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# Treatment of glioblastoma with re-purposed renin-angiotensin system modulators: Results of a phase I clinical trial

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ABSTRACT

Glioblastoma is the most common and most aggressive primary brain cancer in adults. Standard treatment of glioblastoma consisting of maximal safe resection, adjuvant radiotherapy and chemotherapy with temozolomide, results in an overall median survival of 14.6 months. The aggressive nature of glioblastoma has been attributed to the presence of glioblastoma stem cells which express components of the renin-angiotensin system (RAS). This phase I clinical trial investigated the tolerability and efficacy of a treatment targeting the RAS and its converging pathways in patients with glioblastoma. Patients who had relapsed following standard treatment of glioblastoma who met the trial criteria were commenced on dose-escalated oral RAS modulators (propranolol, aliskiren, cilazapril, celecoxib, curcumin with piperine, aspirin, and metformin). Of the 17 patients who were enrolled, ten completed full dose-escalation of the treatment. The overall median survival was 19.9 (95% CI:14.1–25.7) months. Serial FET-PET/CTs showed a reduction in both tumor volume and uptake in one patient, an increase in tumor uptake in nine patients with decreased (n = 1), unchanged (n = 1) and increased (n = 7) tumor volume, in the ten patients who had completed full dose-escalation of the treatment. Two patients experienced mild side effects and all patients had preservation of quality of life and performance status during the treatment. There is a trend towards increased survival by 5.3 months although it was not statistically significant. These encouraging results warrant further clinical trials on this potential novel, well-tolerated and cost-effective therapeutic option for patients with glioblastoma.



## 1. Introduction

Glioblastoma or WHO grade IV astrocytoma is the most common and most aggressive primary brain cancer in humans. It is characterized by microvascular endothelial proliferation and central necrosis [1]. Current standard treatment of glioblastoma consists of maximal safe surgical resection with adjuvant radiotherapy and chemotherapy with temozolomide [2], an alkylating agent that promotes methylation of O[6]-methylguanine-DNA methyltransferase (MGMT) [3] which is associated with an improved survival of glioblastoma patients [4]. Despite this intensive treatment, tumor recurrence in glioblastoma patients is inevitable with an overall median survival time of 14.6 months with a

*Abbreviation:* ACEIs, angiotensin-converting enzyme inhibitors; ARBs, angiotensin receptor blockers; CI, confidence interval; CSCs, cancer stem cells; GSCs, glioblastoma stem cells; IDH, isocitrate dehydrogenase; MGMT, methylation of O [6]-methylguanine-DNA methyltransferase; QoL, quality of life; RAS, renin-angiotensin system.

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mean range of 12–14 months, which has not changed since 2005 [5,6].

The aggressive nature of glioblastoma has been attributed to the presence of glioblastoma stem cells (GSCs), a small sub-population of cancer cells in glioblastoma imbued with pluripotency properties and the capacity for perpetual self-renewal and proliferation [7,8]. These GSCs are responsible for tumor growth and recurrence after serial transplantations [9]. The presence of such GSCs is well-supported in the literature and their interaction with the extracellular matrix and tumor microenvironmental factors, including transforming growth factor-β and hypoxia, may contribute to their resistance to radiotherapy and chemotherapy [10]. Targeting GSCs may open a therapeutic option that may improve both overall survival and progression-free survival of patients with glioblastoma [11].

Multiple links exist between the renin-angiotensin system (RAS) and cancer stem cells (CSCs) including the expression of components of the RAS by GSCs [12]. Pro-renin receptor is crucial for glioma development via the Wnt/β-catenin signaling pathway [13], upstream of the RAS. Expression of components of the RAS by CSCs has also been reported in other cancer types [14–20]. The RAS in the bone marrow can mediate hematopoietic cell production [21] and plays a role in the hemangioblast fate decision to form either blood cells or endothelial cells [22]. Angiotensin II has been found to enhance the CSC phenotype of lung cancer cells [23]. Renin is present in glioblastoma and may contribute to the mechanisms of neo-vascularization in glioblastoma [24]. Furthermore, downregulation of the Ang(1–7)/MAS axis by podocalyxin leads to enhanced glioblastoma cell invasion and proliferation [25]. Cathepsins B, D and G which constitute bypass loops of the RAS, are expressed in glioblastoma [26,27] with cathepsins B and D being expressed by GSCs [28]. The RAS also plays a role in the CSCs [29] and tumor microenvironment [30] in glioblastoma.

Medications such as β-blockers [31], angiotensin-converting enzyme inhibitors (ACEIs) [32], aliskiren that blocks renin [33], angiotensin receptor blockers (ARBs) [34], non-steroidal anti-inflammatory drugs [35,36], that inhibit the RAS, its bypass loops and converging pathways are off-patent and are in common use. Furthermore, metformin that inhibits the insulin growth factor/insulin growth factor receptor-1 pathway [37], and curcumin that inhibits cathepsin B [38,39] result in inhibition of RAS activity (Suppl. Fig. 1). These medications have been proposed as anti-cancer therapies and may offer therapeutic options of targeting GSCs in glioblastoma, and CSCs in other cancers [12,40]. Review of epidemiological studies have demonstrated a reduced risk of cancer and improved survival of cancer patients taking medications that modulate the RAS [41].

There has been interest in using combinations of cytotoxic drugs, vascular endothelial growth factor blockers and checkpoint inhibitors to enhance outcomes of standard treatment whilst maintaining the quality of life (QoL) of patients with glioblastoma, however, to date no particular combination has been shown to prolong overall survival over the Stupp protocol [2,42,43]. The CUSP-9 protocol, using nine re-purposed drugs, following the standard treatment of glioblastoma has also shown no significant improvement of overall survival [44,45].

As GSCs express components of the RAS, and RAS modulators have been found to have anti-cancer properties, we propose that inhibiting the RAS using a combination of oral RAS modulators, may offer a novel therapeutic option for patients with glioblastoma who have exhausted conventional treatment options.

## 2. Methods

We conducted an open-label proof-of-concept phase I clinical trial investigating the safety of a combination of RAS modulating drugs in slowing the progression of glioblastoma and/or preserving the QoL and performance status, and improving the overall median survival of patients with glioblastoma who had exhausted conventional treatment options. Such patients were generally expected to have limited life expectancy with deteriorating QoL. Each patient served as their own control. This study was approved by the Central Health and Disability Ethics Committee (ref. no. 17/CEN/8) and the Standing Committee for Therapeutic Trials (ref. no. cancerstudyRAS1) and was registered with the Australian New Zealand Clinical Trials Registry (ref. no. ACTRN12619001078145). Written consent was obtained from all participants.

The oral medications administered in this study were propranolol, aliskiren, cilazapril, celecoxib, curcumin with piperine, aspirin and metformin, to block the key steps of the RAS (Suppl. Fig. 1). Piperine (an active ingredient of pepper) was included in the curcumin formulation to increase the bioavailability of curcumin [46]. As there are multiple steps within the RAS pathway the treatment regimen was designed to inhibit as many of these steps as possible to reduce the production of angiotensin effector peptides. The treatment regimen was initiated by introducing these RAS modulating drugs in a stepwise fashion and the dosages were escalated over a period of ten weeks (Suppl. Table 1). The fully dose-escalated treatment regimen was then maintained for the entire duration of the study unless there were significant side effects, or if it provided no benefit to the patient(s), or if the patient (s) exited the study.

If the patient was already taking an ACEI and/or a β-blocker, they would be substituted with the equivalent dose of cilazapril and/or propranolol, respectively. If the patient developed a cough associated with cilazapril, then cilazapril was discontinued and losartan (150 mg daily) added. If the patient was already taking metformin prior to the study, then the dosage of the medication would continue and increased to 500 mg twice daily, if necessary. Omeprazole 20 mg daily was administered to mitigate the risk of gastrointestinal bleeding whilst on aspirin.

### 2.1. Eligibility criteria

Patients with glioblastoma who had relapsed and had exhausted conventional treatment options, had a Karnofsky performance score of at least 60, and did not meet the exclusion criteria listed in Supplementary Table 1, were enrolled in this study.

### 2.2. Monitoring

Clinical examination, including blood pressure measurements, was performed and data was collected at 2-weekly intervals during the medication escalation period and thereafter at 3 monthly intervals until exit from the trial. Baseline electrolytes and creatinine levels and full blood count were measured and repeated during escalation of treatment 2 weeks after a change in dosage of either aliskiren or cilazapril (or losartan), and 3-monthly afterwards. The date and the reason(s) for exiting the trial, and the date and cause of death, were documented as appropriate.

### 2.3. Data collection

Patient demographic details including age, gender, co-morbidities, smoking history, alcohol use, medications including the type and dosage of RAS modulators, aspirin and other non-steroidal anti-inflammatory drugs, and anti-diabetic treatment were collected. Allergies and any contraindication to the trial medications, and details of the tumor at the original diagnosis and the response to previous treatment(s) were recorded and entered on the clinical trial database.

Each patient completed the EORTC QLQ-30 and EORTC QLQ-BN20 Questionnaires [58] and Karnofsky score prior to (baseline) and 2-monthly during treatment, to assess and record their QoL and performance status, until death or exit of the study. Patients whose Karnofsky scores fell below 60 were required to exit the study. Questions 1–28 of the EORTC Questionnaires assessed their general health and well-being while Questions 31–50 assessed brain-specific matters. These questionnaires were scored as 1 (not at all) to 4 (very much), i.e., a higher score indicates poorer QoL. For statistical analysis, these scores were aggregated and transformed into a percentage of the total score. Questions 29 and 30 asked the patients to rate their overall health and QoL, from 1 (very poor) to 7 (excellent), i.e., a high score indicates better health or QoL respectively. For statistical analysis, all these responses were also recorded as a percentage score.

Baseline and serial (3, 6, and 12 months following initiation of treatment) FET-PET/CTs were performed. The calculated volume (by multiplying the maximal cranio-caudal, sagittal and lateral dimensions) and the maximal avidity of the tumor(s), were recorded. The date and the reason(s) for exiting the trial, including death, and the cause of death, were documented as appropriate.

### 2.4. Statistical analysis

To calculate the overall survival from diagnosis, survival analysis methods were used, including Kaplan-Meier survival curves and the calculation of mean and median survival with 95% confidence intervals. Other time variables (e.g., time on study) were analyzed in the same way. QoL and performance scores were simply assessed graphically so that changes over time could be examined.

## 3. Results

Of the 28 patients with glioblastoma referred for the study, 11 were excluded for various reasons (Suppl. Table 2) according to the exclusion criteria, including three patients in whom no uptake of the tumor was demonstrated on FET-PET/CT (Suppl. Table 2). 17 (10 male and 7 female) patients, aged 19–75 (mean, 55.4; median, 56) years, were enrolled. Ten patients had full dose-escalation (at least ten weeks) of the treatment. Seven patients did not achieve full dose-escalation of the treatment and had 3–69 (average 16.7) days of treatment. Six of these patients had extensive tumor burden (Fig. 1) at enrolment, suffered rapid deterioration with a Karnofsky score of less than 60, and the remaining patient withdrew from the study 69 days following initiation of treatment. Patients who had full dose-escalation of treatment had, on average, 205 (range 105–398) days of treatment. All patients are now deceased.

The presence of isocitrate dehydrogenase (IDH) mutation and MGMT methylation of the glioblastoma of the participants, where available, are shown in Supplementary Table 3.

Serial FET-PET/CTs of the ten patients who had full dose-escalation of the treatment showed a reduction in both tumor uptake and volume in one patient (Fig. 2), an increase in tumor uptake in nine patients in whom there was decreased tumor volume in one patient, no change in tumor volume in another patient, and increased tumor volume in seven patients.

Fig. 3 shows the cumulative survival of the trial participants. The average time from diagnosis to death was 20.5 (s.d. 2.09; 95% CI: 16.4–24.6) months with a median overall survival of 19.9 (range 8.7–35.3; 95% CI: 14.1–25.7) months for the entire cohort. The average time from diagnosis to death was 23.8 (s.d. 3.8; median 24.0) months for patients who had full dose-escalation of the treatment, and 15.7 (s.d. 9.5; median 13.8) months for patients who did not have full dose-escalation of the treatment.

### 3.1. Quality of life

The patients who had completed full-dose escalation of the trial medications maintained their QoL throughout the study. The mean percentage scores for Q1-28, Q29, Q30 of the EORTC QLQ-30 Questionnaires, and Q31-50 of the EORTC QLQ-BN20 Questionnaires for the trial participants over time are shown in Fig. 4. Patients who did not complete full dose-escalation of the trial medications did not complete QoL measurements beyond their baseline measurements and so are not included in these graphs.

The means were between 0 and 50% at all time points for both percentage scores for Q1-28 and Q31-50, indicating mild to moderate difficulties both in general (Q1-28) and with respect to brain function (Q31-50). For Q29, the means were 50–75%, indicating moderately good general health, and for Q30 the means were 50–75%, up to 12 months before there was a slight decline. The confidence intervals were wider for the later times as fewer patients had data for analysis.

To determine if there was a difference at baseline QoL that was associated with the patient's likelihood of completing the therapy, a *t*-test was used to compare the means of the four summary variables between patients who had full dose-escalation of the treatment and those who did not. At baseline, the means for patients who had full dose-escalation of the treatment were lower than that for patients who did not have full dose-escalation of the treatment, as evidenced by the percentage scores for Q1-28 of 20.4 ± 14.9 vs 27.2 ± 8.1 and Q31-50 of 20.7 ± 10.6 vs 28.3 ± 14.7 indicating that patients who had full dose-escalation of the treatment had a better QoL. In addition, for Q29 and Q30, the means were higher for patients who had full dose-escalation of the treatment (63.3 ± 17.2 vs 59.5 ± 18.9 and 65.0 ± 21.4 vs 61.9 ± 15.9), again indicating a better health and QoL. However, the standard deviations were high, so these results did not achieve statistical significance (p-values 0.229–0.751).

There was no indication of a trend of either improvement or worsening QoL over time (regression analysis).

### 3.2. Performance status

The performance status of all trial participants, measured by Karnofsky scores, was maintained until death or exit from the study. Patients exited the study once their Karnofsky score was less than 60. Therefore, the length of time from diagnosis until study exit measures the time when the patients had good (Karnofsky $\geq$ 60) performance status. Overall, for the entire cohort, the mean time at Karnofsky > 60 was 18.4 months (95% CI: 13.9–22.8) and the median was 16.2 months (95% CI: 12.4–19.9). For patients who had full dose-escalation of the treatment, the mean was 21.7 (17.1–26.3) and the median 18.5 (range 10.5–26.5). Patients who did not have full dose-escalation of the treatment, the mean was 13.6 (range 6.0–21.3) and the median 11.1 (range 9.0–13.2). To further describe the time that patients maintained at Karnofsky > 60, the duration above 60 is grouped in Table 2.

### 3.3. Side effects

One patient developed a marginal bradycardia of 58 beats/minute (60 beats/minute at baseline) attributed to propranolol. One further patient developed indigestion possibly related to curcumin. However, symptoms resolved without the need to cease curcumin. No patient developed hypotension and serial blood tests showed no deterioration of renal function.

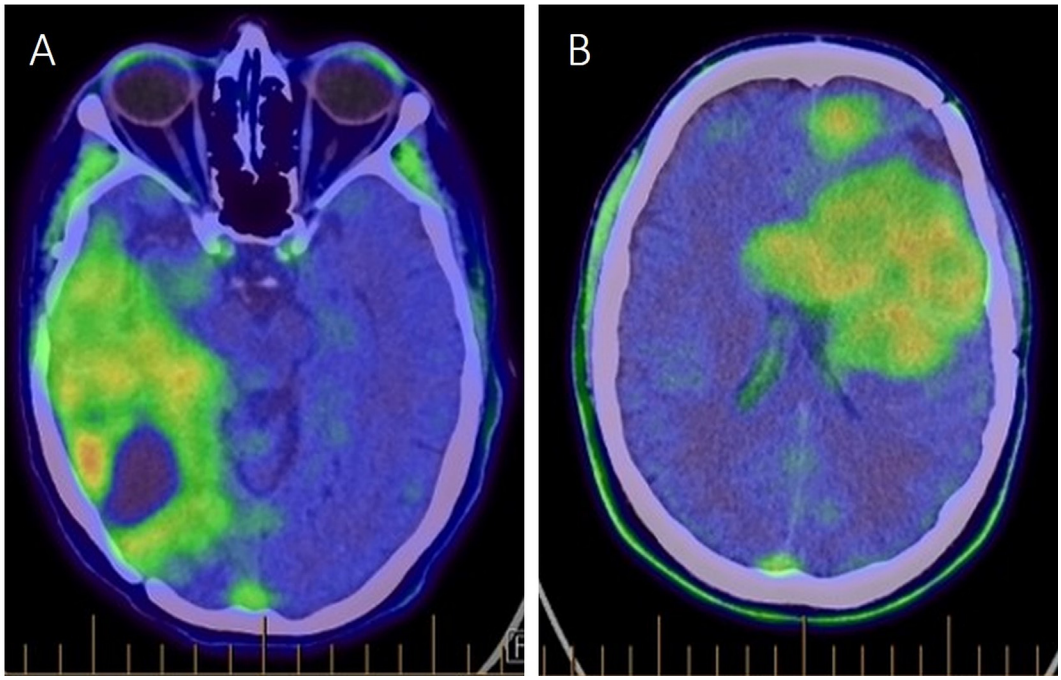


**Fig. 1.** FET-PET/CTs showing extensive recurrent glioblastoma in a 60-year-old female patient (A), and a 31-year-old male patient (B) who did not have full escalation of the trial treatment.

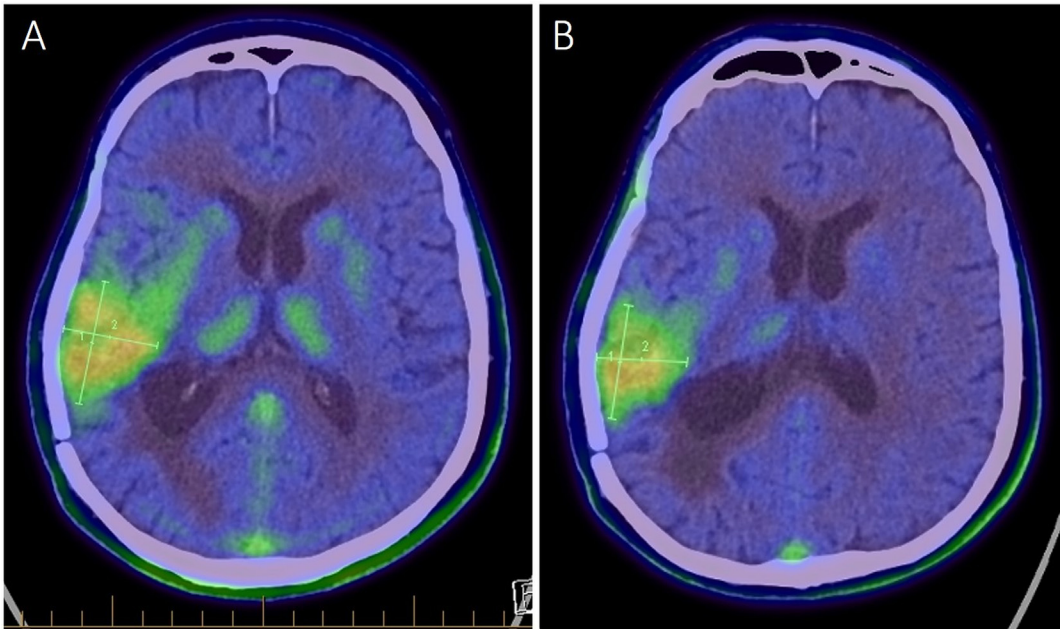


**Fig. 2.** FET-PET/CTs of a 65-year-old male patient with glioblastoma who had full dose-escalation of the trial treatment, beore (A), and 3 months following initiation of the trial medications (B) showing reduced tumor volume (from 32 $cm^3$ to 19 $cm^3$) and the mean standardized uptake values (from 4.1 to 3.8).

## 4. Discussion

This phase I clinical trial demonstrates the treatment regimen consisting of a combination of modulators of the RAS and its converging pathways for patients with glioblastoma is well-tolerated with minimal side effects. The average survival of the entire cohort was 20.5 months, with a median overall survival of 19.9 months. Although this compares favorably to the 12–14 months of median overall survival following standard treatment for glioblastoma patients [6], it is not statistically significant (95% CI: 14.1–25.7 months) given the small sample size. Another limitation of this study is that there may be a potential bias in that median survival time is predicated on the date of diagnosis. As the study was not a randomized control trial, patients acted as their own controls although this is acceptable for a feasibility study. Rapid deterioration of the patients who had advanced disease with a large tumor burden demonstrated on FET-PET/CTs, contributed to the seven patients not completing full dose-escalation of the trial treatment. Despite six of these patients having MGMT methylation they had a mean survival of 15.7 (s.d. 9.5; median 13.8) months, compared

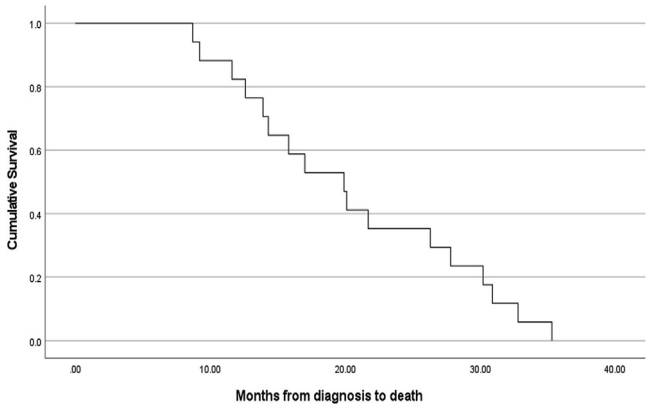


**Fig. 3.** Kaplan-Meier survival curve of 17 patients with glioblastoma treated with the trial medications. All patients entered the trial ≥ 9 months following conventional treatment.

with 23.8 (s.d. 3.8; median 24.0) months for the patients who completed full dose-escalation of the treatment, suggesting a survival benefit for the latter. Moreover, patients who had full dose-escalation of the trial treatment maintained their Karnofsky scores of > 60 for a mean of 21.7 (range 17.1–26.3) months, reflecting reasonable control of their glioblastoma, good tolerance of the trial medications and consequently maintenance of a satisfactory QoL until death. However, these results may be limited by the fact that IDH mutation was present in two patients and MGMT methylation was present in five patients. Consequently, these patients may have been expected to survive longer independent of other factors such as administration of RAS-modulating drugs. Notwithstanding the lack of statistically significant overall survival benefit, the low incidence of adverse effects, and the survival outcomes are favorable compared to other studies using bevacizumab and pembrolizumab which show a higher rate of adverse effects with no survival benefit for patients with recurrent glioblastoma [47,48].

Three of the 28 patients referred for the trial who showed no uptake on FET-PET/CT despite relapse demonstrated on MRI scans, were excluded from this trial. An MRI scan is an adequate imaging tool in documenting treatment-naive glioblastoma and is typically used in the clinical setting to monitor glioblastoma following treatment [49]. However, FET-PET/CTs are increasingly used as they offer advantages in determining active pre-treatment tumor, tumor volume and tumor recurrence following treatment [50]. Current trends value FET-PET/CTs not as a surrogate marker for the tumor but a direct measurement and an anatomical map of greater amino acid metabolism of the tumor cells, compared to normal brain tissue exhibiting less protein synthesis [51]. The cohort of patients who failed to complete full dose-escalation of the trial medications had a greater volume of tumor burden at the time of recruitment seen on FET-PET/CTs. Early FET-PET/CTs in our study protocol could detect early active tumor recurrence, but could also show the absence of active tumor indicated by MRI scan, as with the three patients excluded from our study. Further to early FET-PET/CTs in glioblastoma post-operatively to reduce margins of radiotherapy [52], we would suggest the value of using FET-PET/CTs routinely in the clinical setting.

This study shows the use of a combination of repurposed commonly prescribed oral RAS modulators is well-tolerated in patients with glioblastoma with minimal adverse effects. This study has shown a trend towards an improvement in survival. A phase II/III clinical trial, with earlier introduction of this treatment regimen is warranted to further investigate this novel, well-tolerated and cost-effective therapeutic option for patients with glioblastoma. Furthermore, these results may support the proposed role for the

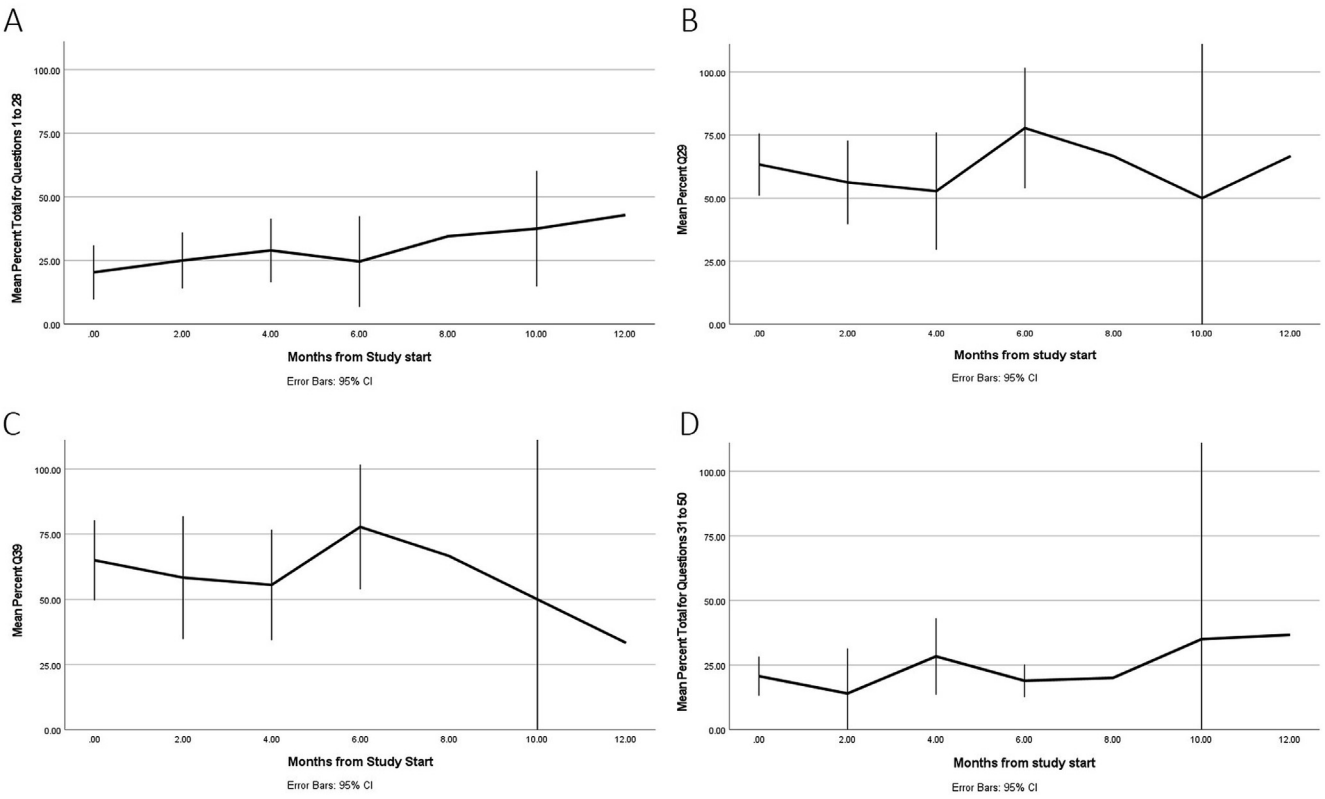


**Fig. 4.** Mean percentage scores for Q1-28 (A), Q29 (B) and Q30 (C) of the EORTC QLQ-30 Questionnaires, and Q31-50 of the EORTC QLQ-BN20 Questionnaires (D), over time for patients who had full dose-escalation of the trial treatment.

RAS in GSCs in glioblastoma and underscores the need for further research from bedside to bench to unravel these complex signal pathways.

## 5. Fundings

The authors thank Pacific Radiology for performing FET-PET/CTs, and Cycloteck Pharmaceuticals for providing the radioisotope for the FET-PET/CTs, gratis for this study. This study was otherwise funded by the general fund from the Gillies McIndoe Research Institute from philanthropic donations.

## 6. Ethics statements

This study was approved by the Central Health and Disability Ethics Committee (ref. no. 17/CEN/8). Written consent was obtained from all participants.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: ST is an inventor of the patents Cancer Diagnosis and Therapy (PCT/NZ2015/050108), Cancer Therapeutic (PCT/NZ2018/050006), Novel Pharmaceutical Compositions for Cancer Therapy (US/62/711709) and Cancer Diagnosis and Therapy (United States Patent No. 10281472). All other authors are not aware of any conflicts of interest.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jocn.2021.11.023.

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