Research

# Targeting glioma stem cells with Zika virus

**Paris Filippidis**
Year 2, Medicine, University of Plymouth
Email: paris.filippidis@students.plymouth.ac.uk

## Abstract

Glioblastoma multiforme (GBM) is the most aggressive type of brain tumour and is widely regarded as incurable. The low therapeutic outcomes of GBM may be associated with glioma stem cells (GSCs) that reside in the tumour and demonstrate stem-like, self-renewing and tumourigenic capabilities. Research into Zika virus (ZIKV) has shown that it displays an oncolytic activity against GSCs. This report will explore the way that ZIKV invades and induces apoptosis in GSCs and its potential for therapeutic application.

## Abbreviations

*AXL - Anexelekto*
*GBM - Glioblastoma multiforme*
*GSC - Glioma stem cells*
*IFN-1 - Type 1 interferon*
*IRF - IFN-regulatory factor*
*ISG - IFN-stimulated gene*
*MAV - Mitochondrial antiviral-signalling protein*
*NF-κB - Necrosis factor κB*
*NPC - Neural progenitor cell*
*OV - Oncolytic viral*
*PAMP - Pathogen-associated molecular pattern*
*PRR - Pattern-recognition receptor*
*RIG - Retinoic acid-inducible gene*
*STAT - Signal transducer and activator of transcription*
*TMZ – Temozolomide*
*WT ZIKV - Wild type Zika virus*
*ZIKV - Zika virus*

## Introduction

GBM is a potentially lethal form of primary brain tumour that starts in neural progenitor cells (NPCs).[1] Around 2,200 cases of GBM are diagnosed in England each year, accounting for 55% of all malignant brain tumours.[2] Despite maximal therapy with surgery, radiation, and chemotherapy, the recurrence rate for GBM remains high and median survival from the point of diagnosis is only 14 months.[2] This may be attributable to GSCs, which have been shown in preclinical models to resist all forms of conventional cancer therapy.[3] Therefore, strategies to target these cells specifically may possibly improve patient response to treatment regimes. Oncolytic viral (OV) treatment with ZIKV might be a potential way to achieve this via stimulation of the intrinsic and extrinsic apoptotic signalling cascades.[4] This potentially devastating virus is known to target NPCs in the foetus and cause microcephaly.[5] However, adults infected with the virus typically develop only mild symptoms and the therapeutic application of an attenuated ZIKV strain has been indicated to further reduce the pathological response.[6,7] Therefore, the aim of this review is to explore the role and mechanism of ZIKV for targeting GSCs in cases of GBM and how patient safety is maintained with the use of the attenuated ZIKV strain.

## Literature search

A literature search was conducted using the NCBI platform (PubMed Central) for the term "Glial Stem Cells" and 52,861 results were retrieved. To obtain the latest scientific data, the publication date was restricted to 2015-2020 and the results were narrowed down to 32,082 articles. Titles and abstracts of retrieved literature were screened for relevance against pre-determined inclusion criteria. Papers were eligible for inclusion if they explored the role of glial stem cells in GBM recurrence. A search in the same database for the term "Zika in glioblastoma" retrieved 535 results. Articles were retained only if they explained the effect of ZIKV on GSCs. Furthermore, the National Cancer Registration and Analysis website was reviewed to obtain valid and reliable values for the incidence and survival rate of GBM patients in the UK. This evidence was obtained from data linkage between the National Cancer Registration Service and the Radiotherapy Data Service.

## Mechanisms of ZIKV internalisation

ZIKV penetrates host cells via receptor mediated endocytosis.[8] Evidence from human glioblastoma specimens has shown that anexelekto (AXL) tyrosine kinase receptors and their ligand, growth arrest-specific 6 (Gas6), play an essential role in this mechanism.[8] Gas6 acts as a co-factor by binding to ZIKV and allowing attachment of the virus to the AXL receptors on GSCs. Once the ZIKV-Gas6 complex activates the receptor, the virus can enter the cell via clathrin-mediated endocytosis.[8] Clathrin proteins coat the pits formed on the inner surface of the plasma membrane during endocytosis of ZIKV. These pits form vesicles that allow transport of the virus into the cytoplasm.[9] Noteworthily, both AXL and Gas6 have been found to be overexpressed in GSCs supporting ZIKV specificity to these cells.[10] However, conflicting evidence has indicated that the virus is able to infect modified GSCs that are deprived of the AXL receptor.[11] Thus, the exact mechanism of ZIKV internalisation is still unclear.

## The role of ZIKV in the induction of the extrinsic apoptotic pathway in GSCs

The first line of defence against invasive pathogens is exerted by the innate immune system, which is central in the extrinsic apoptotic signalling cascade.

> *This cascade is comprised of pattern-recognition receptors (PRRs) that demonstrate a pivotal role in the detection of pathogen-associated molecular patterns (PAMPs).*[12]

In the case of ZIKV infection, retinoic acid-inducible gene (RIG)-like receptors serve as PRRs and activate the mitochondrial antiviral-signalling proteins (MAVs). These proteins induce necrosis factor κB (NF-κB) and IFN-regulatory factor (IRFs), which activate the transcription of type 1 interferon (IFN-1) genes and facilitate IFN-α/β production. Once IFN-α/β are produced, they are secreted from the infected cells into the extracellular space, where they bind to IFN-α/β receptor that may be found on either the same cell or on uninfected cells in the surrounding area. Following the stimulation of this receptor, a second transcriptional cascade is induced by signal transducer and activator of transcription (STAT) 1 and 2 factors that lead to the overexpression of IFN-stimulated genes (ISGs), which are involved in cellular apoptosis.[13,14]

Nevertheless, it has been shown that ZIKV exhibits an inhibitory effect on IFN-1 induction and signalling cascades via the NS5 viral protein (**Figure 1**).[14] Overall, ZIKV acts to suppress the induction of IFN-1 by downregulating NF-κB- and IRF-induced signalling. In addition, ZIKV impedes the activating phosphorylation of both STAT1 and 2, and drives the proteasomal degradation of STAT2, thereby inhibiting IFN-1 signalling.[13,14] This information inspired scientific efforts to introduce mutations in the NS5 gene of ZIKV with the aim of attenuating the virus and making it more susceptible to the IFN-1 innate immune response.[7]

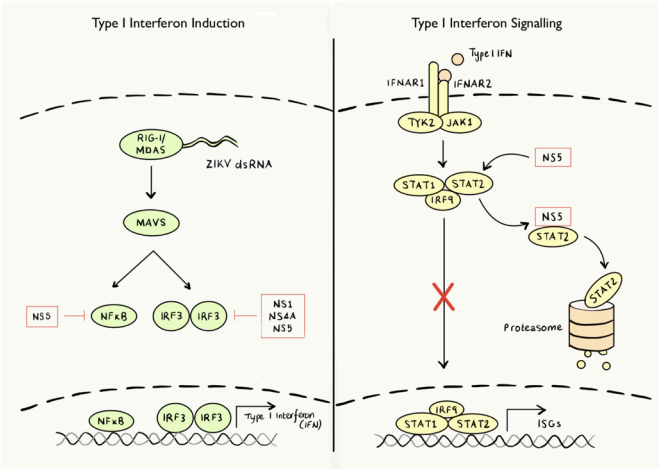


**Figure 1. The impact of NS5 viral proteins on IFN signalling.** NS5 viral proteins inhibit NF-κB and IRFs, interfering with the induction of IFN-1. In addition, they interact with STAT-2, inducing the proteasomal degradation of this protein and constraining IFN-1 signalling. Figure from Kumar et al;[13'] adapted with permission.

## Genetically modified ZIKV strains in OV treatment of GBM

In order to optimise the safety of ZIKV for therapeutic application in GBM patients, the ZIKV viral genome has been altered. The modified ZIKV strain contains two nucleotide changes in the same codon of the NS5 gene. Therefore, the likelihood of regression back to the pathogenic viral form is low as two simultaneous nucleotide changes are required.[7] Moreover, the effect of this modified strain on GSCs can be enhanced with concurrent temozolomide (TMZ) chemotherapy as the virus downregulates expression of ABC transporters. These transporters are cell-membrane proteins that act as molecular pumps in GSCs to expel cytotoxic drugs from the cytosol.[15] Therefore, ZIKV increases the vulnerability of GSCs to chemotherapeutic agents.

A study performed in-vivo on mouse glioma models and in-vitro on glioblastoma tissues obtained from patients compared the tumouricidal effects of the wild type ZIKV (WT-ZIKV) form and its recombinant derivative E218A with NS5 mutations. Both the WT-ZIKV and the E218A strain displayed oncolytic activity against GSCs. However, the WT-ZIKV strain was much more effective than the mutated form in reducing GSC growth. Therefore, ZIKV E218A was tested in combination with TMZ chemotherapy to improve its potency. Although, TMZ alone had a limited effect on GSC growth, the combined treatment showed a great antitumor effect similar to that of the WT-ZIKV strain.[7] This study should be repeated in-vivo with GBM patients to explore whether the results are reproducible in humans and to determine any possible implications arising from the treatment.

## Conclusion

Synthesis of the aforementioned evidence suggests that genetically engineered ZIKV strains may be utilised to target GSCs via OV therapy. The reason for viral tropism towards these specific cells remains unclear as no definitive ZIKV receptor has been found on them. The mechanism through which ZIKV delays the extrinsic apoptotic signalling pathway, improves its replicative capacity and pathogenicity. Fortunately, a genetically modified strain has been formulated to be more vulnerable, especially to the IFN-1 signalling mechanism, making it safer for use in treatment. This, in combination with chemotherapy, has shown great oncolytic effect in vitro. Nevertheless, human trials are fundamental to determine the safety and efficacy of this treatment in vivo. If the same effect is obtained, the survival rate of GBM patients may increase significantly and this will be a breakthrough, especially in this nascent area of cancer treatment.

**Acknowledgements** This article is based on a completed student selected component module as part of the Peninsula Medical School, University of Plymouth curriculum. Many thanks to Dr Claudia Barros (Associate Professor of Neuroscience; University of Plymouth, Plymouth, UK) who supervised this project.

**Contribution statement** The author made significant contributions to the conception of this work, analysis and interpretation of data, drafting and revising its intellectual content and approving the final version to be included in Inspire.



## References

1. Stoyanov GS, Dzhenkov D, Ghenev P, Iliev B, Enchev Y, Tonchev A B. Cell biology of glioblastoma multiforme: from basic science to diagnosis and treatment. Med Oncol, 2018; 35(3): 27.
2. Greenberg D, Winters T, Brodbelt A, et al. Incidence and outcomes for cerebral Glioblastoma in England. Public health England, 2017.
3. Prager B C, Bhargava S, Mahadev V, et al. Glioblastoma Stem Cells: Driving Resilience through Chaos. Trends Cancer, 2020; 6(3): 223-235.
4. Su KY, Balasubramaniam VRMT. Zika Virus as Oncolytic Therapy for Brain Cancer: Myth or Reality?. Front Microbiol, 2019; 10: 2715.
5. Mlakar J, Korva M, Tul N, et al. Zika Virus Associated with Microcephaly. N Engl J Med ,2016; 374(10): 951-8.
6. Souza OIN, Barros-Aragao FGQ, Frost PS, et al. Late Neurological Consequences of Zika Virus Infection: Risk Factors and Pharmaceutical Approaches. Pharmaceuticals, 2019; 12(2): 60.
7. Zhu Z, Gorman JM, McKenzie LD, et al. Zika virus has oncolytic activity against glioblastoma stem cells. J Exp Med, 2017; 214(10).
8. Meertens L, Labeau A, Dejarnac O, et al. Axl Mediates ZIKA Virus Entry in Human Glial Cells and Modulates Innate Immune Responses. Cell Rep, 2017; 18(2): 324-333.
9. Kaksonen M, Roux A. Mechanisms of clathrin-mediated endocytosis. Nat Rev Mol Cell Biol, 2018; 19: 313-326.
10. Wu G, Ma Z, Cheng Y, et al. Targeting Gas6/TAM in cancer cells and tumor microenvironment. Molecular Cancer, 2018; 20.
11. Wells MF, Salick MR, Wiskow O, et al. Genetic Ablation of AXL Does Not Protect Human Neural Progenitor Cells and Cerebral Organoids from Zika Virus infection. Cell Stem Cell, 2016; 19(6): 703-708.
12. Serman TM, Gack MU. Evasion of Innate and Intrinsic Antiviral Pathways by the Zika Virus. Viruses, 2019; 11(10): 970.
13. Kumar A, Hou S, Airo AM, et al. Zika virus inhibits type-I interferon production and downstream signaling. EMBO rep, 2016; 17(12): 1766-1775.
14. Elshahawi H, Hassan SS, Balasubramaniam V. Importance of Zika Virus NS5 Protein for Viral Replication. Pathogens, 2019; 8(4):169
15. Lee CAA, Banerjee P, Wilson BJ, et al. Targeting the ABC transporter ABCB5 sensitizes glioblastoma to temozolomide-induced apoptosis through a cell-cycle checkpoint regulation. H Biol Chem, 2020; 295(22): 7774-7788.