Scientific Research Publishing

Journal of Cancer Therapy, 2018, 9, 522-528
http://www.scirp.org/journal/jct
ISSN Online: 2151-1942
ISSN Print: 2151-1934

Proof of Concept in a Case Study of Glioblastoma Multiforme Successfully Treated with IV Quercetin in Combination with Leading Edge Gamma Knife and Standard Treatments

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How to cite this paper: Nezami, M.A. and Duma, C. (2018) Proof of Concept in a Case Study of Glioblastoma Multiforme Successfully Treated with IV Quercetin in Combination with Leading Edge Gamma Knife and Standard Treatments. *Journal of Cancer Therapy*, **9**, 522-528.
https://doi.org/10.4236/jct.2018.96043

Received: May 23, 2018
Accepted: June 26, 2018
Published: June 29, 2018

Abstract

The Stupp protocol has become standard of care for the treatment of glioblastoma (GBM) (since its publication in 2005) and has led to some limited survival improvements. This protocol, consists of radiotherapy and concomitant chemotherapy with temozolomide, an alkylating agent. Temozolomide + radiation, compared to radiation alone had added in average 3 months additional life span, 16 percent improved survival at 2 years. That said since 2005, the standard of care has not changed in regards to the treatment of early diagnosed aggressive or multifocal GBM, and unfortunately the expected survival is still poor with 75 percent of patients dying in less than 2 years and average survival of 15 months. In patients with multifocal tumors (such as the case below) the average survival is even worse with less than 4 months at her age [1]. Here we present a case study of a patient with advanced multifocal, and rapidly progressing Glioblastoma Multiforme treated with STUPP protocol in combination with IV Quercetin. The patient experienced improved quality of life and response, compared to historical data. It is our recommendation to investigate such combinational approach in patients with Glioblastoma, as in our case it proved to be safe and effective with improved quality of life and performance as well as clinical response and survival.

Keywords

Quercetin, Glioblastoma, Leading Edge Gamma Knife

1. Introduction

According to the original study the Stupp protocol comprises:

- Radiotherapy
  - total 60 Gy
  - 2 Gy per daily fraction (Monday to Friday) over 6 weeks
- Temozolomide
  - during radiotherapy: 75 mg per square meter of body-surface area per day, 7 days per week
  - post-radiotherapy (adjuvant): six cycles consisting of 150 - 200 mg per square meter for 5 days during each 28-day cycle

Here are characteristic features of multifocal disease, which accounts for about 13 percent of cases:

- In one retrospective study [2], researchers reviewed records of 368 newly diagnosed patients with GBM and identified 47 patients with multifocal tumors.
- Tumor progression was defined as increased enhancement on the 2- to 3-month postradiation magnetic resonance imaging scan vs the immediate postoperative scan, together with clinical decline.
- Factors leading to the worst median survival duration were age (>65 years), partial resection or biopsy, and low KPS (≤70) within the multifocal group.
- The median survival duration of older patients with multifocal disease (age > 65 years) was **4 months** vs 12 months in younger patients ($P = 0.0007$).
- Patients with multifocal disease who underwent biopsy or partial resection had a median survival time of 5 months vs the 14-month median survival time seen in patients who underwent gross total or near-gross total resection ($P = 0.04$).
- Patients with multifocal disease with low KPS (<70) had a median survival duration of **3 months** vs patients with high KPS (>70) who survived for 9 months ($P = 0.04$).
- Survival rates at 2 years were 4.3% for patients with multifocal disease and 29.0% for the unifocal cohort.
- Additionally, newly diagnosed patients with multifocal disease had an almost 2-fold increase in the hazard of death vs patients with solitary GBM (hazard ratio, 1.8; 95% CI, 1.1 - 3.1; $P = 0.02$).
- There were no significant differences in expression profile of any of the molecular markers between the multifocal and solitary GBM groups.

Quercetin has been studied extensively in variety of tumor types over decades. For a while it has been used in the form of IV in phase I and II trials with good safety and efficacy profile in cancer and other conditions including cardiovascular disease. Recently it has become a focus of interest, in treatment of Glioblastoma along with different forms of radiation and chemotherapy. Mechanisms of action in Glioblastoma have been identified both as independent as well as synergistic. Targets include HSP27 and COX2, a dual inhibition found in both in vitro and in vivo model [3]-[18].

Quercetin also has been studied by our scientific collaboration with M D Anderson, in 3 dimensional cell culture model and has shown inhibitory effect on

cell migration and glioblastoma spheroid viability (**Figure 2**).

Gamma Knife through leading edge technique showed improved outcome in a large subset of patients with Glioblastoma, due to its antimigratory effect [19].

The rationale of combination therapy relies on the fact that theoretically, the combinational therapy enhances the inhibition on cellular migration and the application of gamma knife could increase the penetration of IV Quercetin in the blood brain barrier. The limiting factor is the bioavailability and the requirement for frequent treatments.

2. Methods and Materials

Quercretin was compounded under sterile conditions at FDA approved facility, and passed required testings for IV injection, further injected intravenously in the dose of 500 mg per treatment. Patient was educated about the therapy and appropriate consent forms obtained. Further she received the IV therapy through her central line at rate of 4 ml per minute, on daily basis per protocol.

Patient was a 70 years old white female (who worked as an accountant with no known exposure to carcinogens, or verifiable family history of cancer),diagnosed with multifocal glioblastoma multiforme in 2/24/17 in right frontal lobe, perisylvian area as well as anterior limb of internal capsule extending to right caudaual head and right frontal white matter, status post MRI guided stereotactic frontal lobe craniotomy on 3/6/17, and further Treatment with leading edge gamma knife radiotherapy and Temodar at 75 mg/m^2 started on 2/24/17 and completed on 5/19/17. She had completed 35 sessions of radiation and further leading gamma knife directed by Dr Chris Duma at Hoag Hospital, and IV Quercetin started on 6/2/17. She also had a history of DVT in March that is currently treated with Eliquis. KPS was less than 70.

She was referred to us for evaluation and treatment on 5/31/17. Initial presentation was fatigue and inability to walk due to ataxia and disrupted gait. Physical examination revealed right foot drop and balance deficiency in walking and coordination.

Her labs indicated positive Circulating tumor cells (CTC) measured by PCR through Biofocus Lab on 6/16/17. Her tumor molecular profiling showed positive CIC, ATRX, TP53, PTEN and MGMT. Her pathology had shown a proliferative index (Ki67) of 90 percent.

This patient suffered from the worse prognostic markers for Glioblastoma, including Ki 67 of 90 percent (more than 22 percent, as cut off) as well as presence of CTC. Both markers are predictive of outcome [20] [21].

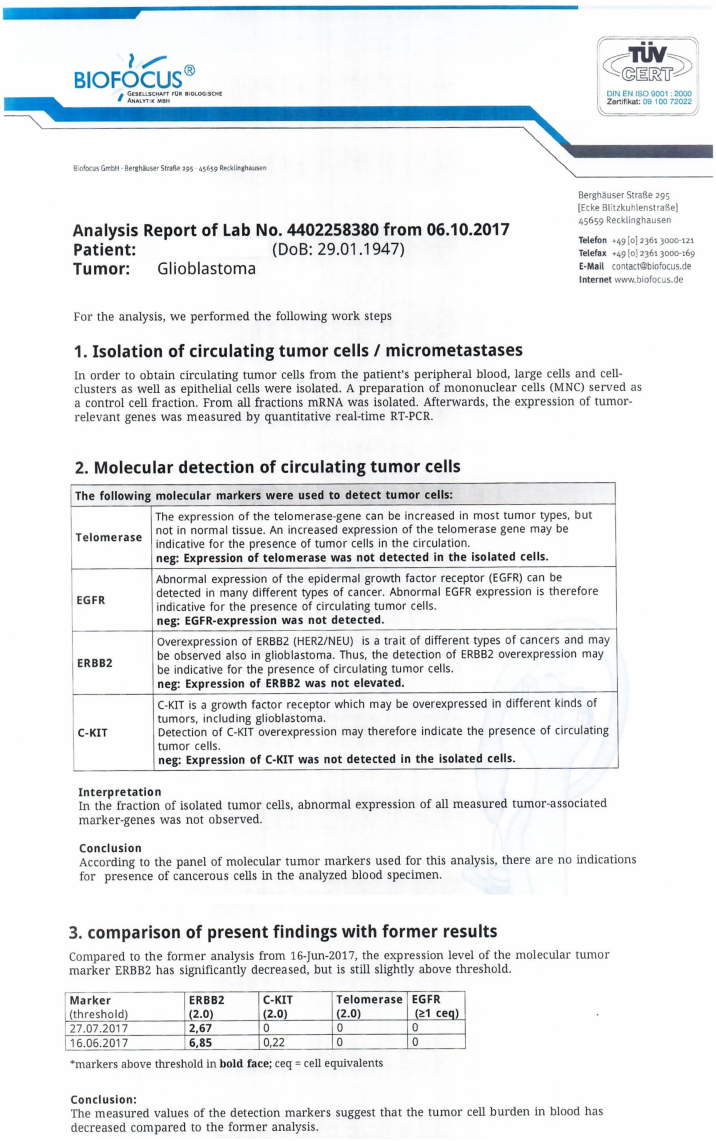
She received 10 IV treatments completed on 6/20/17. All symptoms improved, including ability to walk and her requirement for steroids. Her physical examination post therapy showed improved balance and coordination. Her brain MRI on 6/20/17 showed no progression of disease and no new findings were reported. Post surgical findings were stable. Her ECOG score was reduced two points at this time. She was off Decadron, starting (5/31/17).

She was asymptomatic post treatment, except some heavy left foot (slight drop) otherwise her neurological symptoms have all recovered, including left hand disco-ordination.

By 8/15/17, she had received 28 treatments, where the notes indicate that she was walking better and was more stable, no difficulty with her balance noticed. Her brain MRI on 8/24/17 showed stable.

On 7/27/17 she had repeated circulating tumor cells (CTC) analysis which showed response to the IV Quercetin with reduction of the ERBB and Kit markers (please see **Figure 1**/biofocus1). Further on 10/6/17, she had third sample which showed complete resolution of all circulating tumor cells. (Please see **Figure 2**/biofocus2).

She underwent at least three brain MRIs post therapy, while under our care. (6/20, 7/6/17 8/24/17) Her brain multisequence imaging/MRI on 6/20/17 showed



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Analysis Report of Lab No. 4402258380 from 06.10.2017
Patient: (DoB: 29.01.1947)
Tumor: Glioblastoma

For the analysis, we performed the following work steps

1. Isolation of circulating tumor cells / micrometastases

In order to obtain circulating tumor cells from the patient's peripheral blood, large cells and cell-clusters as well as epithelial cells were isolated. A preparation of mononuclear cells (MNC) served as a control cell fraction. From all fractions mRNA was isolated. Afterwards, the expression of tumor-relevant genes was measured by quantitative real-time RT-PCR.

2. Molecular detection of circulating tumor cells

The following molecular markers were used to detect tumor cells:	
Telomerase	The expression of the telomerase-gene can be increased in most tumor types, but not in normal tissue. An increased expression of the telomerase gene may be indicative for the presence of tumor cells in the circulation. **neg: Expression of telomerase was not detected in the isolated cells.**
EGFR	Abnormal expression of the epidermal growth factor receptor (EGFR) can be detected in many different types of cancer. Abnormal EGFR expression is therefore indicative for the presence of circulating tumor cells. **neg: EGFR-expression was not detected.**
ERBB2	Overexpression of ERBB2 (HER2/NEU) is a trait of different types of cancers and may be observed also in glioblastoma. Thus, the detection of ERBB2 overexpression may be indicative for the presence of circulating tumor cells. **neg: Expression of ERBB2 was not elevated.**
C-KIT	C-KIT is a growth factor receptor which may be overexpressed in different kinds of tumors, including glioblastoma. Detection of C-KIT overexpression may therefore indicate the presence of circulating tumor cells. **neg: Expression of C-KIT was not detected in the isolated cells.**

Interpretation
In the fraction of isolated tumor cells, abnormal expression of all measured tumor-associated marker-genes was not observed.

Conclusion
According to the panel of molecular tumor markers used for this analysis, there are no indications for presence of cancerous cells in the analyzed blood specimen.

3. comparison of present findings with former results

Compared to the former analysis from 16-Jun-2017, the expression level of the molecular tumor marker ERBB2 has significantly decreased, but is still slightly above threshold.

Marker (threshold)	ERBB2 (2.0)	C-KIT (2.0)	Telomerase (2.0)	EGFR (≥1 ceq)
27.07.2017	**2,67**	0	0	0
16.06.2017	**6,85**	0,22	0	0

*markers above threshold in **bold face**; ceq = cell equivalents

Conclusion:
The measured values of the detection markers suggest that the tumor cell burden in blood has decreased compared to the former analysis.

Figure 1. Title is "Circulating tumor cells".

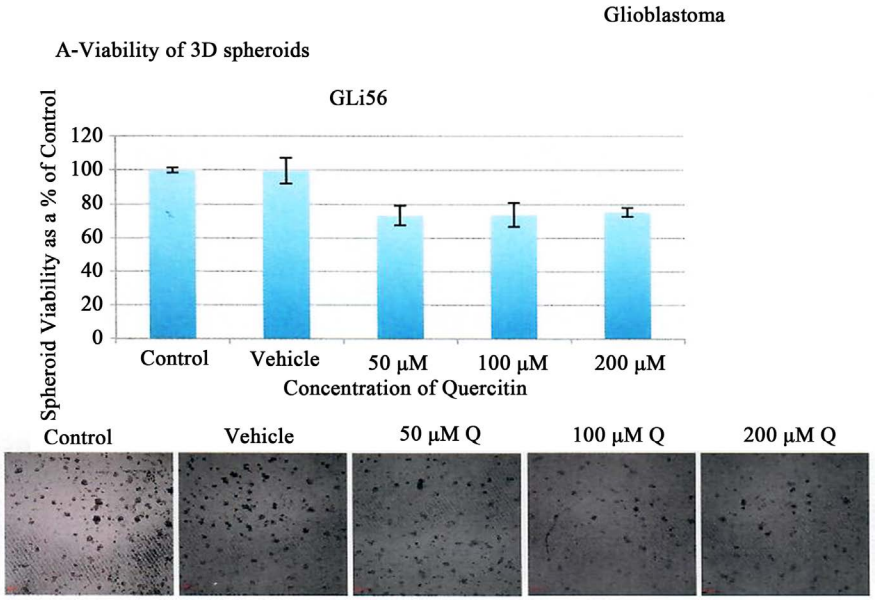

Figure 2. Title is "Glioblastoma spheroid viability assay".

positive response to the therapy, and that her necrotic tumor was decreased in size. Maximal dimension of the post surgical cavity had decreased from 4 to 3.7 cm. Further MRIs continued to show post surgical irregular dominant enhancements and patchy right periventricular satellite enhancements in frontal horn, all stable. Restricted diffusion in right centrum semiovale white matter was contributed to cellular non enhancing tumor.

Her last treatment was provided on 11/3/17. In our experience the therapy was feasible and well tolerated, that said the limiting factor was the requirement for frequent office visits for infusions.

Patient passed away from opportunistic infection and sepsis on 12/9/17, where her disease had regressed in our findings. She lived twice as her expected disease biological prognosis.

3. Discussion

Despite the significant recent efforts in research and drug development aimed at treatment of advanced Glioblastoma, yet the prognosis of the disease is grave. Significant biological barriers are blamed for the failure of therapies, including radiation at it's the best technologies. Cellular migration seems to be a key element responsible for the progressive nature of this disease. Further enhanced drug delivery techniques are also being investigated including nano technology to increase the bioavailability of the antineoplastic agents. Application of gamma knife is documented to increase this penetration. By application of antimigratory agent Quercetin alone or in combination with radiation and chemotherapy, we have shown increased cellular apoptosis, inhibition of angiogenesis and further tumor necrosis and here we believe such therapy has provided a safe and effective measure in improving patient quality of life and survival.

4. Conclusion

Administration of IV quercetin in combination with leading edge gamma knife and STUPP protocol is feasible and in this case provided improved response and quality of life. Patient passed her expected survival. Further studies are required to investigate such combinational approach using Quercetin as an orphan drug designated for treatment of Glioblastoma multiforme.

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