Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 March 2021 doi:10.20944/preprints202103.0093.v1

Review

Contemporary Mouse Models in Glioma Research

William H. Hicks [1], Cylaina E. Bird [1], Jeffrey I. Traylor, MD [1], Diana D. Shi, MD [2], Tarek Y. El Ahmadieh, MD [1], Timothy E. Richardson, DO, PhD [3], Samuel K. McBrayer, PhD [4,5*], and Kalil G. Abdullah, MD, MSc [1,5,6*]

1 Department of Neurological Surgery, University of Texas Southwestern Medical Center, Dallas, Texas (USA)
2 Department of Radiation Oncology, Brigham and Women's Hospital and Dana-Farber Cancer Institute, Boston, MA, United States; Harvard Medical School, Boston, MA, United States
3 Department of Pathology and Glenn Biggs Institute for Alzheimer's and Neurodegenerative Diseases, University of Texas Health San Antonio, San Antonio, Texas (USA)
4 Children's Medical Center Research Institute, University of Texas Southwestern Medical Center, Dallas, Texas (USA)
5 Harrold C. Simmons Comprehensive Cancer Center, University of Texas Southwestern Medical Center, Dallas, Texas (USA)
6 Peter O'Donnell Jr. Brain Institute, University of Texas Southwestern Medical Center, Dallas Texas (USA)
* Correspondence: samuel.mcbrayer@utsouthwestern.edu; kalil.abdullah@utsouthwestern.edu

Abstract: Despite advances in understanding of the molecular pathogenesis of glioma, outcomes remain dismal. Developing successful treatments for glioma requires faithful in vivo disease modeling and rigorous preclinical testing. Murine models, including xenograft, syngeneic, and genetically engineered models, are used to study gliomagenesis, identify methods of tumor progression, and test novel treatment strategies. Since the discovery of highly recurrent isocitrate dehydrogenase (IDH) mutations in lower-grade gliomas, there is increasing emphasis on effective modeling of IDH mutant brain tumors. Improvements in preclinical models that capture the phenotypic and molecular heterogeneity of gliomas are critical for the development of effective new therapies. Herein, we explore the current status, advancements, and challenges with contemporary murine glioma models.

Keywords: glioma; GEMM; isocitrate dehydrogenase; PDX; mouse model

Citation: Lastname, F.; Lastname, F.; Last-name, F. Title. *Cells* **2021**, *10*, x. https://doi.org/10.3390/xxxxx

1. Introduction

Diffuse gliomas are the most common primary tumor of the central nervous system (CNS) and are currently classified as lower-grade (WHO II and III) or glioblastoma (GBM; WHO grade IV) based on a combination of histologic and molecular features [1,2]. Lower-grade astrocytomas tend to be slower growing and are less aggressive than higher grade astrocytomas, with the diagnosis of GBM conferring a dismal prognosis [1]. Despite advances in treatment, patients with GBMs have a median survival of 15 months and a 5-year survival rate of <10% with maximal resection and concomitant chemotherapy and radiation [1]. The intractability of these tumors highlights the need for clinical testing of new therapies that display robust activity in accurate mouse models of glioma.

In vivo cancer modeling provides numerous advantages over in vitro modeling. Over 80% of the genes in the mouse genome have direct human orthologs, thereby leading to adoption of the mouse as the dominant model organism for cancer biology and cancer therapy studies [2]. Recent advances in genetic engineering have enabled the production of mouse models of glioma that increasingly mimic the microenvironmental and genomic characteristics of human brain tumors. The genetic landscape of glioma is characterized by alterations in genes encoding epidermal growth factor receptor (EGFR), phosphate and tensin homolog deleted on chromosome 10 (PTEN), neurofibromatosis 1 (NF1), RAS, TP53, and cyclin dependent kinase inhibitor 2 (CDKN2A/B) genes, among others, leading to cell proliferation and tumorigenesis [3,4]. Recently, mutations in iso-

© 2021 by the author(s). Distributed under a Creative Commons CC BY license.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 March 2021 doi:10.20944/preprints202103.0093.v1

citrate dehydrogenase 1 and 2 (IDH1/2) have been identified in the majority of lower-grade gliomas and a relatively small subset of GBMs [3,5]. As lower-grade gliomas invariable progress to secondary GBMs, evaluating the role of IDH directed therapy is important for patient care.

The genetic diversity, inter- and intra-tumoral heterogeneity, and extensive interaction with brain parenchyma displayed by gliomas lead to late clinical detection, resistance to treatment, and universal tumor recurrence following therapy. These characteristics highlight the need for efficient and representative preclinical mouse models of glioma [6,7]. In this review, the evolution, history, and current status of contemporary glioma mouse models is discussed.

2. Evolution of Cancer Mouse Models

Cancer mouse models evolved alongside advances in molecular and medical technology and vary in cost and immune status (Table 1).

Table 1. Comparison of preclinical animal model features

Model	Tumor Source	Immune status	Cost	Labor/Time
CLX	Human	(-)	$	+
PDX	Human	(-)	$$	++
Syngeneic	Mouse	(+)	$	++
GEMM	Mouse	(+)	$$$	+++

CLX, cell-line xenograft; PDX, patient-derived xenograft; GEMM, genetically engineered mouse model

The first cancer animal model was the xenograft model. Historically, this model achieved tumor growth through heterotransplantation of human cancer cells into immune-privileged sites like the guinea pig eye or hamster cheek-pouch [8]. While effective as an animal culture of the tumor, these early models provided limited opportunity for study of tumor interaction with native tissue cell types and precluded orthotopic transplantation (into the organ of origin). This challenge was overcome in 1953, when cortisone-treated, irradiated rat xenograft models grew transplanted human tumors [9]. Rygaard and Paulson (1969) further established an immune-compromised host as a critical tool for effective tumor xenograft transplantation [10]. Engraftment rates were significantly improved by immune-compromised mice which led to widespread adoption of xenograft models in the cancer research field [11].

Around the time that the first xenograft models were established, syngeneic models were created to facilitate the identification of effective chemotherapies [14,15]. From the 1950s to 1970s, the National Cancer Institute conducted chemotherapeutic screening programs using syngeneic models of sarcoma 180, L1210 leukemia, B16 melanoma, and P388 leukemia, among others [12]. Syngeneic models are created through the use of carcinogens or genetic modification to induce tumorigenesis or by leveraging spontaneous tumor formation in the mouse [13,14]. Malignant transformation can be induced in vitro or in vivo. If primary cells are transformed in vitro, they can be introduced to an organism of the same species. C57BL/6, BALBc, and FVB/N are common mouse species used in syngeneic models and have been critical for the preclinical evaluation of experimental therapeutics [13,14]. Xenograft and syngeneic modeling approaches have been applied extensively to glioma research, as summarized below.

Genetically engineered mouse models (GEMMs) were first established when oncogenic viral DNA was detected in the adult mouse following transfection of the mouse embryo with simian virus 40 (SV40) [15]. In the 1980s, there was a rapid expansion of transgenic GEMMs with the creation of onco-mice [16-18]. These onco-mice have tumorigenic DNA, often known or proposed oncogenes, introduced into their genome to create a mouse predisposed to tumor formation. Subsequent models placed the oncogene under tissue specific promoters, like the pairing of the immunoglobulin enhancer to the

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 March 2021 doi:10.20944/preprints202103.0093.v1

Myc gene to model B-cell lymphomas or the hormone inducible MMTV-Ras mouse to model breast cancer [17,19]. Gu et. al (1993) established the Cre-loxP system as a conditional gene targeted tool for genetic recombination [20]. A similar model was simultaneously developed utilizing the Flp-FRT system [21]. These molecular tools led to the development of conditional inducible mouse models of cancer and are discussed at greater lengths later in the review. As our genetic and molecular understanding of specific cancers, including glioma, continues to grow, individual genetically engineered mice can be bred to generate combinatorial genetic defects that better resemble the multi-allelic abnormalities in human cancer.

3. Xenograft models

Historically, orthotopic high grade glioma (HGG) xenograft models were created with patient-derived cell lines or established cell lines. In cell-line xenograft models (CLX), cells are implanted into the desired location in the mouse (Table 2). For glioma CLXs, immortalized glioma stem cell (GSC) lines commonly used for implantation include U87, U251, T98G and A172 [22,23]. CLXs are a quick and reproducible strategy for studying glioblastoma. However, they often result in well-circumscribed tumors that lack the characteristic infiltrative pattern that is observed in human gliomas [22,23]. Further, the selective pressures of cell culture reduce the subclonal heterogeneity of CLX and its ability to recapitulate the parent tumor [24]. Patient-derived xenografts models (PDX), on the other hand, involve direct xenotransplantation of human biopsy tissue (Table 3). PDX models have been shown to better recapitulate the vascular characteristics and blood brain barrier permeability of patient HGGs as compared to CLX model using the U87 cell line [22,23]. Thus, PDX models are better at recapitulating the stromal and extracellular characteristics of parent tumors than their CLX counterparts.

Several xenograft models have studied gliomagenesis [25-27]. In 1986, Kaye and colleagues were one of the first to create a model using this system by implanting a C6 glioma cell line (a rat glioma cell line) into neonatal and adult mice to demonstrate a reliable murine xenograft glioma model [26]. When creating a reliable xenograft model, the location of cancer stem cell implantation needs to be precise for development of a tumor that accurately recapitulates the human counterpart [27]. Iretenkauf et al. (2017) utilized a glioma xenograft model with nude mice and showed that the implantation location of GSCs can affect the developed tumor characteristics in the murine model [27].

Table 2. Glioma CLX murine models

Mouse species	Brain tumor modeled	Source of genetic material	Reference
BALB/c OlaHsd-Foxn1^{nu}	Glioma	BT4C cells	[28]
C57BL6/N	High Grade Glioma	GL261 cells	[29-36]
	High Grade Glioma	U87 and GL261 cells	[37]
CBA, BALB/c, AKR, C57 black, and RIII	Glioma	Rat C6 cells	[26]
CD-1, Nude, and NOD CRISPR Prkdc IL2Rγ_{null}	Glioma	DAOY and T98G cells	[38]
CIEA-NOG	GBM	Patient derived glioma cell lines	[39]
ICR	High Grade Glioma	C6 rat glioma cells	[40]
NOD/SCID	IDH1 Mutated Glioma	Patient derived IDH1/2 oligoastrocytoma	[41]
	GBM	TG1 human GBM cell line	[42]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 March 2021 doi:10.20944/preprints202103.0093.v1

	GBM	T98 and U87 glioma cell lines	[43]
Not reported	GBM	U87 and U373 glioma cell lines	[44]
Nude	GBM	Ink4a/ARF$^{-/-}$ Id4 astrocyte cells	[45]
	Malignant Astrocytoma	Commercial malignant cell lines	[46]
	High Grade Glioma	BT70 malignant glioma cell line	[47]
	High Grade Glioma	U87 human glioma cell line and C6 rat glioma cell line	[48]
	GBM	LN229 and U87 human glioma cells	[49]
	High Grade Glioma	E98 and E473 glioma cell lines	[50]
	GBM	Mouse GL261 cell line	[51]
	GBM	Human U87 glioma and rat 9L gliosarcoma cell lines	[52]
	GBM	Patient derived GBM cell lines	[53-55]
	High Grade Glioma	Human glioma U87, U251, U373, A172, LN18, LN229, and D54 cell lines	[56]
	High Grade Glioma	Hs683 cells	[57]
	High Grade Glioma	LN229 cells	[58,59]
	High Grade Glioma	SHG44 cells	[60]
	High Grade Glioma	T98G and U373 cells	[61]
	High Grade Glioma	U87, U251 and D566 cells	[62]
	High Grade Glioma	U87 cells	[63-85]
	GBM	U87. LNZ308, LN229 cells	[86]
	High Grade Glioma	U87, U118, N10, U251, A172, and U373 cell lines	[87]
	High Grade Glioma	U251 cell line	[88,89]
	GBM	U87 and LN229 cell line	[90]
	GBM	LN229 cell line	[91]
	Glioma	E102 and E106 glioma cell line	[92]
	Glioma	SNB-19 U87 glioma cell lines with co-transfecting COS-7 cells with pTet-On and treated with doxycycline	[93]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 March 2021 doi:10.20944/preprints202103.0093.v1

	High Grade Glioma	Human T269 4IgB7H3 knockdown or control cells (orthotopic) LN-229 B7H3 knockdown or control cells (subcutaneous)	[94]
	High Grade Glioma	U87 and U251 glioma cell line	[95]
	Glioma	A-172, U343, U87 and T98G glioma cells	[96]
	Glioma	U87 glioma cell line	[97]
	Glioma	U373 human glioma cell line	[98]
SCID	High Grade Glioma	GLI36-EGFRvIII engineered cells	[99]
	Glioma	Patient-derived glioma neurospheres	[100-102]

Table 3. Glioma PDX murine models

Mouse species	Brain tumor modeled	Source of genetic material	Reference
C6B3F1	High Grade Glioma	Mouse Tu2449, Tu9648 and Tu251 mouse glioma cell lines	[103]
eGFP NOD/SCID mice	Oligodendroglioma	Patient-derived tumor cells	[103]
NOD-Prkdc^{SCID} IL2Rγ_{null}	Low Grade Glioma	Patient-derived low grade glioma tissue	[104]
NOD-SCID	Malignant Astrocytoma	Embryonic stem cells	[106]
	Glioma	Patient-derived high grade glioma tissue	[107]
NOG	GBM	Patient-derived GBM cells	[108]
Not reported	Glioblastoma	Patient-derived human GBMs	[109]
NSG	GBM	Patient-derived GBM neurospheres	[109]
Nude	Glioma	Patient-derived IDH mutant glioma tissue	[111]
	GBM	Patient-derived GBM tissue	[112,113]
	High Grade Glioma	Human astroglioma U373 and T98G and oligodendroglioma Hs683 cell line	[113]
	GBM	Patient-derived GBM tissue	[27,115]
Nude/NOD/SCID	High Grade Glioma	U87, U118, LN18, LN229 cell lines	[116]
SCID	IDH-Mutant Glioma	Patient-derived glioma	[117]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 March 2021 doi:10.20944/preprints202103.0093.v1

		neurospheres	
	Glioma	Patient-derived IDH mutant glioma tissue	[118]

3.1 Immunology research in xenograft models

Xenograft models have several benefits including low-cost and fast throughput [118]. A limitation of xenograft models is the required use of immune-deficient mice.

Immune-deficient mice used in xenograft models include nude mice, non-obese/diabetic mice (NOD), severe combined immunodeficient mice (SCID), and the combination NOD/SCID and NOD/SCID/interleukin 2 receptor γ_{null} (NSG) mice. The nude (athymic) mouse has a depleted population of T lymphocytes acquired through mutations in FOXP1 [119]. Nude mice have increased NK cell and macrophage activity as well as intact B cells, dendritic cells, and granulocytes [120]. Thus, while unable to characterize the lymphocyte mediated response, nude mice models can provide information on other immune cell interactions with the tumor [120]. Another common mouse utilized is the SCID mouse which lacks mature B and T lymphocytes [121]. NSG mice carry significant reductions in natural killer cell function to reduce the innate and adaptive immune system for successful grafting of more sensitive tumors [123]. These immune-compromised mice are necessary for the successful engraftment of tumors without risk of short-term rejection. Loss of the immune microenvironment limits study of tumor interaction with the immune system and testing of immune modulating agents [124,125]. Recent studies show that humanized mouse models may help to overcome this challenge [124].

Humanized mouse models are used to generate a mouse with a competent human immune system to study immune responses to anti-cancer immunotherapies [125]. They are created with NSG or NOD/SCID mice undergoing whole body irradiation followed by injection of human CD34+ hematopoietic stem cells intravascularly [121,127]. After 12 weeks of age, engraftment of the human immune system success can be assessed with flow cytometry [125]. These humanized mice are then injected with patient derived tumor tissue to develop into humanized PDX models [125].

An alternate method to study immune systems in PDX models was proposed by Semenkow et al. (2017), who demonstrated that blocking T-cell co-activating signals with immune checkpoint inhibitors, abatacept and MR1, allowed for short term tumor development in orthotopic glioma murine models with intact immune systems [127]. Both models are expensive and time consuming but add to the current and future understanding of immune modulation on tumorigenesis and progression.

4. Syngeneic models

Syngeneic glioma rodent models have been generated via injection of the carcinogen ethyl nitrosourea into the placenta between the 15th and 18th day of murine pregnancy [22]. They can also be propagated with the use of immortalized cell lines, namely GL261 and CT-2A, that were derived after induction with the carcinogen 3-methylcholantrene, forming a tumor that resembled a GBM [22,125,129]. Unlike xenograft models, syngeneic models utilize immune-competent animals. This allows the study of the interaction between the tumor and immune microenvironment and the possibility of testing immunotherapies for cancer treatment. Like other models that are based on cell line propagation, the syngeneic mouse model is subject to genetic drift with long term propagation [22]. In addition, given this model is created entirely from the animal system, it presents challenges in translating findings to human cancer. Gliomas grown through syngeneic induction present as well-circumscribed tumors without infiltration into the surrounding brain parenchyma, which is not the typical growth pattern appreciated in human astrocytoma [22]. Therefore, these models do not fully recapitulate the phenotypic characteristics of the tumor being studied [14,130].

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 March 2021 doi:10.20944/preprints202103.0093.v1

5. Genetically engineered mouse models (GEMMs)

GEMMs involve manipulation of the mouse genome to induce tumor formation [120]. By causing the tumors to grow from endogenous mouse tissue, immune-competent mice can be utilized, a key advantage over xenograft models. The intact immune system and native tumor structure allows for the study of the tumor microenvironment, while the genetic level of control helps with evaluation of the molecular events leading to tumor formation, maintenance, and susceptibility to treatment [120]. Furthermore, GEMMs allow for the ability to activate relevant oncogenes at specific time points in tissue development, and they permit testing of potential therapeutic agents at various stages of tumorigenesis. This offers distinct advantages over PDX models, which are nearly universally derived from advanced tumors.

GEMMs are commonly made with inbred mouse strains. Mice commonly used for GEMMs include C57BL/6 mice, BALBc mice, and FVB/N mice. The C57BL/6 mouse strain, established in the 1920s to study immune responses to cancer, has an increased NK cell activity and high cell-mediated response, but a weak antibody-mediated response [130]. BALB/c mice in comparison to C57BL/6 mice have a better humoral immune reaction [132]. FVB/N mice (also known as friend virus B-type susceptibility), were created in the late 1970s from the 8th inbreed generation of the National institute of Health general purpose mouse [133]. In relation to BALB/c mice, FVB/N mice have been shown to respond with a greater Th2 bias, however, the immune status is poorly defined [134].

Historically, challenges with timing, sufficient tumor development, and inability to recapitulate the heterogenous intra-tumoral findings of gliomas made it difficult to utilize GEMMs for in vivo glioma modeling [120]. Advancements in these GEMMs have created several modeling systems that better recapitulate human gliomas. These systems include the replication competent avian-like sarcoma virus and the corresponding avian tumor virus A (RCAS-tVA), Cre-loxP system, and the sleeping beauty transposon (Table 4).

Table 4. Glioma murine GEMMs

Mouse species	Brain tumor modeled	Source of genetic material	Reference
C57BL/6 and Trp53$^{-/-}$	GBM	PDGFβ, p53 mutations	[135]
C57BL/6	Glioma	Heterozygous TgGZT$_{121}$, KRAS^{G12D}, GFAP-CreER, PP-CreER, NG2-CreER, and Rosa26-tdTomato mice crossed with PTEN, p53, Rb1, and NF1 mice	[136]
	Glioma	Crossing of NF1^{flox+} mice with p53$^{+/-}$ mice and then crossed with wild type F1 C57Bl6 mice	[137]
	High Grade Astrocytoma	RB, PTEN mutations	[138,139]
Crossed IDH1 and Nestin-Cre transgenic mice	IDH1 R132 Mutated Glioma	Nestin-Cre remodeling system	[140]
FVB/N mice	Oligodendrocyte	Ctv-a plasmid was transfected into an immortalized oligodendroglia cell line OLI-neu	[141]
FVB/N, C57BL/6,	GBM	KRas, Akt, Ink4a/Arf	[142,143]

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 March 2021 doi:10.20944/preprints202103.0093.v1

BALB/C, and 129	mutations		
	GBM	PDGFβ, Ink4a/ARF, PTEN mutations	[143]
Gtv-a $Arf^{-/-}$	High Grade Glioma	Induction with RCAS-PDGF-B	[145]
$INK4a^{+/+}$ and $INK4a^{-/-}$	GBM	PDGFβ	[146]
MUT3 (Mice with mixed genetic back-ground of C57BL/6, Sv129 and B6/CBA)	De novo GBM	Introduced PTEN allele and p53 into MUT3 mice	[147]
Not Reported	High Grade Glioma	KRas, p53, Ink4a/Arf mutations	[148]
	GBM	EGFRvIII, Ink4a/ARF, PTEN mutations	[149]
	GBM	PDGFβ mutation	[150]
RasB8	High Grade Glioma	EGFRvIII and V^{12} Ras mutation	[151]
Rosa26-SB11	High Grade Astrocytoma	T2/onc mutagenic transposon	[152]

5.1 Somatic gene delivery model

The RCAS-tVA system allows for oncogenes to be transferred to cells that express the tVA receptor using a cell-type specific promoter [22]. This model has the advantage of not proliferating in mammalian cells. Therefore, the interaction between induced tumor cells and healthy cells remains intact and can be evaluated [22,153]. Genetic mutations arising from single cells and cells selectively undergoing clonal expansion can be demonstrated by this model, including targeting neural specific cells [153]. Nestin positive cells (Ntv-a), glial fibrillary acidic protein (GFAP)-expressing cells (Gtv-a), and CNPase positive oligodendrocyte promoter (Ctv-a) mice models have been created to study glioma formation [152].

Holland and Varmus (1997) were the first to use an RCAS-tVA transgenic mouse model to demonstrate induction, proliferation, and migration of glial cells with β-FGF [153-155]. They also subsequently showed that EGFR mutations in murine glial cells induce lesions that are similar to human gliomas [156]. EGFR induced gliomas also occur in transgenic mice with a INK4a-ARF tumor suppressor locus disruption [156].

While the RCAS-tVA system is limited by the vector capacity of the RCAS virus, other viruses have been used for somatic gene transfer GEMM production, including adenoviruses and lentiviruses [22,154]. The advantage of these viruses in comparison to the RCAS virus is the ability to infect both dividing and non-dividing cells [152].

5.2 Conditional promoter specific model

The Cre-loxP system utilizes the Cre recombinase enzyme to induce recombination between two loxP recognition sites [22]. Conditional models breed a tissue specific transgenic Cre recombinase mouse with a mouse whose gene of interest has been flanked with loxP sites through a knock-in approach [156]. An inducible Cre-loxP system is created by placing Cre protein activity or gene expression under control of tamoxifen (Cre-ER) or tetracycline (Tet-On/Off) [157]. Cre-loxP systems are highly powerful and have been utilized to create mice that develop GBMs through the recombination of EGFRvIII mutations [22,157]. These genes can be placed under the control of brain specific promoters such as Nestin and GFAP for specific neural stem cell targeting [159,160].

Cre-LoxP systems have been utilized to evaluate the relationship between NF1 and glioma formation. C57BL/6 mice with NF1 mutations inbred with C57BL/6 mice with TP53 mutations developed malignant glial neoplasms of the central and peripheral

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 March 2021 doi:10.20944/preprints202103.0093.v1

nervous system [160]. Zhu et al (2005) furthered this understanding demonstrating that mice with NF1 and p53 mutations develop WHO grade II gliomas that progress to anaplastic astrocytoma and GBMs [137,161].

5.3 Transposon/Transposase models

The sleeping beauty system can identify genetic drivers in animal models [22]. These systems are thus important in understanding gliomagenesis [22]. Bender et al. (2010) utilized a T2/onc transposon with a constitutively active sleeping beauty transposase to create a high-grade astrocytoma. The gliomas created displayed an invasive phenotype with positive GFAP and S100 staining, making this an effective system to model human glioma formation [152].

6. Special consideration for IDH1/2 mutations

IDH1/2 mutations are point mutations in the binding pocket of the IDH enzyme with arginine 132 or 172 being substituted with another amino acid (most commonly histidine). This enzyme normally converts isocitrate dehydrogenase to alpha-ketoglutarate (α-KG) [162]. The mutation causes conversion of α-KG into D-2-hydroxyglutarate (D-2-HG), a compound found in small intracellular quantities. Increased quantities of D-2-HG are thought to competitively inhibit α-KG dependent dioxygenases and cause cellular damage including hypermethylation of DNA and suppression of metabolic processes [163]. These mutations also upregulate vascular endothelial growth factor (VEGF) and produce hypoxia-inducible factor-1alpha (HIF-1α) in high levels which promotes gliomagenesis and invasion [164]. Importantly, low grade gliomas with IDH mutations commonly progress to secondary GBMs. Thus, creating an IDH mutated animal model that accurately recapitulates parental tumors to study treatment and prevent progression to higher grade lesions is imperative.

Philip et. al (2018) utilized a RCAS-Ntva system to create IDH1 R132H glioma model with platelet derived growth factor receptor A amplification, loss of CDKN2A, alpha thalassemia/mental retardation syndrome x-linked (ATRX), and PTEN to display the transformation of immortal astrocytes to in vivo glioma development [165]. Heterotopic and orthotopic IDH1 mutant glioma xenografts are also utilized to model this disease state [22]. Borodovsky et al. (2015) utilized fresh patient tissue to create a flank derived IDH1 mutant tumor that was serially propagated [162]. Later, dissociated cells were implanted into nude mice orthotopically and displayed IDH1 mutant anaplastic astrocytoma formation leading to the creation of the JHH-273 murine model [162]. Murine GBM models have also been used to evaluate the effect of NAD+ depletion on IDH1 mutant tumors in SCID mice [166].

Validating and enhancing murine models of IDH mutant low-grade gliomas is important for pre-clinical testing of these new therapeutic strategies. A subcutaneous xenograft murine model with TS603 (a 1p/19q codeletion and IDH1 mutated anaplastic oligodendroglioma patient-derived cell line) has been utilized to test the effects of AGI-5198 (an IDH1-R132H mutant homodimer inhibitor) [166]. It was found that AGI-5198 was able to inhibit colony formation selectively in mutant IDH1 xenograft models and not in wildtype models, a potential benefit of this therapy [166]. Schumacher et al. (2014) utilized a humanized murine model to demonstrate that IDH1 has an immunogenic epitope that can be targeted with a mutation specific vaccination to induce interferon gamma producing T-cells against IDH1 mutant tumor cells [168,169]. There are currently on-going clinical trials to assess if these inhibitors and others will be effective treatment strategies for patients with low grade gliomas [169].

6. Conclusions

Glioma animal models offer an advantage over in vitro two-dimensional cultures as they better recapitulate the genetic, morphologic, and immunologic characteristics of parent tumors. Since the development of the first murine model 70 years ago, there have

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 March 2021 doi:10.20944/preprints202103.0093.v1

been many advances including the creation of PDXs and GEMMs. These advances have allowed for the creation of reliable glioma models to study the genetic and molecular changes preceding gliomagenesis, immunologic reaction, and novel therapeutic reactions. The recent use of glioma murine models to study glioma tumor progression and IDH specific drug therapies provides an opportunity to accurately evaluate the safety and efficacy of compounds in the preclinical setting.

Author Contributions: Conceptualization, S.K.M, K.G.A. Data curation C.E.B. and W.H.H. Writing-original draft preparation, C.E.B. and W.H.H. Writing review and editing, C.E.B., W.H.H., J.I.T., D.D.S., T.Y.E.A., T.E.R., S.K.M., K.G.A. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Eugene P. Frenkel, M.D. Endowed Scholar Program (K.G.A), Cancer Prevention and Research Institute of Texas grant number RR190034 and National Cancer Institute grant number K22CA237752, both awarded to S.K.M.

Informed Consent Statement: Not applicable

Acknowledgments: Special thanks is given to Melissa Logies for creation of Figure 1.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

References

1. Omuro, A.; DeAngelis, L.M. Glioblastoma and other malignant gliomas: a clinical review. *JAMA* **2013**, *310*, 1842-1850, doi:10.1001/jama.2013.280319.
2. Chinwalla, A.T.; Cook, L.L.; Delehaunty, K.D.; Fewell, G.A.; Fulton, L.A.; Fulton, R.S.; Graves, T.A.; Hillier, L.W.; Mardis, E.R.; McPherson, J.D., et al. Initial sequencing and comparative analysis of the mouse genome. *Nature* **2002**, *420*, 520-562, doi:10.1038/nature01262.
3. Noorani, I. Genetically Engineered Mouse Models of Gliomas: Technological Developments for Translational Discoveries. *Cancers (Basel)* **2019**, *11*, doi:10.3390/cancers11091335.
4. Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* **2008**, *455*, 1061-1068, doi:10.1038/nature07385.
5. Chen, R.; Smith-Cohn, M.; Cohen, A.L.; Colman, H. Glioma Subclassifications and Their Clinical Significance. *Neurotherapeutics : the journal of the American Society for Experimental NeuroTherapeutics* **2017**, *14*, 284-297, doi:10.1007/s13311-017-0519-x.
6. Robertson, F.L.; Marques-Torrejon, M.A.; Morrison, G.M.; Pollard, S.M. Experimental models and tools to tackle glioblastoma. *Dis Model Mech* **2019**, *12*, doi:10.1242/dmm.040386.
7. Stylli, S.S.; Luwor, R.B.; Ware, T.M.; Tan, F.; Kaye, A.H. Mouse models of glioma. *J Clin Neurosci* **2015**, *22*, 619-626, doi:10.1016/j.jocn.2014.10.013.
8. Imagi, S. Experimental study on heterotransplantation of Tanaka's rabbit-hepatoma and intestinal cancer into guinea pigs. *Gan* **1950**, *41*, 231-233.
9. Toolan, H.W. Growth of human tumors in cortisone-treated laboratory animals: the possibility of obtaining permanently transplantable human tumors. *Cancer Res* **1953**, *13*, 389-394.
10. Povlsen, C.O.; Spang-Thomsen, M.; Rygaard, J.; Visfeldt, J. Heterotransplantation of human malignant tumours to athymic nude mice. In *Immunodeficient Animals for Cancer Research*, Sparrow, S., Ed. Palgrave Macmillan UK: London, 1980; 10.1007/978-1-349-05014-7_8pp. 95-103.
11. Okada, S.; Vaeteewoottacharn, K.; Kariya, R. Application of Highly Immunocompromised Mice for the Establishment of Patient-Derived Xenograft (PDX) Models. *Cells* **2019**, *8*, doi:10.3390/cells8080889.

12. Talmadge, J.E.; Singh, R.K.; Fidler, I.J.; Raz, A. Murine models to evaluate novel and conventional therapeutic strategies for cancer. *Am J Pathol* **2007**, *170*, 793-804, doi:10.2353/ajpath.2007.060929.
13. Oh, T.; Fakurnejad, S.; Sayegh, E.T.; Clark, A.J.; Ivan, M.E.; Sun, M.Z.; Safaee, M.; Bloch, O.; James, C.D.; Parsa, A.T. Immunocompetent murine models for the study of glioblastoma immunotherapy. *Journal of translational medicine* **2014**, *12*, 107, doi:10.1186/1479-5876-12-107.
14. Chulpanova, D.S.; Kitaeva, K.V.; Rutland, C.S.; Rizvanov, A.A.; Solovyeva, V.V. Mouse Tumor Models for Advanced Cancer Immunotherapy. *Int J Mol Sci* **2020**, *21*, doi:10.3390/ijms21114118.
15. Smith, H.W.; Muller, W.J. Transgenic mouse models--a seminal breakthrough in oncogene research. *Cold Spring Harb Protoc* **2013**, *2013*, 1099-1108, doi:10.1101/pdb.top069765.
16. Hanahan, D.; Wagner, E.F.; Palmiter, R.D. The origins of oncomice: a history of the first transgenic mice genetically engineered to develop cancer. *Genes Dev* **2007**, *21*, 2258-2270, doi:10.1101/gad.1583307.
17. Adams, J.M.; Harris, A.W.; Pinkert, C.A.; Corcoran, L.M.; Alexander, W.S.; Cory, S.; Palmiter, R.D.; Brinster, R.L. The c-myc oncogene driven by immunoglobulin enhancers induces lymphoid malignancy in transgenic mice. *Nature* **1985**, *318*, 533-538, doi:10.1038/318533a0.
18. Brinster, R.L.; Chen, H.Y.; Messing, A.; van Dyke, T.; Levine, A.J.; Palmiter, R.D. Transgenic mice harboring SV40 T-antigen genes develop characteristic brain tumors. *Cell* **1984**, *37*, 367-379, doi:10.1016/0092-8674(84)90367-2.
19. Stewart, T.A.; Pattengale, P.K.; Leder, P. Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion genes. *Cell* **1984**, *38*, 627-637, doi:10.1016/0092-8674(84)90257-5.
20. Gu, H.; Zou, Y.R.; Rajewsky, K. Independent control of immunoglobulin switch recombination at individual switch regions evidenced through Cre-loxP-mediated gene targeting. *Cell* **1993**, *73*, 1155-1164, doi:10.1016/0092-8674(93)90644-6.
21. Jung, S.; Rajewsky, K.; Radbruch, A. Shutdown of class switch recombination by deletion of a switch region control element. *Science* **1993**, *259*, 984-987, doi:10.1126/science.8438159.
22. Akter, F.; Simon, B.; de Boer, N.L.; Redjal, N.; Wakimoto, H.; Shah, K. Pre-clinical tumor models of primary brain tumors: Challenges and opportunities. *Biochimica et biophysica acta. Reviews on cancer* **2021**, *1875*, 188458, doi:10.1016/j.bbcan.2020.188458.
23. Brighi, C.; Reid, L.; Genovesi, L.A.; Kojic, M.; Millar, A.; Bruce, Z.; White, A.L.; Day, B.W.; Rose, S.; Whittaker, A.K., et al. Comparative study of preclinical mouse models of high-grade glioma for nanomedicine research: the importance of reproducing blood-brain barrier heterogeneity. *Theranostics* **2020**, *10*, 6361-6371, doi:10.7150/thno.46468.
24. Frese, K.K.; Tuveson, D.A. Maximizing mouse cancer models. *Nature reviews. Cancer* **2007**, *7*, 645-658, doi:10.1038/nrc2192.
25. Adhikari, B.; Li, J.; Brandel, M.G.; Futalan, D.; Akers, J.; Deming, T.; Chen, C.C.; Carter, B.S. The use of TMZ embedded hydrogels for the treatment of orthotopic human glioma xenografts. *J Clin Neurosci* **2017**, *45*, 288-292, doi:10.1016/j.jocn.2017.07.027.
26. Kaye, A.H.; Morstyn, G.; Gardner, I.; Pyke, K. Development of a xenograft glioma model in mouse brain. *Cancer research* **1986**, *46*, 1367-1373.
27. Irtenkauf, S.M.; Sobiechowski, S.; Hasselbach, L.A.; Nelson, K.K.; Transou, A.D.; Carlton, E.T.; Mikkelsen, T.; deCarvalho, A.C. Optimization of Glioblastoma Mouse Orthotopic Xenograft Models for Translational Research. *Comparative medicine* **2017**, *67*, 300-314.
28. Miner, M.W.; Liljenbäck, H.; Virta, J.; Merisaari, J.; Oikonen, V.; Westermarck, J.; Li, X.G.; Roivainen, A. (2S, 4R)-4-[(18)F]Fluoroglutamine for In vivo PET Imaging of Glioma Xenografts in Mice: an Evaluation of Multiple Pharmacokinetic Models. *Molecular imaging and biology* **2020**, *22*, 969-978, doi:10.1007/s11307-020-01472-1.
29. Pellegatta, S.; Poliani, P.L.; Corno, D.; Menghi, F.; Ghielmetti, F.; Suarez-Merino, B.; Caldera, V.; Nava, S.; Ravanini, M.; Facchetti, F., et al. Neurospheres enriched in cancer stem-like cells are highly effective in eliciting a dendritic cell-mediated

immune response against malignant gliomas. *Cancer research* **2006**, *66*, 10247-10252, doi:10.1158/0008-5472.Can-06-2048.

30. Pellegatta, S.; Finocchiaro, G. Dendritic cell vaccines for cancer stem cells. *Methods in molecular biology (Clifton, N.J.)* **2009**, *568*, 233-247, doi:10.1007/978-1-59745-280-9_15.
31. Wagemakers, M.; van der Wal, G.E.; Cuberes, R.; Alvarez, I.; Andrés, E.M.; Buxens, J.; Vela, J.M.; Moorlag, H.; Mooij, J.J.; Molema, G. COX-2 Inhibition Combined with Radiation Reduces Orthotopic Glioma Outgrowth by Targeting the Tumor Vasculature. *Translational oncology* **2009**, *2*, 1-7, doi:10.1593/tlo.08160.
32. Markovic, D.S.; Vinnakota, K.; van Rooijen, N.; Kiwit, J.; Synowitz, M.; Glass, R.; Kettenmann, H. Minocycline reduces glioma expansion and invasion by attenuating microglial MT1-MMP expression. *Brain, behavior, and immunity* **2011**, *25*, 624-628, doi:10.1016/j.bbi.2011.01.015.
33. Daga, A.; Orengo, A.M.; Gangemi, R.M.; Marubbi, D.; Perera, M.; Comes, A.; Ferrini, S.; Corte, G. Glioma immunotherapy by IL-21 gene-modified cells or by recombinant IL-21 involves antibody responses. *International journal of cancer* **2007**, *121*, 1756-1763, doi:10.1002/ijc.22901.
34. Cantini, G.; Pisati, F.; Mastropietro, A.; Frattini, V.; Iwakura, Y.; Finocchiaro, G.; Pellegatta, S. A critical role for regulatory T cells in driving cytokine profiles of Th17 cells and their modulation of glioma microenvironment. *Cancer immunology, immunotherapy : CII* **2011**, *60*, 1739-1750, doi:10.1007/s00262-011-1069-4.
35. Yamazoe, T.; Koizumi, S.; Yamasaki, T.; Amano, S.; Tokuyama, T.; Namba, H. Potent tumor tropism of induced pluripotent stem cells and induced pluripotent stem cell-derived neural stem cells in the mouse intracerebral glioma model. *International journal of oncology* **2015**, *46*, 147-152, doi:10.3892/ijo.2014.2702.
36. Aguilar-Morante, D.; Cortes-Canteli, M.; Sanz-Sancristobal, M.; Santos, A.; Perez-Castillo, A. Decreased CCAAT/enhancer binding protein β expression inhibits the growth of glioblastoma cells. *Neuroscience* **2011**, *176*, 110-119, doi:10.1016/j.neuroscience.2010.12.025.
37. Hülper, P.; Schulz-Schaeffer, W.; Dullin, C.; Hoffmann, P.; Harper, J.; Kurtzberg, L.; Lonning, S.; Kugler, W.; Lakomek, M.; Erdlenbruch, B. Tumor localization of an anti-TGF-β antibody and its effects on gliomas. *International journal of oncology* **2011**, *38*, 51-59.
38. Madala, H.R.; Punganuru, S.R.; Ali-Osman, F.; Zhang, R.; Srivenugopal, K.S. Brain- and brain tumor-penetrating disulfiram nanoparticles: Sequence of cytotoxic events and efficacy in human glioma cell lines and intracranial xenografts. *Oncotarget* **2018**, *9*, 3459-3482, doi:10.18632/oncotarget.23320.
39. Larsson, S.; Wenger, A.; Dósa, S.; Sabel, M.; Kling, T.; Carén, H. Cell line-based xenograft mouse model of paediatric glioma stem cells mirrors the clinical course of the patient. *Carcinogenesis* **2018**, *39*, 1304-1309, doi:10.1093/carcin/bgy091.
40. Zhang, L.; Zhu, S.; Qian, L.; Pei, Y.; Qiu, Y.; Jiang, Y. RGD-modified PEG-PAMAM-DOX conjugates: in vitro and in vivo studies for glioma. *European journal of pharmaceutics and biopharmaceutics : official journal of Arbeitsgemeinschaft fur Pharmazeutische Verfahrenstechnik e.V* **2011**, *79*, 232-240, doi:10.1016/j.ejpb.2011.03.025.
41. Luchman, H.A.; Stechishin, O.D.; Dang, N.H.; Blough, M.D.; Chesnelong, C.; Kelly, J.J.; Nguyen, S.A.; Chan, J.A.; Weljie, A.M.; Cairncross, J.G., et al. An in vivo patient-derived model of endogenous IDH1-mutant glioma. *Neuro Oncol* **2012**, *14*, 184-191, doi:10.1093/neuonc/nor207.
42. Fareh, M.; Turchi, L.; Virolle, V.; Debruyne, D.; Almairac, F.; de-la-Forest Divonne, S.; Paquis, P.; Preynat-Seauve, O.; Krause, K.H.; Chneiweiss, H., et al. The miR 302-367 cluster drastically affects self-renewal and infiltration properties of glioma-initiating cells through CXCR4 repression and consequent disruption of the SHH-GLI-NANOG network. *Cell death and differentiation* **2012**, *19*, 232-244, doi:10.1038/cdd.2011.89.
43. Jandial, R.; Neman, J.; Lim, P.P.; Tamae, D.; Kowolik, C.M.; Wuenschell, G.E.; Shuck, S.C.; Ciminera, A.K.; De Jesus, L.R.; Ouyang, C., et al. Inhibition of GLO1 in Glioblastoma Multiforme Increases DNA-AGEs, Stimulates RAGE Expression, and

Inhibits Brain Tumor Growth in Orthotopic Mouse Models. *International journal of molecular sciences* **2018**, *19*, doi:10.3390/ijms19020406.

44. Ni, X.R.; Zhao, Y.Y.; Cai, H.P.; Yu, Z.H.; Wang, J.; Chen, F.R.; Yu, Y.J.; Feng, G.K.; Chen, Z.P. Transferrin receptor 1 targeted optical imaging for identifying glioma margin in mouse models. *J Neurooncol* **2020**, *148*, 245-258, doi:10.1007/s11060-020-03527-3.
45. Beck, S.; Jin, X.; Yin, J.; Kim, S.H.; Lee, N.K.; Oh, S.Y.; Jin, X.; Kim, M.K.; Kim, E.B.; Son, J.S., et al. Identification of a peptide that interacts with Nestin protein expressed in brain cancer stem cells. *Biomaterials* **2011**, *32*, 8518-8528, doi:10.1016/j.biomaterials.2011.07.048.
46. Nicolaides, T.P.; Li, H.; Solomon, D.A.; Hariono, S.; Hashizume, R.; Barkovich, K.; Baker, S.J.; Paugh, B.S.; Jones, C.; Forshew, T., et al. Targeted therapy for BRAFV600E malignant astrocytoma. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2011**, *17*, 7595-7604, doi:10.1158/1078-0432.Ccr-11-1456.
47. Santagata, S.; Xu, Y.M.; Wijeratne, E.M.; Kontnik, R.; Rooney, C.; Perley, C.C.; Kwon, H.; Clardy, J.; Kesari, S.; Whitesell, L., et al. Using the heat-shock response to discover anticancer compounds that target protein homeostasis. *ACS chemical biology* **2012**, *7*, 340-349, doi:10.1021/cb200353m.
48. Moroz, M.A.; Huang, R.; Kochetkov, T.; Shi, W.; Thaler, H.; de Stanchina, E.; Gamez, I.; Ryan, R.P.; Blasberg, R.G. Comparison of corticotropin-releasing factor, dexamethasone, and temozolomide: treatment efficacy and toxicity in U87 and C6 intracranial gliomas. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2011**, *17*, 3282-3292, doi:10.1158/1078-0432.Ccr-10-3203.
49. Burden-Gulley, S.M.; Qutaish, M.Q.; Sullivant, K.E.; Lu, H.; Wang, J.; Craig, S.E.; Basilion, J.P.; Wilson, D.L.; Brady-Kalnay, S.M. Novel cryo-imaging of the glioma tumor microenvironment reveals migration and dispersal pathways in vivid three-dimensional detail. *Cancer research* **2011**, *71*, 5932-5940, doi:10.1158/0008-5472.Can-11-1553.
50. Navis, A.C.; Hamans, B.C.; Claes, A.; Heerschap, A.; Jeuken, J.W.; Wesseling, P.; Leenders, W.P. Effects of targeting the VEGF and PDGF pathways in diffuse orthotopic glioma models. *The Journal of pathology* **2011**, *223*, 626-634, doi:10.1002/path.2836.
51. Bai, R.Y.; Staedtke, V.; Aprhys, C.M.; Gallia, G.L.; Riggins, G.J. Antiparasitic mebendazole shows survival benefit in 2 preclinical models of glioblastoma multiforme. *Neuro Oncol* **2011**, *13*, 974-982, doi:10.1093/neuonc/nor077.
52. Emdad, L.; Sarkar, D.; Lee, S.G.; Su, Z.Z.; Yoo, B.K.; Dash, R.; Yacoub, A.; Fuller, C.E.; Shah, K.; Dent, P., et al. Astrocyte elevated gene-1: a novel target for human glioma therapy. *Molecular cancer therapeutics* **2010**, *9*, 79-88, doi:10.1158/1535-7163.Mct-09-0752.
53. Jamal, M.; Rath, B.H.; Williams, E.S.; Camphausen, K.; Tofilon, P.J. Microenvironmental regulation of glioblastoma radioresponse. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2010**, *16*, 6049-6059, doi:10.1158/1078-0432.Ccr-10-2435.
54. Jamal, M.; Rath, B.H.; Tsang, P.S.; Camphausen, K.; Tofilon, P.J. The brain microenvironment preferentially enhances the radioresistance of CD133(+) glioblastoma stem-like cells. *Neoplasia (New York, N.Y.)* **2012**, *14*, 150-158, doi:10.1593/neo.111794.
55. Marian, C.O.; Cho, S.K.; McEllin, B.M.; Maher, E.A.; Hatanpaa, K.J.; Madden, C.J.; Mickey, B.E.; Wright, W.E.; Shay, J.W.; Bachoo, R.M. The telomerase antagonist, imetelstat, efficiently targets glioblastoma tumor-initiating cells leading to decreased proliferation and tumor growth. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2010**, *16*, 154-163, doi:10.1158/1078-0432.Ccr-09-2850.
56. Zhu, H.; Li, J.T.; Zheng, F.; Martin, E.; Kots, A.Y.; Krumenacker, J.S.; Choi, B.K.; McCutcheon, I.E.; Weisbrodt, N.; Bögler, O., et al. Restoring soluble guanylyl cyclase expression and function blocks the aggressive course of glioma. *Molecular pharmacology* **2011**, *80*, 1076-1084, doi:10.1124/mol.111.073585.

57. Bruyère, C.; Madonna, S.; Van Goietsenoven, G.; Mathieu, V.; Dessolin, J.; Kraus, J.L.; Lefranc, F.; Kiss, R. JLK1486, a Bis 8-Hydroxyquinoline-Substituted Benzylamine, Displays Cytostatic Effects in Experimental Gliomas through MyT1 and STAT1 Activation and, to a Lesser Extent, PPARγ Activation. *Translational oncology* **2011**, *4*, 126-137, doi:10.1593/tlo.10253.
58. Qutaish, M.Q.; Sullivant, K.E.; Burden-Gulley, S.M.; Lu, H.; Roy, D.; Wang, J.; Basilion, J.P.; Brady-Kalnay, S.M.; Wilson, D.L. Cryo-image analysis of tumor cell migration, invasion, and dispersal in a mouse xenograft model of human glioblastoma multiforme. *Molecular imaging and biology* **2012**, *14*, 572-583, doi:10.1007/s11307-011-0525-z.
59. Tabatabai, G.; Frank, B.; Wick, A.; Lemke, D.; von Kürthy, G.; Obermüller, U.; Heckl, S.; Christ, G.; Weller, M.; Wick, W. Synergistic antiglioma activity of radiotherapy and enzastaurin. *Annals of neurology* **2007**, *61*, 153-161, doi:10.1002/ana.21057.
60. Dong, J.; Zhou, G.; Tang, D.; Chen, Y.; Cui, B.; Dai, X.; Zhang, J.; Lan, Q.; Huang, Q. Local delivery of slow-releasing temozolomide microspheres inhibits intracranial xenograft glioma growth. *Journal of cancer research and clinical oncology* **2012**, *138*, 2079-2084, doi:10.1007/s00432-012-1290-3.
61. Le Calvé, B.; Rynkowski, M.; Le Mercier, M.; Bruyère, C.; Lonez, C.; Gras, T.; Haibe-Kains, B.; Bontempi, G.; Decaestecker, C.; Ruysschaert, J.M., et al. Long-term in vitro treatment of human glioblastoma cells with temozolomide increases resistance in vivo through up-regulation of GLUT transporter and aldo-keto reductase enzyme AKR1C expression. *Neoplasia (New York, N.Y.)* **2010**, *12*, 727-739, doi:10.1593/neo.10526.
62. Najbauer, J.; Huszthy, P.C.; Barish, M.E.; Garcia, E.; Metz, M.Z.; Myers, S.M.; Gutova, M.; Frank, R.T.; Miletic, H.; Kendall, S.E., et al. Cellular host responses to gliomas. *PloS one* **2012**, *7*, e35150, doi:10.1371/journal.pone.0035150.
63. Staquicini, F.I.; Ozawa, M.G.; Moya, C.A.; Driessen, W.H.; Barbu, E.M.; Nishimori, H.; Soghomonyan, S.; Flores, L.G., 2nd; Liang, X.; Paolillo, V., et al. Systemic combinatorial peptide selection yields a non-canonical iron-mimicry mechanism for targeting tumors in a mouse model of human glioblastoma. *The Journal of clinical investigation* **2011**, *121*, 161-173, doi:10.1172/jci44798.
64. Schauff, A.K.; Kim, E.L.; Leppert, J.; Nadrowitz, R.; Wuestenberg, R.; Brockmann, M.A.; Giese, A. Inhibition of invasion-associated thromboxane synthase sensitizes experimental gliomas to gamma-radiation. *J Neurooncol* **2009**, *91*, 241-249, doi:10.1007/s11060-008-9708-0.
65. McGee, M.C.; Hamner, J.B.; Williams, R.F.; Rosati, S.F.; Sims, T.L.; Ng, C.Y.; Gaber, M.W.; Calabrese, C.; Wu, J.; Nathwani, A.C., et al. Improved intratumoral oxygenation through vascular normalization increases glioma sensitivity to ionizing radiation. *International journal of radiation oncology, biology, physics* **2010**, *76*, 1537-1545, doi:10.1016/j.ijrobp.2009.12.010.
66. Kim, Y.; Kim, K.H.; Lee, J.; Lee, Y.A.; Kim, M.; Lee, S.J.; Park, K.; Yang, H.; Jin, J.; Joo, K.M., et al. Wnt activation is implicated in glioblastoma radioresistance. *Laboratory investigation; a journal of technical methods and pathology* **2012**, *92*, 466-473, doi:10.1038/labinvest.2011.161.
67. Chaponis, D.; Barnes, J.W.; Dellagatta, J.L.; Kesari, S.; Fast, E.; Sauvageot, C.; Panagrahy, D.; Greene, E.R.; Ramakrishna, N.; Wen, P.Y., et al. Lonafarnib (SCH66336) improves the activity of temozolomide and radiation for orthotopic malignant gliomas. *J Neurooncol* **2011**, *104*, 179-189, doi:10.1007/s11060-010-0502-4.
68. Chen, T.C.; Wang, W.; Golden, E.B.; Thomas, S.; Sivakumar, W.; Hofman, F.M.; Louie, S.G.; Schönthal, A.H. Green tea epigallocatechin gallate enhances therapeutic efficacy of temozolomide in orthotopic mouse glioblastoma models. *Cancer letters* **2011**, *302*, 100-108, doi:10.1016/j.canlet.2010.11.008.
69. Matsumura, Y. Preclinical and clinical studies of NK012, an SN-38-incorporating polymeric micelles, which is designed based on EPR effect. *Advanced drug delivery reviews* **2011**, *63*, 184-192, doi:10.1016/j.addr.2010.05.008.
70. Redjal, N.; Chan, J.A.; Segal, R.A.; Kung, A.L. CXCR4 inhibition synergizes with cytotoxic chemotherapy in gliomas. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2006**, *12*, 6765-6771, doi:10.1158/1078-0432.Ccr-06-1372.

71. Nuthalapati, S.; Zhou, Q.; Guo, P.; Lv, H.; Cosenza, S.; Reddy, M.V.; Reddy, E.P.; Gallo, J.M. Preclinical pharmacokinetic and pharmacodynamic evaluation of novel anticancer agents, ON01910.Na (Rigosertib, Estybon™) and ON013105, for brain tumor chemotherapy. *Pharmaceutical research* **2012**, *29*, 2499-2511, doi:10.1007/s11095-012-0780-y.

72. Lamfers, M.L.; Idema, S.; Bosscher, L.; Heukelom, S.; Moeniralm, S.; van der Meulen-Muileman, I.H.; Overmeer, R.M.; van der Valk, P.; van Beusechem, V.W.; Gerritsen, W.R., et al. Differential effects of combined Ad5- delta 24RGD and radiation therapy in in vitro versus in vivo models of malignant glioma. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2007**, *13*, 7451-7458, doi:10.1158/1078-0432.Ccr-07-1265.

73. Bertrand, Y.; Currie, J.C.; Poirier, J.; Demeule, M.; Abulrob, A.; Fatehi, D.; Stanimirovic, D.; Sartelet, H.; Castaigne, J.P.; Béliveau, R. Influence of glioma tumour microenvironment on the transport of ANG1005 via low-density lipoprotein receptor-related protein 1. *British journal of cancer* **2011**, *105*, 1697-1707, doi:10.1038/bjc.2011.427.

74. Chae, S.S.; Kamoun, W.S.; Farrar, C.T.; Kirkpatrick, N.D.; Niemeyer, E.; de Graaf, A.M.; Sorensen, A.G.; Munn, L.L.; Jain, R.K.; Fukumura, D. Angiopoietin-2 interferes with anti-VEGFR2-induced vessel normalization and survival benefit in mice bearing gliomas. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2010**, *16*, 3618-3627, doi:10.1158/1078-0432.Ccr-09-3073.

75. Mukherjee, B.; McEllin, B.; Camacho, C.V.; Tomimatsu, N.; Sirasanagandala, S.; Nannepaga, S.; Hatanpaa, K.J.; Mickey, B.; Madden, C.; Maher, E., et al. EGFRvIII and DNA double-strand break repair: a molecular mechanism for radioresistance in glioblastoma. *Cancer research* **2009**, *69*, 4252-4259, doi:10.1158/0008-5472.Can-08-4853.

76. Momiyama, M.; Zhao, M.; Kimura, H.; Tran, B.; Chishima, T.; Bouvet, M.; Endo, I.; Hoffman, R.M. Inhibition and eradication of human glioma with tumor-targeting Salmonella typhimurium in an orthotopic nude-mouse model. *Cell cycle (Georgetown, Tex.)* **2012**, *11*, 628-632, doi:10.4161/cc.11.3.19116.

77. Menon, L.G.; Pratt, J.; Yang, H.W.; Black, P.M.; Sorensen, G.A.; Carroll, R.S. Imaging of human mesenchymal stromal cells: homing to human brain tumors. *J Neurooncol* **2012**, *107*, 257-267, doi:10.1007/s11060-011-0754-7.

78. Ahmed, A.U.; Thaci, B.; Alexiades, N.G.; Han, Y.; Qian, S.; Liu, F.; Balyasnikova, I.V.; Ulasov, I.Y.; Aboody, K.S.; Lesniak, M.S. Neural stem cell-based cell carriers enhance therapeutic efficacy of an oncolytic adenovirus in an orthotopic mouse model of human glioblastoma. *Molecular therapy : the journal of the American Society of Gene Therapy* **2011**, *19*, 1714-1726, doi:10.1038/mt.2011.100.

79. Fu, Y.; Ong, L.C.; Ranganath, S.H.; Zheng, L.; Kee, I.; Zhan, W.; Yu, S.; Chow, P.K.; Wang, C.H. A Dual Tracer 18F-FCH/18F-FDG PET Imaging of an Orthotopic Brain Tumor Xenograft Model. *PloS one* **2016**, *11*, e0148123, doi:10.1371/journal.pone.0148123.

80. Kirschner, S.; Felix, M.C.; Hartmann, L.; Bierbaum, M.; Maros, M.E.; Kerl, H.U.; Wenz, F.; Glatting, G.; Kramer, M.; Giordano, F.A., et al. In vivo micro-CT imaging of untreated and irradiated orthotopic glioblastoma xenografts in mice: capabilities, limitations and a comparison with bioluminescence imaging. *J Neurooncol* **2015**, *122*, 245-254, doi:10.1007/s11060-014-1708-7.

81. Yin, J.; Kim, J.K.; Moon, J.H.; Beck, S.; Piao, D.; Jin, X.; Kim, S.H.; Lim, Y.C.; Nam, D.H.; You, S., et al. hMSC-mediated concurrent delivery of endostatin and carboxylesterase to mouse xenografts suppresses glioma initiation and recurrence. *Molecular therapy : the journal of the American Society of Gene Therapy* **2011**, *19*, 1161-1169, doi:10.1038/mt.2011.28.

82. von Baumgarten, L.; Brucker, D.; Tirniceru, A.; Kienast, Y.; Grau, S.; Burgold, S.; Herms, J.; Winkler, F. Bevacizumab has differential and dose-dependent effects on glioma blood vessels and tumor cells. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2011**, *17*, 6192-6205, doi:10.1158/1078-0432.Ccr-10-1868.

83. Hayashi, K.; Yamauchi, K.; Yamamoto, N.; Tsuchiya, H.; Tomita, K.; Bouvet, M.; Wessels, J.; Hoffman, R.M. A color-coded orthotopic nude-mouse treatment model of brain-metastatic paralyzing spinal cord cancer that induces angiogenesis and neurogenesis. *Cell proliferation* **2009**, *42*, 75-82, doi:10.1111/j.1365-2184.2008.00574.x.

84. Khan, N.; Mupparaju, S.; Hou, H.; Williams, B.B.; Swartz, H. Repeated assessment of orthotopic glioma pO(2) by multi-site EPR oximetry: a technique with the potential to guide therapeutic optimization by repeated measurements of oxygen. *Journal of neuroscience methods* **2012**, *204*, 111-117, doi:10.1016/j.jneumeth.2011.10.026.

85. Kil, W.J.; Cerna, D.; Burgan, W.E.; Beam, K.; Carter, D.; Steeg, P.S.; Tofilon, P.J.; Camphausen, K. In vitro and in vivo radiosensitization induced by the DNA methylating agent temozolomide. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2008**, *14*, 931-938, doi:10.1158/1078-0432.Ccr-07-1856.

86. Gwak, H.S.; Shingu, T.; Chumbalkar, V.; Hwang, Y.H.; DeJournett, R.; Latha, K.; Koul, D.; Alfred Yung, W.K.; Powis, G.; Farrell, N.P., et al. Combined action of the dinuclear platinum compound BBR3610 with the PI3-K inhibitor PX-866 in glioblastoma. *International journal of cancer* **2011**, *128*, 787-796, doi:10.1002/ijc.25394.

87. Ahmed, A.U.; Tyler, M.A.; Thaci, B.; Alexiades, N.G.; Han, Y.; Ulasov, I.V.; Lesniak, M.S. A comparative study of neural and mesenchymal stem cell-based carriers for oncolytic adenovirus in a model of malignant glioma. *Molecular pharmaceutics* **2011**, *8*, 1559-1572, doi:10.1021/mp200161f.

88. Verhoeff, J.J.; Stalpers, L.J.; Claes, A.; Hovinga, K.E.; Musters, G.D.; Peter Vandertop, W.; Richel, D.J.; Leenders, W.P.; van Furth, W.R. Tumour control by whole brain irradiation of anti-VEGF-treated mice bearing intracerebral glioma. *European journal of cancer (Oxford, England : 1990)* **2009**, *45*, 3074-3080, doi:10.1016/j.ejca.2009.08.004.

89. Verhoeff, J.J.; Stalpers, L.J.; Coumou, A.W.; Koedooder, K.; Lavini, C.; Van Noorden, C.J.; Haveman, J.; Vandertop, W.P.; van Furth, W.R. Experimental iodine-125 seed irradiation of intracerebral brain tumors in nude mice. *Radiation oncology (London, England)* **2007**, *2*, 38, doi:10.1186/1748-717x-2-38.

90. Han, J.H.; Yoon, J.S.; Chang, D.Y.; Cho, K.G.; Lim, J.; Kim, S.S.; Suh-Kim, H. CXCR4-STAT3 Axis Plays a Role in Tumor Cell Infiltration in an Orthotopic Mouse Glioblastoma Model. *Molecules and cells* **2020**, *43*, 539-550, doi:10.14348/molcells.2020.0098.

91. Schötterl, S.; Miemietz, J.T.; Ilina, E.I.; Wirsik, N.M.; Ehrlich, I.; Gall, A.; Huber, S.M.; Lentzen, H.; Mittelbronn, M.; Naumann, U. Mistletoe-Based Drugs Work in Synergy with Radio-Chemotherapy in the Treatment of Glioma In Vitro and In Vivo in Glioblastoma Bearing Mice. *Evidence-based complementary and alternative medicine : eCAM* **2019**, *2019*, 1376140, doi:10.1155/2019/1376140.

92. Verhoye, M.; van der Sanden, B.P.; Rijken, P.F.; Peters, H.P.; Van der Kogel, A.J.; Pée, G.; Vanhoutte, G.; Heerschap, A.; Van der Linden, A. Assessment of the neovascular permeability in glioma xenografts by dynamic T(1) MRI with Gadomer-17. *Magnetic resonance in medicine* **2002**, *47*, 305-313, doi:10.1002/mrm.10072.

93. Wang, S.; Khan, A.; Lang, F.F.; Schaefer, T.S. Conditional gene expression in human intracranial xenograft tumors. *BioTechniques* **2001**, *31*, 196-202, doi:10.2144/01311dd04.

94. Lemke, D.; Pfenning, P.N.; Sahm, F.; Klein, A.C.; Kempf, T.; Warnken, U.; Schnölzer, M.; Tudoran, R.; Weller, M.; Platten, M., et al. Costimulatory protein 4IgB7H3 drives the malignant phenotype of glioblastoma by mediating immune escape and invasiveness. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2012**, *18*, 105-117, doi:10.1158/1078-0432.Ccr-11-0880.

95. Camphausen, K.; Purow, B.; Sproull, M.; Scott, T.; Ozawa, T.; Deen, D.F.; Tofilon, P.J. Orthotopic growth of human glioma cells quantitatively and qualitatively influences radiation-induced changes in gene expression. *Cancer research* **2005**, *65*, 10389-10393, doi:10.1158/0008-5472.Can-05-1904.

96. Xu, Z.F.; Sun, X.K.; Lan, Y.; Han, C.; Zhang, Y.D.; Chen, G. Linarin sensitizes tumor necrosis factor-related apoptosis (TRAIL)-induced ligand-triggered apoptosis in human glioma cells and in xenograft nude mice. *Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie* **2017**, *95*, 1607-1618, doi:10.1016/j.biopha.2017.08.021.

97. Park, C.R.; Kim, H.Y.; Song, M.G.; Lee, Y.S.; Youn, H.; Chung, J.K.; Cheon, G.J.; Kang, K.W. Efficacy and Safety of Human Serum Albumin-Cisplatin Complex in U87MG Xenograft Mouse Models. *International journal of molecular sciences* **2020**, *21*, doi:10.3390/ijms21217932.

98. Palma, C.; Bigioni, M.; Irrissuto, C.; Nardelli, F.; Maggi, C.A.; Manzini, S. Anti-tumour activity of tachykinin NK1 receptor antagonists on human glioma U373 MG xenograft. *British journal of cancer* **2000**, *82*, 480-487, doi:10.1054/bjoc.1999.0946.

99. Bagci-Onder, T.; Wakimoto, H.; Anderegg, M.; Cameron, C.; Shah, K. A dual PI3K/mTOR inhibitor, PI-103, cooperates with stem cell-delivered TRAIL in experimental glioma models. *Cancer research* **2011**, *71*, 154-163, doi:10.1158/0008-5472.Can-10-1601.

100. Yuan, A.L.; Ricks, C.B.; Bohm, A.K.; Lun, X.; Maxwell, L.; Safdar, S.; Bukhari, S.; Gerber, A.; Sayeed, W.; Bering, E.A., et al. ABT-888 restores sensitivity in temozolomide resistant glioma cells and xenografts. *PloS one* **2018**, *13*, e0202860, doi:10.1371/journal.pone.0202860.

101. Wakimoto, H.; Mohapatra, G.; Kanai, R.; Curry, W.T., Jr.; Yip, S.; Nitta, M.; Patel, A.P.; Barnard, Z.R.; Stemmer-Rachamimov, A.O.; Louis, D.N., et al. Maintenance of primary tumor phenotype and genotype in glioblastoma stem cells. *Neuro Oncol* **2012**, *14*, 132-144, doi:10.1093/neuonc/nor195.

102. Tanaka, S.; Luk, S.; Kiyokawa, J.; Onozato, M.L.; Iafrate, A.J.; Shah, K.; Martuza, R.L.; Rabkin, S.D.; Batchelor, T.T.; Cahill, D.P., et al. Genetically distinct glioma stem-like cell xenografts established from paired glioblastoma samples harvested before and after molecularly targeted therapy. *Scientific reports* **2019**, *9*, 139, doi:10.1038/s41598-018-37437-2.

103. Weissenberger, J.; Priester, M.; Bernreuther, C.; Rakel, S.; Glatzel, M.; Seifert, V.; Kögel, D. Dietary curcumin attenuates glioma growth in a syngeneic mouse model by inhibition of the JAK1,2/STAT3 signaling pathway. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2010**, *16*, 5781-5795, doi:10.1158/1078-0432.Ccr-10-0446.

104. Klink, B.; Miletic, H.; Stieber, D.; Huszthy, P.C.; Valenzuela, J.A.C.; Balss, J.; Wang, J.; Schubert, M.; Sakariassen, P.Ø.; Sundstrøm, T., et al. A Novel, Diffusely Infiltrative Xenograft Model of Human Anaplastic Oligodendroglioma with Mutations in FUBP1, CIC, and IDH1. *PloS one* **2013**, *8*, e59773, doi:10.1371/journal.pone.0059773.

105. Zeng, W.; Tang, Z.; Li, Y.; Yin, G.; Liu, Z.; Gao, J.; Chen, Y.; Chen, F. Patient-derived xenografts of different grade gliomas retain the heterogeneous histological and genetic features of human gliomas. *Cancer Cell International* **2020**, *20*, 1, doi:10.1186/s12935-019-1086-5.

106. Ding, H.; Roncari, L.; Shannon, P.; Wu, X.; Lau, N.; Karaskova, J.; Gutmann, D.H.; Squire, J.A.; Nagy, A.; Guha, A. Astrocyte-specific expression of activated p21-ras results in malignant astrocytoma formation in a transgenic mouse model of human gliomas. *Cancer research* **2001**, *61*, 3826-3836.

107. Xue, W.; Ton, H.; Zhang, J.; Xie, T.; Chen, X.; Zhou, B.; Guo, Y.; Fang, J.; Wang, S.; Zhang, W. Patient-derived orthotopic xenograft glioma models fail to replicate the magnetic resonance imaging features of the original patient tumor. *Oncology reports* **2020**, *43*, 1619-1629, doi:10.3892/or.2020.7538.

108. Joo, K.M.; Kim, J.; Jin, J.; Kim, M.; Seol, H.J.; Muradov, J.; Yang, H.; Choi, Y.L.; Park, W.Y.; Kong, D.S., et al. Patient-specific orthotopic glioblastoma xenograft models recapitulate the histopathology and biology of human glioblastomas in situ. *Cell Rep* **2013**, *3*, 260-273, doi:10.1016/j.celrep.2012.12.013.

109. Sasaki, A.; Ishiuchi, S.; Kanda, T.; Hasegawa, M.; Nakazato, Y. Analysis of interleukin-6 gene expression in primary human gliomas, glioblastoma xenografts, and glioblastoma cell lines. *Brain Tumor Pathol* **2001**, *18*, 13-21, doi:10.1007/bf02478920.

110. Kerstetter-Fogle, A.E.; Harris, P.L.R.; Brady-Kalnay, S.M.; Sloan, A.E. Generation of Glioblastoma Patient-Derived Intracranial Xenografts for Preclinical Studies. *International journal of molecular sciences* **2020**, *21*, doi:10.3390/ijms21145113.

111. Yamashita, A.S.; da Costa Rosa, M.; Borodovsky, A.; Festuccia, W.T.; Chan, T.; Riggins, G.J. Demethylation and epigenetic modification with 5-azacytidine reduces IDH1 mutant glioma growth in combination with temozolomide. *Neuro Oncol* **2019**, *21*, 189-200, doi:10.1093/neuonc/noy146.

112. Sarkaria, J.N.; Carlson, B.L.; Schroeder, M.A.; Grogan, P.; Brown, P.D.; Giannini, C.; Ballman, K.V.; Kitange, G.J.; Guha, A.; Pandita, A., et al. Use of an orthotopic xenograft model for assessing the effect of epidermal growth factor receptor

amplification on glioblastoma radiation response. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2006**, *12*, 2264-2271, doi:10.1158/1078-0432.Ccr-05-2510.

113. Kitange, G.J.; Carlson, B.L.; Mladek, A.C.; Decker, P.A.; Schroeder, M.A.; Wu, W.; Grogan, P.T.; Giannini, C.; Ballman, K.V.; Buckner, J.C., et al. Evaluation of MGMT promoter methylation status and correlation with temozolomide response in orthotopic glioblastoma xenograft model. *J Neurooncol* **2009**, *92*, 23-31, doi:10.1007/s11060-008-9737-8.
114. Bruyère, C.; Abeloos, L.; Lamoral-Theys, D.; Senetta, R.; Mathieu, V.; Le Mercier, M.; Kast, R.E.; Cassoni, P.; Vandenbussche, G.; Kiss, R., et al. Temozolomide modifies caveolin-1 expression in experimental malignant gliomas in vitro and in vivo. *Translational oncology* **2011**, *4*, 92-100, doi:10.1593/tlo.10205.
115. Scigliano, S.; Pinel, S.; Poussier, S.; Fouyssac, F.; Plenat, F.; Karcher, G.; Chastagner, P. Measurement of hypoxia using invasive oxygen-sensitive electrode, pimonidazole binding and 18F-FDG uptake in anaemic or erythropoietin-treated mice bearing human glioma xenografts. *International journal of oncology* **2008**, *32*, 69-77.
116. Hlavaty, J.; Jandl, G.; Liszt, M.; Petznek, H.; König-Schuster, M.; Sedlak, J.; Egerbacher, M.; Weissenberger, J.; Salmons, B.; Günzburg, W.H., et al. Comparative evaluation of preclinical in vivo models for the assessment of replicating retroviral vectors for the treatment of glioblastoma. *J Neurooncol* **2011**, *102*, 59-69, doi:10.1007/s11060-010-0295-5.
117. Wakimoto, H.; Tanaka, S.; Curry, W.T.; Loebel, F.; Zhao, D.; Tateishi, K.; Chen, J.; Klofas, L.K.; Lelic, N.; Kim, J.C., et al. Targetable signaling pathway mutations are associated with malignant phenotype in IDH-mutant gliomas. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2014**, *20*, 2898-2909, doi:10.1158/1078-0432.Ccr-13-3052.
118. Tiwari, V.; Mashimo, T.; An, Z.; Vemireddy, V.; Piccirillo, S.; Askari, P.; Hulsey, K.M.; Zhang, S.; de Graaf, R.A.; Patel, T.R., et al. In vivo MRS measurement of 2-hydroxyglutarate in patient-derived IDH-mutant xenograft mouse models versus glioma patients. *Magnetic resonance in medicine* **2020**, *84*, 1152-1160, doi:10.1002/mrm.28183.
119. Day, C.P.; Merlino, G.; Van Dyke, T. Preclinical mouse cancer models: a maze of opportunities and challenges. *Cell* **2015**, *163*, 39-53, doi:10.1016/j.cell.2015.08.068.
120. Huszthy, P.C.; Daphu, I.; Niclou, S.P.; Stieber, D.; Nigro, J.M.; Sakariassen, P.; Miletic, H.; Thorsen, F.; Bjerkvig, R. In vivo models of primary brain tumors: pitfalls and perspectives. *Neuro Oncol* **2012**, *14*, 979-993, doi:10.1093/neuonc/nos135.
121. Richmond, A.; Su, Y. Mouse xenograft models vs GEM models for human cancer therapeutics. *Dis Model Mech* **2008**, *1*, 78-82, doi:10.1242/dmm.000976.
122. Bosma, G.C.; Custer, R.P.; Bosma, M.J. A severe combined immunodeficiency mutation in the mouse. *Nature* **1983**, *301*, 527-530, doi:10.1038/301527a0.
123. Shultz, L.D.; Lyons, B.L.; Burzenski, L.M.; Gott, B.; Chen, X.; Chaleff, S.; Kotb, M.; Gillies, S.D.; King, M.; Mangada, J., et al. Human lymphoid and myeloid cell development in NOD/LtSz-scid IL2R gamma null mice engrafted with mobilized human hemopoietic stem cells. *J Immunol* **2005**, *174*, 6477-6489, doi:10.4049/jimmunol.174.10.6477.
124. da Hora, C.C.; Schweiger, M.W.; Wurdinger, T.; Tannous, B.A. Patient-Derived Glioma Models: From Patients to Dish to Animals. *Cells* **2019**, *8*, doi:10.3390/cells8101177.
125. Miyai, M.; Tomita, H.; Soeda, A.; Yano, H.; Iwama, T.; Hara, A. Current trends in mouse models of glioblastoma. *J Neurooncol* **2017**, *135*, 423-432, doi:10.1007/s11060-017-2626-2.
126. Choi, Y.; Lee, S.; Kim, K.; Kim, S.-H.; Chung, Y.-J.; Lee, C. Studying cancer immunotherapy using patient-derived xenografts (PDXs) in humanized mice. *Experimental & Molecular Medicine* **2018**, *50*, 99, doi:10.1038/s12276-018-0115-0.
127. Morton, J.J.; Bird, G.; Refaeli, Y.; Jimeno, A. Humanized Mouse Xenograft Models: Narrowing the Tumor-Microenvironment Gap. *Cancer Res* **2016**, *76*, 6153-6158, doi:10.1158/0008-5472.CAN-16-1260.
128. Semenkow, S.; Li, S.; Kahlert, U.D.; Raabe, E.H.; Xu, J.; Arnold, A.; Janowski, M.; Chol Oh, B.; Brandacher, G.; Bulte, J.W.M., et al. An immunocompetent mouse model of human glioblastoma. *Oncotarget* **2017**, *8*.

129. House, C.D.; Hernandez, L.; Annunziata, C.M. Recent technological advances in using mouse models to study ovarian cancer. *Front Oncol* **2014**, *4*, 26, doi:10.3389/fonc.2014.00026.

130. da Silva, B.; Mathew, R.K.; Polson, E.S.; Williams, J.; Wurdak, H. Spontaneous Glioblastoma Spheroid Infiltration of Early-Stage Cerebral Organoids Models Brain Tumor Invasion. *SLAS discovery : advancing life sciences R & D* **2018**, *23*, 862-868, doi:10.1177/2472555218764623.

131. Song, H.K.; Hwang, D.Y. Use of C57BL/6N mice on the variety of immunological researches. *Laboratory animal research* **2017**, *33*, 119-123, doi:10.5625/lar.2017.33.2.119.

132. Trunova, G.V.; Makarova, O.V.; Diatroptov, M.E.; Bogdanova, I.M.; Mikchailova, L.P.; Abdulaeva, S.O. Morphofunctional characteristic of the immune system in BALB/c and C57BL/6 mice. *Bulletin of experimental biology and medicine* **2011**, *151*, 99-102, doi:10.1007/s10517-011-1268-1.

133. Taketo, M.; Schroeder, A.C.; Mobraaten, L.E.; Gunning, K.B.; Hanten, G.; Fox, R.R.; Roderick, T.H.; Stewart, C.L.; Lilly, F.; Hansen, C.T., et al. FVB/N: an inbred mouse strain preferable for transgenic analyses. *Proceedings of the National Academy of Sciences of the United States of America* **1991**, *88*, 2065-2069, doi:10.1073/pnas.88.6.2065.

134. Kim, E.M.; Bae, Y.M.; Choi, M.H.; Hong, S.T. Cyst formation, increased anti-inflammatory cytokines and expression of chemokines support for Clonorchis sinensis infection in FVB mice. *Parasitol Int* **2012**, *61*, 124-129, doi:10.1016/j.parint.2011.07.001.

135. Hede, S.M.; Hansson, I.; Afink, G.B.; Eriksson, A.; Nazarenko, I.; Andrae, J.; Genove, G.; Westermark, B.; Nistér, M. GFAP promoter driven transgenic expression of PDGFB in the mouse brain leads to glioblastoma in a Trp53 null background. *Glia* **2009**, *57*, 1143-1153, doi:10.1002/glia.20837.

136. Vitucci, M.; Irvin, D.M.; McNeill, R.S.; Schmid, R.S.; Simon, J.M.; Dhruv, H.D.; Siegel, M.B.; Werneke, A.M.; Bash, R.E.; Kim, S., et al. Genomic profiles of low-grade murine gliomas evolve during progression to glioblastoma. *Neuro Oncol* **2017**, *19*, 1237-1247, doi:10.1093/neuonc/nox050.

137. Zhu, Y.; Guignard, F.; Zhao, D.; Liu, L.; Burns, D.K.; Mason, R.P.; Messing, A.; Parada, L.F. Early inactivation of p53 tumor suppressor gene cooperating with NF1 loss induces malignant astrocytoma. *Cancer cell* **2005**, *8*, 119-130, doi:10.1016/j.ccr.2005.07.004.

138. Xiao, A.; Wu, H.; Pandolfi, P.P.; Louis, D.N.; Van Dyke, T. Astrocyte inactivation of the pRb pathway predisposes mice to malignant astrocytoma development that is accelerated by PTEN mutation. *Cancer cell* **2002**, *1*, 157-168, doi:10.1016/s1535-6108(02)00029-6.

139. Xiao, A.; Yin, C.; Yang, C.; Di Cristofano, A.; Pandolfi, P.P.; Van Dyke, T. Somatic induction of Pten loss in a preclinical astrocytoma model reveals major roles in disease progression and avenues for target discovery and validation. *Cancer research* **2005**, *65*, 5172-5180, doi:10.1158/0008-5472.Can-04-3902.

140. Sasaki, M.; Knobbe, C.B.; Itsumi, M.; Elia, A.J.; Harris, I.S.; Chio, II; Cairns, R.A.; McCracken, S.; Wakeham, A.; Haight, J., et al. D-2-hydroxyglutarate produced by mutant IDH1 perturbs collagen maturation and basement membrane function. *Genes & development* **2012**, *26*, 2038-2049, doi:10.1101/gad.198200.112.

141. Lindberg, N.; Kastemar, M.; Olofsson, T.; Smits, A.; Uhrbom, L. Oligodendrocyte progenitor cells can act as cell of origin for experimental glioma. *Oncogene* **2009**, *28*, 2266-2275, doi:10.1038/onc.2009.76.

142. Uhrbom, L.; Kastemar, M.; Johansson, F.K.; Westermark, B.; Holland, E.C. Cell type-specific tumor suppression by Ink4a and Arf in Kras-induced mouse gliomagenesis. *Cancer research* **2005**, *65*, 2065-2069, doi:10.1158/0008-5472.Can-04-3588.

143. Uhrbom, L.; Dai, C.; Celestino, J.C.; Rosenblum, M.K.; Fuller, G.N.; Holland, E.C. Ink4a-Arf loss cooperates with KRas activation in astrocytes and neural progenitors to generate glioblastomas of various morphologies depending on activated Akt. *Cancer research* **2002**, *62*, 5551-5558.

144. Hambardzumyan, D.; Amankulor, N.M.; Helmy, K.Y.; Becher, O.J.; Holland, E.C. Modeling Adult Gliomas Using RCAS/t-va Technology. *Translational oncology* **2009**, *2*, 89-95, doi:10.1593/tlo.09100.

145. Jiang, Y.; Boije, M.; Westermark, B.; Uhrbom, L. PDGF-B Can sustain self-renewal and tumorigenicity of experimental glioma-derived cancer-initiating cells by preventing oligodendrocyte differentiation. *Neoplasia (New York, N.Y.)* **2011**, *13*, 492-503, doi:10.1593/neo.11314.

146. Uhrbom, L.; Hesselager, G.; Ostman, A.; Nistér, M.; Westermark, B. Dependence of autocrine growth factor stimulation in platelet-derived growth factor-B-induced mouse brain tumor cells. *International journal of cancer* **2000**, *85*, 398-406, doi:10.1002/(sici)1097-0215(20000201)85:3<398::aid-ijc17>3.0.co;2-l.

147. Kwon, C.H.; Zhao, D.; Chen, J.; Alcantara, S.; Li, Y.; Burns, D.K.; Mason, R.P.; Lee, E.Y.; Wu, H.; Parada, L.F. Pten haploinsufficiency accelerates formation of high-grade astrocytomas. *Cancer research* **2008**, *68*, 3286-3294, doi:10.1158/0008-5472.Can-07-6867.

148. de Vries, N.A.; Bruggeman, S.W.; Hulsman, D.; de Vries, H.I.; Zevenhoven, J.; Buckle, T.; Hamans, B.C.; Leenders, W.P.; Beijnen, J.H.; van Lohuizen, M., et al. Rapid and robust transgenic high-grade glioma mouse models for therapy intervention studies. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2010**, *16*, 3431-3441, doi:10.1158/1078-0432.Ccr-09-3414.

149. Zhu, H.; Acquaviva, J.; Ramachandran, P.; Boskovitz, A.; Woolfenden, S.; Pfannl, R.; Bronson, R.T.; Chen, J.W.; Weissleder, R.; Housman, D.E., et al. Oncogenic EGFR signaling cooperates with loss of tumor suppressor gene functions in gliomagenesis. *Proceedings of the National Academy of Sciences of the United States of America* **2009**, *106*, 2712-2716, doi:10.1073/pnas.0813314106.

150. Uhrbom, L.; Hesselager, G.; Nistér, M.; Westermark, B. Induction of brain tumors in mice using a recombinant platelet-derived growth factor B-chain retrovirus. *Cancer research* **1998**, *58*, 5275-5279.

151. Wei, Q.; Clarke, L.; Scheidenhelm, D.K.; Qian, B.; Tong, A.; Sabha, N.; Karim, Z.; Bock, N.A.; Reti, R.; Swoboda, R., et al. High-grade glioma formation results from postnatal pten loss or mutant epidermal growth factor receptor expression in a transgenic mouse glioma model. *Cancer research* **2006**, *66*, 7429-7437, doi:10.1158/0008-5472.Can-06-0712.

152. Bender, A.M.; Collier, L.S.; Rodriguez, F.J.; Tieu, C.; Larson, J.D.; Halder, C.; Mahlum, E.; Kollmeyer, T.M.; Akagi, K.; Sarkar, G., et al. Sleeping beauty-mediated somatic mutagenesis implicates CSF1 in the formation of high-grade astrocytomas. *Cancer research* **2010**, *70*, 3557-3565, doi:10.1158/0008-5472.Can-09-4674.

153. Rankin, S.L.; Zhu, G.; Baker, S.J. Review: insights gained from modelling high-grade glioma in the mouse. *Neuropathology and applied neurobiology* **2012**, *38*, 254-270, doi:10.1111/j.1365-2990.2011.01231.x.

154. Ahronian, L.G.; Lewis, B.C. Using the RCAS-TVA system to model human cancer in mice. *Cold Spring Harbor protocols* **2014**, *2014*, 1128-1135, doi:10.1101/pdb.top069831.

155. Holland, E.C.; Varmus, H.E. Basic fibroblast growth factor induces cell migration and proliferation after glia-specific gene transfer in mice. *Proceedings of the National Academy of Sciences of the United States of America* **1998**, *95*, 1218-1223, doi:10.1073/pnas.95.3.1218.

156. Holland, E.C.; Hively, W.P.; DePinho, R.A.; Varmus, H.E. A constitutively active epidermal growth factor receptor cooperates with disruption of G1 cell-cycle arrest pathways to induce glioma-like lesions in mice. *Genes & development* **1998**, *12*, 3675-3685, doi:10.1101/gad.12.23.3675.

157. Bouabe, H.; Okkenhaug, K. Gene targeting in mice: a review. *Methods in molecular biology (Clifton, N.J.)* **2013**, *1064*, 315-336, doi:10.1007/978-1-62703-601-6_23.

158. Kim, A.H.; Kim, S.P. Surviving travel or travelling to survive: the association of travel distance with survival in muscle invasive bladder cancer. *Translational andrology and urology* **2018**, *7*, S83-s85, doi:10.21037/tau.2018.01.16.

159. Mignone, J.L.; Kukekov, V.; Chiang, A.S.; Steindler, D.; Enikolopov, G. Neural stem and progenitor cells in nestin-GFP transgenic mice. *J Comp Neurol* **2004**, *469*, 311-324, doi:10.1002/cne.10964.

160. Weissenberger, J.; Steinbach, J.; Malin, G.; Spada, S.; Rülicke, T.; Aguzzi, A. Development and malignant progression of astrocytomas in GFAP-v. *Oncogene* **1997**, *14*, 2005-2013, doi:10.1038/sj.onc.1201168.

Preprints (www.preprints.org) | NOT PEER-REVIEWED | Posted: 2 March 2021 doi:10.20944/preprints202103.0093.v1

161. Reilly, K.M.; Loisel, D.A.; Bronson, R.T.; McLaughlin, M.E.; Jacks, T. Nf1;Trp53 mutant mice develop glioblastoma with evidence of strain-specific effects. *Nature Genetics* **2000**, *26*, 109-113, doi:10.1038/79075.

162. Borodovsky, A.; Meeker, A.K.; Kirkness, E.F.; Zhao, Q.; Eberhart, C.G.; Gallia, G.L.; Riggins, G.J. A model of a patient-derived IDH1 mutant anaplastic astrocytoma with alternative lengthening of telomeres. *J Neurooncol* **2015**, *121*, 479-487, doi:10.1007/s11060-014-1672-2.

163. Yang, H.; Ye, D.; Guan, K.L.; Xiong, Y. IDH1 and IDH2 mutations in tumorigenesis: mechanistic insights and clinical perspectives. *Clinical cancer research : an official journal of the American Association for Cancer Research* **2012**, *18*, 5562-5571, doi:10.1158/1078-0432.Ccr-12-1773.

164. Parsons, D.W.; Jones, S.; Zhang, X.; Lin, J.C.; Leary, R.J.; Angenendt, P.; Mankoo, P.; Carter, H.; Siu, I.M.; Gallia, G.L., et al. An integrated genomic analysis of human glioblastoma multiforme. *Science (New York, N.Y.)* **2008**, *321*, 1807-1812, doi:10.1126/science.1164382.

165. Philip, B.; Yu, D.X.; Silvis, M.R.; Shin, C.H.; Robinson, J.P.; Robinson, G.L.; Welker, A.E.; Angel, S.N.; Tripp, S.R.; Sonnen, J.A., et al. Mutant IDH1 Promotes Glioma Formation In Vivo. *Cell Rep* **2018**, *23*, 1553-1564, doi:10.1016/j.celrep.2018.03.133.

166. Tateishi, K.; Wakimoto, H.; Iafrate, A.J.; Tanaka, S.; Loebel, F.; Lelic, N.; Wiederschain, D.; Bedel, O.; Deng, G.; Zhang, B., et al. Extreme Vulnerability of IDH1 Mutant Cancers to NAD+ Depletion. *Cancer cell* **2015**, *28*, 773-784, doi:10.1016/j.ccell.2015.11.006.

167. Rohle, D.; Popovici-Muller, J.; Palaskas, N.; Turcan, S.; Grommes, C.; Campos, C.; Tsoi, J.; Clark, O.; Oldrini, B.; Komisopoulou, E., et al. An Inhibitor of Mutant IDH1 Delays Growth and Promotes Differentiation of Glioma Cells. *Science (New York, N.Y.)* **2013**, *340*, 626-630, doi:10.1126/science.1236062.

168. Schumacher, T.; Bunse, L.; Pusch, S.; Sahm, F.; Wiestler, B.; Quandt, J.; Menn, O.; Osswald, M.; Oezen, I.; Ott, M., et al. A vaccine targeting mutant IDH1 induces antitumour immunity. *Nature* **2014**, *512*, 324-327, doi:10.1038/nature13387.

169. Huang, J.; Yu, J.; Tu, L.; Huang, N.; Li, H.; Luo, Y. Isocitrate Dehydrogenase Mutations in Glioma: From Basic Discovery to Therapeutics Development. *Frontiers in oncology* **2019**, *9*, 506, doi:10.3389/fonc.2019.00506.