

Enhancing Treatment of Diffuse Intrinsic Pontine Glioma: Synergistic Effect of Focused Ultrasound and Anti-CD47 Immunotherapy

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INTRODUCTION

Diffuse Intrinsic Pontine Glioma (DIPG), a fatal pediatric brain cancer with few treatment options,¹ faces drug delivery challenges due to the blood-brain barrier (BBB).² Focused ultrasound (FUS) is a novel method to open the BBB, improving therapeutic agent penetration.³ Anti-CD47 (aCD47) immunotherapy has shown promising results in various solid cancers by promoting phagocytosis of cancer cells by the immune system.⁴ This study investigates combining FUS BBB opening with systemic aCD47 administration to improve DIPG treatment efficacy.

METHODS

The aCD47 antibody was conjugated to deferoxamine (DFO), and subsequently radiolabeled with zirconium-89 (⁸⁹Zr) for imaging purposes. We utilized instant thin-layer chromatography (iTLC), high-performance liquid chromatography (HPLC) combined with a radioflowmonitor, mass spectrometry, and surface plasmon resonance (SPR) to validate the conjugation, radiochemical purity, and binding affinity of our construct. In vitro studies were conducted using luciferase-positive DIPG cells co-cultured with mouse bone marrow-derived macrophages to assess the therapeutic impact of CD47 blockade. In vivo, a DIPG mouse model was created using a previously established method,⁵ with groups assigned to various treatments: IV aCD47 antibody, FUS alone, and a combination of FUS and IV aCD47 antibody, with the initial two animals treated in this group. ⁸⁹Zr-aCD47 delivery to the brain was assessed with positron-emission tomography (PET) and a biodistribution study. Outcome measures included bioluminescence imaging (BLI), magnetic resonance imaging (MRI), and overall animal survival.

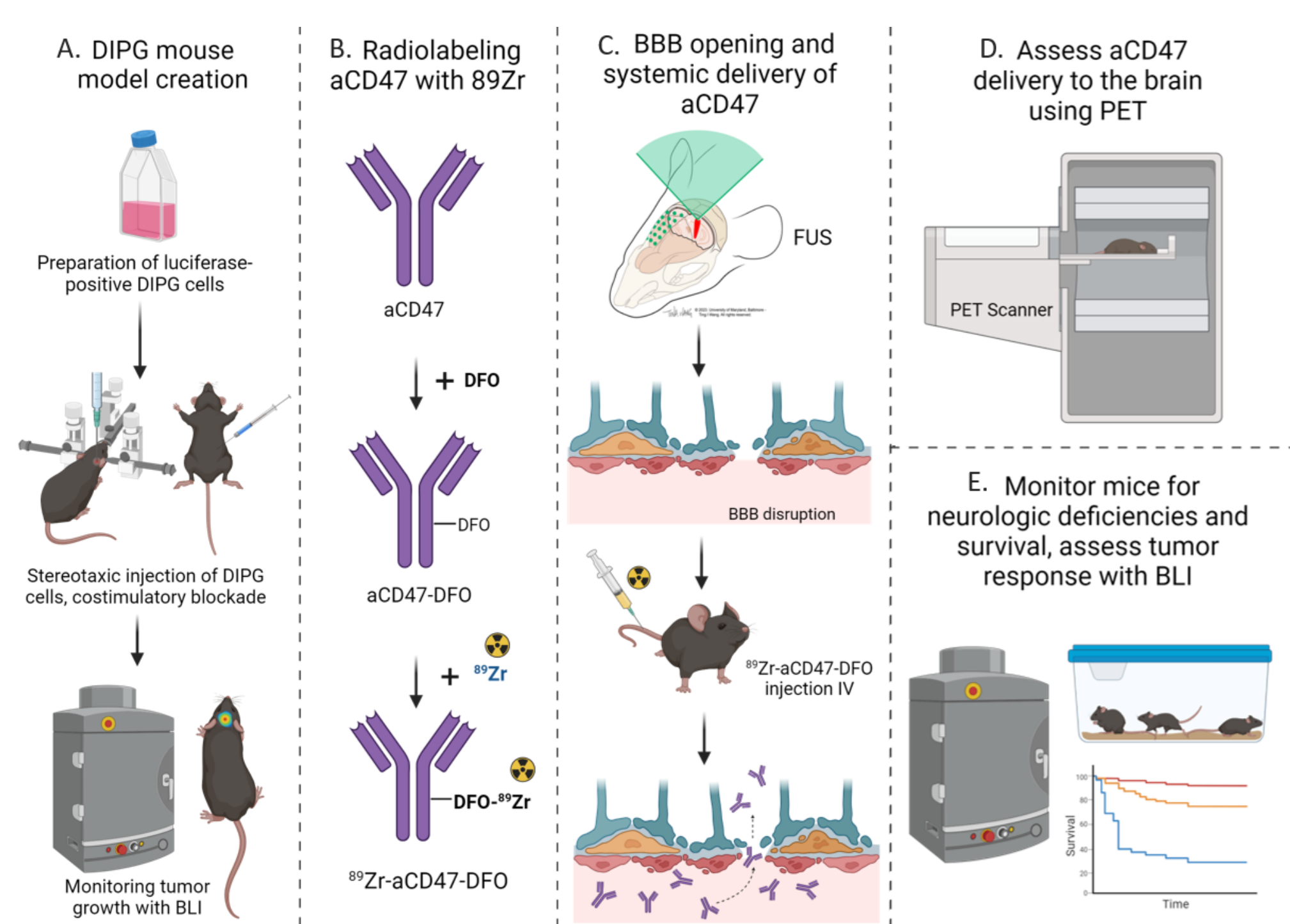


Figure 1. Overview of the study. (A) We produced DIPG mouse models by intracerebral injection of luciferase-positive DIPG cells and confirmed tumor growth with BLI. (B) We radiolabeled our aCD47 antibody to be able to track it using PET imaging. (C) DIPG tumor-bearing mice underwent FUS treatment followed by injection of radiolabeled aCD47 antibody. (D) We used PET imaging to assess the delivery of the antibody to the brain. (E) We followed the mice with BLI studies to assess tumor response and monitored them for neuro deficiencies and survival.

RESULTS

Mass spectrometry revealed the binding of up to four DFO molecules per antibody (Fig. 2A, 2B), and SPR confirmed unaffected affinity to CD47 (Fig. 2C, 2D). ITLC revealed 97.4% radiochemical purity (Fig. 3A) and HPLC-radioflowmonitor showed specific binding of ⁸⁹Zr to the antibody (Fig. 3B, 3C). In vitro, CD47 blockade activated macrophages, reducing bioluminescence of DIPG cells (Fig. 4). PET imaging after FUS and systemic delivery of radiolabeled aCD47 showed successful accumulation of the antibody in the targeted brain area (Fig. 5). The mice treated with IV aCD47 antibody alone showed no response to treatment, and no change in survival was observed (Fig. 6A, 6B). Similarly, the mice treated with FUS alone showed no response to treatment (Fig. 6C, 6D). Mice treated with both FUS and IV aCD47 showed BLI (Fig. 6E, 6F) and MRI (Fig. 7) findings suggestive of tumor eradication. These mice were followed for three months after the treatment and no tumor recurrence or neurologic deficits were observed.

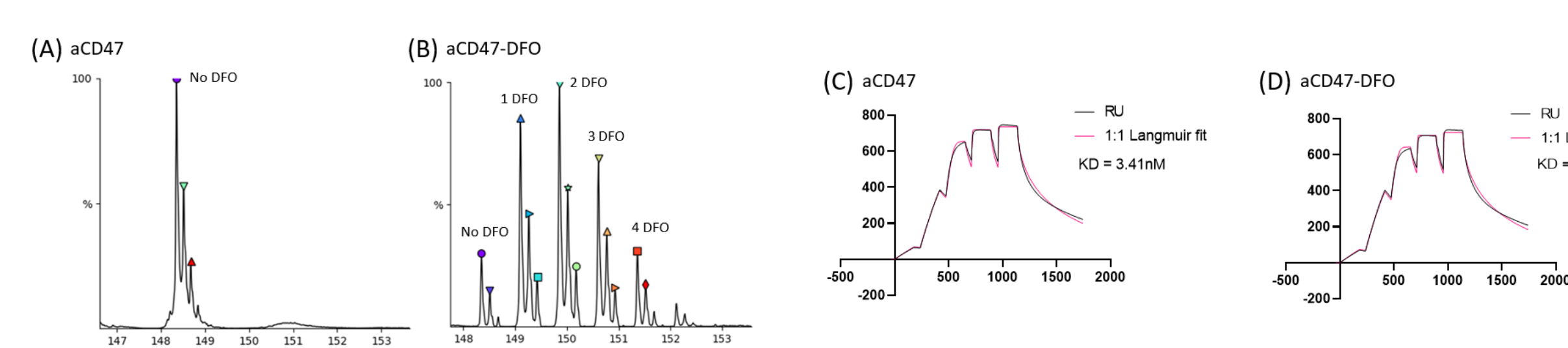


Figure 2. Mass spectrometry and SPR analysis. (A) aCD47 antibody without DFO attachment showing a single peak at ~148.3kDa with its glycoforms. (B) DFO-tagged aCD47 showing up to 4 DFO attachments per antibody molecule. (C) Unchanged aCD47 binding to CD47 with a KD of 3.41. (D) DFO-tagged aCD47 binding to CD47 reveals similar KD of 3.43, signifying no changes in antibody binding following DFO attachment.

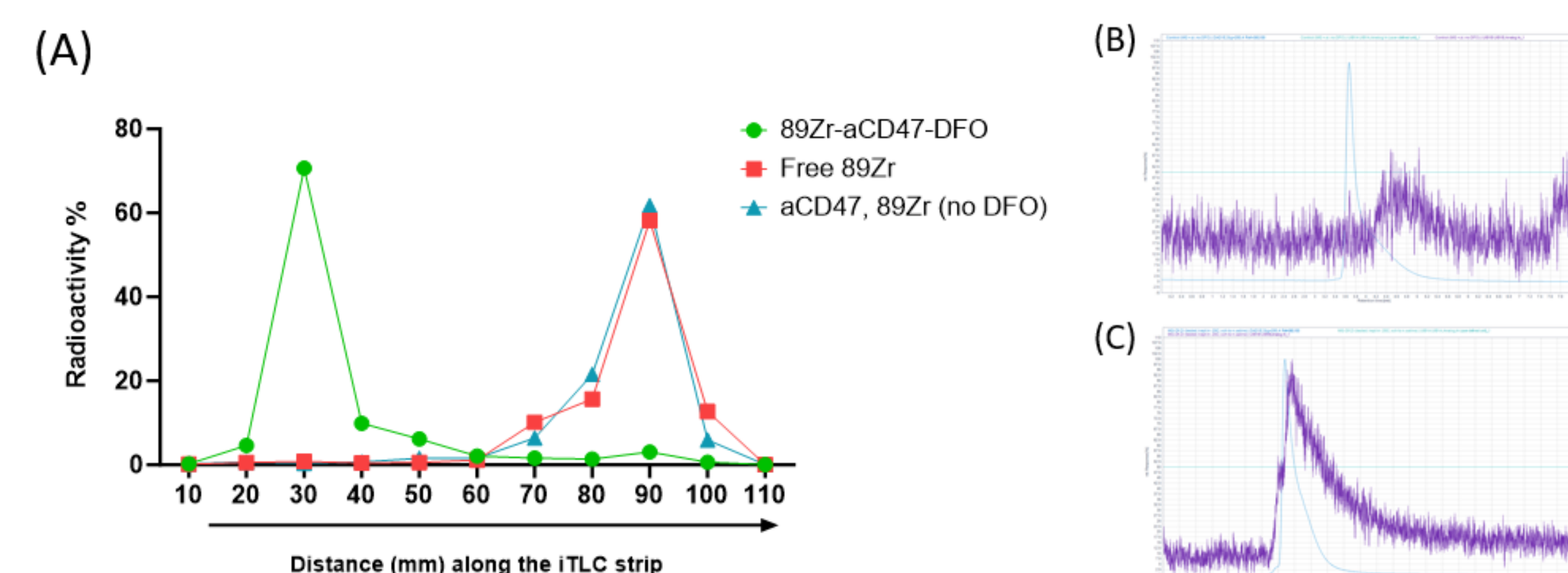


Figure 3. Radiolabeling quality assessment. (A) ITLC results showing successful chelation of free ⁸⁹Zr by aCD47-DFO, as evidenced by the ⁸⁹Zr-aCD47-DFO complex remaining at the bottom of the iTLC strip. (B) HPLC-radioflowmetry of aCD47 mixed with free ⁸⁹Zr without DFO. (C) HPLC-radioflowmetry of ⁸⁹Zr-aCD47-DFO complex showing overlapping peaks of radioactivity and antibody, suggesting specific binding of ⁸⁹Zr to aCD47.

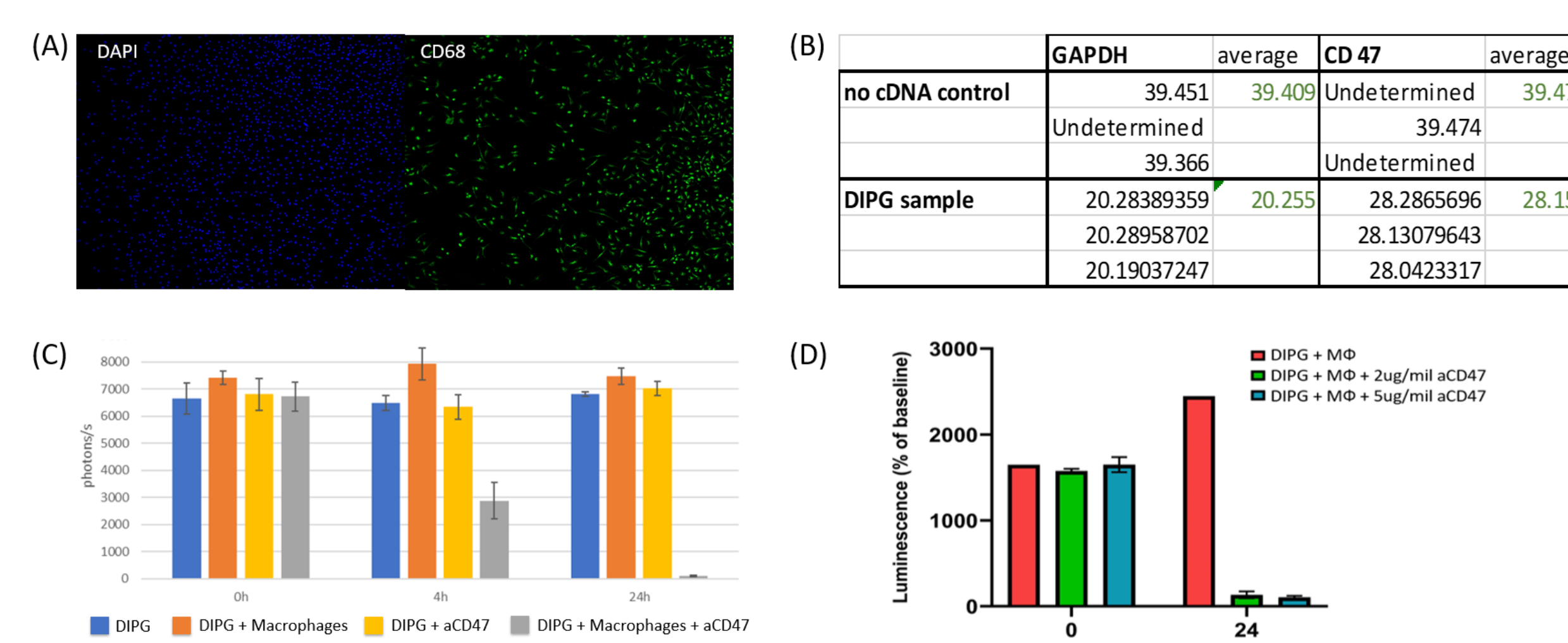


Figure 4. In vitro assay of macrophage-mediated DIPG cell destruction in the presence of aCD47 antibody. (A) Macrophages isolated from mouse bone marrow stained for CD68. (B) PCR results showing the presence of CD47 on DIPG cells. (C) DIPG cell bioluminescence is reduced only when both macrophages and aCD47 antibody are present, suggesting macrophage-mediated DIPG cell phagocytosis mediated by CD47. (D) Result is similar regardless of the administered dose of aCD47, suggesting no aCD47 dose-dependency in the assessed dose range.

RESULTS

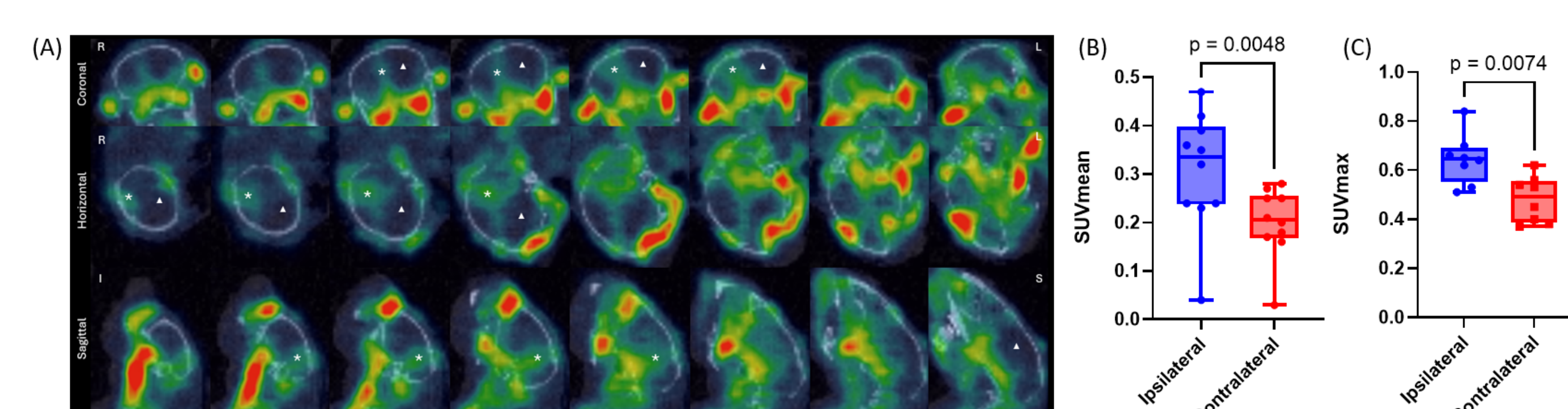


Figure 5. PET imaging of DIPG mouse after FUS and systemic ⁸⁹Zr-aCD47 injection. (A) Images show more uptake of radiolabeled aCD47 in the ipsilateral hemisphere (white asterisk) compared to contralateral hemisphere (white triangle). (B, C) Quantitative PET-based calculations of SUVmean and SUVmax, respectively, showing significantly more signal from the ipsilateral hemisphere compared to contralateral hemisphere.

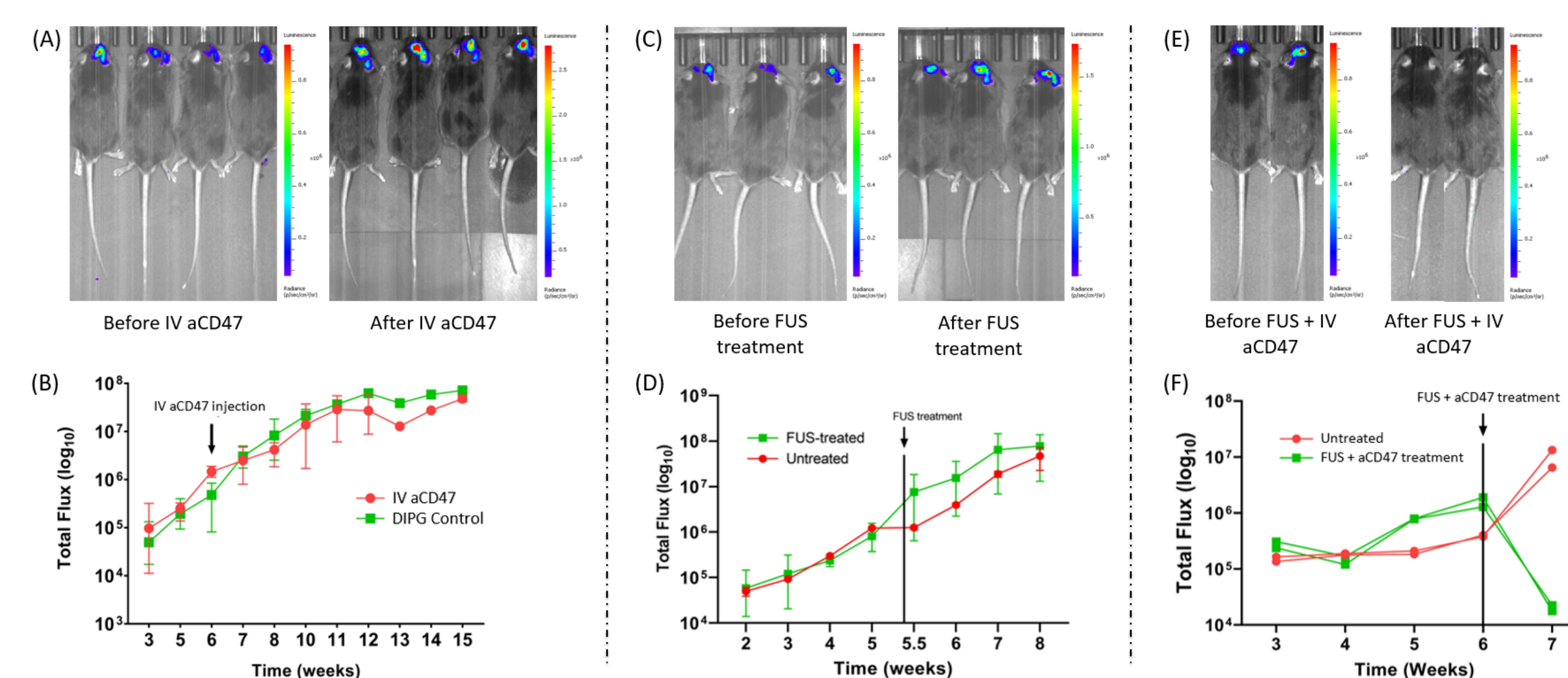


Figure 6. Therapeutic effect of FUS followed by systemic aCD47 administration on mouse DIPG models assessed by BLI. (A, B) DIPG mice that received only systemic aCD47 with no preceding FUS treatment did not show any response per BLI. (C, D) DIPG mice that received only FUS treatment without aCD47 did not show any response per BLI. (E, F) DIPG mice that received both FUS and systemic aCD47 showed reduction of the BLI signal to baseline, suggesting elimination of the tumor cells.

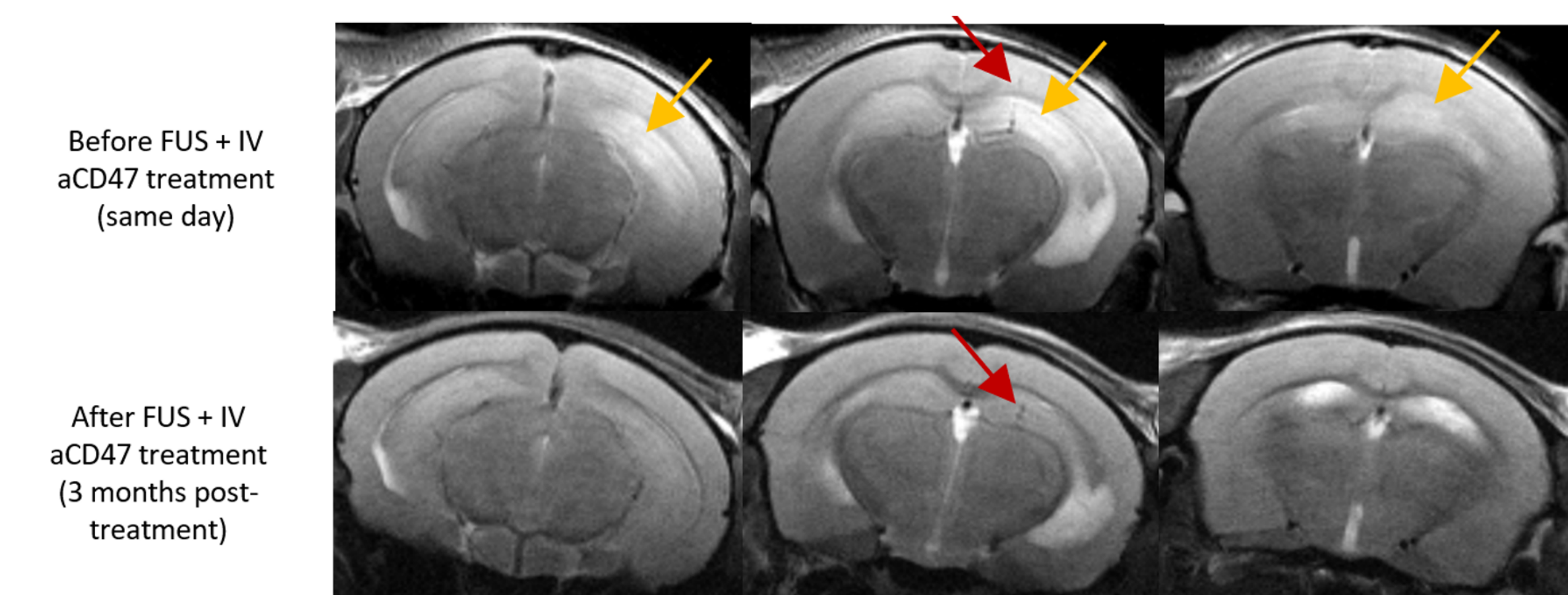


Figure 7. MRI brain of a DIPG mouse before (top row) and 3 months after (bottom row) treatment with FUS and systemic aCD47. Yellow arrows point to a T2-hyperintensity adjacent to the needle track (red arrows), which is believed to represent tumor cells. Three months after the treatment the hyperintensity disappears, suggesting successful tumor cell elimination.

CONCLUSIONS

Our findings provide evidence that combining FUS-mediated BBB opening with systemic administration of aCD47 antibody holds potential as a therapeutic strategy for DIPG. PET imaging served as a tool to validate the delivery of the antibody to the brain, underscoring the synergy between FUS and immunotherapy. Studies with larger sample sizes are warranted to further validate these promising results and explore the full therapeutic potential of this approach.

ACKNOWLEDGEMENTS

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