TITLE: A Phase I study of pegylated arginine deiminase (pegargiminase), cisplatin and pemetrexed in argininosuccinate synthetase 1-deficient recurrent high-grade glioma

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RUNNING TITLE:

Arginine deprivation therapy in recurrent high-grade gliomas

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CONFLICT OF INTEREST DISCLOSURE STATEMENT:

PEH has received honoraria from MSD. XF, AJ, JT, JB and B-WW are employees of Polaris Pharmaceuticals Inc. MTS has an advisory role with Roche Molecular Diagnostics. SP has received research funding from AstraZeneca. PWS has received honoraria from Merck & Co Inc., Merck KGaA, Roche and Bristol-Myers Squibb, has an advisory role with Roche and is a recipient of research funding from Polaris Pharmaceuticals, Inc. All remaining authors have declared no conflicts of interest.

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STATEMENT OF TRANSLATIONAL RELEVANCE:

Recurrent high-grade gliomas (HGGs) have a dismal prognosis and limited treatment options. Loss of the urea cycle enzymes argininosuccinate synthetase 1 (ASS1) and/or argininosuccinate lyase (ASL), that synthesize arginine from citrulline, occurs in 70% of HGGs and sensitizes them to arginine deprivation by pegylated arginine deiminase (ADI-PEG20, pegargiminase) in pre-clinical models. Furthermore, ADI-PEG20 synergises with the antifolate agent pemetrexed to increase HGG cell death *in vitro*. Translating these data, we show that ADI-PEG20 in combination with cisplatin and pemetrexed in heavily pre-treated patients with ASS1-deficient recurrent HGGs was well tolerated. Progression-free survival (PFS) and overall survival (OS) compared favourably with historical data in recurrent HGG. Notably, a patient with primary isocitrate dehydrogenase (IDH) wildtype glioblastoma had a PFS of 20.8 months and OS of 24+ months, uncovering a potential role for quinine sulfate as an ADI-PEG20 re-sensitizer via autophagy inhibition. Further ADI-PEG20 trials in recurrent HGG are planned.

ABSTRACT:

Background: Patients with recurrent high-grade gliomas (HGGs) are usually managed with alkylating chemotherapy +/- bevacizumab. However, prognosis remains very poor. Pre-clinically, we showed that HGGs are a target for arginine depletion with pegargiminase (ADI-PEG20) due to epimutations of argininosuccinate synthetase (ASS1) and/or argininosuccinate lyase (ASL). Moreover, ADI-PEG20 disrupts pyrimidine pools in ASS1-deficient HGGs, thereby impacting sensitivity to the antifolate, pemetrexed.

Patients and methods: We expanded a Phase I trial of ADI-PEG20 with pemetrexed and cisplatin (ADIPEMCIS) to patients with ASS1-deficient recurrent HGGs (NCT02029690). Patients were enrolled (01/16 – 06/17) to receive weekly ADI-PEG20 36 mg/m^2 intramuscularly plus pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2 intravenously once every three weeks for up to six cycles. Patients with disease control were allowed ADI-PEG20 maintenance. The primary endpoints were safety, tolerability and preliminary estimates of efficacy.

Results: Ten ASS1-deficient heavily pre-treated patients were treated with ADIPEMCIS therapy. Treatment was well tolerated with the majority of adverse events being CTCAE v4.03 grade 1-2. The best overall response was stable disease in 8 patients (80%). Plasma arginine was suppressed significantly below baseline with a reciprocal increase in citrulline during the sampling period. The anti-ADI-PEG20 antibody titer rose during the first four weeks of treatment before reaching a plateau. Median progression-free survival (PFS) was 5.2 months (95% CI 2.5-20.8) and overall survival (OS) was 6.3 months (95% CI 1.8-9.7).

Conclusions: In this recurrent HGG study, ADIPEMCIS was well tolerated and compares favorably to historical controls. Additional trials of ADI-PEG20 in HGG are planned.

INTRODUCTION:

High-grade gliomas (HGGs) are usually treated with a combination of surgery and chemoradiotherapy in the first-line setting, but, despite these measures, recurrence is nearly universal (1). At this point, treatment options are limited with no defined standard of care (2). Alkylating chemotherapy is most commonly used, but efficacy is poor with a progression-free survival (PFS) of around 1.5-3 months and an overall survival of 6-9 months (2). New treatment options are urgently required.

Arginine is a conditionally essential amino acid involved in the regulation of numerous cellular processes (3). These include cell signaling, proliferation (by modulating polyamine and nucleotide synthesis), vasodilatation (via nitric oxide) and hormone synthesis (3). Arginine also plays a crucial role in regulating the immune system in health and disease (4).

Auxotrophy is the inability of an organism to synthesise an organic compound necessary for its growth (5). Normal cells express the urea cycle enzymes argininosuccinate synthetase 1 (ASS1) and argininosuccinate lyase (ASL), which convert citrulline to arginine thereby providing an endogenous supply. However, ASS1 is downregulated in many tumors, commonly through hypermethylation of the *ASS1* promoter (6,7). This leads to increased pyrimidine synthesis and consequent tumor proliferation, but renders the tumor dependent on exogenous arginine (6,8,9). This can be exploited therapeutically using pegylated arginine deiminase (ADI-PEG20/pegargiminase) which catalyses the conversion of L-arginine to citrulline and an ammonium ion (10). Importantly, low ASS1 expression is associated with a more aggressive clinical phenotype and a worse clinical outcome in several different cancer types (11-14).

Initial clinical trials showed that ADI-PEG20 is well tolerated with injection site tenderness being the most commonly reported adverse event. Allergic reactions have occurred, but anaphylaxis is rare (<1%). The most common laboratory toxicities were mild (grade 1-2) asymptomatic elevations in various serum chemistry values, while neutropenia has been described infrequently (13,15,16).

ADI-PEG20 has biological efficacy as a single agent in a range of tumors including melanoma, hepatocellular carcinoma and mesothelioma (13,15,16). Furthermore, patients deficient in ASS1 gained the most benefit from monotherapy (17). In addition, pre-clinical studies of ADI-PEG20 showed that arginine deprivation suppressed the folate-dependent enzymes thymidylate synthase (TS) and dihydrofolate reductase (DHFR) (12). This leads to the potentiation of pemetrexed, an inhibitor of TS and DHFR, when combined with ADI-PEG20 (12). Also, ADI-PEG20 potentiates the activity of cisplatin in melanoma cells by inhibiting synthesis of DNA repair enzymes that attempt to abrogate the effects of cisplatin (18). Therefore, ADI-PEG20 enhances the activity of both folate inhibitors and platinum agents. Furthermore, a Phase I trial combining ADI-PEG20, pemetrexed and cisplatin (ADIPEMCIS) has shown efficacy in patients with treatment-refractory ASS1-deficient mesothelioma or non-small cell lung cancer, thus validating this clinical approach (19).

Pre-clinical findings have shown that 70% of HGG patient tissue samples were deficient in ASS1 (20). Arginine deprivation, either by exposure to arginine deiminase or arginase, inhibited the proliferation of patient-derived ASS1-deficient HGG cell lines and xenografts (20,21). These pre-clinical studies established that targeting arginine auxotrophy is a potential therapeutic strategy in HGG that would have the benefit of partially overcoming the limitations imposed by the blood-brain barrier by acting in the peripheral blood rather than the tumor (22). This Phase I expansion cohort

assessed the safety and preliminary evidence for clinical activity using ADIPEMCIS in patients with recurrent or refractory HGG.

PATIENTS AND METHODS:

Patients

Eligible patients were 18 years of age or older with histologically confirmed HGG in which >50% of tumor cells did not express ASS1 as determined by immunohistochemistry (IHC; 0 or 1+ by ASS1 immunohistochemistry 1:500; from Polaris Pharmaceuticals, Inc.) (7,13,19). Archived tissue was used for ASS1 assessment where the initial diagnosis was HGG. Patients who were initially diagnosed with a low-grade glioma were required to undergo a biopsy to confirm transformation to a HGG which was subsequently used for ASS1 assessment. Patients must have failed first-line treatment with radiotherapy ± temozolomide (progressive disease and/or side-effects) and have evaluable disease by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 (23). Any steroid dose must have been stable for at least 5 days prior to the patient receiving the first ADI-PEG20 dose. Patients had an Eastern Co-operative Oncology Group (ECOG) performance status of 0-1 as well as adequate haematological, renal and hepatic function. Exclusion criteria included radiotherapy or surgery within 4 weeks of first study treatment, ongoing toxic manifestations of previous treatments and significant concomitant or uncontrolled intercurrent illness. Prior treatment with ADI-PEG20 was not allowed.

The study was performed in accordance with good clinical practice and the European Union Clinical Trials Directive, with approval from Leeds East (Yorkshire and The Humber) ethical review board. All patients provided written informed consent.

Study design and treatment

This was an open-label, single arm, expansion cohort of a Phase I study conducted across two centers in the United Kingdom once the recommended Phase II dose (RP2D) of ADIPEMCIS had been determined by the dose escalation cohort (19). The planned number of patients to be enrolled was ten. The RP2D was weekly ADI-PEG20 36 mg/m^2 intramuscularly (i.m.) plus pemetrexed 500 mg/m^2 and cisplatin 75 mg/m^2 intravenously once every three weeks (19). The initial dose of ADI-PEG20 was given at least 48 hours in advance of the first dose of cytotoxic drugs. To reduce pemetrexed toxicity, daily oral folic acid 400 µg and hydroxycobalamin 1000 µg i.m. every nine weeks were administered, starting at least seven days prior to first dose. Oral dexamethasone 4 mg twice daily was given for three days with each cycle, starting the day before pemetrexed was due, if the patients were not already taking steroids or were on a lower dose. Treatment continued for a maximum of six cycles (18 weeks) after which patients who had stable disease or better were eligible to continue on single-agent ADI-PEG20 until disease progression.

Endpoints and assessment

A data cut-off of 15 November 2018 was used. The primary endpoints were safety, tolerability and preliminary estimates of efficacy by best overall response rate (ORR) as assessed by RECIST 1.1 (23). Secondary endpoints included PFS, OS, pharmacodynamics, immunogenicity. Exploratory endpoints included analysis of ASS1/ASL epimutations.

The safety and tolerability of ADIPEMCIS was assessed using National Cancer Institute Common Terminology Criteria for Adverse Events (NCI-CTCAE) version 4.03.

Baseline imaging by diffusion-weighted magnetic resonance imaging (DW-MRI) was undertaken within 21 days prior to the patient receiving the first ADI-PEG20 dose. MRI scans were performed every six weeks during ADIPEMCIS dosing and after every eighth weekly dose of ADI-PEG20 during the maintenance phase.

During combination treatment with ADIPEMCIS, blood samples were taken weekly for cycle 1 and then on days 1 and 15 for cycles 2-6 to analyse arginine and citrulline levels and for immunogenicity analyses, as described previously (13).

The methylation status of *ASS1* and *ASL* were determined by methylation-specific PCR as described previously (20).

RESULTS:

Patients

Between January 2016 and June 2017, ten patients with HGG were enrolled from two centers in the United Kingdom (Table 1). The age range was 43-63 years with a median age at enrolment of 51 years. Ninety percent of patients were male and 80% were Caucasian. Twenty patients were screened with 18 (90%) being ASS1 deficient (Supplementary Fig. S1). It was not possible to determine the ASS1 score for two patients who were therefore excluded. The reasons for screen-failure in the other eight patients were clinical deterioration (6, 60%) and withdrawal of consent (2, 20%).

For the ten treated patients, six had glioblastoma, one of which had transformed from a grade II astrocytoma (Table 2). Three patients had anaplastic astrocytoma, one of which had transformed from a grade II astrocytoma. One patient had an anaplastic oligodendroglioma (1p/19q codeleted) which had transformed from a grade II oligodendroglioma. All patients with glioblastoma were isocitrate dehydrogenase (IDH) wildtype as assessed by R132H immunohistochemistry as was one patient with anaplastic astrocytoma. Six patients had unmethylated *O^6-methylguanine-DNA methyltransferase* (*MGMT*) promoters, three had methylated ones and it was not determined for one patient. Ninety percent of patients had received two or more lines of systemic therapy prior to enrolment, including temozolomide, lomustine and procarbazine/lomustine/vincristine (PCV).

Safety

The majority (74%) of adverse events (AEs) were grade 1 or 2 (Table 3). The commonest grade 1-2 AEs seen in more than one patient related to at least one treatment agent were thrombocytopenia (5 patients, 50%), fatigue (3 patients, 30%), raised alanine aminotransferase (3 patients, 30%), nausea (2 patients, 20%) and dyspepsia (2 patients, 20%). Eight patients (80%) experienced at least one grade 3-4 AE with neutropenia (4 patients, 40%) and thrombocytopenia (3 patients, 30%) being the most frequent. There was one case of grade 3 neutropenic sepsis and one grade 3 rise in alanine aminotransferase level, both of which were treatment-related.

Two patients did not have any follow-up scans after the first cycle of treatment: patient E chose to withdraw from the study; and patient C suffered a combination of adverse events, clinical progression and performance status deterioration.

Pharmacodynamics

Complete data were available for five patients over the 18-week period of blood sampling. Circulating plasma arginine levels fell rapidly upon commencing ADI-PEG20 and remained below 30% of baseline for the duration of the sampling period (Fig. 1a). A reciprocal rise in citrulline as expected was also maintained throughout the sampling period. Anti-ADI-PEG20 antibody titres rose during the first four weeks of ADI-PEG20 before plateauing at 10^{-2} for the remainder of the study (Fig. 1b).

Efficacy

The best response by RECIST 1.1 was stable disease seen in eight (80%) patients with two patients being non-evaluable due to lack of follow-up scans. No complete or partial responses were seen. The median PFS was 5.2 months (95% confidence interval [CI] 2.6-20.8) and the median OS was 6.3 months (95% CI 1.8-9.7). Progression-free survival at six months (PFS6) was 20%. Individual patient responses are summarised in Fig. 2 with Kaplan-Meier plots shown in Supplementary Figure S2. Notably, one patient with primary IDH wildtype, *MGMT* promoter unmethylated glioblastoma had a PFS of 20.8 months and remains alive currently (OS 24+ months). Moreover, re-resection of his glioblastoma at disease progression continued to demonstrate ASS1 deficiency by IHC (Supplementary Fig. S1). A retrospective analysis by Response Assessment in Neuro-Oncology (RANO) criteria was undertaken (24): two patients had a partial response as their best response to treatment, namely the two patients with the longest PFS of 20.8 and 9.3 months; one patient had stable disease; two patients had progressive disease; and five patients were non-evaluable owing to extensive necrosis. Median PFS by RANO was 2.5 months (95% CI 0.0-9.2).

ASS1 and ASL1 epimutations

The promoter methylation status of *ASS1* and *ASL* was determined for eight of the patients on treatment using archived tissue, except where a HGG had transformed from a low-grade glioma when new biopsy tissue was used (insufficient material for two patients). There were no cases of *ASL* promoter methylation. Three patients had at least one tumor sample that demonstrated methylation of *ASS1* promoter and there were five patients with unmethylated tumors (Table 2). Two samples from the same tumor were tested for patient G. Interestingly, one sample showed *ASS1* methylation and the other did not, illustrating heterogeneity within the tumor.

DISCUSSION:

This is the first reported use of arginine deprivation therapy in combination with cisplatin and pemetrexed (ADIPEMCIS) in recurrent HGGs, a biomarker-led approach. Patients enrolled in the trial had been heavily pre-treated with 90% having received at least two prior lines of chemotherapy. Unsurprisingly, the commonest AEs were related to bone marrow suppression, including grade 4 neutropenia and thrombocytopenia, consistent with the chemotherapy. However, no bleeding events occurred and there was only one episode of neutropenic sepsis, in line with the expected rate. Overall, treatment was well tolerated.

Arginine suppression was observed for the duration of ADIPEMCIS treatment together with a concomitant rise in citrulline. The anti-ADI-PEG20 antibody titre rose to a peak of 10^{-2} by week four and then plateaued at this level for the remainder of treatment. This is consistent with findings in thoracic tumor patients treated with ADIPEMCIS (19).

Preliminary efficacy results showed PFS 5.2 months, OS 6.3 months and PFS6 20% by RECIST 1.1. These are in line with historical findings for recurrent HGG, albeit towards the lower end of the range. This may be due to our population being more heavily pre-treated as 90% had received at least two prior lines of chemotherapy, compared to only one line in most studies (25-28). In addition, ASS1 deficiency is associated with a worse prognosis in several different cancer types (11-14). However, given that our mixed population comprised grade III gliomas (better prognosis) and glioblastomas (worse prognosis), larger studies will be required to determine efficacy in these subtypes.

One patient with primary glioblastoma who had poor prognostic features (IDH-wildtype, unmethylated *MGMT* promoter) had a PFS of 20.8 months and remains alive currently (OS 24+ months). He had progressed after three cycles of adjuvant temozolomide and then again after two cycles (or 12 weeks) of PCV chemotherapy. He commenced ADIPEMCIS in November 2016 and completed the first two cycles at 100% dose, but required dose reductions in cisplatin and pemetrexed for cycles three and four before they were stopped from cycle five onwards owing to recurrent grade 3-4 thrombocytopenia. He continued on ADI-PEG20 monotherapy for a further 18 months. Radiologically, there had been a progressive increase in the enhancing component of the tumor from his first on-treatment MRI scan to 18% above baseline on the July 2017 scan (Fig. 3). However, arginine levels were suppressed in June 2018 after 16 months on ADI-PEG20 maintenance compared to baseline (data not shown) suggesting another mechanism causing disease progression. In July 2017, he was started on quinine sulfate 200 mg once daily by his physician for leg cramps. Subsequent scans showed that his tumor had stabilised before reducing in size which was sustained for a further twelve months and was associated with behavioural improvements. Although only one patient with circumstantial evidence for an interaction, there is a pre-clinical basis for the effect. Autophagy is a known resistance mechanism of arginine deprivation therapy, and quinolone autophagy inhibitors have shown efficacy in pre-clinical tumor models treated with ADI-PEG20, including glioblastoma (20,29). Indeed, recent studies confirm quinolone re-sensitization of glioma cell lines to targeted agents such as BRAF inhibitors, with clinical evidence of efficacy (30,31). Thus, the potential synergy between ADI-PEG20 and autophagy inhibitors warrants further clinical investigation. At disease progression, he underwent debulking surgery where the histology reconfirmed glioblastoma and molecular analysis showed no mutations in *IDH1/2*, although interestingly *MGMT* promoter methylation was now present at a low level (>0-5%) and may reflect prior treatment with cisplatin (32). Intriguingly, his recurrent tumor on ADI-PEG20 monotherapy remained ASS1-deficient (Supplementary Fig. S1) implicating as yet unknown mechanisms of drug resistance to ADI-PEG20 that may include stromal provision of arginine or its precursors (9).

Platinum agents are an established treatment option in recurrent HGG (33,34). However, to our knowledge, this is the first time pemetrexed has been trialled in this setting in more than one patient (clinic trial NCT00276783 recruited one patient only with HGG – J. Raizer, personal communication). This is despite pre-clinical evidence showing pemetrexed has activity in a xenograft model of glioblastoma and clinical studies showing that cisplatin/pemetrexed has efficacy in brain metastases (35-37). Furthermore, pre-clinical data has shown that ADI-PEG20 suppresses the folate-dependent enzymes TS and DHFR in primary GBM cells (Supplementary Fig. S3a) and when combined with pemetrexed synergizes to decrease proliferation in an ASS1 negative glioma cell line, but not in an ASS1 positive cell line as has been seen previously in bladder cancer and malignant mesothelioma cell lines (Supplementary Fig. S3b) (12).

This was an expansion cohort of the initial phase I TRAP trial which was conducted in thoracic tumors (19). As such, RECIST 1.1 was used to evaluate progression-free survival (23). This involves a unidirectional measurement of the target lesion, rather than a bidirectional one used by RANO (24). Despite these differences, the correlation between the two sets of criteria is usually good, although in our small sample set differences were seen (38). Patient A had a PFS that was around two months shorter by RANO than RECIST 1.1 criteria. In contrast, patient J had a sustained partial response by RANO but did have a progression event according to RECIST 1.1. Future studies will employ RANO criteria exclusively. Two patients had clinical progression, rather than radiological progression, which led to their data being censored by RECIST 1.1. Patient A appeared to have disease progression on the July 2017 MRI scan with new left frontal enhancement (Fig. 3a). However, this region was not measurable by RANO criteria owing to central necrosis and thin walls. These findings highlight the well-documented issues with measuring PFS in HGGs.

Ninety percent of patients screened were found to be deficient for ASS1 expression by immunohistochemistry using a less stringent threshold of >50% ASS1 loss than used in earlier studies that mandated >95-99% ASS1 loss (20). Therefore, it is proposed that ASS1 expression status will not be required at study enrolment to minimise time to treatment, however subgroup analysis will be performed retrospectively. In our small patient cohort, *ASS1* promoter methylation occurred in only 30% of patients and did not correlate with treatment response in contrast to *in vitro* findings (20) (Table 2). Heterogeneity was seen in one patient indicating that this may be an underrepresentation of the true rate. Alternatively, methylation of *ASS1* intron 1 may be a more important determinant than promoter methylation in clinical samples as shown in mesothelioma (11). Also, ASS1-deficiency appears to be less important for medical benefit when ADI-PEG20 is combined with chemotherapy, as has been shown with gemcitabine and nab-paclitaxel in pancreatic cancer and with folinic acid, fluorouracil and oxaliplatin in hepatocellular carcinoma (39,40).

In summary, the combination of ADI-PEG20, pemetrexed and cisplatin was well tolerated in patients with recurrent aggressive gliomas. Preliminary estimates of efficacy were encouraging with the intriguing role of a quinolone-based autophagy inhibitor in combination with ADI-PEG20 needing further clinical investigation. Additional trials of ADI-PEG20 are planned in patients with HGGs.

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Characteristic	No. of Patients
Age, years	43-63
Sex	
Male	9
Female	1
Diagnosis	
Glioblastoma	6
Anaplastic astrocytoma	3
Anaplastic oligodendroglioma	1
Performance status	
0	0
1	10
Prior systemic therapy	
1	1
2	7
3	2
ASS1 screening	
Patients screened	20
Negative for ASS1 expression	18 (90%)
ASS1 expression not determine	2 (10%)
Patients ineligible / rapid disease progression / unknown	8
Patients enrolled	10

Table 1: Patient characteristics. Abbreviations: ASS1, argininosuccinate synthetase 1.

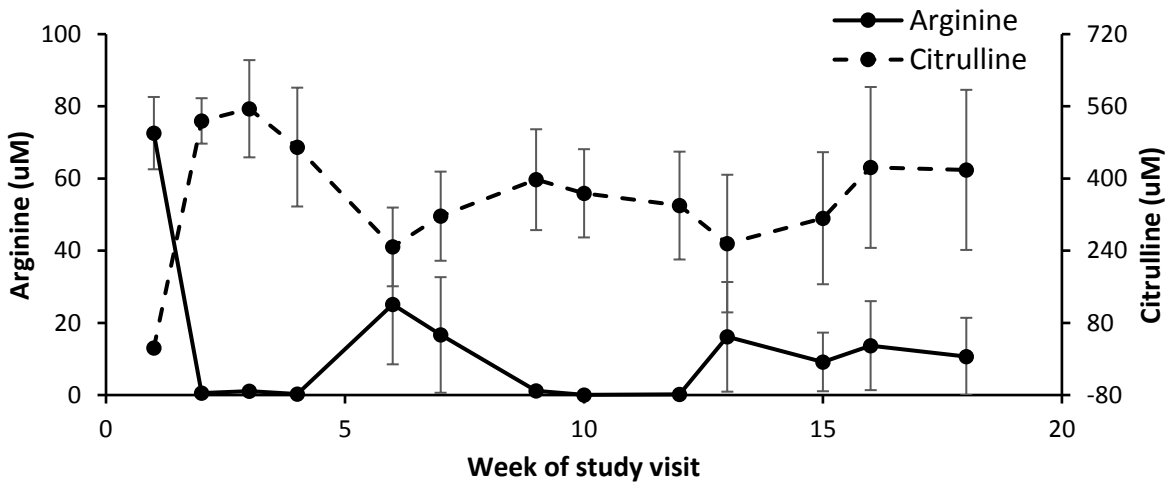
Toxicity	Grade 1 or 2	Grade 3	Grade 4
Thrombocytopenia*	4	3	1
Fatigue	3		
ALT increased	2	1	
Nausea	2		
Neutropenia*	1	4	4
Neutropenic sepsis		1	
Dehydration	1		
Headache	1		
Dysaesthesia	1		
Constipation	1		
Dyspepsia	1		
Dysgeusia	1		
Decreased appetite	1		
Purpura	1		
Convulsion	1		
Hypoalbuminaemia	1		

Table 2: Summary of treatment-related toxicities. Number of patients reporting an adverse event related to at least one of the agents in treatment. ALT, alanine aminotransferase. *includes decreased platelet count and decreased neutrophil count, respectively.

Patient	Histology	IDH status	*MGMT* promoter status	*ASS1* promoter status	No. lines of prior systemic therapy*
1	Glioblastoma	Wildtype	Unmeth	Unmeth	2
2	Glioblastoma	Wildtype	Unmeth	Unmeth	2
3	Glioblastoma	Wildtype	Unmeth	ND	2
4	Glioblastoma	Wildtype	Meth	Unmeth	2
5	Glioblastoma	Wildtype	Meth	Unmeth	2
6	Glioblastoma (transformed)	Wildtype	Unmeth	Meth	3
7	Anaplastic Astrocytoma	Wildtype	Unmeth	Meth	1
8	Anaplastic Astrocytoma	Mutant	Unmeth	ND	2
9	Anaplastic Astrocytoma (transformed)	Mutant	ND	Meth	3
10	Anaplastic Oligodendroglioma (transformed)	Mutant	Meth	Unmeth	2

Table 3: Individual patient tumoor characteristics. Unmeth, unmethylated; meth, methylated; ND, not determined; PFS, progression-free survival; OS, overall survival. *concomitant and adjuvant temozolomide (Stupp protocol) counted as one line of treatment.

A



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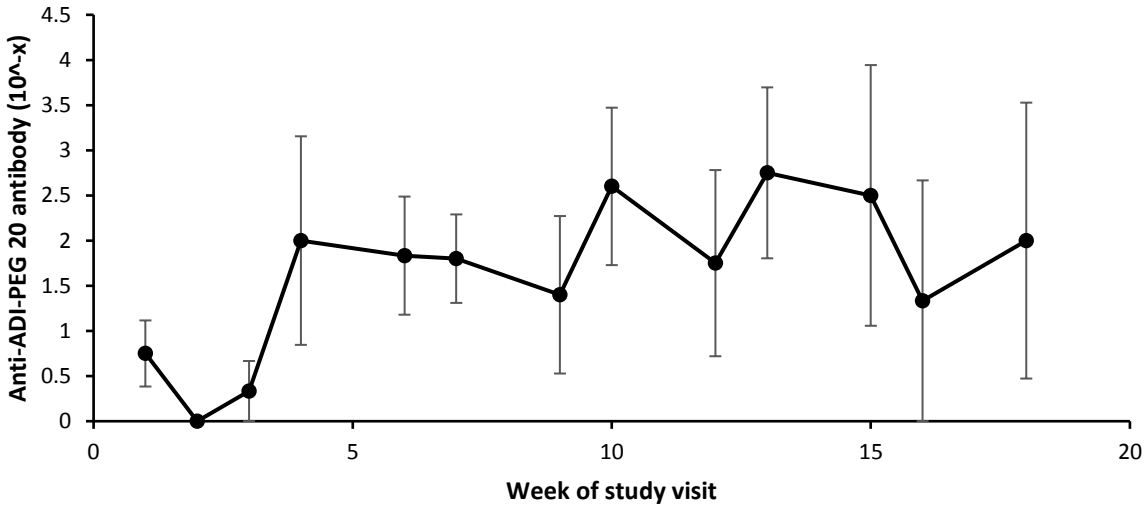
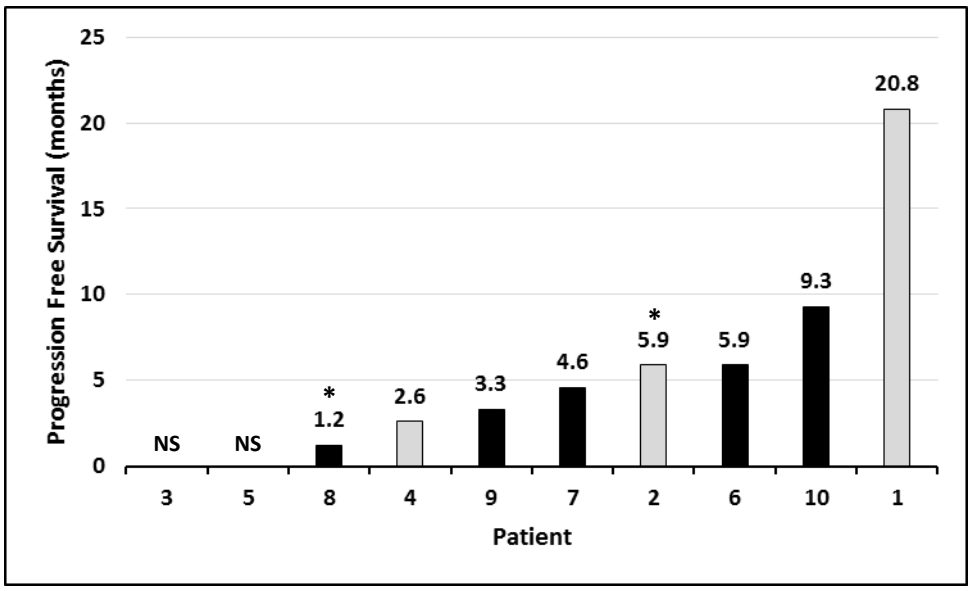


Figure 1: Pharmacodynamic and immunogenicity studies. (A) Median serum concentrations of both arginine and citrulline and (B) mean serum levels of anti–ADI-PEG20 antibodies in patients treated with pegylated arginine deiminase combined with pemetrexed and cisplatin are shown by week of treatment. Error bars shown are ±SEM. ADI-PEG20, pegylated arginine deiminase.

A



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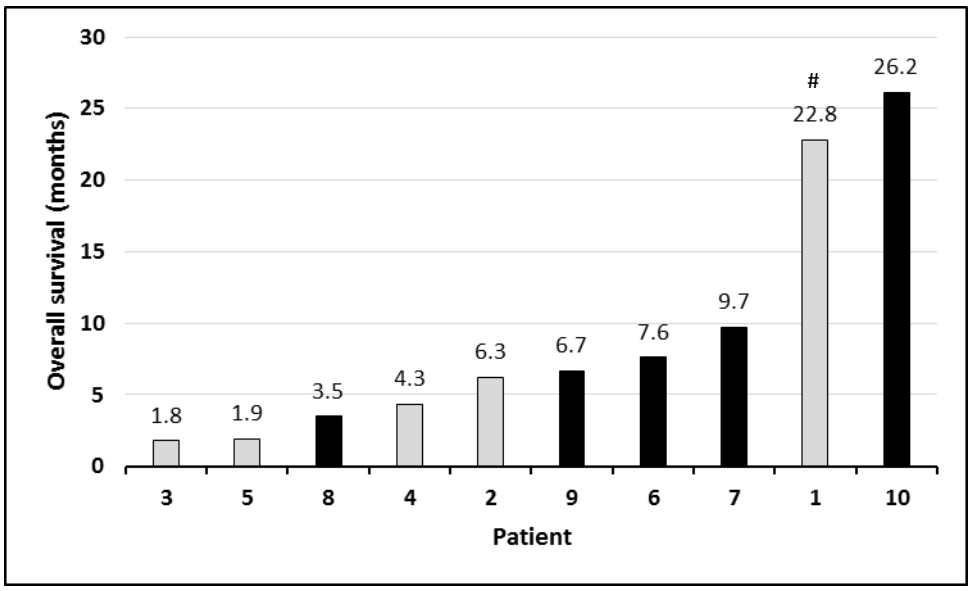


Figure 2: Survival data for individual patients. (A) Progression free survival and (B) Overall survival. Grey bars, isocitrate dehydrogenase wildtype glioblastoma; Black bars, other high-grade gliomas. See Table 3 for further information about each patient. NS, no scans on treatment; *censored, from date of last scan as clinical progression only; #censored, remained alive at data cut-off.

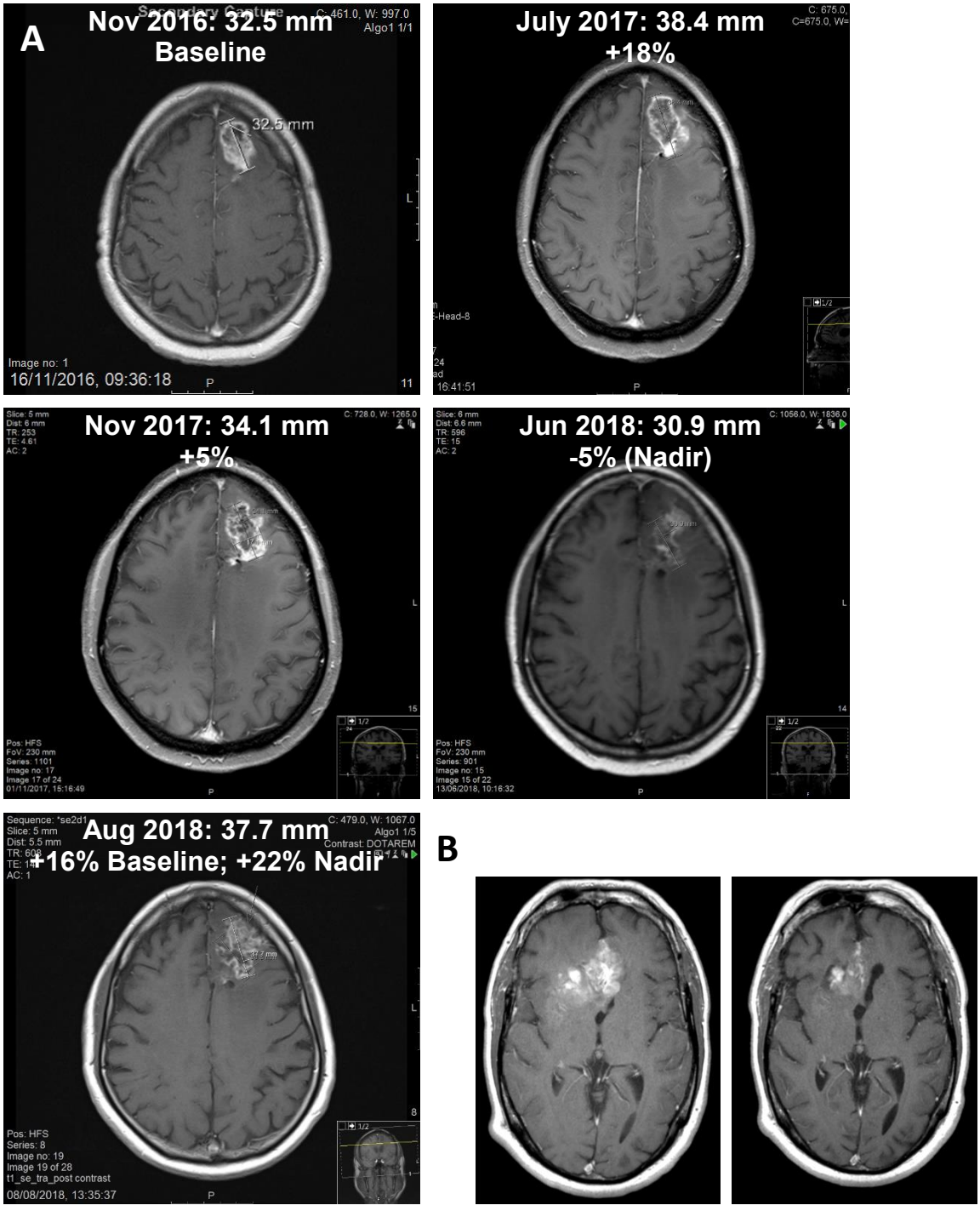
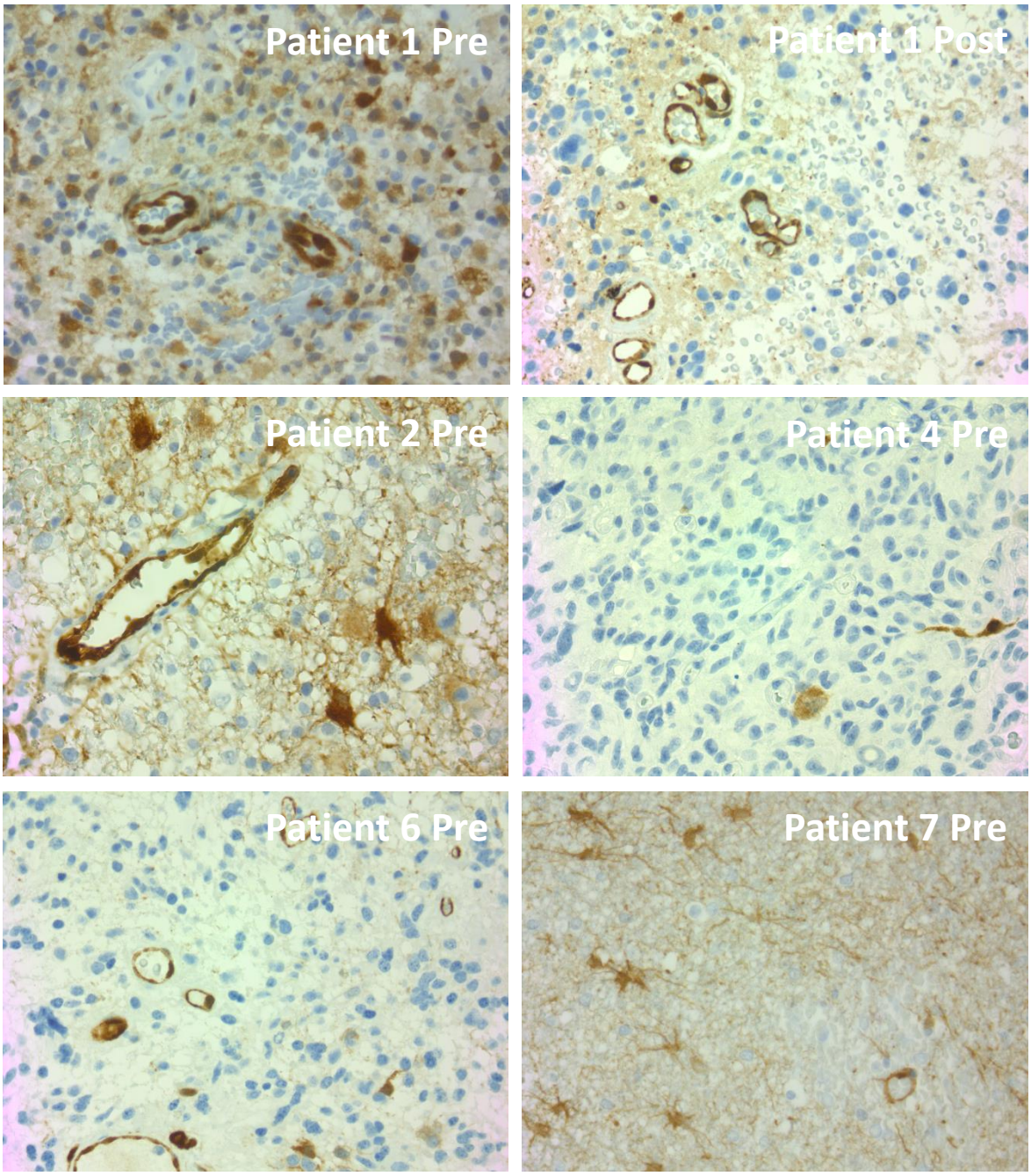
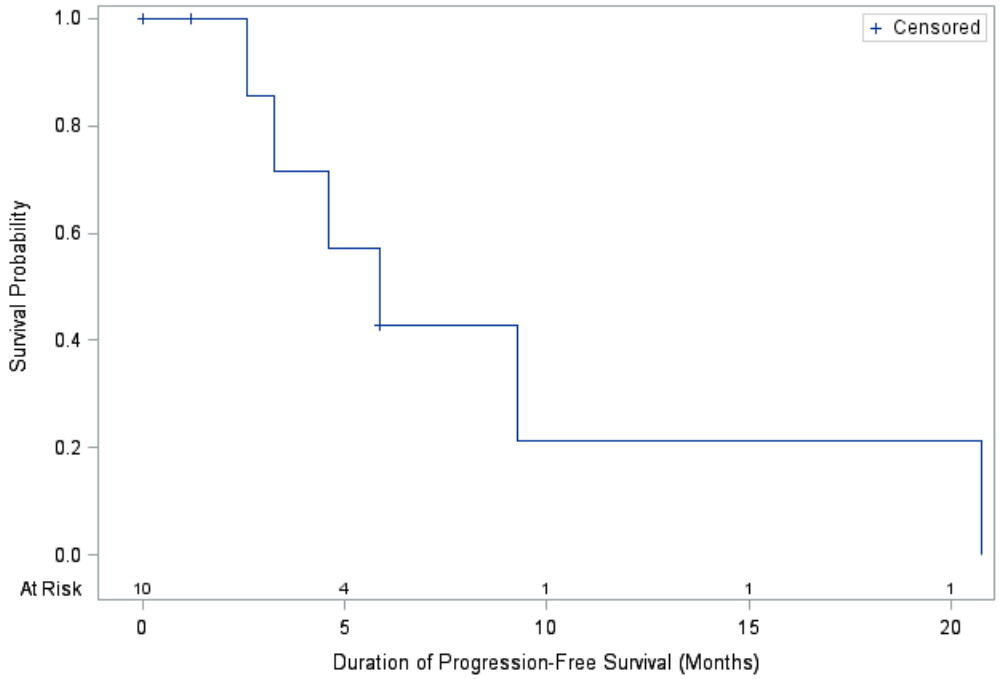


Figure 3: MRI scan images showing response to treatment in (A) Patient 1 and (B) Patient 10. (A) Representative images and RECIST 1.1 measurements for Patient 1 pre (November 2016 and July 2017) and post (November 2017, June 2018 and August 2018) starting quinine sulfate. (B) Representative images for Patient 10 showing baseline (left) and partial response to treatment by RANO criteria. Note, the best response by RECIST 1.1 in Patient 10 was stable disease.



Supplementary Figure S1: ASS1 immunohistochemistry. Representative images of ASS1 staining in tissue from patients' glioma, showing strong expression in endothelial and microglial cells, but negative/weak expression in the glioma cells. Pre, before trial treatment started; Post, after completion of trial (re-resection done for progressive disease). For experimental methods, please see Szlosarek et al., 2017 (16). All images were taken at x400.

A



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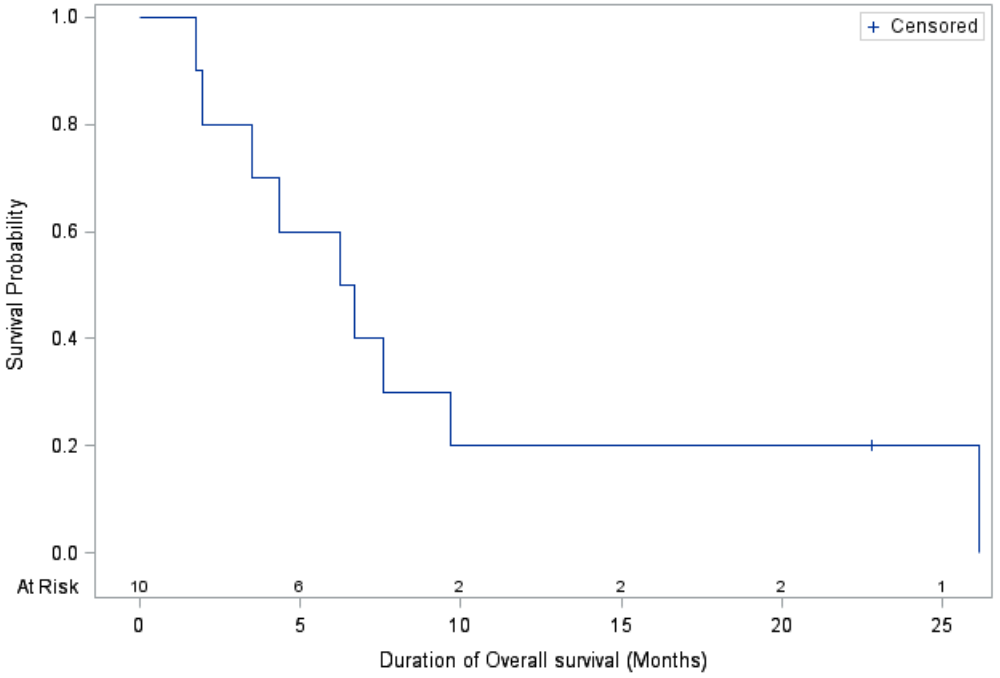
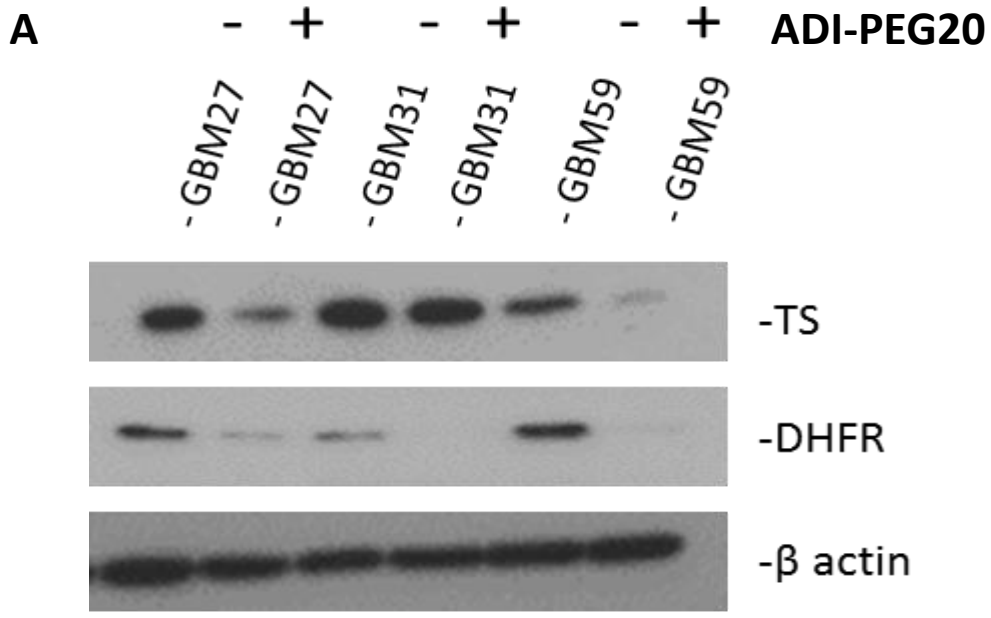
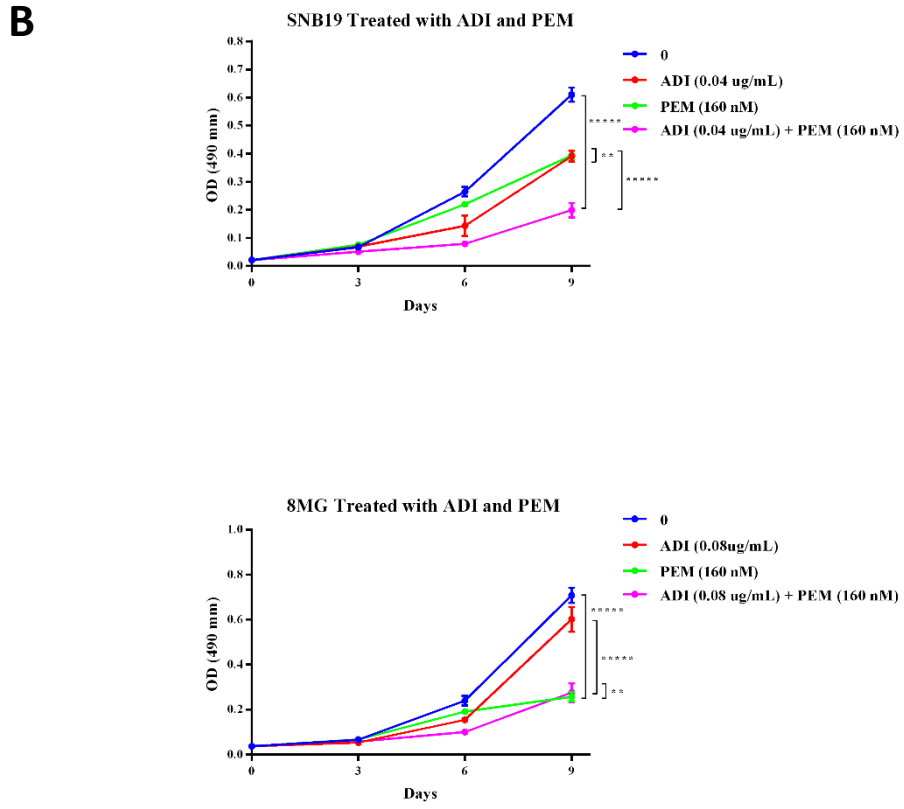


Supplementary Figure S2: Kaplan-Meier plots for (A) Progression free survival and (B) Overall survival.





Supplementary Figure S3: Effects of ADI-PEG20 on the folate pathway. (A) Representative western blot showing the addition of ADI-PEG20 reduces the expression of thymidylate synthase (TS) and dihydrofolate reductase (DHFR) in ASS1 negative glioblastoma primary cell lines. (B) Pemetrexed (PEM) and ADI-PEG 20 (ADI) synergize to reduce proliferation in ASS1 negative glioblastoma cell line (SNB19) but not in ASS1 positive glioma cell line (8MG). Experiments were repeated in triplicate. $^{**}P<0.01$, $^{*****}P<0.001$. For experimental methods, please see Syed et al., 2013 (20).