INTRACRANIAL ZIKA VIRUS INOCULATION IN A PATIENT WITH RECURRENT GLIOBLASTOMA:

A BRIEF REPORT OF THE CLINICAL AND TECHNICAL ASPECTS IN A CASE OF COMPASSIONATE USE

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Introduction

High-grade glioma is a primary malignant brain tumor for which there is no current curative treatment. It is characterized by a high rate of recurrence and a limited survival, thus encouraging development of novel therapies (Reni et al.). Current standard treatment is based on maximal safe surgical resection followed by temozolomide and radiation therapy (Stupp et al.). Additional treatments for the recurrence are still limited and oncolytic viral treatments are under study in both, basic and clinical settings. Recently, it was shown that Zika virus (ZIKV) has an in vitro potential for selective destruction of glial cells with stem cell properties, but so far there have been no reports of this potential treatment approach in humans (Kaid et al., Zhu et al., Lima et al.).

We report the first application of the intracerebral injection of ZIKV in a 67-year-old female patient presenting with recurrence of a left fronto-parietal wild-type glioblastoma, previously submitted to standard treatment.

Case summary

A 67-year-old female diagnosed in June 2017 with a left-frontal-parietal high-grade glioma after episodes of confusion, was initially submitted to an open biopsy elsewhere and 6 weeks later referred to our institution for extended resection of the residual tumor. The patient had aphasia and right-sided motor deficit, with limitations for daily activities. Following reoperation, the patient was submitted from September to December 2017 to adjuvant treatment with radiotherapy (60 Gy) and concurrent temozolomide, followed by seven cycles of adjuvant temozolomide (50 mg/m2), concluded in May 2018, as standard of care.

Radiological signs of progression were noted from July to October 2018, with increase in the dimensions of the tumor on gadolinium-enhanced magnetic resonance imaging (MRI) – Fig.1. Clinically, the patient was progressive worse, with right-sided hemiplegia and decreased consciousness. A daily regimen of oral temozolomide was initiated, also at 50 mg/m^2.

Gadolinium-enhanced magnetic resonance imaging before the proposed resection and inoculation

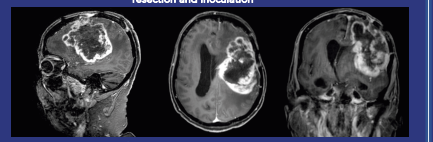

Fig 1. Preoperative gadolinium-enhanced MRI after progression was diagnosed. Note the extensive contrast-enhancement at sagittal (A), axial (B) and coronal (C) planes, with extension to the left frontal, parietal and temporal lobes, with a necrotic center compatible with the diagnosis of glioblastoma. There are also indirect signs of raised intracranial pressure, with marked midline shift.

In September 2018 an experimental protocol was proposed by the multidisciplinary team to the family members, which agreed to the enrollment of the patient. Institutional Ethics Board approval was granted on a compassionate basis. In October 2018 the patient was submitted to a subtotal resection of the tumor with the objectives to reduce the volume of the tumor, reduce intracranial pressure and to obtain samples for the culture of the tumor and in vitro inoculation of the Zika virus. The surgery underwent without remarks, the patient showed signs of improved level of consciousness (spontaneous eye opening and following commands), while keeping the previous neurological deficits (i.e., hemiplegia and aphasia).

Nineteen days after the previous surgery and after culture of the tumor sample, the patient was submitted to an open craniotomy for the intra-tumoral and intraventricular injection of 2,5 x 10^5 viral particles obtained from the inoculated in vitro culture of the tumor. The patient was then assessed daily in the first week for serum and CSF markers and clinical signs viral infection. Clinical signs of Zika infection were noticed on the eight postoperative day, which consisted of cutaneous rash on the extremities, face and neck and mild fever. No other adverse effects were noticed. The clinical response of raised alertness was sustained for approximately 5 weeks post-inoculation, after which the patient showed clinical and radiological signs of tumor progression, being admitted to the palliative care infirmary 2 weeks later and 10 weeks after the inoculation the patient died.

Proposed intervention

N-of-1 intervention (on compassionate basis) to assess both the *in vitro* and clinical effects of the inoculation of Zika virus for the potential treatment of recurrent high-grade glioma.

The patient was reoperated after clinical and radiological diagnosis of recurrence, while on standard of care. A macroscopic subtotal resection of the tumor with posterior in vitro culture and inoculation of the Zika virus in the tumor sample were performed.

After *in vitro* response (oncolytic effects of the inoculation) was noticed, 2,5x10^5 viral particles were obtained, and the patient was inoculated targeting both the left lateral ventricle and the tumor bed.

The patient was then assessed for clinical and laboratorial markers of Zika virus infection, as well as for potential adverse effects from the intervention and for the behavior of the tumor (radiological and clinical response).

Intervention design

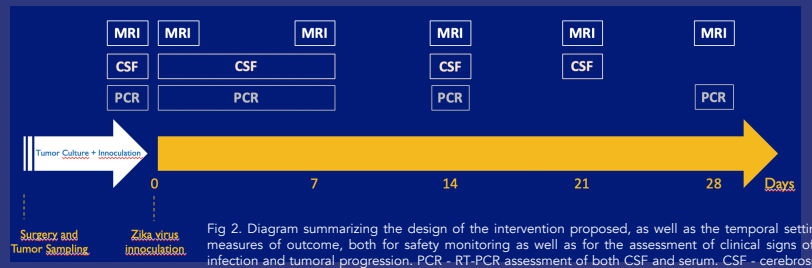

Fig 2. Diagram summarizing the design of the intervention proposed, as well as the temporal setting for the measures of outcome, both for safety monitoring as well as for the assessment of clinical signs of systemic infection and tumoral progression. PCR - RT-PCR assessment of both CSF and serum. CSF - cerebrospinal fluid collection via spinal tap. MRI - gadolinium-enhanced magnetic resonance imaging.

Results

A. Tumor culture 24h (A) and 72h (B) after ZIKV inoculation

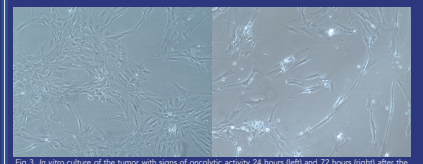

Fig 3. *In vitro* culture of the tumor with signs of oncolytic activity 24 hours (left) and 72 hours (right) after the inoculation of Zika virus. Note the progressive derangement of the glioblastoma cells following the inoculation. The glioblastoma cells were obtained following the reoperation for tumor resection and tissue sampling and were culture in a medium with DMEM-12 + 10% SBF.

B. Clinical signs of ZIKV infection eight days after inoculation

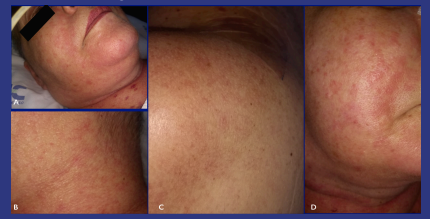

Fig 4. Photographs of the patient at the 8th day post-inoculation, showing clinical signs of Zika virus systemic infection with diffuse cutaneous rash at the face (A), neck (B), shoulder and back (C), with a zoomed-in image of the lesions more noticeable in the face. Written informed consent was granted by the patient and family members.

C. Serum and CSF biomarkers for ZIKV

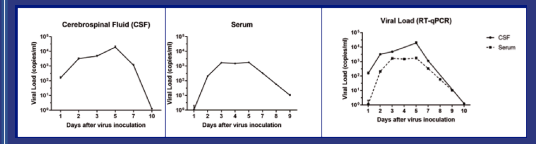

Fig 5. RT-qPCR quantification of the viral load after the intracranial inoculation of the ZIKV. Note the high viral load in the CSF from the first post-inoculation day, with a peak between the 5th and 7th days and a marked decrease after the 7th day, both in serum and CSF. 200µL were extracted from the sample (total RNA). RT-qPCR with TaqMan® Fast Virus1-Step kit.

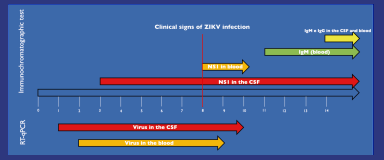

Fig 6. Temporal summary of the periods of detection of the virus, NS1 surface protein and IgM and IgG in both blood and CSF of the patient, following inoculation with the Zika virus. Note that the virus was detected in the CSF from the first post-inoculation day, followed by detection in the blood the day after. The appearance of clinical signs of ZIKV infection were concurrent with the detection of the NS1 surface protein in the blood, at the 8th post-inoculation day. Immune response was noted at the 11th post-inoculation day, with IgM detected in blood followed by the detection of both IgM and IgG in CSF and blood.

D. Post-inoculation MRI

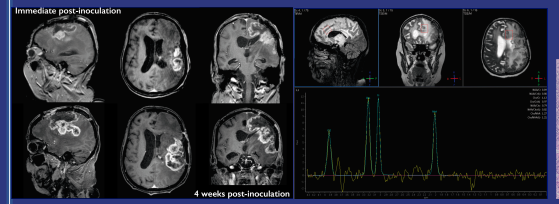

Fig 7. Radiological progression of the lesion from the immediate post-inoculation gadolinium-enhanced MRI (above), after subtotal resection with tumoral sampling for the *in vitro* culture, to the control MRI 4 weeks post-inoculation (bellow). Note the increase of both enhancing and non-enhancing areas, particularly towards the temporal lobe and basal ganglia, denoting radiological progression of the tumor.

Fig 8. MRI spectroscopy 4 weeks post-inoculation. The volume of interest was positioned at the anterior-superior-mesial aspect of the lesion, at the left-frontal lobe and the profile was compatible with a high-grade glioma, with diffuse tissular infiltration: Choline (Cho), creatine (Cr) and N-acetyl aspartate (NAA) presented peaks, also the presence of lactate was noted in all the volumes of interest (not shown).

E. Histopathological changes

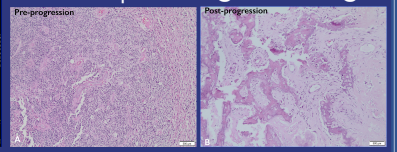

Fig 9. Microscopic images of samples of the tumor on hematoxylin and eosin, obtained before (A) and after (B) tumor progression. The initial histopathological diagnosis was of wild-type glioblastoma, with diffuse positivity for GFAP, p53 and ATRX and negativity for IDH1 (R132H), without co-deletion for 1p19q (non-shown). The second sample, obtained for in vitro culture showed additional characteristics compatible with sarcomatous differentiation, thus establishing the diagnosis of gliossarcoma, a variant of the IDH-wild type glioblastoma.

Discussion

Although there are reports of the potential oncolytic effects of the Zika virus from both animal and *in vitro* studies (Kaid, Lima and Zhu et al.), this is the first case report of the ZIKV inoculation in a patient with a diagnosis of high-grade glioma.

This intervention was performed in a compassionate basis, since the intervention haven't still been tested for safety and clinical outcomes.

The objective of this pilot study was to assess the feasibility of obtaining viable samples of the tumor, culturing it *in vitro* and posteriorly inoculating with ZIKV to both evaluate the occurrence of the effects in the tumor cells *in vitro* and to increase the number of copies of the ZIKV to inoculate intracranially in the patient above mentioned.

It was possible to culture the tumor cells *in vitro* and there were marked signs of tumor cells infection, with viral replication and oncolytic effects. It was possible to later recover 2,5x10^5 copies of the tumor for intratumor and intraventricular injection, in a subsequent surgery. The patient was then assessed for clinical signs of tumor progression, as well as for potential adverse side effects and monitored for the status of the inoculation and potential systemic infection with Zika (Fig.2).

There were signs of oncolytic effects over the cultured tumoral cells, from 24h to 72h post-inoculation (Fig. 3), in accordance with the previous literature reports.

The patient presented mild clinical signs of Zika systemic infection, as reported in the literature (Petersen, 2016), at the 8th post-inoculation day, consisting mostly of a mild-fever and diffuse cutaneous rash (Fig. 4). This was in accordance with what was observed from the serological and CSF samples obtained in the first days post-inoculation (Fig. 5). Although the virus was detected in the CSF from the first day post-inoculation, it was only in the 8th day that the NS1 protein was detected on the blood of the patient - a non-structural protein that is essential for viral replication (Fig. 6).

No further clinical effects from the Zika infection were noted during the observation period and the rash disappeared after approximately 3 days. Also, the immune response was assessed with the detection of IgM and IgG antibodies in both CSF and blood, respectively IgM at the 11th day and IgG at the 14th post-inoculation day, compatible with the resolution of the infection.

After the surgeries for both tumor resection and later ZIKV inoculation, the patient sustained a marked improvement in the level of consciousness, being able to interact with family members, while maintaining the previous motor and speech deficits. Since this outcome was highly subjective, it could also be attributed to the effect of the tumor resection with the subsequent decompression of the mass effect. Radiological progression of the tumor was noted from the 4th post-inoculation week (Fig. 7 and 8), in accordance with the clinical decline in the 5th post-inoculation week.

Although there were *in vitro* signs of oncolytic effects from the ZIKV inoculation and the virus was detected both in CSF and serum of the patient, with clinical signs of infection, we hypothesize that the effects were transient after the intracranial inoculation due to: (1) immune response directed at the ZIKV, from the 11th post-inoculation day and (2) differentiation of the tumor, from a glioblastoma with glial features to a sub-type deemed gliossarcoma, which presented marked glial and a prominent sarcomatous component in the subsequent samples obtained (Fig. 9), thus suggesting the destruction of the glial components by the ZIKV and proliferation of the not affected sarcomatous components, leading to the progression of the tumor to a more aggressive sub-type (Han, 2010). This must be further assessed from the *in vitro* analysis of the effects of the inoculation in the different sub-types of high-grade gliomas, to allow for the potential design of a clinical trial. Also, autopsy yielded no signs of encephalitis or vasculitis.

Conclusions

It is feasible to culture samples of tumor cells, posteriorly inoculate *in vitro* with Zika virus with assessment of the presence or not of cytopathic effects.

Also, it is possible to recover and filter ZIKV copies from the culture of the patient for posterior intracranial (tumor bed and intraventricular) inoculation.

Although there was an initial clinical and radiological response following the inoculation with ZIKV, the tumor eventually progressed, particularly the sarcomatous component (compatible with a diagnosis of gliossarcoma), thus potentially discouraging the recruitment of patients with diagnosis of this subtype for initial trials.

There was no serious adverse side effects from the intervention proposed, thus warranting future clinical trials designed at assessing this paradigm for the treatment of recurring high-grade gliomas, after standard of care is employed, and for selected adult patients.

References