

# Vaccine-based immunotherapy for glioblastoma using tumor cells, Zika virus, and a checkpoint inhibitor

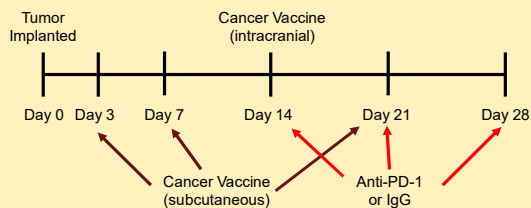
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## Introduction

- Glioblastoma multiforme (GBM) is the most common and malignant form of brain tumor, with a survival rate of less than 5% at 5 years following diagnosis.<sup>1</sup>
- Current treatment involves surgical resection, radiation therapy, and chemotherapy with temozolomide.<sup>1</sup>
- Pharmacological treatments are hindered by the blood brain barrier (BBB) and the multidrug resistance (MDR) of tumor cells.<sup>2</sup>
- The tumor microenvironment is strongly immunosuppressed; thus, immunotherapy is a promising new approach that enhances the immune system's ability to combat tumors.<sup>3</sup>
- Examples of immunotherapy include oncolytic viruses which infect tumor cells, checkpoint inhibitors which disinhibit the immune system, and cancer vaccines which train immune cells to target tumor cells. These methods yield moderate improvements in survival against GBM.<sup>4</sup>
- Zika virus is an oncolytic virus that can specifically target glioma stem cells that contribute to proliferation of GBM cells.<sup>5</sup>
- Previous work has shown larger improvements in survival following treatment with two immunotherapies (e.g. two checkpoint inhibitors, oncolytic virus + cancer vaccine).<sup>6,7</sup>
- Here we investigate the efficacy of a treatment combining a 1) checkpoint inhibitor and a 2) cancer vaccine containing irradiated Zika virus.

## Methodology

Figure 1. Experimental Procedure



### Tumor Implantation and Treatment Regimen:

Mice were implanted with mouse glioma cells at day 0. All mice not in the control group were treated with injections of a cancer vaccine containing irradiated mouse glioma cells that were infected with Zika virus (ZIKV), which was coupled with granulocyte macrophage-colony stimulating factor (GM-CSF) in subcutaneous, but not intracranial injections. Mice were injected with the cancer vaccine subcutaneously on days 3, 7, and 21 after tumor induction, and intracranially on day 14 after tumor induction. The PD-1+Vaccine and IgG+Vaccine groups were also given subcutaneous injections of anti-PD-1 or a non-reactive IgG antibody, respectively. On day 14 after tumor implantation, these mice were injected with 500 µg of either anti-PD-1 or IgG. On days 21 and 28, these mice were injected with 200 µg of either anti-PD-1 or IgG.

## Results

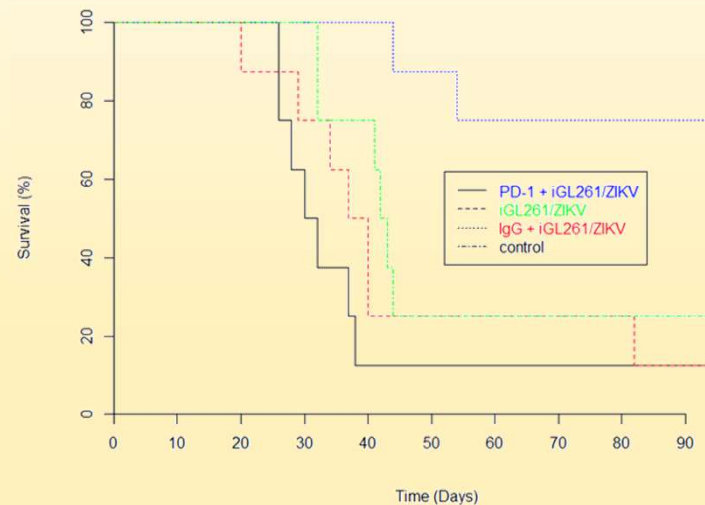


Figure 2. Survival of mice following glioblastoma (GBM) tumor implantation and treatments involving a checkpoint inhibitor (anti-PD-1L) and a "Vaccine" (irradiated tumor cells and ZIKV).

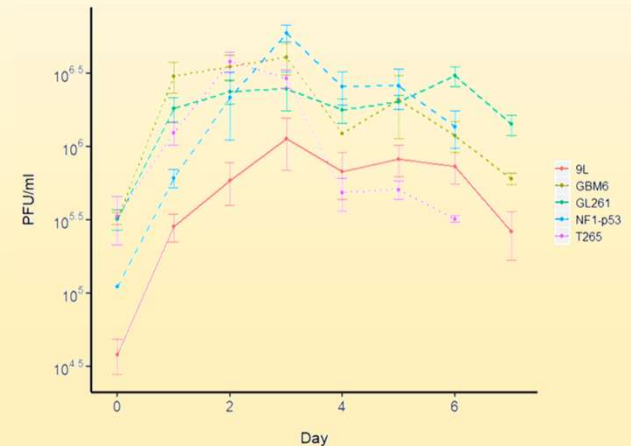


Figure 3. Infectivity of Zika virus in human (GBM6), rat (9L), and mouse (GL261) glioma cell lines, as well as human (T265) and mouse (NF1-p53) neurofibroma cell lines, using a viral plaque assay. Values are the concentration of plaque forming units (PFU) released by infected cell lines, measured each day for 7 days. Zika virus can infect and release viral particles from multiple glioma and neurofibroma tumor lines.

## Results

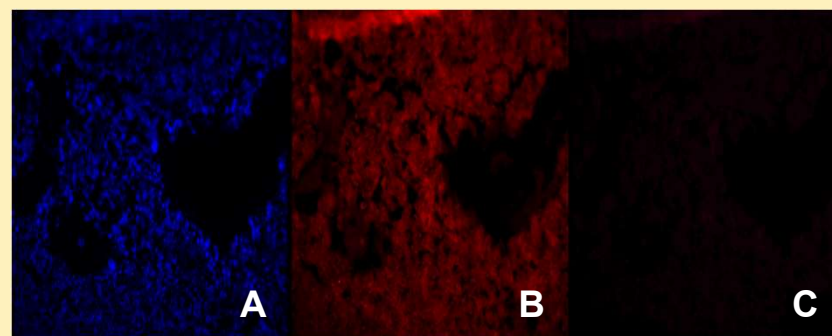


Figure 4. Expression of DAPI (A), SOX2 (B), and CD3 (C) in the tumor region of a control mouse brain. DAPI and SOX2 were co-expressed in the tumor region without CD3.

## Conclusion

- Adding a checkpoint inhibitor greatly increases the efficacy of a cancer vaccine and enhances survival (Fig 2).
- Zika virus successfully targets the intended glioma cell population and contributes to the cancer vaccine (Fig 3).
- In control mice, immune cells do not infiltrate the tumor region (Fig 4).
- The checkpoint inhibitor may promote survival by increasing immune cell infiltration and activating the recruited immune cells.
- Viability of this treatment should be tested further in animal, then clinical trials.

## References & Acknowledgements

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