Personalized Medicine and Imaging

Clinical Cancer Research

Administration of Non-Torsadogenic human Ether-à-go-go-Related Gene Inhibitors Is Associated with Better Survival for High hERG–Expressing Glioblastoma Patients

Kelli B. Pointer[1,2,3,4], Paul A. Clark[1], Kevin W. Eliceiri[4,5], M. Shahriar Salamat[6], Gail A. Robertson[7,8], and John S. Kuo[1,2,3,5,9]

Abstract

Purpose: Glioblastoma is the most malignant primary brain tumor, with a median survival of less than 2 years. More effective therapeutic approaches are needed to improve clinical outcomes.

Experimental Design: Glioblastoma patient-derived cells (GPDC) were isolated from patient glioblastomas and implanted in mice to form xenografts. IHC was performed for *human Ether-à-go-go-Related Gene* (hERG) expression and tumor proliferation. Sphere-forming assays with the hERG blocker E-4031 were performed on a high and low hERG–expressing lines. A glioblastoma tissue microarray (TMA; 115 patients) was used to correlate hERG expression with patient survival. Clinical data were analyzed to determine whether patient survival was affected by incidental administration of hERG inhibitory drugs and the correlative effect of patient glioblastoma hERG expression levels.

Results: hERG expression was upregulated in glioblastoma xenografts with higher proliferative indices. High hERG–expressing GPDCs showed a reduction in sphere formation when treated with hERG inhibitors compared with low hERG–expressing GPDCs. Glioblastoma TMA analysis showed worse survival for glioblastoma patients with high hERG expression versus low expression—43.5 weeks versus 60.9 weeks, respectively ($P = 0.022$). Furthermore, patients who received at least one hERG blocker had a better survival rate compared with patients who did not ($P = 0.0015$). Subgroup analysis showed that glioblastoma patients with high hERG expression who received hERG blockers had improved survival ($P = 0.0458$). There was no difference in survival for low hERG–expressing glioblastoma patients who received hERG blockers ($P = 0.4136$).

Conclusions: Our findings suggest that hERG is a potential glioblastoma survival marker, and that already approved drugs with non-torsadogenic hERG inhibitory activity may potentially be repurposed as adjuvant glioblastoma therapy in high hERG–expressing glioblastoma patients. *Clin Cancer Res; 23(1); 73–80. ©2016 AACR.*

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[1]Department of Neurological Surgery, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, Wisconsin. [2]Medical Scientist Training Program, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, Wisconsin. [3]Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, Wisconsin. [4]Laboratory for Optical and Computational Instrumentation (LOCI), University of Wisconsin-Madison, Madison, Wisconsin. [5]Carbone Cancer Center, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, Wisconsin. [6]Department of Pathology and Laboratory Medicine, University of Wisconsin-Madison, Madison, Wisconsin. [7]Department of Neuroscience, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, Wisconsin. [8]Cardiovascular Research Center, University of Wisconsin-Madison, Madison, Wisconsin. [9]Department of Surgery, National University of Singapore, Singapore.

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Corresponding Authors: John S. Kuo, University of Wisconsin-Madison, Box 8660 Clinical Science Center, 600 Highland Avenue, Madison, WI 53792. Phone: 608-261-1877; Fax: 608-263-1728; E-mail: j.kuo@neurosurgery.wisc.edu; and Gail A. Robertson, 9459 Wisconsin Institute Medical Research, 1111 Highland Avenue, Madison, WI 53705. Phone: 608-265-3339; Fax: 608-263-1728; E-mail: garobert@wisc.edu

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Introduction

In the United States, more than 14,000 new cases of glioblastoma, the World Health Organization grade IV brain cancer, will be diagnosed each year (1). Despite the current standard of maximal safe surgical resection followed by radiotherapy and chemotherapy with the alkylating agent temozolomide, patients have an overall median survival of less than 2 years (2). Thus, there is an urgent need to find new prognostic biomarkers to inform clinical management and therapies to improve outcome.

Ion channels, transmembrane proteins regulating ion flux across the plasma membrane, have been reported to influence progression of glioblastoma and other cancers (3–8). Kv.11.1, encoded by the *human Ether-à-go-go-Related Gene* (*hERG* or *KCNH2*), is well characterized for its role in cardiac repolarization (9, 10). It is the primary target for acquired or drug-induced long QT syndrome, leading in some cases to sudden cardiac death. However, many hERG blockers are in clinical use and known to be generally safe (11). *hERG* was shown to be overexpressed in leukemia, gastric cancer, colon cancer, and glioblastoma cell lines (8, 12–15). In other tumor cells, altered

Translational Relevance

Glioblastoma is the most malignant and deadly primary brain tumor, with a median survival of less than 2 years despite maximal therapy. The *human Ether-à-go-go-Related Gene* (hERG) is a voltage-dependent K^+ channel found overexpressed in glioblastoma cell lines and linked to aberrant proliferation in other cancers. We analyzed hERG expression in glioblastoma patient-derived cells (GPDC) and a clinically annotated human glioblastoma tissue microarray (TMA) to determine correlation with patient survival. Because all FDA-approved drugs undergo cardiotoxicity profiling that includes hERG inhibition, glioblastoma patient survival was also analyzed after subgrouping based on glioblastoma hERG expression and whether patients received hERG inhibitory drugs (phenytoin, haloperidol, fluoxetine, tamoxifen, amitriptyline, and/or ketoconazole). Our research demonstrates that hERG is a potential glioblastoma biomarker, and that non-torsadogenic hERG blockers may be repurposed as potential adjuvant therapies for high hERG–expressing glioblastoma.

hERG expression was associated with cell-cycle regulation and proliferation (16, 17). Recent work was reported showing a possible role for hERG inhibition in pediatric medulloblastoma, a type of brain cancer (18). The few studies that implicate *hERG* in glioblastoma have used traditional cell lines or serum-cultured cells that lack the diverse genotypic and phenotypic characteristics observed in patient-derived tumors (19). Furthermore, many prior studies were conducted *in vitro* and direct consequences of hERG expression on glioblastoma tumors *in situ* or patient survival have not been reported.

We set out to determine whether *in vivo hERG* expression of tumor xenografts initiated from glioblastoma patient-derived cells (GPDC; ref. 20). We also sought to determine whether GPDC proliferation rates and glioblastoma patient survival were affected by hERG channel inhibition. We show that hERG expression levels are correlated with higher proliferation rates in GPDC-derived xenografts and that patients with high hERG–expressing glioblastoma had worse survival rates. We also demonstrate that hERG blockers reduced GPDC proliferation, and improved survival in patients who received one or more hERG-blocking drugs, but only if their tumors exhibited high hERG expression levels.

Materials and Methods

Isolation of GPDCs

GPDCs were isolated following protocols previously reported (19, 21–23), with some modifications. Tumor tissue was collected directly from the operating room, weighed, coarsely minced with a scalpel blade, and subsequently chopped twice to 200 μm using a tissue chopper (Sorvall TC-2 Smith & Farquhar). Chopped tissue was directly plated in suspension at 10 mg/mL in growth medium [passage medium (PM): 70% DMEM-high glucose, 30% Ham's F12, 1× B27 supplement, 5 μg/mL heparin, penicillin–streptomycin–amphotericin (PSA), and 20 ng/mL each of EGF and bFGF]. Cultures were passaged approximately every 7 to 10 days by tissue chopping twice at 200 μm. For these studies, we used four different GPDC lines: 12.1, 22, 112, and 114. 12.1 and 22 displayed markers consistent with a mesenchymal phenotype, whereas 112 and 114 displayed markers consistent with a proneural phenotype (unpublished data). Each cell line was validated for self-renewal by neurosphere formation, multipotency, and tumor initiation before experiments were performed. Establishment of cell cultures came from cryopreservation of cell cultures ranging from passages 10 to 22. Cells used for experiments ranged from passage 20 to 25.

Sphere formation assay

GPDCs were enzymatically dissociated to single cells and seeded at 100 cells per well into 96-well plates. After cell recovery overnight, media was exchanged with drug-containing media at increasing concentrations of E-4031. After 2 weeks, GPDC spheres were counted, with drug-treated conditions normalized to vehicle controls.

GPDC orthotopic xenograft model

Glioblastoma orthotopic xenografts were initiated, as previously described (24). GPDCs were enzymatically dissociated to single cells, and 2×10^5 cells were suspended in 5 μL of media. Using a Hamilton syringe, the cells were stereotactically implanted into the right striatum of anesthetized NOD-SCID mice between the ages of 8 and 10 weeks at 0.33 μL/minute at the following coordinates referenced from bregma: 0 mm anteroposterior, +2.5 mm mediolateral, and −3.5 mm dorsoventral. At either 3 months or the onset of neurologic symptoms, tumor formation was verified using MRI. Mice were anesthetized, contrast enhanced using 10 mmol/kg of intraperitoneal gadolinium (Omniscan; GE Healthcare), and placed onto a small-animal MRI scanner (4.7-T horizontal bore imaging/spectroscopy system; Varian), and then T1- and T2-weighted images were obtained. As per our approved animal protocol, once neurologic symptoms were observed and the mice were moribund, which occurred between 80 and 120 days, implanted NOD-SCID mice were immediately euthanized by perfusion fixation with 4% paraformaldehyde. Brains were then excised, embedded in paraffin, and processed for general histology.

Immunohistochemistry

IHC was performed on glioblastoma mouse xenografts and a human tissue microarray (TMA), as described previously (22). IHC was performed on NOD-SCID mouse brains implanted with 200,000 GPDCs from lines 12.1, 22, 112, and 114. IHC was also performed on the human glioblastoma TMA. Formalin-fixed, paraffin-embedded tissue sections were mounted on positively charged microscope slides. Tissue sections were then deparaffinized and rehydrated to water, microwaved in antigen unmasking solution (Vector Laboratories) to retrieve epitopes, and blocked for endogenous peroxidase and biotin before the application of the primary antibody. Incubation of antibodies was performed overnight at 4°C. The anti-hERG primary antibody was used at the concentration of 1:1,600 (ALX-215-049-R100; Enzo Life Sciences). Subsequent immunodetection was performed using the Elite Vector Stain ABC System (Vector Laboratories). Color visualization was performed using 3-3′-diaminobenzidine (DAB) as the chromagen substrate (Sigma Chemical Company). Tissues were counterstained with hematoxylin to visualize cellular morphology. Images were acquired with Nikon TE-2000 and EVOS XL Core

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(Advanced Microscopy Group) brightfield microscopes. To determine hERG expression levels, positive cells (brown) were counted at 100× and compared with the total number of cells present in three random fields in the tumor. Black boxes in whole-brain images indicate the corresponding location of acquisition for high-magnification photomicrographs.

Ki-67 indexing

After Ki-67 immunolabeling, positive cells were defined as those with nuclei of any brown color, regardless of the intensity or pattern of staining. Human versus mouse nuclei were distinguished using 4′,6-diamidino-2-phenylindole (DAPI) labeling. Mouse nuclei were small, regular, and round, displaying concentrated aggregate DAPI labeling, indicative of characteristic mouse chromocenters. Human GPDC nuclei were comparatively large and atypically oblong with homogenous DAPI labeling. Five random high power fields were chosen using a 100× objective, and the number of Ki-67–positive cells was counted as well as the total number of human cells. Ki-67 percentage was determined from the average of the five random fields chosen for each GPDC xenograft. High hERG expression xenografts and low hERG expression xenografts were then grouped together to determine the Ki-67–positive percentage in each group. Counting of Ki-67–positive nuclei began at the site of implantation until 500 random human nuclei were identified with a 60× objective.

Tissue microarray

With institutional review board (IRB) approval, we created a clinically annotated glioblastoma TMA from 205 glioblastomas resected between 1999 and 2009, archived in the University of Wisconsin-Madison (UW) Department of Pathology and Laboratory Medicine and clinical data accessed from UW patient records. One to three representative tissue punches/cores were obtained for each tumor sample, depending on morphologic heterogeneity and tissue availability. Neuropathology-designated patient diagnosis and tissue punch locations were determined on the basis of the most representative section of the whole glioblastoma specimen. Each additional tissue punch contained classic glioblastoma features (nuclear atypia, high mitotic indices, vascular endothelial proliferation, and/or necrosis). Grades II and III astrocytoma, grade II oligodendroglioma, meningioma, hippocampus and neocortex tissue punches were used as controls. Of the 205 patients on the TMA, 149 of them have corresponding clinical records including survival information. On the basis of histologic processing 115 samples of 149 were usable. IHC was performed using the aforementioned anti-hERG antibody. Punches were blindly scored as having high hERG expression or low hERG expression. To take into account the heterogeneity of glioblastoma samples even from the same tumor, for patients that had multiple punches, if one punch had high hERG expression, the patient was denoted as having high expression levels. Survival analysis was done using a log-rank test and presented as a Kaplan–Meier survival plot with the addition of HR analysis. *P* values <0.05 were considered statistically significant. Plots were generated using GraphPad Prism 6.

Study approval

All procedures performed in studies involving human participants were in accordance with the ethical standards of the UW IRB and in compliance with the 1964 Declaration of Helsinki and its later amendments. Informed consent was obtained from all individual participants included in the study. All procedures performed in studies involving animals were in accordance with the ethical standards of UW and approved by the Animal Care and Use Committee.

Results

hERG expression levels in glioblastomas correlate with tumor proliferation rates

We evaluated whether hERG expression levels *in vivo* correlated with proliferation in a tumor xenograft assay where four distinct GPDC lines were orthotopically implanted into the right striatum of NOD-SCID mice. H&E staining was used to visualize tumor xenografts in mouse brains (Fig. 1A1–D1), and tumor proliferation rate was determined via IHC for the proliferation protein marker, Ki-67, on sections of glioblastoma xenografts. Five random fields at 100× were chosen, and Ki-67–positive cells were counted and compared with the total number of tumor cells. On the basis of the Ki-67 index, two groups emerged from the mouse xenografts: a higher proliferation group (mean of 70.29 ± 4.837% Ki-67–positive cells) and a lower proliferation group (mean of 46.60 ± 2.744% Ki-67–positive cells; $P = 0.0005$; Fig. 1E). Then, IHC using a pan-hERG antibody on serial xenograft sections (Fig. 1A2–D2) was performed on both groups. Higher Ki-67 index xenografts [12.1 GPDC (66%) and 22 GPDC (75%)] were also positive for high hERG levels [12.1 GPDC (91% of cells) and 22 GPDC (85% of cells)], whereas lower Ki-67 index xenografts [112 GPDC (47%) and 114 GPDC (46%)] had low hERG levels [112 GPDC (21% of cells) and 114 GPDC (33% of cells)], suggesting that hERG expression is associated with glioblastoma proliferation (Fig. 1F). Interestingly, these data also show that although hERG is overexpressed in glioblastomas with higher proliferation rates, not all glioblastomas exhibit high hERG expression levels.

Lower patient survival rates associated with hERG expression

To determine whether hERG expression correlated with patient survival, we examined a human TMA consisting of glioblastomas resected from the years 1999 to 2009 with 149 patients that had clinically accessible records for correlation. Of note, many of these patients were treated before the current standard of care consisting of temozolomide adjuvant chemoradiotherapy was established and many current molecular markers (e.g., MGMT methylation, IDH mutation) were tested. Each patient had one to three 1-mm glioblastoma specimen punches that were chosen by a neuropathologist to be the most representative areas of the entire patient glioblastoma specimen. IHC was performed on the TMA using the same pan-hERG antibody mentioned above, followed by blind scoring for low or high hERG expression levels. The blind scoring revealed that high hERG expression correlated with a worse survival rate compared with low hERG expression, based on the log-rank test ($P = 0.022$), with a median survival of 43.5 weeks ($N = 45$) compared with 60.9 weeks ($N = 70$), respectively (Fig. 2). Furthermore, patients with high hERG levels had a 61% greater chance of dying before patients with low hERG levels [log-rank hazard ratio (HR) of 1.536]. These findings indicate that hERG may be an informative survival biomarker for patients with glioblastoma.

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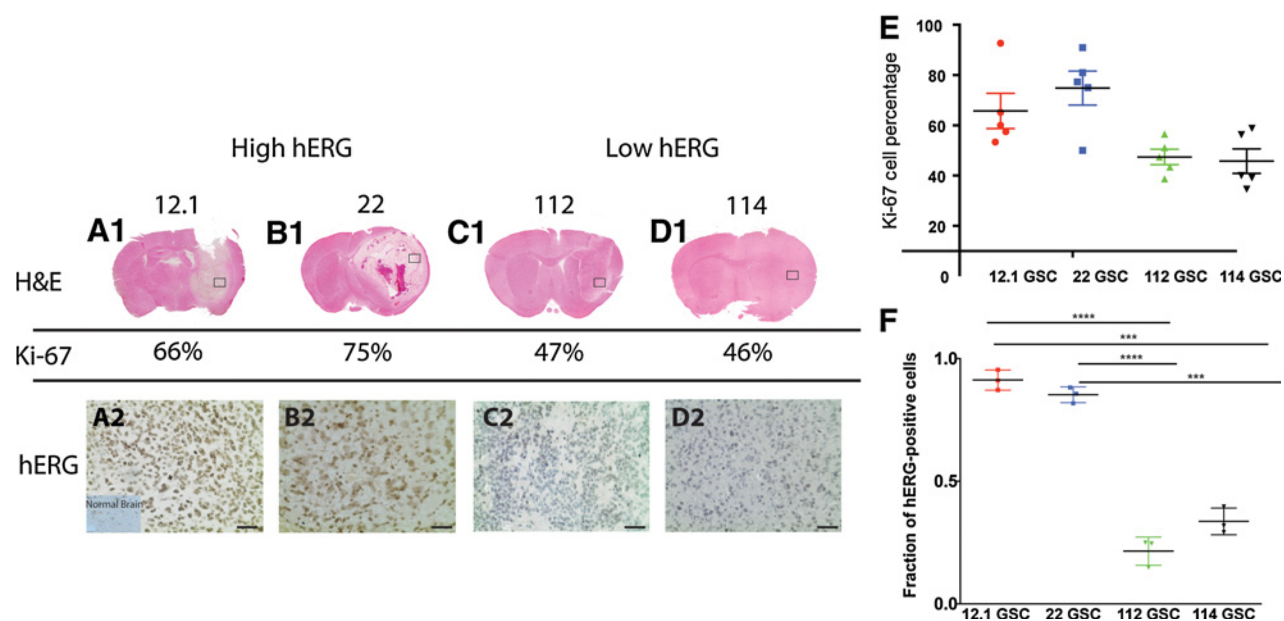

Figure 1.
hERG expression levels are correlated with proliferation. **A1–D1,** Mouse brains from GPDC xenografts were stained with H&E. **A2–D2,** An anti-hERG antibody was used on GPDC mouse xenograft sections. The scale bar represents 250 μm. **E,** Ki-67 cell percentages from five random fields for each GPDC xenograft, with Ki-67 cell percentages grouped based on high hERG levels and low hERG levels. **F,** Fraction of hERG positive cells for each xenograft. ***, <0.001; ****, <0.0001. GSC stands for GPDC.

Univariate and multivariate analyses of hERG expression

Univariate and multivariate analyses were used to determine whether the correlation of hERG expression with survival was influenced by any confounding variables. Patient gender, age, Karnofsky performance status (KPS) score, temozolomide treatment, radiation treatment, and tobacco use were assessed as potential confounding variables. Univariate analysis was conducted to determine which factors influenced patient survival rates. There was not a statistically significant difference in survival based on patient gender, age, or tobacco use; however, there was a statistically significant difference in survival based on KPS score (0.024), temozolomide treatment (<0.001), and radiation treatment (<0.001). Multivariate survival analysis using the Cox regression model was used to determine whether hERG expression was an independent biomarker for survival, taking into account KPS score, temozolomide treatment, and radiation treatment. This analysis indicated that hERG expression level is an independent marker for glioblastoma patient survival, with reduced survival rates for patients exhibiting high hERG expression levels compared with those exhibiting low hERG expression levels ($P = 0.003$; Table 1).

hERG blockers decreased sphere formation in high hERG–expressing glioblastoma cell lines

On the basis of hERG expression levels found in GPDC-derived xenografts, we wanted to determine whether hERG

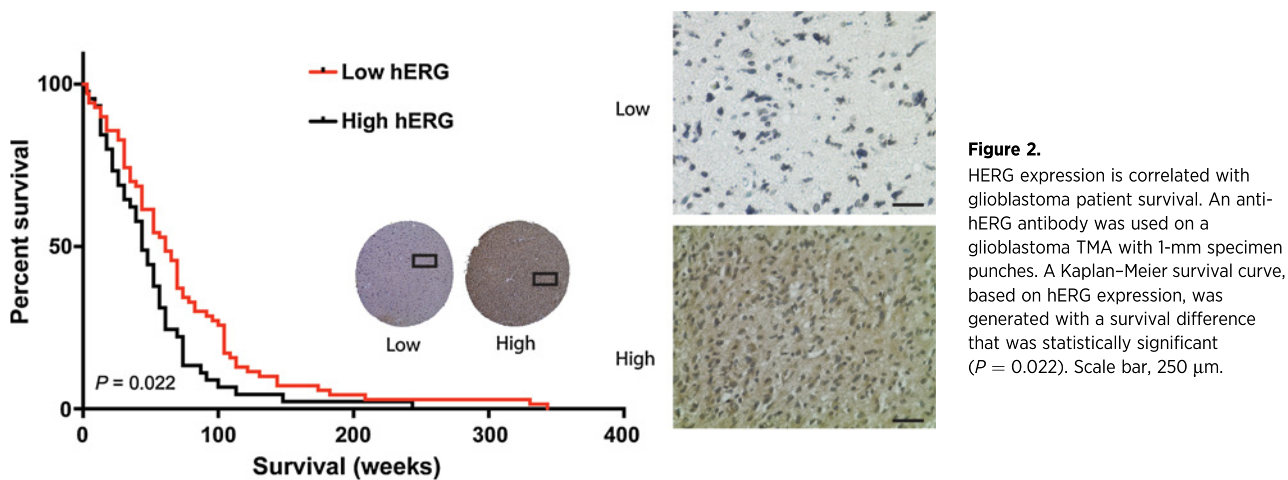

Figure 2.
HERG expression is correlated with glioblastoma patient survival. An anti-hERG antibody was used on a glioblastoma TMA with 1-mm specimen punches. A Kaplan–Meier survival curve, based on hERG expression, was generated with a survival difference that was statistically significant ($P = 0.022$). Scale bar, 250 μm.

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Table 1. Univariate and multivariate Cox regression analyses of glioblastoma hERG expression and confounding variables

	Univariate analysis			Multivariate analysis	
	N	**Median survival, weeks (95% CI)**	***P* (log-rank)**	**HR (95% CI)**	***P***
hERG expression			0.022	2.122 (1.247–3.610)	0.003
High	45	43.5 (33.8–53.1)			
Low	71	60.9 (48.2–73.6)			
Gender			0.463		NS
Male	81	52.2 (44.5–59.9)			
Female	35	56.5 (34.5–78.5)			
Age			0.957		NS
≤55	51	52.2 (39.5–64.8)			
>55	58	52.2 (44.1–60.3)			
KPS			0.024	1.143 (0.769–1.700)	0.508
≤70	55	60.9 (48.3–73.5)			
>70	60	43.5 (29.7–57.3)			
Temozolomide			<0.001	2.845 (1.849–4.378)	<0.001
Yes	66	69.6 (61.6–77.6)			
No	50	34.8 (27.3–42.3)			
Radiation			<0.001	2.122 (1.247–3.610)	0.006
Yes	95	60.9 (9.7–25.1)			
No	21	17.4 (49.6–72.2)			
Tobacco use			0.519		NS
Yes	46	52.2 (41.4–63.0)			
No	70	52.2 (37.9–66.5)			

Abbreviation: CI, confidence interval; NS, not significant.

blockers could decrease proliferation rates in high hERG–expressing GPDC lines more than low hERG–expressing GPDC lines. GPDCs were dissociated into single cells, and 100 cells were seeded into a 96-well plate. E-4031, a known hERG-specificblocker, was added at increasing drug concentrations (25). After 2 weeks, spheres were counted and normalized to vehicle control. E-4031 decreased sphere formation at increasing drug concentrations, with a statistically significant difference in sphere formation seen at drug concentration 5 μmol/L (Fig. 3). This suggests that hERG blockers have the ability to decrease GPDC sphere formation and proliferation in an hERG-specific manner, and hERG inhibition might be a potentially useful glioblastoma therapeutic strategy.

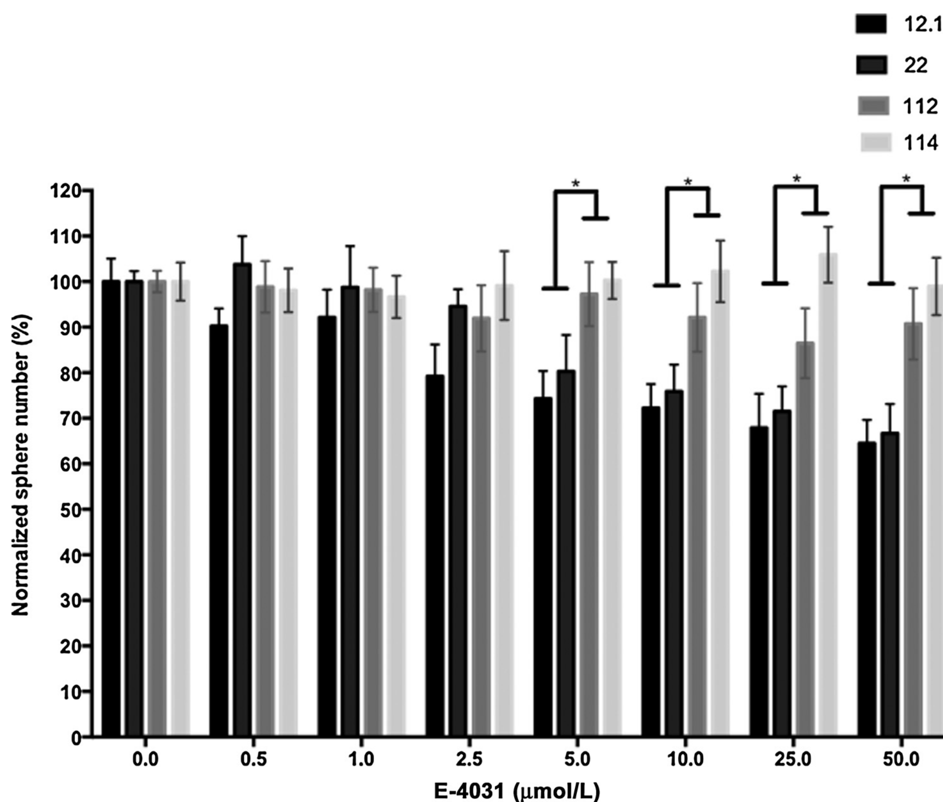

Figure 3.
Sphere formation is reduced with hERG blockers. The hERG-specific blocker E-4031 was used in increasing concentrations on 12.1, 22, 112, and 114 GPDCs. After 2 weeks of drug treatment, the number of spheres were counted and normalized to vehicle control (*, $P < 0.05$).

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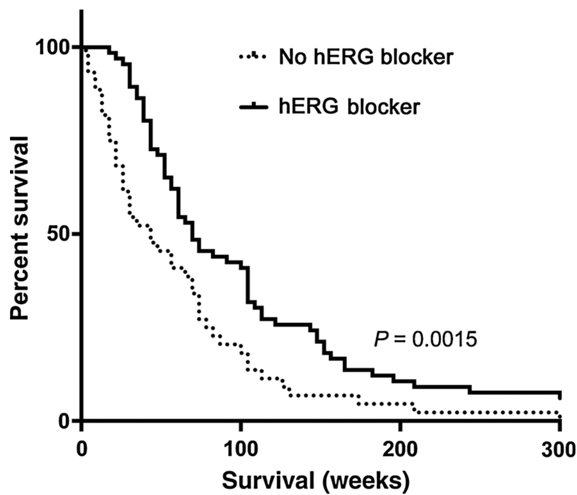

Figure 4.

Receipt of hERG blockers correlates with a better glioblastoma patient survival. Patients who received more than one hERG blocker were compared with patients who had not received hERG blockers, and there was a statistically significant difference in their survival ($P = 0.0015$).

Patients who received hERG blockers had a better overall survival rate

To determine whether hERG blockers affected patient survival, we examined whether patients represented on the glioblastoma TMA received commonly prescribed hERG blockers after their glioblastoma diagnosis (26). Of the 110 patients analyzed, 66 patients received at least one of the following drugs known to exhibit off-target hERG block: phenytoin, haloperidol, fluoxetine, tamoxifen, amitriptyline, and ketoconazole (Supplementary Fig. S1). Patients prescribed at least one hERG blocker exhibited significantly enhanced survival compared with those who did not receive an hERG blocker, based on the log-rank test ($P = 0.0015$), with a median survival of 69.6 weeks ($N = 66$) and 43.5 weeks ($N = 44$), respectively (Fig. 4). Patients who did not receive an hERG blocker had a 64% greater chance of dying before patients who did receive an hERG blocker (log-rank HR of 1.790). This data suggest that hERG blockers may increase patient survival. Interestingly, of the 66 patients who received at least one hERG blocker, seven patients received two or more hERG blockers and had a median survival of 73.9 weeks, also a statistically significant improvement in survival compared with those patients who did not receive an hERG blocker ($P = 0.0117$). Patients who did not receive an hERG blocker were 71% more likely to die before patients who received two or more hERG blockers (log-rank HR of 2.344). This suggests a patient survival benefit with greater hERG blockade.

hERG blockers enhance survival specifically for patients with high hERG expression levels

Interestingly, the correlation of hERG blockers with survival was not statistically significant for patients on the TMA with low hERG–expressing glioblastoma ($P = 0.4136$; Fig. 5A). However, patients with high hERG–expressing glioblastoma who received an hERG blocker fared better than those who did not ($P = 0.0458$; Fig. 5B), with a median survival of 56.5 weeks ($N = 15$) compared with 34.8 weeks ($N = 16$), respectively. Patients with high hERG–expressing glioblastoma who did not receive hERG blockers had a 66% greater chance of dying first (log-rank HR of 1.906) compared with patients with high hERG–expressing glioblastoma who received a hERG blocker. This suggests that hERG blockers are beneficial for patients with high hERG–expressing glioblastomas, and it supports the idea that hERG blockers improve survival times via a direct action on hERG channels rather than other targets for these drugs. Moreover, these findings suggest the potential of tailoring glioblastoma treatments according to hERG expression level stratification.

Discussion

In this study, we found that upregulation of hERG expression correlated with greater tumor proliferation in heterogeneous, GPDC-derived mouse xenografts and a worse patient survival rate. We found that the hERG blocker E-4031 decreased proliferation as assayed by sphere formation in a GPDC line

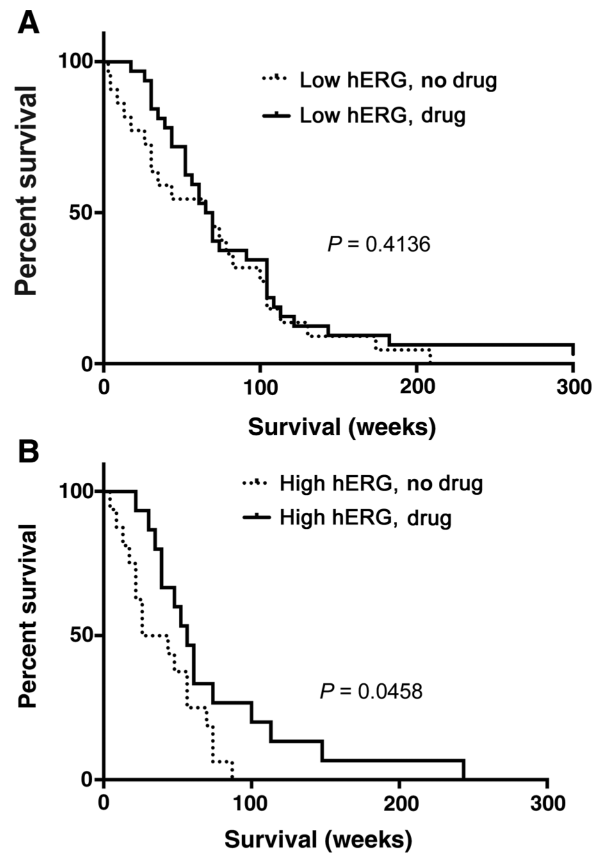

Figure 5.

Patient hERG expression levels correlate with the benefit of receiving an hERG. Patients were stratified based on whether or not they had high or low hERG expression levels. In each group, patients were further stratified on the basis of whether they had received an hERG blocker. **A,** No statistically significant difference was found in survival between patients who had low hERG expression levels ($P = 0.4136$). **B,** There was a statistically significant difference in survival for patients with high hERG expression levels based on whether they received an hERG blocker ($P = 0.0458$).

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exhibiting high levels of hERG expression, whereas blocking hERG in low hERG–expressing lines did not decrease proliferation to the same extent. Survival was enhanced in patients receiving one or more hERG blockers but only when their tumors exhibited high levels of hERG expression. These findings suggest that hERG may be a useful biomarker for glioblastoma survival, and that non-torsadogenic hERG blockers may be potentially repurposed for adjuvant glioblastoma therapy.

Glioblastomas are heterogeneous, and a variety of subtypes have been described (24, 27). Research to characterize differences in glioblastomas has led to a better understanding of glioblastoma progression and new therapeutic targets (28, 29). For example, the efficacy of temozolomide is affected by the methylation status of MGMT, an enzyme that repairs the DNA damage resulting from temozolomide's chemotherapeutic action (30). Methylation of the MGMT promoter silences protein production, preventing repair of DNA damage and leading to tumor cell death (31). MGMT status is now commonly tested in patients, and it is well documented that glioblastoma with MGMT promoter methylation are associated with higher median survival (30). However, because this study was performed with a TMA of glioblastoma patients treated before the current standard of care consisting of adjuvant temozomolide chemoradiation was established and markers such as MGMT methylation and IDH mutation status were known, we are working to confirm our findings with an updated glioblastoma TMA. Other researchers have discussed the role of hERG as a novel biomarker for several other cancers, and our work further supports this approach in glioblastoma (32, 33). If validated, hERG expression may be used in the future to stratify glioblastoma patients for possibly hERG inhibitor adjuvant therapy.

Many drugs inhibit hERG channels off-target, but some of these lack the proarrhythmic risk associated with hERG block. Phenytoin, prescribed to control seizures for most patients in this study, poses little risk for torsades de pointes arrhythmia. However, other troubling side effects have led to substitution of other antiepileptic drugs, such as levetiracetam, which does not block hERG channels (34). Whether this trend will affect survival of patients, especially those with tumors expressing high hERG levels, is unknown. Our study provides the rationale for clinical trials to determine whether other drugs targeting hERG may provide benefit as adjuvant therapies by repurposing drugs such as fluoxetine (Prozac; Eli Lilly), also a hERG blocker, to reduce tumor proliferation in a subset of patients with the added benefit of also treating depression.

Although many FDA-approved hERG blockers exist, some have the potential to cause arrhythmias due to cardiac hERG blockade, especially under conditions of polypharmacy (35). One way to avoid off-target effects of hERG blockade might be combination with tumor-targeting agents, such as alkylphosphocholine (APC) analogues (36, 37). Tumor cells are selectively targeted by long-term APC retention, whereas normal tissues metabolize APCs. APC analogues can be modified by addition other moieties, such as radioactive iodine or optical fluorescence for diagnostic or therapeutic purposes (27, 28). On the basis of our findings, hERG blockers that are already FDA approved for other purposes may be repurposed as new therapeutic options for patients with glioblastoma. Studies in other cancers, such as leukemia, have shown that specific hERG blockers can have therapeutic effects without causing cardiac toxicities (38). Prospective clinical trials will be needed to test and validate hERG as a glioblastoma biomarker, and potentially stratify glioblastoma patients for hERG inhibitor adjuvant therapy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: K.B. Pointer, K.W. Eliceiri, G.A. Robertson, J.S. Kuo
Development of methodology: K.B. Pointer, P.A. Clark, J.S. Kuo
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K.B. Pointer, P.A. Clark, M.S. Salamat
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K.B. Pointer, J.S. Kuo
Writing, review, and/or revision of the manuscript: K.B. Pointer, K.W. Eliceiri, G.A. Robertson, J.S. Kuo
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): K.B. Pointer, P.A. Clark, M.S. Salamat, J.S. Kuo
Study supervision: J.S. Kuo

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