: EGFRvIII-CARs Molecular Target: EGFRvIII	Intracerebral EGFR-vIII CAR-T Cells for Recurrent GBM (INTERCEPT)	Phase 1	NCT03283631
	Biological: IL13Ralpha2-specific Hinge-optimized 4-1BB-co-stimulatory CAR/Truncated CD19-expressing Autologous TN/MEM Cells Biological: IL13Ralpha2-specific Hinge-optimized 4-1BB-co-stimulatory CAR Truncated CD19-expressing Autologous T-Lymphocytes Other: Laboratory Biomarker Analysis Procedure: MRI Procedure: Magnetic Resonance Spectroscopic Imaging Other: Quality-of-Life Assessment Molecular Target: IL13Ra2	Genetically Modified T-cells in Treating Patients With Recurrent or Refractory Malignant Glioma	Phase 1	NCT02208362
	Biological: IL13Ralpha2-specific Hinge-optimized 4-1BB-co-stimulatory CAR/Truncated CD19-expressing Autologous TN/MEM Cells Biological: Ipilimumab, Biological: Nivolumab Molecular Target: IL13Ra2	IL13Ra2-CAR T Cells With or Without Nivolumab and Ipilimumab in Treating Patients With GBM	Phase 1	NCT04003649
	Biological: Chlorotoxin (EQ)-CD28-CD3zeta-CD19t-expressing CAR T-lymphocytes (ICT delivery) Biological: Chlorotoxin (EQ)-CD28-CD3zeta-CD19t-expressing CAR T-lymphocytes (via ICT/ICV dual delivery) Molecular Target: MMP2 (Chlorotoxin)	Chimeric Antigen Receptor (CAR) T Cells With a Chlorotoxin Tumor-Targeting Domain for the Treatment of MMP2+ Recurrent or Progressive Glioblastoma	Phase 1	NCT04214392
	Biological: chimeric antigen receptor T cells	Personalized Chimeric Antigen Receptor T Cell Immunotherapy for Patients With Recurrent Malignant Gliomas	Phase 1	NCT03423992

Ongoing Vaccination Clinical Trials	ADU-623	Phase I Study of Safety and Immunogenicity of ADU-623, a Live-attenuated <i>Listeria Monocytogenes</i> Strain (Δ actA/ Δ inlB) Expressing the EGFRvIII-NY-ESO-1 Vaccine, in Patients With Treated and Recurrent WHO Grade III/IV Astrocytomas	Phase 1	NCT01967758
	Biological: HSPPC-96 Drug: Temozolomide Procedure: Standard Surgical Resection	PHASE 2, Multi-center, Single Arm Investigation of HSPPC-96 Vaccine With Temozolomide in Patients With Newly Diagnosed Glioblastoma Multiforme	Phase 2	NCT00905060
	HSPPC-96, Biological: gp96	Research for Immunotherapy of Glioblastoma With Autologous Heat Shock Protein gp96	Phase 1	NCT02122822
	Biological: HSPPC-96 Drug: bevacizumab	A Phase II Randomized Trial Comparing the Efficacy of Heat Shock Protein-Peptide Complex-96 (HSPPC-96) (NSC #725085, ALLIANCE IND # 15380) Vaccine Given With Bevacizumab Versus Bevacizumab Alone in the Treatment of Surgically Resectable Recurrent Glioblastoma Multiforme (GBM)	Phase 2	NCT01814813
	Drug: Pembrolizumab Biological: HSPPC-96 Drug: Temozolomide Other: Placebo	A Randomized, Double Blind Phase II Trial of Surgery, Radiation Therapy Plus Temozolomide and Pembrolizumab With and Without HSPPC-96 in Newly Diagnosed Glioblastoma (GBM)	Phase 2	NCT03018288
	Drug: IDH1 peptide vaccine	Targeting IDH1R132H in WHO Grade III-IV IDH1R132H-mutated Gliomas by a Peptide Vaccine - a Phase I Safety, Tolerability and Immunogenicity Multicenter Trial (NOA-16)	Phase 1	NCT02454634
	Biological: K27M peptide Drug: Nivolumab	H3.3K27M Specific Peptide Vaccine Combined With Poly-ICLC With and Without PD-1 Inhibition Using Nivolumab for the Treatment of Newly Diagnosed HLA-A2 (02:01)+ H3.3K27M Positive Diffuse Intrinsic Pontine Glioma (DIPG) and Newly Diagnosed HLA-A2 (02:01)+ H3.3K27M Positive Gliomas	Phase 1/ Phase 2	NCT02960230
	Other: Laboratory Biomarker Analysis Drug: Montanide ISA 51 VG Biological: Sargramostim Biological: SVN53-67/M57-KLH Peptide Vaccine Drug: Temozolomide	A Phase II Study of the Safety and Efficacy of SVN53-67/M57-KLH (SurVaxM) in Survivin-Positive Newly Diagnosed Glioblastoma	Phase 2	NCT02455557
	DCs vaccine (DCVax)	A Phase III Clinical Trial Evaluating DCVax®-L, Autologous Dendritic Cells Pulsed With Tumor Lysate Antigen For The Treatment Of Glioblastoma Multiforme (GBM)	Phase 3	NCT00045968

	Biological: BTSC mRNA-loaded DCs	Recurrent GBM Stem Cell Tumor Amplified RNA Immunotherapy Trial	Phase 1	NCT00890032
	Biological: Dendritic Cells Drug: Imiquimod	Phase I Study of Vaccination With Dendritic Cells Loaded With Brain Tumor Stem Cells for Recurrent or Progressive Malignant Gliomas	Phase 1	NCT01171469
	Biological: DEC-205/NY-ESO-1 Fusion Protein CDX-1401 Other: Laboratory Biomarker Analysis Other: Pharmacological Study Drug: Sirolimus	A Phase I Clinical Trial of mTOR Inhibition With Rapamycin for Enhancing Intranodal Dendritic Cell Vaccine Induced Anti-tumor Immunity in Patients With NY-ESO-1 Expressing Solid Tumors	Phase 1	NCT01522820
	Biological: tetanus toxoid Biological: therapeutic autologous dendritic cells Biological: therapeutic autologous lymphocytes	Anti-Tumor Immunotherapy Targeted Against Cytomegalovirus in Patients With Newly-Diagnosed Glioblastoma Multiforme During Recovery From Therapeutic Temozolomide-induced Lymphopenia	Phase 1	NCT00639639
	Biological: pp65-shLAMP DC with GM-CSF Biological: unpulsed PBMC and saline Drug: Td Drug: Saline Biological: pp65-fILAMP DC with GM-CSF	A Phase II Randomized, Blinded, and Placebo-controlled Trial of CMV RNA-Pulsed Dendritic Cells With Tetanus-Diphtheria Toxoid Vaccine in Patients With Newly-Diagnosed Glioblastoma	Phase 2	NCT02465268
	Biological: Unpulsed DCs Biological: Td Biological: Human CMV pp65-LAMP mRNA-pulsed autologous DCs Biological: 111In-labeled DCs Drug: Temozolomide Drug: Saline Drug: Basiliximab	Evaluation of Overcoming Limited Migration and Enhancing Cytomegalovirus-specific Dendritic Cell Vaccines With Adjuvant TEtanus Pre-conditioning in Patients With Newly-diagnosed Glioblastoma	Phase 2	NCT02366728
	Biological: autologous tumor lysate-pulsed DC vaccination Biological: Tumor lysate-pulsed DC vaccination+0.2% resiquimod Biological: Tumor-lysate pulsed DC vaccination +adjuvant polyICLC	A Phase II Clinical Trial Evaluating Autologous Dendritic Cells Pulsed With Tumor Lysate Antigen +/- Toll-like Receptor Agonists for the Treatment of Malignant Glioma	Phase 2	NCT01204684
	Biological: RNA-loaded dendritic cell vaccine Drug: basiliximab	REGULATory T-Cell Inhibition With Basiliximab (Simulect®) During Recovery From Therapeutic Temozolomide-induced Lymphopenia During Antitumor Immunotherapy Targeted Against Cytomegalovirus in Patients With Newly-Diagnosed Glioblastoma Multiforme	Phase 1	NCT00626483

	Other: Laboratory Biomarker Analysis Biological: Malignant Glioma Tumor Lysate-Pulsed Autologous Dendritic Cell Vaccine Drug: Temozolomide	Pilot Clinical Trial of Allogeneic Tumor Lysate-Pulsed Autologous Dendritic Cell Vaccination in Newly Diagnosed Glioblastoma	Phase 1	NCT01957956
	Biological: ICT-121 DC vaccine	Immunological Targeting of CD-133 in Recurrent Glioblastoma: A Multi-center Phase I Translational and Clinical Study of an Autologous CD-133 DC Vaccine	Phase 1	NCT02049489
	Biological: Dendritic Cell Vaccine Biological: Tumor Lysate Drug: Imiquimod, Procedure: Leukapheresis	Dendritic Cell Vaccine For Malignant Glioma and Glioblastoma Multiforme in Adult and Pediatric Subjects	Phase 1	NCT01808820
	Biological: Dendritic cell vaccination + temozolomide chemotherapy + involved field radiation therapy Biological: Dendritic cell vaccination, + optional bevacizumab treatment	Phase I Trial of Vaccination With Autologous Dendritic Cells Pulsed With Lysate Derived From an Allogeneic Glioblastoma Stem-like Cell Line for Patients With Newly Diagnosed or Recurrent Glioblastoma	Phase 1	NCT02010606
	Biological: SL-701; poly-ICLC (polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethyl cellulose) Drug: Bevacizumab	A Phase 1/2 Study of SL-701, a Subcutaneously Injected Multivalent Glioma-Associated Antigen Vaccine, in Adult Patients With Recurrent Glioblastoma Multiforme (GBM)	Phase 1/ Phase 2	NCT02078648
	Biological: IMA 950 Biological: Poly ICLC Other: Immunomonitoring	Phase I/II Study of Intradermal IMA950 Peptide-based Vaccine Adjuvanted With Intra Muscular Poly-ICLC in Combination With Temozolomide in Newly Diagnosed HLA-A2 Glioblastoma Patients	Phase 1/ Phase 2	NCT01920191
	Biological: glioblastoma multiforme multi-peptide vaccine IMA950 Biological: sargramostim, Drug: temozolomide Other: laboratory biomarker analysis Other: pharmacological study Procedure: adjuvant therapy Radiation: radiation therapy	A Cancer Research UK Phase I Trial of IMA950 (A Novel Multi-Peptide Vaccine) Plus GM-CSF in Patients With Newly Diagnosed Glioblastoma	Phase I	NCT01222221
	Drug: Bevacizumab Drug: Rindopepimut (CDX-110) with GM-CSF Drug: KLH	A Phase II Study of Rindopepimut/GM-CSF in Patients With Relapsed EGFRvIII-Positive Glioblastoma	Phase 2	NCT01498328
	Drug: CDX-110 with GM-CSF Drug: Temozolomide	A Phase II Study of CDX-110 With Radiation and Temozolomide in Patients With Newly Diagnosed Glioblastoma Multiforme	Phase 2	NCT00458601

	Drug: Rindopepimut (CDX-110) with GM-CSF Drug: Temozolomide Drug: KLH	An International, Randomized, Double-Blind, Controlled Study of Rindopepimut/GM-CSF With Adjuvant Temozolomide in Patients With Newly Diagnosed, Surgically Resected, EGFRvIII-positive Glioblastoma	Phase 3	NCT01480479
	Biological: PEP-3 vaccine Biological: sargramostim Drug: Temozolomide	A Complementary Trial of an Immunotherapy Vaccine Against Tumor-Specific EGFRvIII	Phase 2	NCT00643097
	Biological: Dendritic vaccine pulsed with multiple peptides Biological: First booster vaccine phase Biological: Second booster vaccine phase	A Phase I/II Evaluation of Vaccination With Type 1 Dendritic Cells Pulsed With Multiple Peptides in the Treatment of HLA-A2 Positive Patients With Recurrent Malignant Gliomas	Phase 1/ Phase 2	NCT00766753
	Biological: SL-701; poly-ICLC (polyinosinic-polycytidylic acid stabilized with polylysine and carboxymethyl cellulose) Drug: Bevacizumab	A Phase 1/2 Study of SL-701, a Subcutaneously Injected Multivalent Glioma-Associated Antigen Vaccine, in Adult Patients With Recurrent Glioblastoma Multiforme (GBM)	Phase 1/ Phase 2	NCT00293423
Ongoing Oncolytic Virotherapy Clinical Trials	Drug: Biological G207	Phase II Clinical Trial of HSV G207 With a Single 5 Gy Radiation Dose in Children With Recurrent High-Grade Glioma	Phase 2	NCT04482933
	Biological: DNX-2401, Biological: pembrolizumab	Combination Adenovirus + Pembrolizumab to Trigger Immune Virus Effects (CAPTIVE)	Phase 2	NCT02798406
	Biological: PVSRIPO	A Multicenter Phase 2 Study of Oncolytic Polio/Rhinovirus Recombinant (PVSRIPO) in Recurrent WHO Grade IV Malignant Glioma Patients	Phase 2	NCT02986178
	Biological: lerapolturev, Biological: pembrolizumab	A Phase 2, Open-label, Single-arm Study Evaluating the Efficacy, Safety and Tolerability of Lerapolturev (PVSRIPO) and the Immune Checkpoint Inhibitor Pembrolizumab in the Treatment of Patients With Recurrent Glioblastoma	Phase 2	NCT04479241
	Drug: DNX-2401, Procedure: Tumor Removal	Phase I Trial of Conditionally Replication-Competent Adenovirus (DNX-2401, Formerly Known as Delta-24-RGD-4C) for Recurrent Malignant Gliomas	Phase 1	NCT00805376
	Biological: delta-24-RGD adenovirus	A Phase I/II Trial of a Conditionally Replication-competent Adenovirus (Delta-24-rgd) Administered by Convection Enhanced Delivery in Patients With Recurrent Glioblastoma	Phase 1/ Phase 2	NCT01582516
	Procedure: DNX2401 and Temozolomide	Phase I Trial of Combination of DNX-2401 (Formerly Named Delta-24-RGD) Oncolytic Adenovirus With a Short Course of Temozolomide for Treatment of Glioblastoma at First Recurrent	Phase 1	NCT01956734

	Drug: Single intratumoral injection of DNX-2401 Drug: Interferon-gamma	A Phase 1b, Randomized, Multi-center, Open-label Study of a Conditionally Replicative Adenovirus (DNX-2401) and Interferon Gamma (IFN- γ) for Recurrent Glioblastoma or Gliosarcoma (TARGET-I)	Phase 1	NCT02197169
	Drug: DNX-2440 injection	Phase I Trial of DNX-2440 Oncolytic Adenovirus in Patients With Recurrent Glioblastoma	Phase 1	NCT03714334
	Biological: Oncolytic Adenovirus Ad5-DNX-2401, Procedure: Therapeutic Conventional Surgery	Phase I Clinical Trial of Allogeneic Bone Marrow Human Mesenchymal Stem Cells Loaded With A Tumor Selective Oncolytic Adenovirus, DNX-2401, Administered Via Intra-Arterial Injection in Patients With Recurrent High-Grade Glioma	Phase 1	NCT03896568
	Drug: G207, an oncolytic virus	An Open-Label Phase Ib/II Study of the Safety, Tolerability and Efficacy of G207, a Genetically Engineered Herpes Simplex Type-1 Virus, Administered Intracerebrally to Patients With Recurrent Malignant Glioma	Phase 1/ Phase 2	NCT00036699
	Drug: G207	A Staged Phase 1 Study of the Treatment of Malignant Glioma With G207, a Genetically Engineered HSV-1, Followed by Radiation Therapy	Phase 1	NCT00157703
	Biological: M032 (NSC 733972)	A Phase 1 Study of M032 (NSC 733972), a Genetically Engineered HSV-1 Expressing IL-12, in Patients With Recurrent/Progressive Glioblastoma Multiforme, Anaplastic Astrocytoma, or Gliosarcoma	Phase 1	NCT02062827
	Biological: G207	Phase I Clinical Trial of HSV G207 Alone or With a Single Radiation Dose in Children With Recurrent Supratentorial Brain Tumors	Phase 1	NCT02457845
	Drug: rQNestin, Drug: Cyclophosphamide, Procedure: Stereotactic biopsy	A Phase I Study of the Treatment of Recurrent Malignant Glioma With rQNestin34.5v.2, a Genetically Engineered HSV-1 Virus, and Immunomodulation With Cyclophosphamide	Phase 1	NCT03152318
	Biological: G207	Phase 1 Trial of Engineered HSV G207 in Children With Recurrent or Refractory Cerebellar Brain Tumors	Phase 1	NCT03911388
	Biological: C134	A Phase I Trial of IRS-1 HSV C134 Administered Intratumorally in Patients With Recurrent Malignant Glioma	Phase 1	NCT03657576
	Biological: Carcinoembryonic Antigen-Expressing Measles Virus, Other: Laboratory Biomarker Analysis, Procedure: Therapeutic Conventional Surgery	Phase I Trial of a Measles Virus Derivative Producing CEA (MV-CEA) in Patients With Recurrent Glioblastoma Multiforme (GBM)	Phase 1	NCT00390299

	Biological: Recombinant nonpathogenic polio-rhinovirus chimera (PVSRIPO)	Dose-finding and Safety Study of an Oncolytic Polio/Rhinovirus Recombinant Against Recurrent WHO Grade IV Malignant Glioma	Phase 1	NCT01491893
	Biological: Polio/Rhinovirus Recombinant (PVSRIPO)	Phase Ib Study of Oncolytic Polio/Rhinovirus Recombinant Against Recurrent Malignant Glioma in Children	Phase 1	NCT03043391
	Drug: H-1PV	Phase I/IIa Study of Intratumoral/Intracerebral or Intravenous/Intracerebral Administration of Parvovirus H-1 (ParvOryx) in Patients With Progressive Primary or Recurrent Glioblastoma Multiforme.	Phase 1/ Phase 2	NCT01301430
	Biological: REOLYSIN®	A Phase I/II Clinical Trial to Evaluate Dose Limiting Toxicity and Efficacy of Intralesional Administration of REOLYSIN® for the Treatment of Patients With Histologically Confirmed Recurrent Malignant Gliomas	Phase 1	NCT00528684
	Other: Laboratory Biomarker Analysis Biological: Sargramostim, Biological: Wild-type Reovirus	Phase 1 Study of Replication Competent Reovirus (Reolysin®) in Combination With GM-CSF in Pediatric Patients With Relapsed or Refractory Brain Tumors	Phase 1	NCT02444546
	Drug: Combination of TG6002 and 5-flucytosine (5-FC, Ancotil®)	Safety and Efficacy of the ONCOlytic VIRus Armed for Local Chemotherapy, TG6002/5-FC, in Recurrent Glioblastoma Patients	Phase 1/ Phase 2	NCT03294486

References

1. U.S. National Library of Medicine. **ClinicalTrials.gov background**. U.S. National Library of Medicine, Bethesda, MD (2014). Available at <https://clinicaltrials.gov/ct2/about-site/background> . Accessed April 20th, 2023