Title

A study of the focal adhesion kinase inhibitor GSK2256098 in patients with recurrent glioblastoma with evaluation of tumor penetration of [^{11}C]GSK2256098

Authors

Nicholas F. Brown, Matthew Williams, Hendrik-Tobias Arkenau, Ronald A. Fleming, Jerry Tolson, Li Yan, Jianping Zhang, Lisa Swartz, Rajendra Singh, Kurt R. Auger, Laurie Lenox, David Cox, Yvonne Lewis, Christophe Plisson, Graham Searle, Azeem Saleem, Sarah Blagden, Paul Mulholland

Affiliations

NIHR UCLH Clinical Research Facility, University College London Hospitals NHS Foundation Trust, 4th Floor, 170 Tottenham Court Road, London, W1T 7HA (NB)(PM)

Department of Oncology, UCL Cancer Institute, 72 Huntley Street, London, WC1E 6DD. UK (NB)(PM)(TA)

Computational Oncology Lab, Institute of Global Health Innovation, First floor, Faculty Building, South Kensington Campus, Imperial College, London, SW7 2AZ. UK (MW)

Radiotherapy Department, Charing Cross Hospital, Fulham Palace Road, London, W6 8RF. UK (MW)

Sarah Cannon Research Institute UK, 93 Harley Street, London, W1G 6AD. UK (TA)

GlaxoSmithKline, Research Triangle Park, Five Moore Drive, NC 27709-3398, USA (RF)(JT)(LY)

PAREXEL International, 2520 Meridian Parkway, Suite 200, Durham, NC 27713, USA (JZ)(LS)

GlaxoSmithKline, 1250 S Collegeville Road, Collegeville, PA 19426, USA (RS)(KA)(LL)

GlaxoSmithKline, 980 Great West Road, Middlesex, TW8 9GS. UK (DC)

Imanova Ltd, Centre for Imaging Sciences, Burlington Danes Building, Du Cane Road, London W12 0NN (YL)(CP)(GS)(AS)

NIHR/Wellcome Trust Imperial CRF, Imperial Centre for Translational and Experimental Medicine, Hammersmith Hospital, W12 0HS. United Kingdom. (SB)

Running Title

GSK2256098 in recurrent glioblastoma

Corresponding Author

Dr. Paul Mulholland, NIHR UCLH Clinical Research Facility, University College London Hospitals NHS Foundation Trust, 4th Floor, 170 Tottenham Court Road, London, W1T 7HA

Telephone: +44 20 3447 4437 *Fax:* +44 20 3447 0955 *E-mail*: paul.mulholland@nhs.net

Funding

This study was sponsored by GlaxoSmithKline

Conflicts of interest

JT, LY, KA are employees of GlaxoSmithKline (GSK). RA, AZ are former employees of GSK. RA, AZ, JT own stock in GSK. KA has a pending patent related to this study (WO 2013/003575 A1). CP, GS are employees of Immanova UK (previously part of GSK) which performed and was paid for scanning services for this study and performed work for GSK outside of this study.

Total Word Count

5199

Abstract

Background GSK2256098 is a novel oral focal adhesion kinase (FAK) inhibitor. Preclinical studies demonstrate growth inhibition in glioblastoma cell lines. However, studies in non-diseased rodents indicate limited blood-brain barrier penetration. In this expansion cohort within a phase I study the safety, tolerability, pharmacokinetics and clinical activity of GSK2256098 was evaluated in patients with recurrent glioblastoma. Biodistribution and kinetics of [^{11}C]GSK2256098 in tumors and normal brain was assessed in a sub-study using positron-emission tomography (PET).

Methods Patients were treated with GSK2256098 until disease progression or withdrawal due to adverse events (AEs). Serial pharmacokinetic samples were collected on day 1. On a single day between days 9-20, patients received a microdose of intravenous [^{11}C]GSK2256098 and scanned with PET over 90 minutes. Response was assessed by MRI scans every 6 weeks.

Results 13 patients were treated in three dose cohorts (1000mg, 750mg, 500mg, all dosed twice daily). The maximum tolerated dose was 1000mg twice daily. Dose-limiting toxicities were related to cerebral edema. Treatment-related AEs (>25%) were diarrhea, fatigue and nausea. Eight patients participated in the imaging sub-study, with [^{11}C]GSK2256098 V_T estimates of around 0.4 in normal brain, and 0.9 in tumor tissue. Best response of stable disease was observed in 3 patients, including one patient on treatment for 11.3 months.

Conclusions GSK2256098 was tolerable in patients with relapsed glioblastoma. GSK2256098 crosses the blood-brain barrier at low levels into normal brain, but at markedly higher levels into tumor consistent with tumor associated blood-brain barrier disruption. Further clinical trials of GSK2256098 are ongoing.

Keywords

GSK2256098, focal adhesion kinase, glioblastoma, PET

Importance of the Study

This is the first clinical trial to evaluate a focal adhesion kinase (FAK) inhibitor in glioblastoma. Increased FAK expression is observed in glioblastoma and is associated with a negative prognosis. Whilst the FAK inhibitor GSK2256098 demonstrates preclinical activity in glioblastoma cell lines and xenografts, pharmacokinetic studies in rodents demonstrate limited central nervous system penetration in the presence of an intact blood-brain barrier. We evaluated blood-brain barrier penetration through assessment of [^{11}C]GSK2256098 bio-distribution and kinetics. Tumor penetration of [^{11}C]GSK2256098 was observed in all participants with limited penetration into surrounding normal brain. Tumo~~u~~r concentrations of [^{11}C]GSK2256098 exceeded those association with antitumor activity in preclinical studies. GSK2256098 was well tolerated in patients with recurrent glioblastoma. These data support further clinical trials of GSK2256098 in patients with central nervous system tumors, which are ongoing.

Introduction

Glioblastoma is the most common malignant primary brain tumor~~, with approximately 2,200 cases diagnosed each year in England, and over 11,500 cases diagnosed annually in the USA (1, 2)~~. Median survival is less than a year and treatment with ~~The~~ standard ~~treatment~~ therapy of surgery followed by radiotherapy and temozolomide chemotherapy in clinical trials results in a median survival of 14.6 months and 5-year overall survival of less than 10%.[1-3] The addition of tumor treating fields has recently been reported to prolong survival.[4] No clinical trials have demonstrated a survival improvement for second-line therapies. ~~Improved treatments are required both in the first-line setting and at relapse.~~

Focal adhesion kinase (FAK) is a non-receptor tyrosine kinase that functions at the site of attachment between cells and the extracellular matrix. It supports focal-adhesion scaffolding and integrates signals from integrins and growth factor receptors.[5,6] FAK is phosphorylated at tyrosine 397 during activation and phosphorylated FAK (pFAK) forms a binary complex with the Src family kinases. Downstream signal transduction of FAK can trigger multiple intracellular signaling pathways that are implicated in cell survival, signaling, growth, adhesion, migration and invasion.[5-7] In addition, FAK is also associated with the protection of cells from anoikis through the sequestration of receptor-interacting protein from the death-receptor machinery, and with regulation of chemokine transcription.[5,8]

Overexpression of FAK mRNA or protein is documented in many solid tumors, including glioblastoma.[5,6,9-14] FAK expression is increased in metastatic disease compared with normal and early stage disease indicating that it may be a marker of invasive potential.[15,16] FAK protein expression is elevated in human glioblastoma relative to normal brain tissue.[9,17] There is increased FAK and pFAK expression in higher-grade versus lower-grade gliomas which are associated with poorer survival.[18] FAK expression is associated with angiogenesis in high grade gliomas but not in normal brain tissue, and there is evidence that FAK promotes angiogenesis in glioma by activating endothelial cell migration.[10]

GSK2256098 is a potent, ATP-competitive, reversible inhibitor of FAK with enzymatic half maximal inhibitory concentration values of 1.5 nmol/L. It has approximately 1000-fold specificity for FAK over the closest family member Pyk2.[19] In assays of 95 cell lines, glioblastoma cell lines were some of the most sensitive to GSK2256098 (GSK internal data). In preclinical studies, dose and time-dependent inhibition of pFAK was observed in subcutaneous xenograft models with mice bearing U87MG (glioma) human tumors, with correlation between pFAK inhibition and blood concentration of GSK2256098.[19] Preclinical studies in ~~mice~~ rats indicate limited central nervous system (CNS) penetration of GSK2256098 in the presence of an intact blood-brain barrier, with brain:plasma concentrations of 0.08, 0.06 and 0.07 at 20,40, and 60 minutes post a single oral dose, and 0.12, 0.35 and 0.45 at 6 hours post dose following a 6 hour IV infusion (6ml/kg/hr). GSK2256098 is a substrate of p-glycoprotein (Pgp) an efflux pump implicated in poor blood-brain barrier penetration of many drugs (efflux ratio of 5.0 with 3μM GSK2256098 and 47% inhibition of digoxin transport [probe Pgp substrate] with 100μM in MDCKII-MDR1 cell lines).~~an efflux pump implicated in poor blood-brain barrier penetration of many drugs~~ [20] However, in glioblastoma the blood-brain barrier is disrupted,[21-23] potentially permitting tumor penetration of GSK2256098.

A phase I open-label clinical trial of GSK2256098 has been conducted in patients with advanced non-CNS cancers.[24] It enrolled 62 patients with advanced cancers in dose-escalation and expansion cohorts. GSK2256098 was well tolerated, and the declared maximum tolerated dose was 1000 mg twice daily. Dose-limiting toxicities (DLTs) were Grade 2 proteinuria (1000 mg), Grade 2 nausea, vomiting, fatigue (1250 mg), and Grade 3 asthenia and Grade 2 fatigue (1500 mg). Minor responses were observed in four patients. These findings, combined with the encouraging preclinical evidence, raised the question of whether the drug was active in patients with glioblastoma. Here we report the safety, systemic pharmacokinetics, and CNS pharmacokinetics of GSK2256098 in an expansion cohort of patients with glioblastoma. Evidence of CNS and tumor penetration of [^{11}C]GSK2256098 in participants was obtained using PET imaging.

Methods

Ethics

This study was conducted in accordance with the Declaration of Helsinki, the principles of Good Clinical Practice (GCP) and applicable clinical trials regulations. Study conduct was approved by the London (Chelsea) Research Ethics Committee (11/LO/0551) and the Medicines and Healthcare Regulatory Agency (MHRA). All participants provided written informed consent prior to participation in the study. The trial was registered with Clinicaltrials.gov (NCT01138033).

Study design & patients

FAK113517 was a phase I open-label, non-randomized, multicenter study of GSK2256098. In this expansion cohort patients were recruited at three sites within the United Kingdom. GSK2256098 was initially administered at the maximum tolerated dose (MTD) of 1000 mg twice daily [24].

Key eligibility criteria for recruitment: glioblastoma at first or second recurrence; progressive disease by RANO criteria; prior temozolomide-based chemoradiotherapy; have measurable disease by RANO criteria; age ≥ 18 years; Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1; and adequate hematologic, hepatic, and renal function. Key exclusion criteria included active gastrointestinal disease that may impair drug absorption, prolonged QTc, history of acute coronary syndrome, and heart failure with limitation of physical activity. CYP3A4 inhibitors and inducers were excluded within the prior 7 and 14 days respectively. Sensitive substrates of CYP3A4, CYP2C8, CYP2C9, and OATP1B1 were excluded within the prior 7 days.

Endpoints

The primary objectives of the study were to assess the safety and tolerability of GSK2256098 in patients with relapsed glioblastoma at the MTD determined in systemic cancers, with secondary objectives of characterizing PK and anti-tumor activity. The objectives of the PET imaging substudy were to assess the biodistribution and kinetics of [^{11}C]GSK2256098, and to estimate the quantity of [^{11}C]GSK2256098 in tumors and normal brain.

Assessments

Safety and tolerability. Pretreatment patient evaluation included medical history, physical examination, ECOG performance status, vital signs, blood tests (haematology, biochemistry, coagulation profiles), ECG, and urinalysis. These were repeated at specified timepoints throughout the study. Toxicities were graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE), version 4.0. Toxicities occurring in the first 21 days of treatment were considered for determining dose-limiting toxicities (DLTs).

Tumor Assessment. Brain MRI with contrast was performed at baseline and every 6 weeks. Response evaluations were made according to the RANO criteria.[25]

Pharmacokinetics. Blood samples were collected for PK assessment pre-dose, and at 1, 1.5, 2, 3, and 4 hours following Day-1 dose; and pre-dose on days 8, 15, 22 and 43. GSK2256098 concentrations were determined by ultra high performance liquid chromatography-tandem mass spectrometry (assay range 10-10,000 ng/mL).

Imaging sub-study. A positron emission tomography (PET)- computed tomography (CT) scan was performed on a single day between day 9 and 20, after intravenous injection of carbon-11 radiolabeled [^{11}C]GSK2256098, 2 hours after oral dosing of GSK2256098 (to coincide with expected peak plasma concentration). A CT scan of the head was performed in order to estimate tissue attenuation. A 90-minute cranial dynamic PET scan was then performed during and following a bolus intravenous administration of a microdose (<10 μg) of [^{11}C]GSK2256098. Continuous arterial blood sampling was performed for the first 15 minutes of the scan for blood radioactivity measurements. Additionally, arterial blood samples for radioactivity counting and radio-HPLC metabolite analysis were collected during the the PET scan. Blood was collected for PK sampling prior to oral dosing, and at 1, 2 and 4 hours post oral dosing. Whole blood and plasma radioactivity measurements were used to calculate time-activity curves (TACs) of radioactivity concentration in blood and plasma. Measured radiometabolite data were used to correct these curves and derive the time course of

[^{11}C]GSK2256098 in plasma. The supplementary materials (S1, Table S1, Figures S2-3) give additional PET methodologies.

The dynamic PET images were reconstructed and corrected for attenuation, randoms, and scatter. Dynamic PET images were registered to each subject's MR images and corrected for motion using a frame to frame registration process with a normalized mutual information cost function. Regions of interest (ROIs) were manually delineated on the [^{11}C]GSK2256098 PET scan corresponding visually to areas of [^{11}C]GSK2256098 uptake in the PET scan (*PET-enhanced* regions) and in the normal brain tissue (*normal brain region*) away from the tumor. ROIs were also manually delineated on MR images obtained for clinical purposes on a day prior to the PET-CT scan and to correspond with gadolinium enhancing regions on T1 post-contrast sequences (*T1 Gd-enhanced region*) and regions with increased T2 FLAIR signal (*T2-FLAIR enhanced region*) indicating edematous regions. Given the T1 Gd-enhanced region was typically a subset of the T2 FLAIR enhanced region, a third ROI was derived of the T2 FLAIR enhanced region that was not also enhanced on T1 post-gadolinium (*T2 FLAIR exc. Gd region*).

All the manually delineated regions were applied to the dynamic PET images to derive TACs for each of the regions defined. Tissue and plasma TACs were modelled to estimate the PET volume of distribution (V_T), which is the equilibrium partition coefficient of radioactivity between tissue and plasma for the tissue regions delineated.

Statistical analysis

Descriptive statistics were used for safety, response, and PK data. Sample size was based on feasibility and not on power to test a statistical hypothesis. PET imaging data was analyzed using MIAKAT™ software version 3.3.8.

Results

Patients

Thirteen patients (median age 53 years) from three hospitals were enrolled and received at least one dose of GSK2256098 (Table 1). Eleven patients had a histological diagnosis of glioblastoma, one had a histological diagnosis of gliomatosis cerebri, and one had a previous histological diagnosis of anaplastic oligoastrocytoma with radioliogical evidence of subsequent ~~radiological~~ transformation to glioblastoma. The median time from diagnosis of glioblastoma to study entry was 17 months, with a median time from last progression to study entry of 42 days. The median number of lines of previous chemotherapy was 2 (range 1-3). All patients had measurable disease at baseline. All patients discontinued study treatment, most frequently due to disease progression (69%). Two patients died whilst on study after discontinuation of study treatment, both due to glioblastoma.

Safety and tolerability

Patients were treated with GSK22560981000mg (n=6), 750mg (n=4), or 500mg (n=3) twice daily. A single DLT was observed in the 1000mg cohort (n=6), and 1000mg twice daily was deemed to be the MTD. The DLT in this patient was Grade 4 cerebral edema with associated Grade 3 somnolence. Due to the observed cerebral edema, the investigators decided to explore treatment at dose levels below the MTD, to further characterize the safety and tolerability and to investigate tumor penetration of [^{11}C]GSK2256098. One patient in the 750mg cohort (n=4) experienced Grade 4 cerebral edema with associated Grade 3 somnolence and Grade 3 headache. There were no DLTs in patients in the 500mg cohort (n=3).

All patients reported at least one treatment-related AE. The most frequent treatment-related AEs were diarrhea (38%), fatigue (31%), and nausea (31%). Treatment-related AEs by maximum toxicity grade are shown in Table 2. The majority of treatment-related AEs were Grade 1 or 2. Brain edema was the only treatment-related Grade 4 AE and was reported in two subjects. In addition, there were two subjects with Grade 3 AEs of fatigue and two with Grade 2 AEs of somnolence that were reported as related to treatment. All other Grade 3 and Grade 4 AEs related to treatment were reported for only one subject. All Grade 3 or Grade 4 treatment related AEs that occurred in more

than one subject were reported for subjects in the 750 mg or the 1000 mg dose cohorts. No Grade 3 or Grade 4 treatment related AEs occurred in more than one subject in the 500mg cohort.

Discontinuation of GSK2256098 due to treatment-related AEs occurred in two patients due to Grade 3 fatigue (n=1) and Grade 3 rise in alanine transaminase (n=1). Dose reductions of GSK2256098 due to treatment-related AEs occurred in two patients in the 1000mg cohort due to fatigue (n=1) and somnolence (n=1), and in one patient in the 750mg cohort due to fatigue (n=1). Two other patients had dose reductions (1 due to dosing error, and one per sponsor request). Twelve serious AEs occurred in seven patients (54%), most unrelated to GSK2256098 (58%). Treatment related serious AEs included raised alanine transaminase (Grade 3), somnolence (Grade 3), lower respiratory tract infection (Grade 3), and myalgia (Grade 3).

Pharmacokinetics

All subjects participated in PK assessments on day 1 (Table 3). There was moderate to high inter-subject variability. The results were not meaningfully different from those estimated from subjects in solid tumors in previous cohorts of this phase I trial with a difference in Cmax within 42% between the two groups following single or repeated doses across 750mg and 1000mg [24].

Clinical Activity

Eleven patients had imaging subsequent to commencing GSK2256098 and were evaluable for assessment of clinical activity. A best response of stable disease by RANO criteria was observed in 3 patients (27%), with progressive disease in 8 patients (73%). The median progression free survival was 5.7 weeks (95% confidence interval 3.1-8.3 weeks). Two patients were on study for over 90 days: one patient for 3.3 months, and one patient for 11.3 months.

[^{11}C]GSK2256098 biodistribution and kinetics

Eight patients participated in the PET imaging substudy. Tracer metabolism was moderate, with around 30-55% of radioactivity in plasma at 90 minutes attributable to intact parent radiotracer

(Supplementary Materials, Figure S3). Estimates of the regional PET volume of distribution (V_T) and images from a representative patient are shown in Table 4 and Figure 1. Estimate of [^{11}C]GSK2256098 V_T was around 0.4 (range 0.2 to 0.6) in normal brain, and 0.9 (range 0.5 to 1.7) in tumor tissue demonstrating [^{11}C]GSK2256098 uptake (PET-enhanced regions). This indicates that at steady state, the concentration of GSK2256098 was approximately 0.4 times the corresponding concentration in plasma, and markedly higher (0.9) in tumor. Areas of high PET signal (indicating [^{11}C]GSK2256098 concentration) were spatially consistent with areas that were gadolinium enhancing on T1 MRI. Based on an estimate of GSK2256098 ~~bound~~ blood to plasma ratio of 0.84 from human *in vitro* experiments, the measured blood concentrations of GSK2256098 were combined with estimated V_T values to produce estimates of tumor concentrations ranging from 448 ng/mL to 3482 ng/mL (Table 4). No clear relationship was observed between dose of GSK2256098 and V_T. There was no significant association between estimated tumor concentration of GSK2256098 and radiological response or time on study, although of note the patient on treatment for 11.3 months had the highest estimated tumor concentration.

Discussion

This study demonstrates that [^{11}C]GSK2256098 penetrates through the blood-brain barrier at low levels into normal brain but at markedly higher levels into tumor, and that GSK2256098 is tolerable in patients with recurrent glioblastoma. One patient with recurrent glioblastoma in the 750mg twice daily cohort received GSK2256098 treatment for 11.3 months until disease progression.

The MTD of GSK2256098 in patients with recurrent glioblastoma was 1000 mg twice daily, consistent with that declared in previous cohorts of this trial which evaluated GSK2256098 in patients with advanced systemic cancer. The observed DLTs in patients with glioblastoma were both related to cerebral edema, which had not been observed in previous studies of GSK2256098. However, previous cohorts had excluded patients with primary CNS malignancy and patients with brain metastases that were symptomatic, untreated, or required corticosteroids or P450-inducing antiepileptics. Cerebral

edema is common in patients with glioblastoma and is primarily vasogenic rather than cytotoxic in nature due to blood-brain barrier disruption.[26,27] Cerebral edema (both vasogenic and cytotoxic) is associated with focal treatments for glioblastoma, including radiotherapy and carmustine wafers.[28,29] Given the potential for cerebral edema, in future studies in patients with glioblastoma we would advocate initiating treatment with GSK2256098 at 500mg twice daily, and uptitrating to 1000 mg twice daily as tolerated.

As yet, no small molecule targeted inhibitor therapy has demonstrated sufficient clinical efficacy in glioblastoma to be approved for routine use.[30-38] Effective treatment of glioblastoma requires a target that is present throughout the tumor, and adequate drug penetration through the blood-brain barrier and into the tumor (not just to cells adjacent to blood vessels).[39,40] This is challenging to confirm clinically due to the difficulty in obtaining invasive tumor biopsies. Despite preclinical data indicating limited penetration and distribution of GSK2256098 into the intact rodent brain, PET scans of participants in this study demonstrated significant penetration of [^{11}C]GSK2256098 into tumors following microdose administration. Additionally, there was limited penetration of [^{11}C]GSK2256098 into normal brain tissue. The PET volume of distribution did not appear to be impacted by different doses of non-radioactive GSK2256098, which suggests that within the range of doses explored there was no significant variation in the extent to which GSK2256098 saturated transporters (e.g. p-glycoprotein) that prevent blood-brain barrier penetration. Combined with the co-localization of the gadolinium enhancing tissue on MRI, the PET signal is consistent with penetration of GSK2256098 being associated with a locally compromised blood-brain barrier. However, given the infiltrating nature of glioblastoma, more limited penetration of GSK2256098 outside of contrast-enhancing areas may limit clinical effectiveness. This, along with the cytostatic nature of FAK inhibitors, supports combination therapy in future studies of GSK2256098 in glioblastoma.

In conclusion, this is the first study of a FAK inhibitor in glioblastoma. GSK2256098 achieved high tumor penetration in study participants, consistent with tumor-related blood-brain

barrier disruption. It was present at lower levels into normal brain tissue. Diarrhea, fatigue, and nausea were the most frequently observed treatment-related adverse events. Whilst the MTD of GSK2256098 in patients with glioblastoma was 1000 mg twice daily, due to the potential for cerebral edema we would advocate initiating treatment at 500 mg twice daily and uptitrating as tolerated. ~~These data support further clinical investigation of GSK2256098 in patients with glioblastoma. The cytostatic nature of FAK inhibitors supports combination therapy approaches in glioblastoma, either with temozolomide chemoradiotherapy in the primary setting, or with other cytotoxic chemotherapeutics at relapse.~~ Clinical trials of GSK2256098 as monotherapy and in combination with other agents in patients with brain tumors and others cancers are currently ongoing (NCT01938443, NCT02428270, NCT02523014).

Acknowledgements

We are thankful to staff at participating hospitals, Imanova, patients, and their family and carers for their contributions to the study. The study center at UCL is supported by the National Institute of Health Research/Wellcome University College Hospital Clinical Research Facility, the University College Hospital/University College London Biomedical Research Centre and UCL Experimental Cancer Medicine Centre, and the National Brain Appeal. Imperial College acknowledges infrastructural funding from the Experimental Cancer Medicine Centres, Cancer Research UK and NIHR Biomedical Research Centre initiatives.

References

1. Brodbelt A, Greenberg D, Winters T, et al. Glioblastoma in England: 2007-2011. *Eur J Cancer*. 2015; 51(4):533-542.

2. Ostrom QT, Gittleman H, Fulop J, et al. CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008-2012. *Neuro-oncology*. 2015; 17 Suppl 4:iv1-iv62.

3. Stupp R, Hegi ME, Mason WP, et al. Effects of radiotherapy with concomitant and adjuvant temozolomide versus radiotherapy alone on survival in glioblastoma in a randomised phase III study: 5-year analysis of the EORTC-NCIC trial. *The Lancet. Oncology*. 2009; 10(5):459-466.

4. Stupp RH, ME; Idbaih, A; Steinberg, DM; Lhermitte, B; Read, W; Toms, SA; Barnett, GH; Nicholas, G; Kim, C; Fink, K; Salmaggi, A; Lieberman, FS; Zhu, J; Taylor, L; Stragliotto, G; Hottinger, AF; Kirson, ED; Weinberg, U; Palti Y; Ram, Z; . CT007 - Tumor treating fields added to standard chemotherapy in newly diagnosed glioblastoma (GBM): Final results of a randomized, multi-center, phase III trial. Paper presented at: AACR Annual Meeting 20172017; Washington D.C, USA.

5. McLean GW, Carragher NO, Avizienyte E, Evans J, Brunton VG, Frame MC. The role of focal-adhesion kinase in cancer - a new therapeutic opportunity. *Nat Rev Cancer.* 2005; 5(7):505-515.

6. Zhao J, Guan JL. Signal transduction by focal adhesion kinase in cancer. *Cancer metastasis reviews.* 2009; 28(1-2):35-49.

7. Schlaepfer DD, Hauck CR, Sieg DJ. Signaling through focal adhesion kinase. *Progress in biophysics and molecular biology.* 1999; 71(3-4):435-478.

8. Serrels A, Lund T, Serrels B, et al. Nuclear FAK controls chemokine transcription, Tregs, and evasion of anti-tumor immunity. *Cell.* 2015; 163(1):160-173.

9. Natarajan M, Hecker TP, Gladson CL. FAK signaling in anaplastic astrocytoma and glioblastoma tumors. *Cancer journal (Sudbury, Mass.).* 2003; 9(2):126-133.

10. Haskell H, Natarajan M, Hecker TP, et al. Focal adhesion kinase is expressed in the angiogenic blood vessels of malignant astrocytic tumors in vivo and promotes capillary tube formation of brain microvascular endothelial cells. *Clinical cancer research : an official journal of the American Association for Cancer Research.* 2003; 9(6):2157-2165.

11. Jones G, Machado J, Jr., Merlo A. Loss of focal adhesion kinase (FAK) inhibits epidermal growth factor receptor-dependent migration and induces aggregation of nh(2)-terminal FAK in the nuclei of apoptotic glioblastoma cells. *Cancer research.* 2001; 61(13):4978-4981.

12. Ozkal S, Paterson JC, Tedoldi S, et al. Focal adhesion kinase (FAK) expression in normal and neoplastic lymphoid tissues. *Pathology, research and practice.* 2009; 205(11):781-788.

13. Recher C, Ysebaert L, Beyne-Rauzy O, et al. Expression of focal adhesion kinase in acute myeloid leukemia is associated with enhanced blast migration, increased cellularity, and poor prognosis. *Cancer research.* 2004; 64(9):3191-3197.

14. Giaginis CT, Vgenopoulou S, Tsourouflis GS, Politi EN, Kouraklis GP, Theocharis SE. Expression and clinical significance of focal adhesion kinase in the two distinct histological types, intestinal and diffuse, of human gastric adenocarcinoma. *Pathology oncology research : POR.* 2009; 15(2):173-181.

15. Weiner TM, Liu ET, Craven RJ, Cance WG. Expression of focal adhesion kinase gene and invasive cancer. *Lancet (London, England).* 1993; 342(8878):1024-1025.

16. Owens LV, Xu L, Craven RJ, et al. Overexpression of the focal adhesion kinase (p125FAK) in invasive human tumors. *Cancer research.* 1995; 55(13):2752-2755.

17. Riemenschneider MJ, Mueller W, Betensky RA, Mohapatra G, Louis DN. In situ analysis of integrin and growth factor receptor signaling pathways in human glioblastomas suggests overlapping relationships with focal adhesion kinase activation. *The American journal of pathology.* 2005; 167(5):1379-1387.

18. Ding L, Sun X, You Y, Liu N, Fu Z. Expression of focal adhesion kinase and phosphorylated focal adhesion kinase in human gliomas is associated with unfavorable overall survival. *Translational research : the journal of laboratory and clinical medicine.* 2010; 156(1):45-52.

19. Auger KR, Smitheman KN, Korenchuk S, et al. 387 The Focal Adhesion Kinase Inhibitor GSK2256098: a Potent and Selective Inhibitor for the Treatment of Cancer. *European Journal of Cancer.* 48:118.

20. Schinkel AH. P-Glycoprotein, a gatekeeper in the blood-brain barrier. *Advanced drug delivery reviews.* 1999; 36(2-3):179-194.

21. Schneider SW, Ludwig T, Tatenhorst L, et al. Glioblastoma cells release factors that disrupt blood-brain barrier features. *Acta Neuropathol.* 2004; 107(3):272-276.

22. Roberts HC, Roberts TP, Brasch RC, Dillon WP. Quantitative measurement of microvascular permeability in human brain tumors achieved using dynamic contrast-enhanced MR imaging: correlation with histologic grade. *AJNR. American journal of neuroradiology.* 2000; 21(5):891-899.

23. Seitz RJ, Wechsler W. Immunohistochemical demonstration of serum proteins in human cerebral gliomas. *Acta Neuropathol.* 1987; 73(2):145-152.

24. Soria JC, Gan HK, Blagden SP, et al. A phase I, pharmacokinetic and pharmacodynamic study of GSK2256098, a focal adhesion kinase inhibitor, in patients with advanced solid tumors. *Annals of oncology : official journal of the European Society for Medical Oncology / ESMO.* 2016.

25. Wen PY, Macdonald DR, Reardon DA, et al. Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group. *J Clin Oncol.* 2010; 28(11):1963-1972.

26. Papadopoulos MC, Saadoun S, Binder DK, Manley GT, Krishna S, Verkman AS. Molecular mechanisms of brain tumor edema. *Neuroscience.* 2004; 129(4):1009-1018.

27. Klatzo I. Evolution of Brain Edema Concepts. In: Ito U, Baethmann A, Hossmann K-A, et al., eds. *Brain Edema IX: Proceedings of the Ninth International Symposium Tokyo, May 16–19, 1993*. Vienna: Springer Vienna; 1994:3-6.

28. Siu A, Wind JJ, Iorgulescu JB, Chan TA, Yamada Y, Sherman JH. Radiation necrosis following treatment of high grade glioma--a review of the literature and current understanding. *Acta Neurochir (Wien).* 2012; 154(2):191-201; discussion 201.

29. Westphal M, Ram Z, Riddle V, Hilt D, Bortey E, Executive Committee of the Gliadel Study G. Gliadel wafer in initial surgery for malignant glioma: long-term follow-up of a multicenter controlled trial. *Acta Neurochir (Wien).* 2006; 148(3):269-275; discussion 275.

30. Brown N, McBain C, Nash S, et al. Multi-Center Randomized Phase II Study Comparing Cediranib plus Gefitinib with Cediranib plus Placebo in Subjects with Recurrent/Progressive Glioblastoma. *PLoS ONE.* 2016; 11(5):e0156369.

31. Mulholland PJ, Thirlwell C, Brock CS, Newlands ES. Emerging targeted treatments for malignant glioma. *Expert opinion on emerging drugs.* 2005; 10(4):845-854.

32. Wick W, Puduvalli VK, Chamberlain MC, et al. Phase III study of enzastaurin compared with lomustine in the treatment of recurrent intracranial glioblastoma. *J Clin Oncol.* 2010; 28(7):1168-1174.

33. Batchelor TT, Mulholland P, Neyns B, et al. Phase III randomized trial comparing the efficacy of cediranib as monotherapy, and in combination with lomustine, versus lomustine alone in patients with recurrent glioblastoma. *J Clin Oncol.* 2013; 31(26):3212-3218.

34. Stupp R, Hegi ME, Gorlia T, et al. Cilengitide combined with standard treatment for patients with newly diagnosed glioblastoma with methylated MGMT promoter (CENTRIC EORTC 26071-22072 study): a multicentre, randomised, open-label, phase 3 trial. *The Lancet. Oncology.* 2014; 15(10):1100-1108.

35. Wen PY, Chang SM, Lamborn KR, et al. Phase I/II study of erlotinib and temsirolimus for patients with recurrent malignant gliomas: North American Brain Tumor Consortium trial 04-02. *Neuro-oncology.* 2014; 16(4):567-578.

36. Lassen U, Sorensen M, Gaziel TB, Hasselbalch B, Poulsen HS. Phase II study of bevacizumab and temsirolimus combination therapy for recurrent glioblastoma multiforme. *Anticancer research.* 2013; 33(4):1657-1660.

37. Muhic A, Poulsen HS, Sorensen M, Grunnet K, Lassen U. Phase II open-label study of nintedanib in patients with recurrent glioblastoma multiforme. *Journal of neuro-oncology.* 2013; 111(2):205-212.

38. Chen R, Cohen AL, Colman H. Targeted Therapeutics in Patients With High-Grade Gliomas: Past, Present, and Future. *Current treatment options in oncology.* 2016; 17(8):42.

39. Minchinton AI, Tannock IF. Drug penetration in solid tumours. *Nat Rev Cancer.* 2006; 6(8):583-592.

40. Jain RK. Vascular and interstitial barriers to delivery of therapeutic agents in tumors. *Cancer and Metastasis Reviews.* 1990; 9(3):253-266.

Tables

Figures

Figure 1 **PET data: (A) Aligned PET and MR images in a representative patient ~~(subject 411)~~ with ROIs highlighted as colored overlays in images on right side. (B) Time activity curves in a representative patient ~~(subject 409)~~**

	500 mg BID (N=3)	750 mg BID (N=4)	1000 mg BID (N=6)	Total (N=13)
Age, years				
Mean	52.0	43.0	52.0	49.2
Standard deviation	11.36	12.75	8.99	10.70
Median (min, max)	57.0 (39, 60)	39.0 (33, 61)	53.0 (40, 65)	53.0 (33, 65)
Age group, n (%)				
18 – 64 years	3 (100)	4 (100)	5 (83)	12 (92)
65 – 74 years	0	0	1 (17)	1 (8)
Sex, n (%)				
Female	0	2 (50)	1 (17)	3 (23)
Male	3 (100)	2 (50)	5 (83)	10 (77)
Race, n (%)				
White - Arabic/North African Heritage	1 (33)	0	0	1 (8)
White - White/Caucasian/European Heritage	2 (67)	4 (100)	6 (100)	12 (92)
Number of prior chemotherapy regimens, n (%)				
1	0	0	2 (33)	2 (15)
2	2 (67)	3 (75)	3 (50)	8 (62)
3	1 (33)	1 (25)	1 (17)	3 (23)
Receiving Steroids at Baseline, n (%)				
Yes	3 (100)	2 (50)	5(83)	10 (77)
No	0	2 (50)	1 (17)	3 (23)

Table 1: Baseline Patient Characteristics

Preferred Term	Maximum CTCAE Toxicity Grade, n (%)								
	500 mg BID (N=3)			750 mg BID (N=4)			1000 mg BID (N=6)		
	3	4	Any Grade	3	4	Any Grade	3	4	Any Grade
Any event	**1 (33)**	**0**	**3 (100)**	**1 (25)**	**1 (25)**	**4 (100)**	**2 (33)**	**1 (17)**	**6 (100)**
Diarrhea	0	0	0	1 (25)	0	3 (75)	0	0	2 (33)
Fatigue	0	0	1 (33)	1 (25)	0	1 (25)	1 (17)	0	2 (33)
Nausea	0	0	1 (33)	0	0	2 (50)	0	0	1 (17)
Blood bilirubin increased	0	0	1 (33)	0	0	0	0	0	2 (33)
Somnolence	0	0	0	1 (25)	0	1 (25)	1 (17)	0	2 (33)
Cerebral edema	0	0	0	0	1 (25)	1 (25)	0	1 (17)	1 (17)
Hypercholesterolemia	0	0	0	0	0	1 (25)	0	0	1 (17)
Vomiting	0	0	0	0	0	1 (25)	0	0	1 (17)
ALT increased	1 (33)	0	1 (33)	0	0	0	0	0	0
Aphasia	0	0	0	0	0	0	1 (17)	0	1 (17)
AST increased	0	0	1 (33)	0	0	0	0	0	0
Asthenia	0	0	0	0	0	0	0	0	1 (17)
Blood cholesterol increased	0	0	0	0	0	1 (25)	0	0	0
Headache	0	0	0	1 (25)	0	1 (25)	0	0	0
Hypokalaemia	0	0	0	0	0	1 (25)	0	0	0
Fall	0	0	0	0	0	0	0	0	1 (17)
Lower respiratory tract infection	0	0	0	0	0	0	1 (17)	0	1 (17)
Lymphopenia	0	0	0	0	0	1 (25)	0	0	0
Muscular weakness	0	0	1 (33)	0	0	0	0	0	0
Myalgia	0	0	0	0	0	0	1 (17)	0	1 (17)
Myoclonus	0	0	1 (33)	0	0	0	0	0	0
Oropharyngeal pain	0	0	0	0	0	0	0	0	1 (17)
Seizure	0	0	0	0	0	1 (25)	0	0	0

ALT=alanine aminotransferase; AST=aspartate aminotransferase.

Table 2: Treatment-Related Adverse Events

	Dose (mg)	C_{max} (ng/mL), Geometric Mean (%CVb)	C_{avg} (ng/mL)	t_{max} (h), Median (Range)	$AUC_{(0-4)}$ (ng*h/mL), Geometric Mean (%CVb)
Day 1	500 (n=3)	3075 (108)		1.5 (1.0, 2.2)	7038 (78.0)
	750 (n=4)	4912 (14.7)		3.0 (1.5, 4.0)	8644 (49.0)
	1000 (n=6)	4079 (80.1)		2.0 (1.5, 4.0)	9330 (102)
Day of imaging procedure	500 (n=3)	3262 (113)	1548 (78.6)		8608 (93.7)
	750 (n=2)	5860 (32.9)	2338 (40.3)		10,639 (11.7)
	1000 (n=3)	3803 (82.8)	1753 (60.3)		10,286 (67.3)

%CVb=between-subject coefficient of variation; $AUC_{(0-4)}$=area under the DBS concentration-time curve to the last quantifiable concentration; C_{avg}= average concentration over the dose interval; C_{max}=maximum observed concentration; DBS=dried blood spot.

Data reported as geometric mean (%CVb).

Table 3: Summary of GSK2256098 Pharmacokinetic Parameter Values

Subject	Age (years)	Gender	Weight (kg)	Dose (mg):	Injected radioactivity (MBq)	Injected mass (μg)	V_T in region of interest (ml.cm^{-3}):					Blood concentration C_{avg} (ng/ml)	Estimated tumor concentration (ng.ml)
							T1 Gd-enhanced	T2 FLAIR-enhanced	T2 FLAIR exc. Gd	PET-enhanced	Normal brain		
409	53	Male	81.2	1000	468	6.65	0.63	0.44	0.42	0.83	0.39	2312	2273
410	54	Female	118.5	1000	95	1.55	0.64	0.64	0.64	0.82	0.55	2522	2449
411	61	Male	79.9	750	298	2.90	0.86	0.57	0.52	0.75	0.29	3076	2760
1002	57	Male	86.0	1000	429	5.17	1.68	0.67	0.74	1.72	0.44	923	1888
1008	34	Female	84.9	750	398	4.52	1.66	0.69	0.62	1.65	0.51	1777	3482
1012	61	Male	72.0	500	314	4.66	0.35	0.26	0.26	0.39	0.23	3147	2455
1015	40	Male	65.2	500	130	1.49	0.88	0.64	0.57	0.83	0.41	1496	1487
1017	57	Male	126.3	500	431	5.15	0.52	0.40	0.35	0.48	0.28	787	448

Table 4: Estimates of regional PET volume of distribution V_T ~~(ml.cm^{-3})~~and tumor concentration

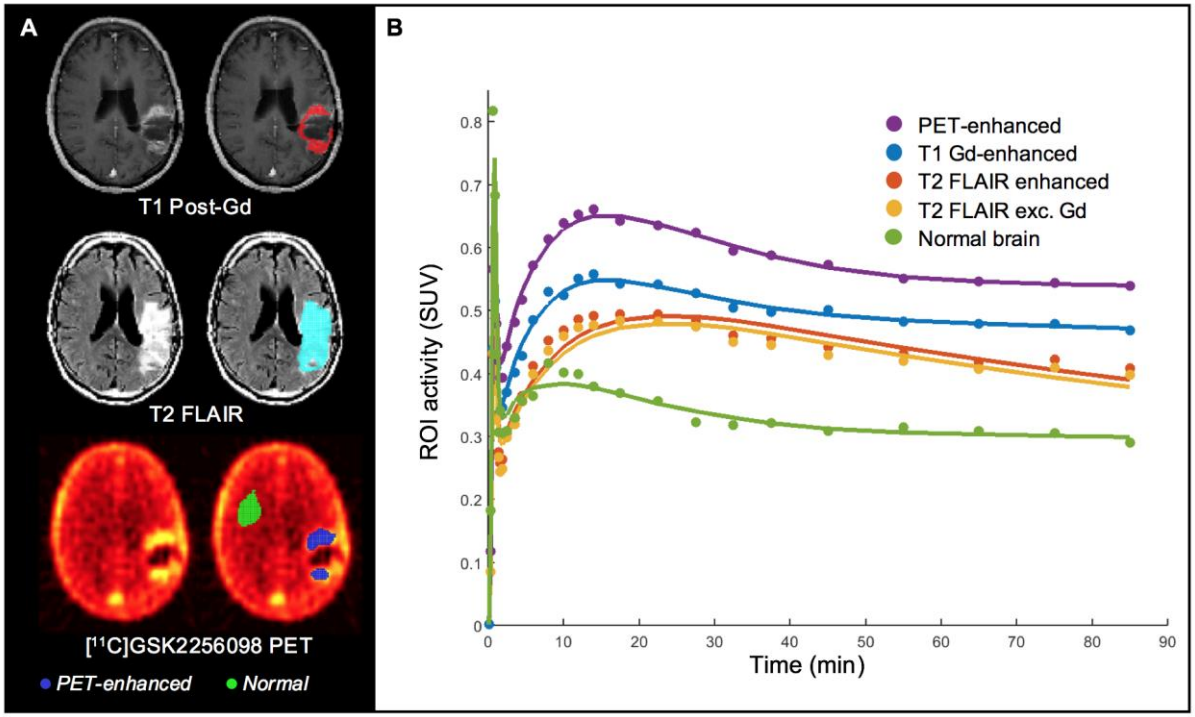

Figure 1: PET data: (A) Aligned PET and MR images in a representative patient ~~(subject 411)~~ with ROIs highlighted as colored overlays in images on right side. (B) Time activity curves in a representative patient ~~(subject 409)~~