RESEARCH ARTICLE

hERG channel agonist NS1643 strongly inhibits invasive astrocytoma cell line SMA-560

Kieran W. Benn[1]ʘ, Patrick H. Yuan[1]ʘ, Harvey K. Chong[1], Stanley S. Stylii[2,3], Rodney B. Luwor[2], Christopher R. French[1,4]*

1 Neural Dynamics Laboratory, Department of Medicine, University of Melbourne, Melbourne, Victoria, Australia, 2 Department of Surgery, The Royal Melbourne Hospital, The University of Melbourne, Melbourne, Victoria, Australia, 3 Department of Neurosurgery, Royal Melbourne Hospital, The University of Melbourne, Victoria, Australia, 4 Department of Medicine, Royal Melbourne Hospital, The University of Melbourne, Melbourne, Victoria, Australia

ʘ These authors contributed equally to this work.
* frenchc@unimelb.edu.au

Abstract

Gliomas are highly malignant brain tumours that remain refractory to treatment. Treatment is typically surgical intervention followed by concomitant temozolomide and radiotherapy; however patient prognosis remains poor. Voltage gated ion channels have emerged as novel targets in cancer therapy and inhibition of a potassium selective subtype (hERG, Kv11.1) has demonstrated antitumour activity. Unfortunately blockade of hERG has been limited by cardiotoxicity, however hERG channel agonists have produced similar chemotherapeutic benefit without significant side effects. In this study, electrophysiological recordings suggest the presence of hERG channels in the anaplastic astrocytoma cell line SMA-560, and treatment with the hERG channel agonist NS1643, resulted in a significant reduction in the proliferation of SMA-560 cells. In addition, NS1643 treatment also resulted in a reduction of the secretion of matrix metalloproteinase-9 and SMA-560 cell migration. When combined with temozolomide, an additive impact was observed, suggesting that NS1643 may be a suitable adjuvant to temozolomide and limit the invasiveness of glioma.

Introduction

Gliomas are the most common primary central nervous system tumour and one of the most deadly [1]. Advancement in the standard of care has failed to translate into improved prognosis for glioma patients, with five-year survival improving only 1% over the last three decades for glioblastoma (GBM) [2, 3]. The current standard of care for the treatment of brain cancer involves maximal resection of the tumour, followed by radiotherapy and concomitant temozolomide (TMZ), known as the Stupp protocol [4, 5]. This treatment protocol extends median survival to only 15 months for GBM patients underpinning the need for new therapeutics to improve both prognosis and quality of life amongst this population [6].

There is increasing evidence that ion channels play a significant role in tumorigenesis and cancer progression [7, 8]. The human Ether-à-go-go Related Gene (hERG), also known as KCNH2, encodes for a component of Kv11.1, a voltage-gated potassium channel involved in homeostatic regulation, as well as rhythmogenesis in cardiac and neural tissue [9–13]. Overexpression of hERG channels is implicated in driving both malignant transformation and tumour progression [14–20]. It has also been demonstrated to be associated with neo-angiogenesis, upregulation of cell proliferation, and increases in cell motility and invasion [12, 21]. Importantly, the overexpression of hERG channels is a marker for poor patient prognosis [15, 18, 22]. In GBM patients, high hERG expression resulted in poorer overall survival; 43.5 weeks compared to 60.9 weeks in patients with low hERG expression [23]. Notably, this difference in survival was reduced when GBM patients with hERG overexpression were incidentally treated with non-torsadogenic hERG channel blockers, demonstrating potential antitumor efficacy through the modulation of hERG channels. Expression of KCNH2 is further variable by tumour histological subtype and may be examined through microarray data from gene expression database Oncomine [24].

Through inhibition of hERG channel function, tumour cells with high hERG expression can undergo apoptosis and show a decline in their rate of proliferation [14, 25–28]. However, therapies targeting hERG channel function are limited due to the cardiac risk profile of hERG channel antagonists, posed through drug-induced arrhythmias and sudden cardiac death [29]. Interestingly, the narrow range of function of hERG channels enables hyperstimulation *via* hERG channel agonists as a viable method to irreversibly inhibit the proliferation of tumour cells that are ectopically expressing the channel, while simultaneously minimising cardiotoxic risk [30, 31].

The hERG channel agonist NS1643 has been reported to reduce cell proliferation in rodent and human glioma cell lines [32]. Herein, the SMA-560 mouse glioma cell line was treated with the hERG channel agonist NS1643 and in combination with TMZ, resulting in a strong inhibition of cell proliferation. Our data supports further research into the potential use of N1643 in the clinical management of glioma patients.

Materials and methods

SMA-560 cell culture

The SMA-560 tumour cell line, which was generously provided by Dr. Hui Lau (Royal Melbourne Hospital), was cultured in DMEM (10313–021, Gibco) supplemented with (in v/v) 10% FBS (12003C, SAFC Biosciences, Australia), 1% penicillin-streptomycin (15140122, Gibco) and 1% GlutaMax (35050061, Gibco) and incubated at 37˚C with 5% CO_2 in a humidified environment [33].

Cell proliferation assays

Cells were seeded in 96-well plates and allowed to adhere overnight. Triplicate wells were treated with varying concentrations of NS1643 as indicated for 72 h. Cells were subsequently lysed and cell viability determined relative to the vehicle control using a commercially available Cell Titer-Glo kit (Promega) following manufacturer's instructions.

Scratch assay

Cells were seeded into 6-well plates and allowed to adhere for 48 h. A 1 mL pipet tip was used to create the scratch. NS1643 (Sigma Aldrich; CAS 448895-37-2) or temozolomide (Xi'an

D-Sung Health Biotechnology; CAS 85622-93-1) were prepared in the cell medium at a stock concentration of 20 mM and 100 mM, respectively.

Cell migration was monitored using an IX50 inverted system microscope (Olympus) and DFC3000 G microscope camera (Leica) controlled by Leica Application Suite Version 4.6. Images were taken with a 4X objective at 0, 24 and 48 h post-scratch and ImageJ (Schneider et al., 2012; version 1.53t) was used to analyse the closure of the scratch to determine the cell migration rate.

Gelatinase zymography

Cells were seeded in six-well plates (Corning) and were allowed to adhere overnight before washing with sterile PBS and subsequent incubation in serum-free OptiMEM (Thermofisher Scientific) for 24 h. One-hundred-microliter aliquots of the conditioned OptiMEM medium was then sampled and centrifuged at $1000 \times g$ ($4°C$) for 10 min. Gelatin-based zymography was performed with the conditioned OptiMEM media samples using 10% gelatin-substrate zymography NuPAGE precast gels (Invitrogen, Australia) at 125 V for 1.5 h and the gels were stained with Simply-Blue Stain (Life Technologies), followed by washing in distilled water until clear gelatinolytic bands were visible. The gels were scanned using a flatbed scanner for further densitometric analysis using Image J (version 1.51f).

Identification of hERG channels *via* patch clamp

Healthy SMA-560 cells were selected for recordings. Fire polished borosilicate micropipettes (1.5–2.5 MΩ) were fabricated with a Sutter Instrument model P-1000 micropipette puller and filled with a KF-based intracellular solution (in mM: 130 KF, 1 $CaCl_2$, 1 $MgCl_2$, 5 NaCl, 10 HEPES, 10 phosphocreatine, 4 MgATP, 0.3 GTP, 5 EGTA) (pH 7.25) and whole cell voltage clamp recordings were carried out in a temperature controlled room at $22°C$ in a high K^+ external solution (in mM: 150 KCl, 4 NaCl, 10 HEPES, 2 $MgCl_2$, 2 $CaCl_2$). Currents were recorded with an AM systems model 2400 patch clamp amplifier with a sampling interval of 10µs and Clampfit 10 software with a Digidata 1322A interface. The inhibitor-sensitive currents were obtained by subtracting the recorded currents in the presence of a hERG-channel inhibitor E4031 from those recorded in the absence of the drug.

At the start of experimentation on each neuron, the high K^+ external solution was perfused into the recording chamber. From a holding potential of -100 mV the cell was depolarised to +40 mV for 500 ms followed by step changes in voltage with 500 ms negative repolarising pulses from -80 mV to 170 mV with a 10 mV decrement at each sweep. Cells were perfused with hERG channel inhibitor E4031 in high K^+ external solution and the hERG protocol repeated to obtain current tracing following treatment with E4031. E4031 was washed out at a holding voltage of -100 mV, and the hERG protocol repeated to obtain current tracing following washout. Analysis of current tracing was performed using Clampfit 10 software.

Oncomine data mining

Oncomine was used to compare expression levels of KCNH2 in different histological grades of glioma relative to normal brain tissue from different studies [24]. Search criteria examined primary articles only from the previous 10 years, relevant to KCNH2 expression in human gliomas.
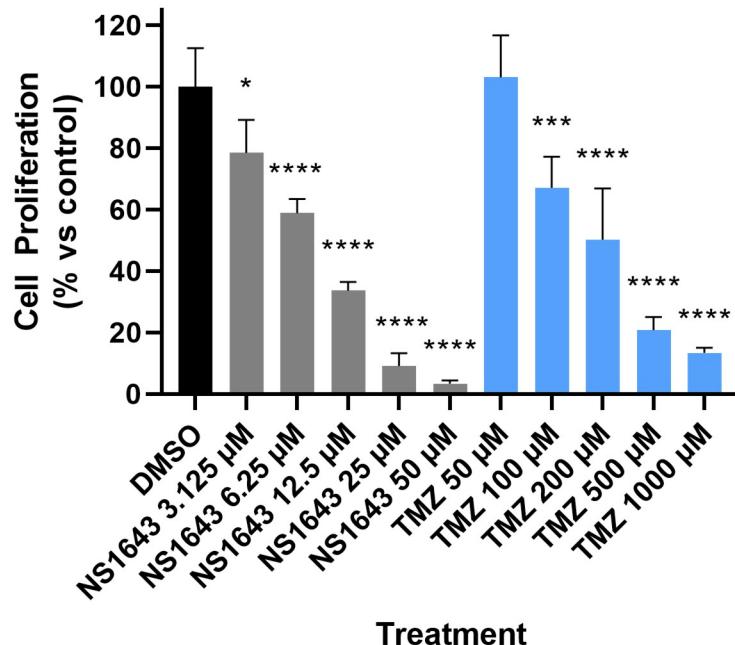
Fig 1. Cell proliferation assays: NS1643 inhibits cell proliferation. Cells were treated with indicated concentrations of NS1643 (n = 5) or TMZ (n = 5) for 72 h. Cell proliferation determined *via* Cell Titer-Glo kit. All statistical analyses were performed using GraphPad Prism 10. Data was analysed using repeated measures one-way ANOVA followed by a Dunnett's test relative to DMSO control cells. Values were determined to be significant if p<0.05 (*), p<0.01 (**), p<0.001, and p<0.0001 (***).

https://doi.org/10.1371/journal.pone.0309438.g001

Results

NS1643 inhibits cell proliferation

When SMA-560 cells were treated with NS1643 at concentrations greater than or equal to 3.125 μM over a 72 h period a statistically significant reduction in cell proliferation was observed, relative to the control (Fig 1). Notably, when treated with NS1643 concentrations greater than 25 μM, cell proliferation was almost arrested; 3.385%. ± 1.034 for 50 μM NS1643. Comparatively, high dose temozolomide treatment at 1000 μM resulted in 13.4353% ± 1.658 cell proliferation, relative to the control.

NS1643 reduces matrix metalloproteinase-9 secretion and migration in SMA-560 cells

Treatment of SMA-560 cells with a 40 μM concentration of NS1643 significantly reduces matrix metalloproteinase-9 (MMP) secretion (37.67% ± 6.49) (Fig 2).

NS1643 was effective in reducing wound healing of SMA-560 cells relative to the untreated control (Fig 3). When treated with 40 μM of NS1643, SMA-560 exhibit statistically significant reduction in wound healing capacity resulting in an average remaining wound area at 48 h of 46.30% ± 3.08 (mean ± SEM). Compared to untreated control cells, which displays 16.78% ± 1.34 (mean ± SEM) of the original wound area, this amounts to a 29.53% difference in wound area. Treatment with 200 μM TMZ resulted in 31.05% ± 1.267 (mean ± SEM) wound area remaining, representative of a 15.25% difference in wound area when compared to 40 μM NS1643.
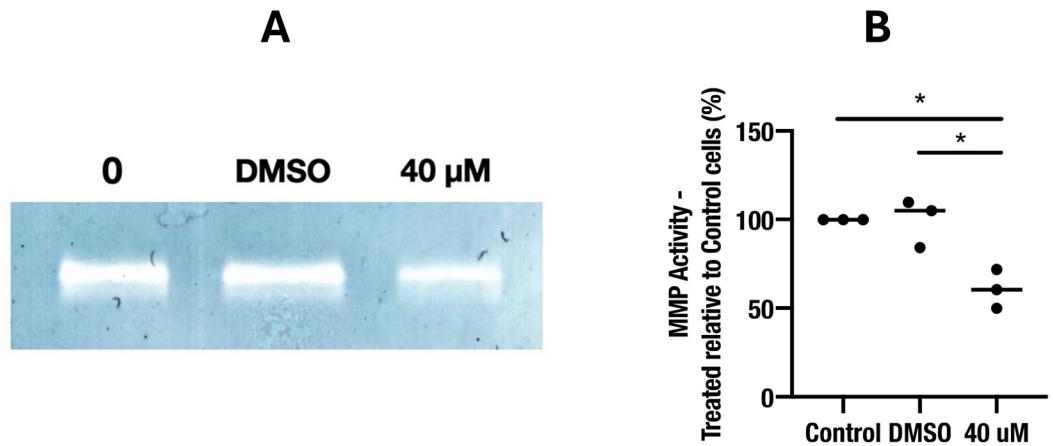
Fig 2. NS1643 (40 µM) reduces MMP-9 secretion in SMA-560 cells: A) Gelatinase zymogram analysis of serum-reduced Optimem conditioned medium showing MMP-9 secretion SMA-560 cells (0 µM NS1643, DMSO control, 40 µM NS1643). B) Densitometric analysis of 3 zymogram experiments. All statistical analyses were performed using GraphPad Prism 10. Data was analysed using repeated measures one-way ANOVA followed by a Tuckey's post hoc test. Values were determined to be significant if $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$, and $p < 0.0001$ (***).

NS1643 and temozolomide combination treatment displays additivity in SMA-560 cells

The IC_{50} values for NS1643 and TMZ for cell proliferation in the SMA-560 cells were determined to be 7.6 µM and 167.5 µM, respectively (Fig 4). Following this, a range of paired concentrations below for IC_{50} for both NS1643 and TMZ were assessed to determine a possible synergistic relationship as previously described [34]. Superadditivity indicative of synergism was not observed, however low dose combination treatment produced a substantial reduction in cell proliferation, relative to DMSO control, suggestive of additivity (Fig 4)

hERG channel inhibitor E4031 reduces hERG channel characteristic tail current amplitude in SMA-560 cells

Patch clamp investigation of healthy SMA-560 cells, when subject to the hERG protocol and dosed with E-4031, at concentrations of 25 µM or 1 µM, revealed clear inhibition compatible with hERG currents as previously described [35]. Current subtraction between the currents recorded in the presence and the absence of the E4031 was carried out to obtain the E-4031-sensitive currents (Fig 5). A steady outward component, as well as a hook shaped tail current was observed; highly characteristic of hERG channels.

Gene KCNH2 is expressed differentially independent of brain tumour histological subtype

Microarray data from publicly available gene expression database *Oncomine* was extracted to examine the expression level of KCNH2 in different histological subtypes of human brain cancers, relative to normal brain tissue (Table 1) [24]. Independent studies showed a significant difference in the value of gene expression ($p \leq 0.05$) in human brain cancers compared with normal human brain tissues [36–39].

In the Beroukhim study, KCNH2 was significantly overexpressed in primary and secondary glioblastoma compared to normal brain and was among the top 3% and 6% overexpressed
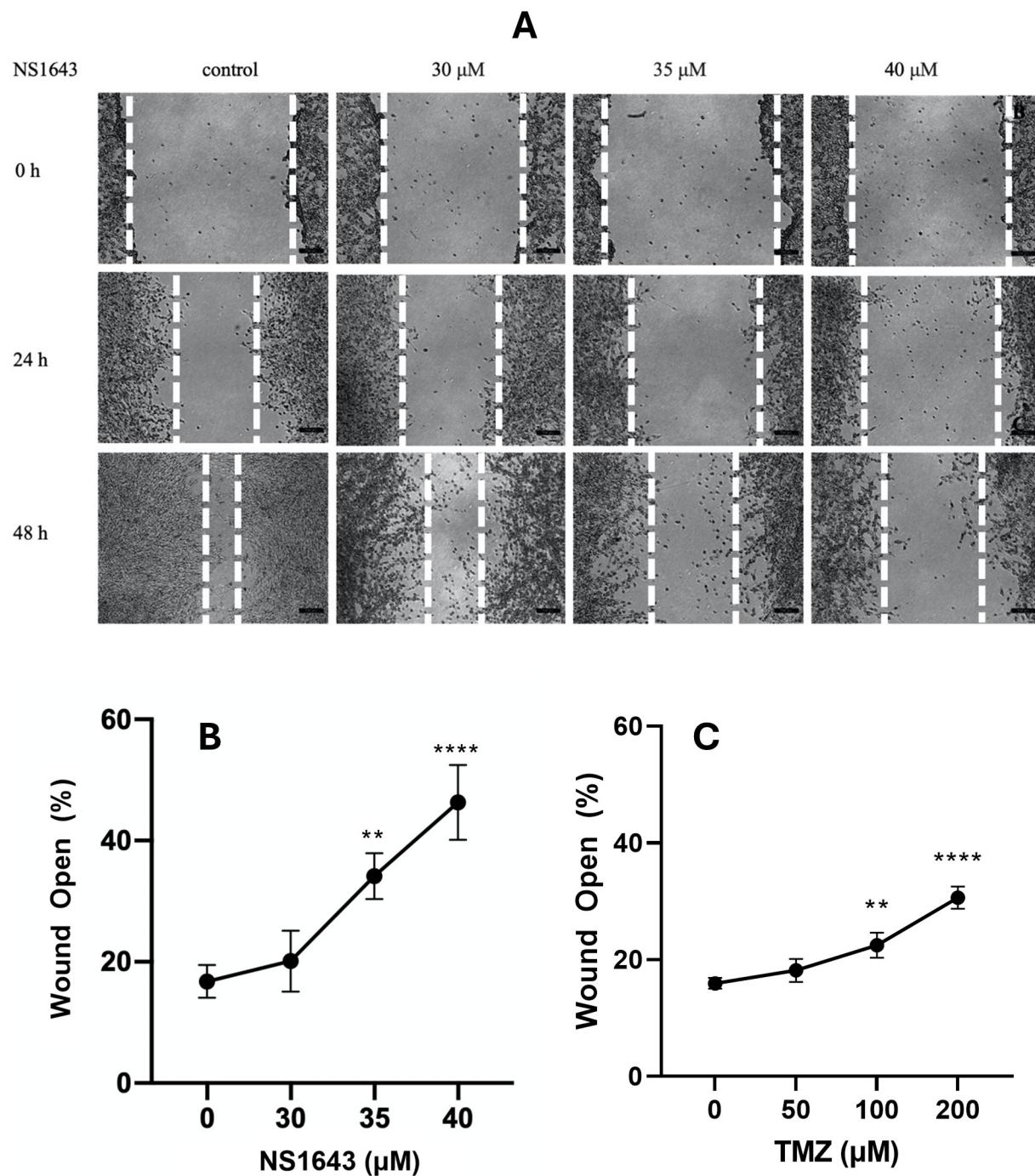
Fig 3. Scratch assays of SMA-560 cells following 48 h treatment with NS1643 or TMZ: A) Images of SMA-560 scratch assay at varying timepoints following NS1643 treatment (n = 4; Dotted lines mark the borders of the wound area). A,B) Quantification of scratch assays with pixel analysis demonstrating % of wound area remaining open at 48 h following treatment with either NS1643 or TMZ at indicated concentration. Data was analysed using repeated measures one-way ANOVA followed by a Dunnett's post hoc test relative to DMSO control cells. Values were determined to be significant if $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****).
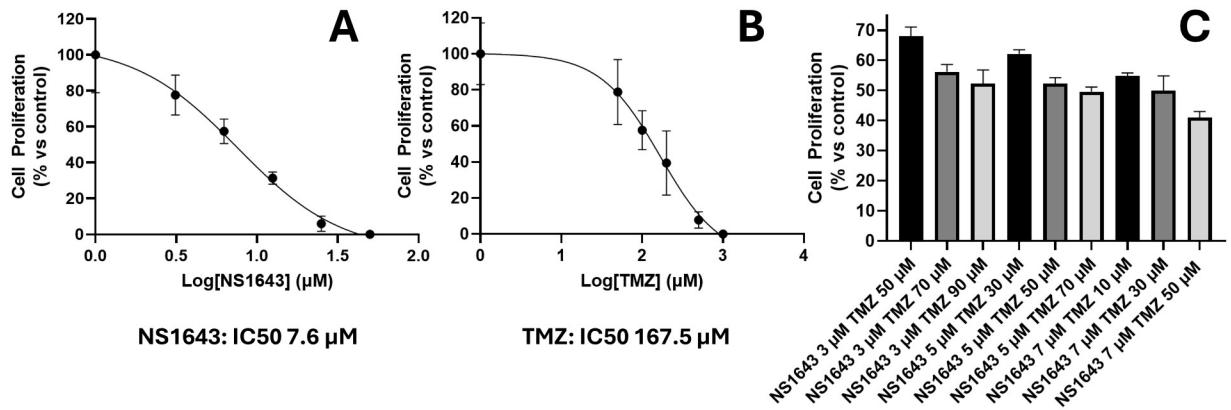
https://doi.org/10.1371/journal.pone.0309438.g003

Fig 4. NS1643 and TMZ display simple additivity: A) IC_{50} of NS1643 for SMA-560 as determined by cell proliferation assay. B) IC_{50} of temozolomide for SMA-560 cells as determined by cell proliferation assay. C) Cell proliferation following combination treatment of SMA-560 cells with NS1643 and TMZ at indicated concentrations. Cell proliferation determined *via* Cell Titre Glo kit following 72h treatment.

genes, respectively (Table 1). The study also showed that KCNH2 was significantly overexpressed in anaplastic astrocytoma and anaplastic oligodendroglioma (Fig 6) [36] Similarly, in the study conducted by French, KCNH2 was also found to be significantly overexpressed and was ranked among the top 9–15% overexpressed genes in anaplastic oligoastrocytoma and
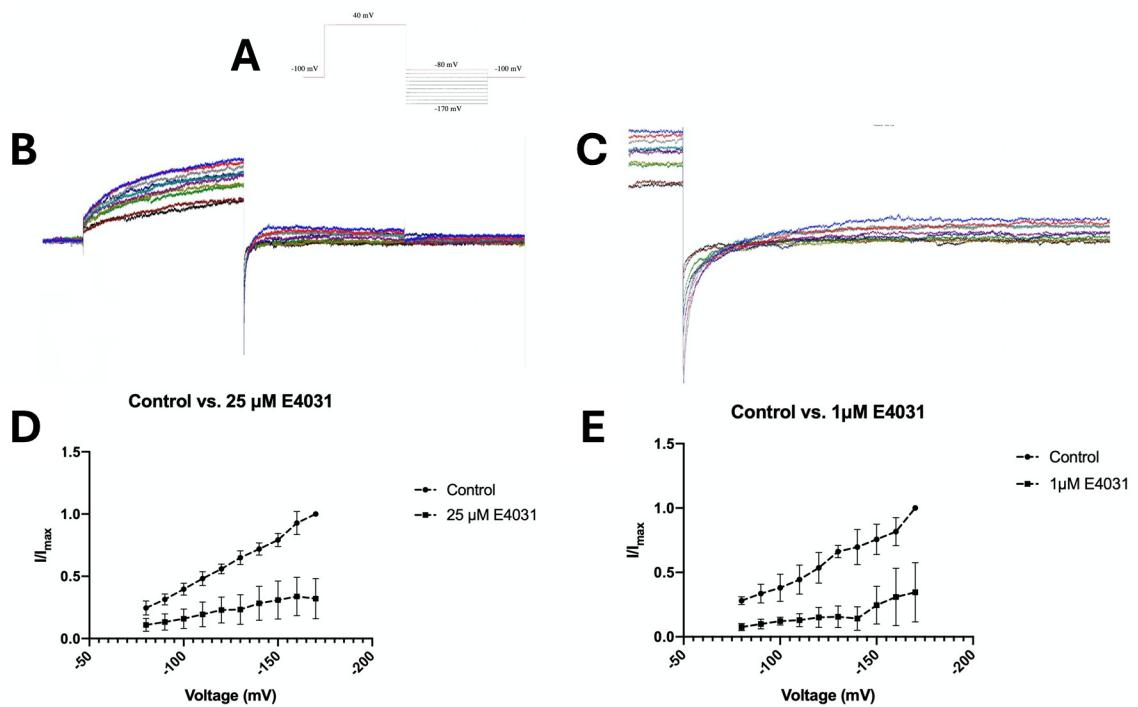

Fig 5. Characterisation of E-4031-senstive currents in the SMA-560 cells (n = 5): A) hERG protocol; cells were held at -100 mV prior to depolarisation the cell at +40 mV for 500 ms and stepping down to a series of 500 ms repolarising pulses from -80 mV to -170 mV, with a 10 mV decrement each sweep. B) Whole current tracing of hERG channel inhibitor E-4031-subtracted (25μM) currents elicited by a hERG channel-specific voltage protocol. C) Enlarged representative E-4031-substracted tail currents. D, E) Normalised tail current amplitude elicited by repolarising currents. Peak tail currents were measured and normalised to the maximal tail current produced at -170 mV. Amplitude is compared.

Table 1. Analysis of KCNH2 mRNA levels in human brain cancer tissues.

Study	Beroukhim [36] [187]				French [37] [101]		Bredel [38] [54]		Murat [39] [84]
Percentile	97	94	92	90	91	85	87	85	78
Tumour Type	GBM	GBM*	AA	AO	AOA	AO	AOA	AO	GBM
Percentage Change (%)	117.5	113.9	109.5	112.6	185.2	171.9	281.5	296.9	125.2
P-value	3.49E-13	5.88E-4	0.023	0.038	0.005	8.98E-4	0.027	0.031	0.005

Percentile–percentile of overexpression for KCNH2 mRNA. GBM–glioblastoma; AA–anaplastic astrocytoma; AO–anaplastic oligodendroglioma; AOA–anaplastic oligoastrocytoma.

*—Secondary GBM

anaplastic oligodendroglioma [37]. A study by Murat revealed that KCNH2 was overexpressed in GBM and was ranked among the top 22% of overexpressed genes [39].

Discussion

Malignant gliomas are among the deadliest cancers and represent a disproportionately high percentage of all cancer-related deaths annually, despite their low prevalence [40]. The five-year relative survival of GBM patients has only improved by 1% over the last three decades, signifying that despite some appreciable advances in the treatment of gliomas, a dearth of efficacious therapeutics that can effectively improve the prognosis for glioma patients still remains [2].
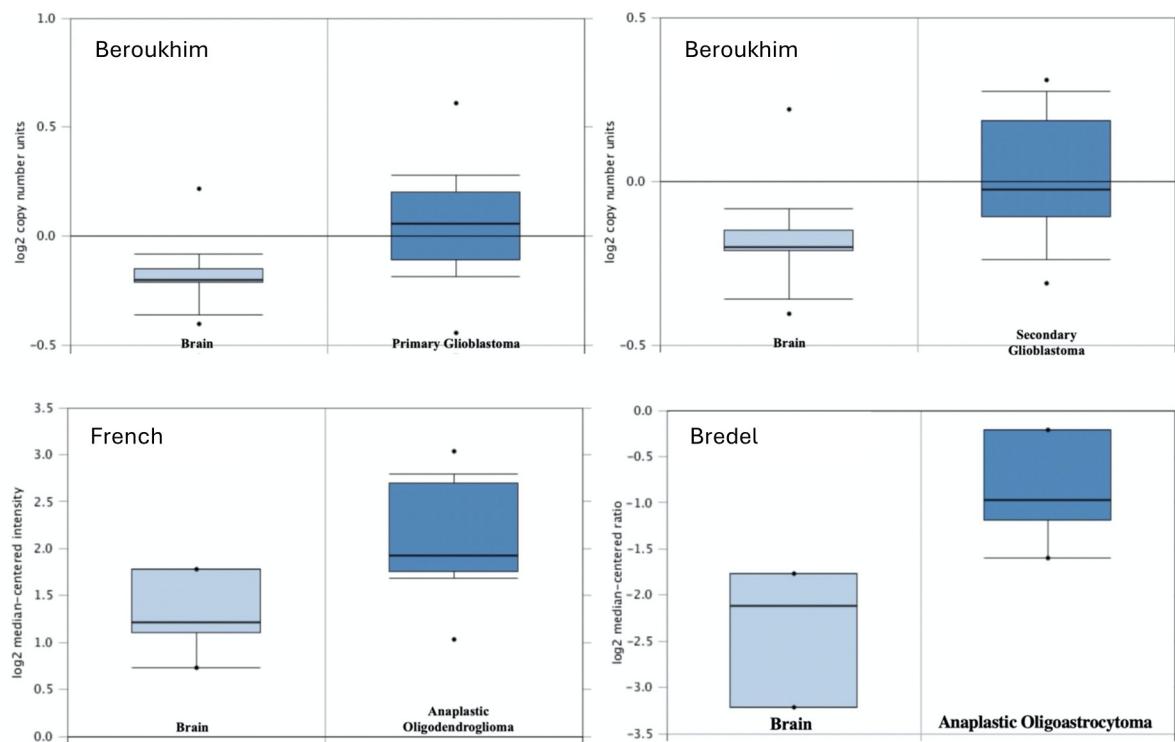

Fig 6. Box plot indicating KCNH2 expression level in different studies: Oncomine dataset; threshold by P-Value: 0.05; Fold Change: 1; Gene rank: Top 20%.

Increasing evidence suggests that potassium channels play many fundamental roles in the pathogenesis of glioma [41]. Overexpression or disruption of ion channel function can promote cell proliferation and cell migration [42–44]. The voltage-gated potassium channel, hERG, has been shown to be involved in the upregulation of cell proliferation, cell motility, cell invasion as well as in the process of angiogenesis [12, 21]. Treatment with hERG channel antagonists has been shown to increase apoptosis and reduce proliferation in melanoma, non-small cell lung cancer, and GBM [14, 25, 26]. However, the therapeutic action of hERG channel blockade is limited by severe cardiotoxicity [45]. Off-target blockade of hERG channels is known to produce drug-induced long QT syndrome, which increases the risk of cardiac arrhythmias, namely *torsade de pointes*, and sudden cardiac death [46]. Due to the cardiac risk profile, several hERG channel antagonists have been discontinued [13].

Further research has demonstrated that hERG channels have a narrow range of function during cell cycle progression [47]. Consequently, both constitutive inhibition and hyperstimulation of hERG channels have been proposed to be capable of inducing senescence [30]. This has presented an alternative route to extract chemotherapeutic utility from hERG channel modulation and significant cardiotoxicity has yet to observed in animal models [31]. Treatment of breast cancer cells expressing hERG channels with NS1643, irreversibly inhibited proliferation [30]. Although they were not able to detect an apoptotic event, it was however observed that NS1643 treated cells were preferentially arrested in the G0/G1 phase with increased expression of the tumour suppressor proteins p21 and p16. Similar results are seen in this study where treatment of SMA-560 cells with NS1643 results in potently decreased cell proliferation. Notably, at concentrations of NS1643 greater than 25 µM, cell proliferation of SMA-560 cells was reduced to approximately 9% of the control.

The mechanism of inhibition is purported to be through the downregulation of cyclin E2 [48, 49]. Cyclin E2 plays a critical role in cell cycle phase G1 progression and is described as rate limiting for S phase entry [49]. Treatment with NS1643 has been demonstrated to reduce levels of cyclin E2, possibly through increased rates of degradation through the ubiquitin-proteasome pathway [49]. It also has been reported that down-regulation of cell proliferation may occur *via* a calcineurin-dependent transcription of p21$^{waf/cip}$ induced by treatment with NS1643 [50]. Notably, an *in vivo* model of breast cancer demonstrated that mice treated with NS1643 did not exhibit significant cardiac dysfunction, affirming a reduced cardiac risk profile of hERG channel hyper-stimulation, comparatively to blockade [31]. Treatment with NS1643 did produce a small elevation in the heart rate of some mice, however, this can be appropriately managed with routine pharmacotherapy. Treatment of SMA-560 cells with NS1643 at 50 µM, also resulted in a more significant decrease in cell proliferation compared to high dose treatment with TMZ at 1000 µM (Fig 1). Notably, the observed sensitivity of SMA-560 cells to temozolomide appears to be significantly greater than previously reported, perhaps due to differing methods of viability assessment [51]. The absence of reported cardiotoxicity in animal models, and potent anti-tumour activity, suggests treatment with NS1643 may offer therapeutic utility for the management of glioma.

Treatment of glioma is intrinsically challenging due to the inherent limitations of surgical intervention only providing a maximal safe resection of the tumour and often leaving residual tumour tissue [52]. Furthermore, glioma cells surviving subsequent chemotherapy and radiotherapy see a shift towards a more invasive phenotype through increased MMP activity [53–56]. Invasion is facilitated by cell membrane bound, dynamic, actin-rich structures known as invadopodia that secrete MMP-2 and MMP-9 [56]. MMP secretion results in degradation of the surrounding extracellular matrix, thereby facilitating migration, invasion, and metastasis [57]. Adjuvant therapies for glioma should aim to kill cells surviving the Stupp protocol, as well as inhibit the invasive capability of glioma. Treatment of SMA-560 cells with 40 µM

NS1643 reduced MMP-9 secretion over a 24 h period suggesting NS1643 may reduce the invasivity of glioma. Furthermore, reduced wound healing, and migratory capacity, of SMA-560 cells was observed following treatment with NS1643.

The diverse microenvironment of gliomas is driving current research toward combinatorial therapy [58]. TMZ alkylates purine bases within DNA generating a persistent methyl adduct in the template strand [59]. This adduct is clastogenic, leading to collapse of the replication fork and cell cycle arrest. Senescence follows and concomitant action of downstream apoptotic proteins results in cell death. This mechanism of action is distinct from NS1643, suggesting combinatorial therapy may be a viable treatment strategy [30, 31]. Combination treatment of SMA-560 cells with NS1643 and TMZ displayed simple additivity as determined by cell proliferation assay, suggesting NS1643 may be a suitable adjuvant.

Bioinformatics data, obtained from Oncomine, indicates that KCNH2 gene is expressed differentially independent of brain cancer histological subtypes [36–39]. The degree of overexpression is most prominent in diffuse astrocytic and oligodendroglial tumours. Herein, patch-clamp investigation of SMA-560 cells is highly suggestive of hERG channel expression. The hERG channel shares structural similarities with the depolarisation-activated potassium channel family, however displays inward rectification expected of potassium channels with two transmembrane segments [60]. Upon repolarisation to a negative membrane potential, channel conductance increases dramatically to a large value before returning slowly to the resting closed state; this produces a prominent tail characteristic of hERG channels. The underlying mechanism for the gating kinetics of hERG channels is proposed to be the result of an inactivation mechanism that deactivates channels at positive potentials, but quickly recovers at negative potentials [61–63]. SMA-560 cells were treated with hERG channel antagonist E-4031 to visualise underlying hERG channel kinetics. Treatment with the inhibitor produced currents with hook-like characteristics consistent with classical hERG channel blockade [62, 64]. Amplitude of tail currents was proportional to increasingly negative potential, suggesting a dependence on repolarising potential. This is due to rapid recovery of hERG channels from inactivation to an open state, before slowly returning to the deactivated state [64]. SMA-560 cells are a well-established glioma model, representative of differentiated anaplastic astrocytoma and the patch-clamp data provides constitutive evidence for the presence of hERG channels [65]. This suggests a direct mechanism of action for NS1643 on SMA-560 cells, instead of non-specific toxicity.

Conclusion

In summary, the presence of hERG channels is recognised in SMA-560 cells through patch clamp investigation and hERG channel agonist NS1643 is demonstrated to almost entirely halt proliferation of anaplastic astrocytoma cell line SMA-560 at concentrations greater than 25 μM. The invasive capability of SMA-560 would also be impacted through the reduced secretion of MMP-9. In addition, the migratory ability of the SMA-560 cell line appears to be affected due to the observed decrease in wound closure. Furthermore, NS1643 displays additivity for combination treatment with TMZ, suggesting NS1643 may be a useful adjuvant to the current standard of care. Future work should aim to expand these findings to human cell lines and *in vivo* study.

Supporting information

S1 Fig. Gelatinase zymogram measuring MMP-9 secretion following NS1643 treatment: Image taken 24 h after treatment with 40 μM NS1643.
(TIF)

Acknowledgments

Author Contributions

References

1. Walker C, Baborie A, Crooks D, Wilkins S, Jenkinson MD. Biology, genetics and imaging of glial cell tumours. Br J Radiol. 2011; 84 Spec No 2(Spec Iss 2):S90–106. https://doi.org/10.1259/bjr/23430927 PMID: 22433833

2. AIHW AIoHaW. Cancer in Australia 2017. In: Welfare AIoHa, editor. Website: Australian Government; 2017.

3. Ozdemir-Kaynak E, Qutub AA, Yesil-Celiktas O. Advances in Glioblastoma Multiforme Treatment: New Models for Nanoparticle Therapy. Frontiers in physiology. 2018; 9:170. https://doi.org/10.3389/fphys.2018.00170 PMID: 29615917

4. Weller M, Cloughesy T, Perry JR, Wick W. Standards of care for treatment of recurrent glioblastoma—are we there yet? Neuro-oncology. 2013; 15(1):4–27. https://doi.org/10.1093/neuonc/nos273 PMID: 23136223

5. Stupp R, Mason WP, van den Bent MJ, Weller M, Fisher B, Taphoorn MJB, et al. Radiotherapy plus Concomitant and Adjuvant Temozolomide for Glioblastoma. New England Journal of Medicine. 2005; 352(10):987–96. https://doi.org/10.1056/NEJMoa043330 PMID: 15758009

6. De Vleeschouwer S. Glioblastoma. 2017.

7. Griffin M, Khan R, Basu S, Smith S. Ion Channels as Therapeutic Targets in High Grade Gliomas. Cancers (Basel). 2020; 12(10). https://doi.org/10.3390/cancers12103068 PMID: 33096667

8. Molenaar RJ. Ion channels in glioblastoma. ISRN Neurol. 2011; 2011:590249. https://doi.org/10.5402/2011/590249 PMID: 22389824

9. Grunnet M, Hansen RS, Olesen SP. hERG1 channel activators: a new anti-arrhythmic principle. Progress in biophysics and molecular biology. 2008; 98(2–3):347–62. https://doi.org/10.1016/j.pbiomolbio.2009.01.002 PMID: 19351523

10. Vandenberg JI, Perry MD, Perrin MJ, Mann SA, Ke Y, Hill AP. hERG K(+) channels: structure, function, and clinical significance. Physiological reviews. 2012; 92(3):1393–478. https://doi.org/10.1152/physrev.00036.2011 PMID: 22988594

11. Mitcheson J, Arcangeli A. CHAPTER 11 The Therapeutic Potential of hERG1 K+ Channels for Treating Cancer and Cardiac Arrhythmias. Ion Channel Drug Discovery. 2015:258–96.

12. Masi A, Becchetti A, Restano-Cassulini R, Polvani S, Hofmann G, Buccoliero AM, et al. hERG1 channels are overexpressed in glioblastoma multiforme and modulate VEGF secretion in glioblastoma cell lines. British journal of cancer. 2005; 93(7):781–92. https://doi.org/10.1038/sj.bjc.6602775 PMID: 16175187

13. Sanguinetti MC, Tristani-Firouzi M. hERG potassium channels and cardiac arrhythmia. Nature. 2006; 440(7083):463–9. https://doi.org/10.1038/nature04710 PMID: 16554806

14. Afrasiabi E, Hietamaki M, Viitanen T, Sukumaran P, Bergelin N, Tornquist K. Expression and significance of HERG (KCNH2) potassium channels in the regulation of MDA-MB-435S melanoma cell proliferation and migration. Cellular signalling. 2010; 22(1):57–64. https://doi.org/10.1016/j.cellsig.2009.09.010 PMID: 19765650

15. Ding XW, Luo HS, Luo B, Xu DQ, Gao S. Overexpression of hERG1 in resected esophageal squamous cell carcinomas: a marker for poor prognosis. Journal of surgical oncology. 2008; 97(1):57–62. https://doi.org/10.1002/jso.20891 PMID: 17786970

16. Nilius B, Wohlrab W. Potassium channels and regulation of proliferation of human melanoma cells. The Journal of physiology. 1992; 445:537–48. https://doi.org/10.1113/jphysiol.1992.sp018938 PMID: 1323670

17. Strobl JS, Wonderlin WF, Flynn DC. Mitogenic signal transduction in human breast cancer cells. General pharmacology. 1995; 26(8):1643–9. https://doi.org/10.1016/0306-3623(95)00062-3 PMID: 8745151

18. Lastraioli E, Guasti L, Crociani O, Polvani S, Hofmann G, Witchel H, et al. herg1 gene and HERG1 protein are overexpressed in colorectal cancers and regulate cell invasion of tumor cells. Cancer research. 2004; 64(2):606–11. https://doi.org/10.1158/0008-5472.can-03-2360 PMID: 14744775

19. Pillozzi S, Brizzi MF, Balzi M, Crociani O, Cherubini A, Guasti L, et al. HERG potassium channels are constitutively expressed in primary human acute myeloid leukemias and regulate cell proliferation of normal and leukemic hemopoietic progenitors. Leukemia. 2002; 16(9):1791–8. https://doi.org/10.1038/sj.leu.2402572 PMID: 12200695

20. Patt S, Preussat K, Beetz C, Kraft R, Schrey M, Kalff R, et al. Expression of ether à go-go potassium channels in human gliomas. Neurosci Lett. 2004; 368(3):249–53.

21. Pillozzi S, Arcangeli A. Physical and functional interaction between integrins and hERG1 channels in cancer cells. Advances in experimental medicine and biology. 2010; 674:55–67. https://doi.org/10.1007/978-1-4419-6066-5_6 PMID: 20549940

22. Pillozzi S, Brizzi MF, Bernabei PA, Bartolozzi B, Caporale R, Basile V, et al. VEGFR-1 (FLT-1), beta1 integrin, and hERG K+ channel for a macromolecular signaling complex in acute myeloid leukemia: role in cell migration and clinical outcome. Blood. 2007; 110(4):1238–50. https://doi.org/10.1182/blood-2006-02-003772 PMID: 17420287

23. Pointer KB, Clark PA, Eliceiri KW, Salamat MS, Robertson GA, Kuo JS. Administration of Non-Torsadogenic human Ether-à-go-go-Related Gene Inhibitors Is Associated with Better Survival for High hERG-Expressing Glioblastoma Patients. Clin Cancer Res. 2017; 23(1):73–80.

24. Rhodes DR, Yu J, Shanker K, Deshpande N, Varambally R, Ghosh D, et al. ONCOMINE: a cancer microarray database and integrated data-mining platform. Neoplasia. 2004; 6(1):1–6. https://doi.org/10.1016/s1476-5586(04)80047-2 PMID: 15068665

25. Glassmeier G, Hempel K, Wulfsen I, Bauer CK, Schumacher U, Schwarz JR. Inhibition of HERG1 K+ channel protein expression decreases cell proliferation of human small cell lung cancer cells. Pflügers Archiv—European Journal of Physiology. 2012; 463(2):365–76. https://doi.org/10.1007/s00424-011-1045-z PMID: 22075718

26. Staudacher I, Jehle J, Staudacher K, Pledl HW, Lemke D, Schweizer PA, et al. HERG K+ channel-dependent apoptosis and cell cycle arrest in human glioblastoma cells. PloS one. 2014; 9(2):e88164. https://doi.org/10.1371/journal.pone.0088164 PMID: 24516604

27. Saadat F, Zareighane Z, Safavifar F, Jalali SZ, Berahmeh A, Khorramizadeh MR. The Repression of Matrix Metalloproteinases and Cytokine Secretion in Glioblastoma by Targeting K+ Channel. Basic Clin Neurosci. 2021; 12(6):737–44. https://doi.org/10.32598/bcn.2021.1693.1 PMID: 35693140

28. Shugg T, Dave N, Amarh E, Assiri AA, Pollok KE, Overholser BR. Letrozole targets the human ether-a-go-go-related gene potassium current in glioblastoma. Basic Clin Pharmacol Toxicol. 2021; 128 (3):357–65. https://doi.org/10.1111/bcpt.13515 PMID: 33040444

29. De Bruin ML, Pettersson M, Meyboom RH, Hoes AW, Leufkens HG. Anti-HERG activity and the risk of drug-induced arrhythmias and sudden death. Eur Heart J. 2005; 26(6):590–7. https://doi.org/10.1093/eurheartj/ehi092 PMID: 15637086

30. Lansu K, Gentile S. Potassium channel activation inhibits proliferation of breast cancer cells by activating a senescence program. Cell death & disease. 2013; 4(6):e652–e. https://doi.org/10.1038/cddis.2013.174 PMID: 23744352

31. Fukushiro-Lopes DF, Hegel AD, Rao V, Wyatt D, Baker A, Breuer E-K, et al. Preclinical study of a Kv11.1 potassium channel activator as antineoplastic approach for breast cancer. Oncotarget. 2017; 9 (3):3321–37. https://doi.org/10.18632/oncotarget.22925 PMID: 29423049

32. Mathews J, Kuchling F, Baez-Nieto D, Diberardinis M, Pan JQ, Levin M. Ion Channel Drugs Suppress Cancer Phenotype in NG108-15 and U87 Cells: Toward Novel Electroceuticals for Glioblastoma. Cancers. 2022; 14(6):1499. https://doi.org/10.3390/cancers14061499 PMID: 35326650

33. Serano RD, Pegram CN, Bigner DD. Tumorigenic cell culture lines from a spontaneous VM/Dk murine astrocytoma (SMA). Acta Neuropathol. 1980; 51(1):53–64. https://doi.org/10.1007/BF00688850 PMID: 7435141

34. Tallarida RJ. Quantitative methods for assessing drug synergism. Genes Cancer. 2011; 2(11):1003–8. https://doi.org/10.1177/1947601912440575 PMID: 22737266

35. Sanguinetti MC, Jurkiewicz NK. Two components of cardiac delayed rectifier K+ current. Differential sensitivity to block by class III antiarrhythmic agents. Journal of General Physiology. 1990; 96(1):195–215. https://doi.org/10.1085/jgp.96.1.195 PMID: 2170562

36. Beroukhim R, Getz G, Nghiemphu L, Barretina J, Hsueh T, Linhart D, et al. Assessing the significance of chromosomal aberrations in cancer: methodology and application to glioma. Proc Natl Acad Sci U S A. 2007; 104(50):20007–12. https://doi.org/10.1073/pnas.0710052104 PMID: 18077431

37. French PJ, Swagemakers SM, Nagel JH, Kouwenhoven MC, Brouwer E, van der Spek P, et al. Gene expression profiles associated with treatment response in oligodendrogliomas. Cancer research. 2005; 65(24):11335–44. https://doi.org/10.1158/0008-5472.CAN-05-1886 PMID: 16357140

38. Bredel M, Bredel C, Juric D, Harsh GR, Vogel H, Recht LD, et al. Functional network analysis reveals extended gliomagenesis pathway maps and three novel MYC-interacting genes in human gliomas. Cancer research. 2005; 65(19):8679–89. https://doi.org/10.1158/0008-5472.CAN-05-1204 PMID: 16204036

39. Murat A, Migliavacca E, Gorlia T, Lambiv WL, Shay T, Hamou MF, et al. Stem cell-related "self-renewal" signature and high epidermal growth factor receptor expression associated with resistance to concomitant chemoradiotherapy in glioblastoma. J Clin Oncol. 2008; 26(18):3015–24. https://doi.org/10.1200/JCO.2007.15.7164 PMID: 18565887

40. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018; 68(1):7–30. https://doi.org/10.3322/caac.21442 PMID: 29313949

41. Takayasu T, Kurisu K, Esquenazi Y, Ballester LY. Ion Channels and Their Role in the Pathophysiology of Gliomas. Mol Cancer Ther. 2020; 19(10):1959–69. https://doi.org/10.1158/1535-7163.MCT-19-0929 PMID: 33008831

42. Bates E. Ion channels in development and cancer. Annu Rev Cell Dev Biol. 2015; 31:231–47. https://doi.org/10.1146/annurev-cellbio-100814-125338 PMID: 26566112

43. Urrego D, Tomczak AP, Zahed F, Stühmer W, Pardo LA. Potassium channels in cell cycle and cell proliferation. Philos Trans R Soc Lond B Biol Sci. 2014; 369(1638):20130094. https://doi.org/10.1098/rstb.2013.0094 PMID: 24493742

44. Pardo LA, Stuhmer W. The roles of K(+) channels in cancer. Nat Rev Cancer. 2014; 14(1):39–48. https://doi.org/10.1038/nrc3635 PMID: 24336491

45. Wulff H, Castle NA, Pardo LA. Voltage-gated potassium channels as therapeutic targets. Nature Reviews Drug Discovery. 2009; 8:982. https://doi.org/10.1038/nrd2983 PMID: 19949402

46. Staudacher I, Schweizer PA, Katus HA, Thomas D. hERG: protein trafficking and potential for therapy and drug side effects. Current opinion in drug discovery & development. 2010; 13(1):23–30. PMID: 20047143

47. Wang H, Zhang Y, Cao L, Han H, Wang J, Yang B, et al. HERG K+ channel, a regulator of tumor cell apoptosis and proliferation. Cancer research. 2002; 62(17):4843–8. PMID: 12208728

48. Perez-Neut M, Shum A, Cuevas BD, Miller R, Gentile S. Stimulation of hERG1 channel activity promotes a calcium-dependent degradation of cyclin E2, but not cyclin E1, in breast cancer cells. Oncotarget. 2015; 6(3):1631–9. https://doi.org/10.18632/oncotarget.2829 PMID: 25596745

49. Hwang HC, Clurman BE. Cyclin E in normal and neoplastic cell cycles. Oncogene. 2005; 24(17):2776–86. https://doi.org/10.1038/sj.onc.1208613 PMID: 15838514

50. Perez-Neut M, Rao VR, Gentile S. hERG1/Kv11.1 activation stimulates transcription of p21waf/cip in breast cancer cells via a calcineurin-dependent mechanism. Oncotarget. 2016; 7(37):58893–902. https://doi.org/10.18632/oncotarget.3797 PMID: 25945833

51. Weiss T, Schneider H, Silginer M, Steinle A, Pruschy M, Polić B, et al. NKG2D-Dependent Antitumor Effects of Chemotherapy and Radiotherapy against Glioblastoma. Clinical Cancer Research. 2018; 24(4):882–95. https://doi.org/10.1158/1078-0432.CCR-17-1766 PMID: 29162646

52. Diksin M, Smith SJ, Rahman R. The Molecular and Phenotypic Basis of the Glioma Invasive Perivascular Niche. Int J Mol Sci. 2017; 18(11). https://doi.org/10.3390/ijms18112342 PMID: 29113105

53. Sawaya R, Tofilon PJ, Mohanam S, Ali-Osman F, Liotta LA, Stetler-Stevenson WG, et al. Induction of tissue-type plasminogen activator and 72-kDa type-IV collagenase by ionizing radiation in rat astrocytes. Int J Cancer. 1994; 56(2):214–8. https://doi.org/10.1002/ijc.2910560212 PMID: 8314304

54. Trog D, Fountoulakis M, Friedlein A, Golubnitschaja O. Is current therapy of malignant gliomas beneficial for patients? Proteomics evidence of shifts in glioma cells expression patterns under clinically relevant treatment conditions. Proteomics. 2006; 6(9):2924–30. https://doi.org/10.1002/pmic.200500587 PMID: 16596702

55. Trog D, Yeghiazaryan K, Fountoulakis M, Friedlein A, Moenkemann H, Haertel N, et al. Pro-invasive gene regulating effect of irradiation and combined temozolomide-radiation treatment on surviving human malignant glioma cells. Eur J Pharmacol. 2006; 542(1–3):8–15. https://doi.org/10.1016/j.ejphar.2006.05.026 PMID: 16806166

56. Whitehead CA, Nguyen HPT, Morokoff AP, Luwor RB, Paradiso L, Kaye AH, et al. Inhibition of Radiation and Temozolomide-Induced Invadopodia Activity in Glioma Cells Using FDA-Approved Drugs. Transl Oncol. 2018; 11(6):1406–18. https://doi.org/10.1016/j.tranon.2018.08.012 PMID: 30219696

57. Huang H. Matrix Metalloproteinase-9 (MMP-9) as a Cancer Biomarker and MMP-9 Biosensors: Recent Advances. Sensors (Basel). 2018; 18(10). https://doi.org/10.3390/s18103249 PMID: 30262739

58. Gieryng A, Pszczolkowska D, Walentynowicz KA, Rajan WD, Kaminska B. Immune microenvironment of gliomas. Laboratory Investigation. 2017; 97(5):498–518. https://doi.org/10.1038/labinvest.2017.19 PMID: 28287634

59. Zhang J, Stevens MF, Bradshaw TD. Temozolomide: mechanisms of action, repair and resistance. Curr Mol Pharmacol. 2012; 5(1):102–14. https://doi.org/10.2174/1874467211205010102 PMID: 22122467

60. Smith PL, Baukrowitz T, Yellen G. The inward rectification mechanism of the HERG cardiac potassium channel. Nature. 1996; 379(6568):833–6. https://doi.org/10.1038/379833a0 PMID: 8587608

61. Sanguinetti MC, Jiang C, Curran ME, Keating MT. A mechanistic link between an inherited and an acquired cardiac arrhythmia: HERG encodes the IKr potassium channel. Cell. 1995; 81(2):299–307. https://doi.org/10.1016/0092-8674(95)90340-2 PMID: 7736582

62. Trudeau MC, Warmke JW, Ganetzky B, Robertson GA. HERG, a human inward rectifier in the voltage-gated potassium channel family. Science. 1995; 269(5220):92–5. https://doi.org/10.1126/science.7604285 PMID: 7604285

63. Shibasaki T. Conductance and kinetics of delayed rectifier potassium channels in nodal cells of the rabbit heart. J Physiol. 1987; 387:227–50. https://doi.org/10.1113/jphysiol.1987.sp016571 PMID: 2443680

64. Danker T, Möller C. Early identification of hERG liability in drug discovery programs by automated patch clamp. Frontiers in Pharmacology. 2014; 5(203). https://doi.org/10.3389/fphar.2014.00203 PMID: 25228880

65. Sampson JH, Ashley DM, Archer GE, Fuchs HE, Dranoff G, Hale LP, et al. Characterization of a spontaneous murine astrocytoma and abrogation of its tumorigenicity by cytokine secretion. Neurosurgery. 1997; 41(6):1365–72; discussion 72–3. https://doi.org/10.1097/00006123-199712000-00024 PMID: 9402588