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# Targeted opening of the blood–brain barrier facilitates doxorubicin/anti-PD-1-based chemoimmunotherapy of glioblastoma

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**ABSTRACT**

Doxorubicin is a prototypical inducer of immunogenic cell death (ICD) that sensitizes to subsequent immunotherapy by PD-1 blockade. However, this systemic drug combination fails against glioblastoma, hidden behind the blood–brain barrier (BBB). A recent work delineates a biophysical method for BBB permeabilization that yields effective preclinical effects of chemoimmunotherapy.

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Immunotherapy has achieved remarkable success across multiple cancers.[1] However, resistance remains frequent. Combining immunotherapy with agents that promote antitumor immune cell infiltration is a strategy to overcome resistance. Chemotherapeutic drugs inducing immunogenic cell death (ICD), notably anthracyclines like doxorubicin (DOX), are among these agents.[2] In preclinical studies, ICD-inducing interventions enhanced sensitivity to immune checkpoint inhibitors (ICIs), such as programmed cell death 1 (PDCD1, best known as PD-1)-blocking antibodies (αPD1).[3]

Glioblastoma (GBM) is the most aggressive brain cancer. Immunotherapy for GBM is challenging due to the blood–brain barrier (BBB) and the immune-privileged status of the brain.[4,5] These factors hinder drug delivery and immune cell trafficking, reducing treatment efficacy. Previous attempts to treat GBM with αPD1, either alone or with standard chemotherapy, or with liposome-embedded DOX plus an anti-angiogenic drug, failed in clinical trials.[6–8]

A recent study by Arrieta et al. published in *Nature Communications*, exploited a novel strategy to transiently open the BBB utilizing low-intensity pulsed ultrasound (LIPU) combined with intravenously-administered microbubbles (MB). This technique aimed to enhance the delivery of therapeutic agents, specifically liposomal DOX, and αPD1, into the brain (Figure 1).[9]

Four GBM patients received DOX/αPD1 treatment before surgery. DOX concentrations appeared twice higher in sonicated regions of resected tissues than non-sonicated areas. Consistently, a 4-fold increase in DOX concentrations was detected in brain tissues of naïve mice following LIPU/MB compared to controls. This demonstrated LIPU/MB's ability to enhance drug penetration into the brain.[9]

In clinical samples, enhanced DOX delivery led to higher expression of class-I and II antigen-presenting major histocompatibility complex (MHC) molecules in tumor cells. In contrast, temozolomide, a standard GBM treatment, did not stimulate MHC expression. The DOX treatment also facilitated recognition of murine glioma cells by $CD8^+$ T cells, stimulating their activation and expansion, indicating the immunogenicity of DOX-accumulated GBM tissues.[9]

In an intracranial murine GBM model, microglia and monocyte-derived macrophages produced more interferon-gamma (IFNG) following high-dose DOX treatment. This immunomodulatory effect included upregulation of surface MHC-I and CD274 (best known as programmed cell death 1 ligand 1, PD-L1), both IFNG-inducible genes. In GBM patients, microglial cells positive for IFNG and MHC-I were more abundant in post-treatment samples compared to pre-treatment tissues. Thus, LIPU/MB-mediated DOX delivery modulated the phenotype of myeloid cells constitutive of GBM microenvironment.[9]

While LIPU/MB facilitated αPD1 brain penetration in mice, it was ineffective alone to treat GBM. In patient tissues, αPD1 was more concentrated in sonicated peritumoral areas. In the CT2A mouse model of intracranial GBM, combining αPD1 with liposomal DOX was more effective than either therapy alone, achieving 40% long-term survival. LIPU/MB further improved survival, reaching an 80% cure rate.[9]

Mice cured of GBM were protected against tumor rechallenge, indicating immune memory establishment. Depleting microglia and bone marrow-derived macrophages impeded

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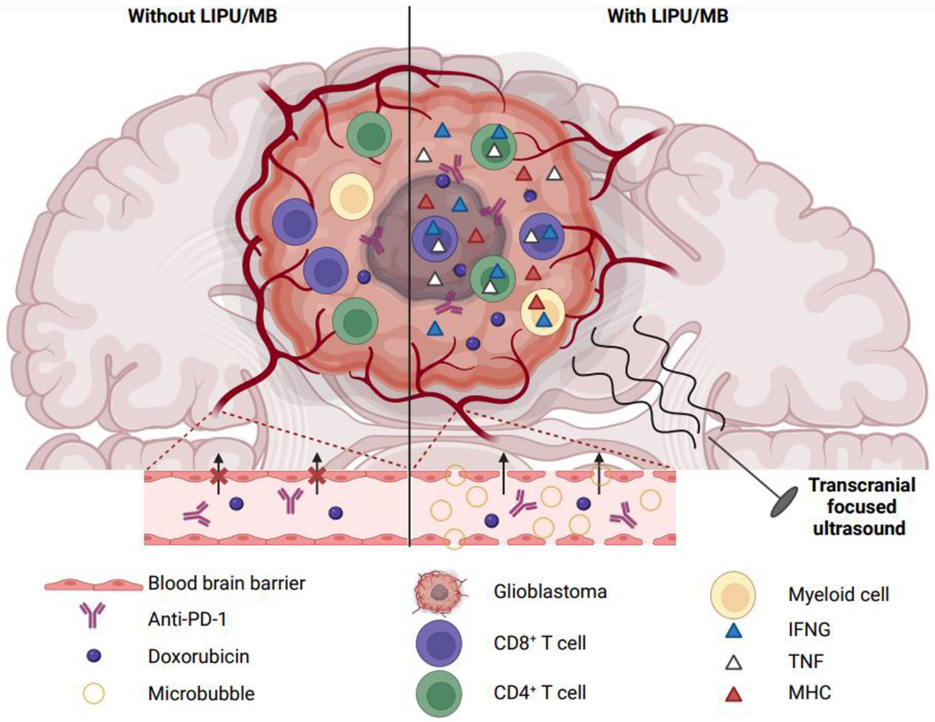


**Figure 1.** Ultrasounds combined with administration of microbubbles facilitate the delivery of doxorubicin/anti-PD-1 to glioblastoma and improve therapeutic efficacy. LIPU/MB transiently opens the blood-brain barrier, facilitating the access of liposomal doxorubicin and anti-PD-1 to glioblastoma. Locally, concentration of the dual therapeutic agents stimulates IFNG production by cerebral myeloid cells and upregulation of MHC molecules by surrounding cells like malignant cells. This pro-inflammatory environment enhances the recognition of cancer cells by T lymphocytes. These latter show polyfunctionality, secreting both IFNG and TNF, improved antitumor activity, and persist in treated mice surviving the disease. IFNG, interferon-gamma; LIPU, low-intensity pulsed ultrasound; MB, microbubble; MHC, major histocompatibility complex; PD-1, programmed cell death 1; TNF, tumor-necrosis factor-alpha.

the antitumor activity of LIPU/MB-delivered liposomal DOX plus αPD1 and abrogated protection against tumor recurrence, supporting previously reported memory by cerebral myeloid cells.[9,10]

Liposomal DOX promoted accumulation of T cells co-producing IFNG and tumor-necrosis factor-alpha (TNF) in the brain of mice surviving orthotopic GBM. Sonication expanded polyfunctional $CD4^+$ T cells without affecting $CD8^+$ subsets. Depleting $CD8^+$ T cells abrogated the therapeutic effect, highlighting their critical contribution to antitumor immunity induced by LIPU/MB-mediated liposomal DOX/αPD1 delivery. Correspondingly, GBM patients treated with this strategy exhibited higher IFNG production by tumor-infiltrating T lymphocytes than subjects without neoadjuvant treatment.

These findings by Arrieta and colleagues suggest that LIPU/MB-mediated drug delivery systems could significantly improve GBM treatment outcomes and potentially extend to other intracranial cancers limited by the BBB and local immunosuppression. Continued research and clinical trials will be essential to optimize this procedure for becoming the standard-of-care in managing GBM and other challenging cancers.

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## Disclosure statement

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