Mouse models of uveal melanoma: Strengths, weaknesses, and future directions
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INTRODUCTION

Uveal melanoma is a rare (estimated incidence of 6 cases per million) and unique subtype of melanoma that arises in the uveal tract of the eye, most commonly in the choroid (Damato & Damato, 2012; McLaughlin et al., 2005). Local interventions, such as radiation therapy and enucleation, are effective at treating the primary tumor (Krantz, Dave, Komatsubara, Marr, & Carvajal, 2017). However, up to half of the patients will develop metastatic disease, predominantly to the liver (Rietschel et al., 2005). For these patients, liver-directed therapy and participation in clinical trials are recommended, but most die from their disease, and median survival is only 10.2 months (Khoja et al., 2019; Kujala, Makitie, & Kivela, 2003; National Comprehensive Cancer Network).

Despite this, great strides have been made in understanding the molecular features of uveal melanoma. In the past decade, the collective work from several groups has led to the identification of important recurrent mutations and overactive signaling pathways in this cancer. Early oncogenic driver mutations occur in a nearly mutually exclusive pattern in the guanine nucleotide-binding protein subunit alpha-q/11 signaling pathway (Field et al., 2018; Moore et al., 2016; Robertson et al., 2017). This includes constitutively active variants of GNAQ and GNA11, which are found in over 90% of
cases (Van Raamsdonk et al., 2009, 2010). A smaller subset of tumors harbor activating mutations in the G protein-coupled receptor cysteinyl leukotriene receptor 2 (CYSLTR2) or phospholipase C beta 4 (PLCB4) (Johansson et al., 2016; Moore et al., 2016). There is a second node of nearly mutually exclusive mutations that classifies uveal melanomas and affects prognosis. Inactivating mutations are found in BRCA1-associated protein 1 (BAP1), while recurrent point mutations are observed in the eukaryotic translation initiation factor 1A X-linked (EIF1AX) or a splicing factor such as SF3B1 (Field et al., 2018; Harbour et al., 2010, 2013; Martin et al., 2013).

The molecular makeup of a particular uveal melanoma has significant implications for predicting metastasis. Most importantly, tumors with loss-of-function BAP1 mutations carry the worst prognosis, as approximately 84% of metastatic uveal melanomas are of this subtype (Harbour et al., 2010; Shain et al., 2019). Specific cytogenetic alterations have also been well described in this cancer (Aalto, Eriksson, Seregard, Larsson, & Knuttila, 2001; Anbunathan, Verstraten, Singh, Harbour, & Bowcock, 2019). Monosomy 3 co-occurs with BAP1 mutation, thereby eliminating both functional alleles (Field et al., 2018; Robertson et al., 2017). 6q loss, 1q gain, and 8q gain are also significantly enriched in uveal melanoma metastases (Ehlers, Worley, Onken, & Harbour, 2005; Hammond et al., 2015; Shain et al., 2019).

These discoveries were largely enabled by the analysis of patient tumor specimens and have greatly advanced our understanding of the molecular underpinnings of uveal melanoma tumorigenesis and their prognostic significance. Various animal models have likewise been indispensable in elucidating the biology and potential therapeutic vulnerabilities of this cancer (Cao & Jager, 2015; Stei, Loeffler, Holz, & Herwig, 2016; Yang, Cao, & Grossniklaus, 2015). In the past several years, there have been many promising preclinical studies that have used these models to identify novel treatment strategies, several of which are now in the early stages of clinical trials (Vivet-Noguer, Tarin, Roman-Roman, & Alsafadi, 2019; Yang, Manson, Marr, & Carvajal, 2018).

In this review article, we discuss the strengths and weaknesses of existing animal models of uveal melanoma, with an emphasis on mouse models. We also identify unmet needs that will require future model development and refinement. The goal of any animal model of uveal melanoma should be to faithfully recapitulate the processes of tumor initiation, growth, metastasis, and response to therapy as observed in patients with this disease.

2 | ANIMAL MODELS OF UVEAL MELANOMA

Though the focus of this review is mouse models of uveal melanoma, other species certainly have their advantages. Rabbits (Oryctolagus cuniculus), for example, have large eyes that facilitate the implantation of tumor cells and subsequent monitoring using techniques such as fundoscopy, ultrasound, and magnetic resonance imaging (Bontzos & Detorakis, 2017; Gao, Tang, Liu, Yang, & Liu, 2018). The zebrafish (Danio rerio) is a model organism that has been used more widely in many scientific fields in recent years (Meyers, 2018). Both xenograft (Fornabaio et al., 2018; van der Ent et al., 2014) and transgenic (Mouti, Dee, Coupland, & Hurlstone, 2016; Perez, Henle, Amsterdam, Hagen, & Lees, 2018) zebrafish models of uveal melanoma have been developed. These models are excellent for high-throughput pharmacologic screening and in vivo microscopy. The genetic models have yielded valuable insights into uveal melanoma signaling, such as the establishment of the importance of YAP activation in the initiation of this cancer. However, tumorigenesis in these models required mutation of p53, and metastasis was difficult to assess because of the induction of multiple primary tumors (Mouti et al., 2016; Perez et al., 2018).

Mice (Mus musculus) are the most widely used laboratory animal in the study of uveal melanoma (Cao & Jager, 2015). Their fecundity, gestation time, and size make them the most cost-effective mammalian model (Zuberi & Lutz, 2016). Furthermore, genetic manipulation of mice has produced various strains that are used in many uveal melanoma models. The primary goals of this review are to compare the different types of mouse models of uveal melanoma and propose directions for further development.

3 | INOCULATION SITES

The majority of murine models of uveal melanoma require the inoculation of cells or tumors into mice. Some uveal melanoma cell lines can be grown subcutaneously, which is convenient for measuring growth and response to therapy. However, others grow poorly subcutaneously but flourish in the tissue from which they were derived (Ozaki et al., 2016). In these cases, orthotopic models are preferable and may better model the human disease. Models of primary uveal melanoma in which the route of inoculation results in growth in the iris, ciliary body, or choroid.
are considered orthotopic (Figure 1). Inoculation of cells into the anterior chamber of the eye was one of the first techniques developed and reliably produces tumors in the iris that are capable of metastasis (Niederkorn, 1984). In 2000, a suprachoroidal injection technique was described in which cells are deposited into the posterior compartment (not to be confused with posterior chamber) of the eye (Dithmar, Rusciano, & Grossniklaus, 2000). In this approach, the needle is inserted through the limbus and into the choroid. Injected cells occupy the suprachoroidal space and likely spill into the subretinal space and vitreous. This technique is advantageous because it rapidly produces tumors in the choroid and ciliary body, the sites at which uveal melanoma most commonly occurs in patients. Furthermore, it reduces extraocular growth as compared to transconjunctival inoculations and consistently produces distant metastases (Tables 1 and 2a,b). A third type of orthotopic model is intravitreal injection. Although uveal melanoma does not arise in the vitreous humor, this environment is supportive of tumor growth and injected cells mimic human disease by invading and involving the uveal tract (Kilian et al., 2016; Yoo et al., 2016). All three of the above inoculation methods are amenable to combination with enucleation, which allows for longer follow-up and the study of metastatic outgrowth.

The eye is bypassed in some models in order to more quickly and reliably produce large tumors in visceral organs, especially the liver. Intravenous injection into either the retro-orbital sinus or tail vein mimics the latter part of the metastatic cascade—hematogenous growth.

| Cell line | Source | Original publication (Laboratory of origin) | Inoculation method | Metastasis | References |
|-----------|--------|--------------------------------------------|--------------------|------------|------------|
| B16LS9 Mouse cutaneous melanoma | Rusciano et al. (1994) (Max Burger) | Suprachoroidal | Liver, lungs, and lymph nodes | Jones et al. (2019); Dong et al. (2019); Xue et al. (2015); Yang et al. (2016), Yang and Grossniklaus (2010), Yang, Jager, and Grossniklaus (2010), Yang, Xu, luvone, and Grossniklaus (2006); Lattier et al. (2013); Zhang et al. (2011); Alizadeh et al. (2003); Dithmar, Rusciano, and Grossniklaus (2000), Dithmar, Rusciano, Lynn, et al. (2000); Diaz, Rusciano, Dithmar, and Grossniklaus (1999) |
| | | Ant. chamber | Not reported | Han, Brown, and Niederkorn (2016) |
| | | Intraocular | Liver | Han et al. (2016); Yang et al. (2011) |
| | | Intrasplenic | Not reported | Xue et al. (2015) |
| B16F10 Mouse cutaneous melanoma | Fidler, Gersten, and Budmen (1976) (Marilyn Budmen) | Suprachoroidal | None | Grossniklaus, Barron, and Wilson (1995) |
| | | Ant. chamber | Not reported | el Filali et al. (2012); de Lange et al. (2012); Ly et al. (2010); Grossniklaus et al. (1995); Knisely and Niederkorn (1990); |
| | | | Lungs | Harning and Szalay (1987); Niederkorn, Sanborn, and Gamel (1987), Niederkorn (1984) |
| | | Tail vein | Lungs | Sanborn, Niederkorn, and Gamel (1992); Niederkorn et al. (1987) |
| Queens Mouse cutaneous melanoma | Harning et al. (1987) (Jeanne Szalay) | Suprachoroidal | Lungs | Rajaii et al. (2014); Grossniklaus et al. (1995) |
| | | Ant. chamber | None | Grossniklaus et al. (1995) |
| | | | Lungs | Sanborn, Niederkorn, Kan-Mitchell, and Albert (1992), Sanborn, Niederkorn, and Gamel (1992); Harning and Szalay (1987) |
| | | Tail vein | Lungs | Sanborn, Niederkorn, and Gamel (1992) |
| HCmel12 Mouse cutaneous melanoma | Kilian et al. (2016) (Thomas Tüting) | Intravitreal | Lungs and lymph nodes | Stei, Loeffler, Kurts, et al. (2016); Kilian et al. (2016) |
| Oncogene-transduced melan-A cells | Immortalized mouse melanocyte | Subcutaneous | Not reported | Moore et al. (2016) |
| | | | Lungs and liver | Van Raamsdonk et al. (2010) |

Abbreviation: Ant. chamber, anterior chamber.
dissemination, arrest and extravasation in distant sites, and metastatic colony formation and growth. The liver and lungs are the most frequently reported sites of experimental metastasis with these routes of injection (Tables 1 and 2a,b). Others have developed the intrasplenic inoculation, which consistently produces tumors in the liver (Barisione et al., 2015; Gangemi et al., 2014, 2012; Jin et al., 2018). Finally, direct implantation of cells or tumors into the liver also results in florid growth in an orthotopic model of metastatic uveal melanoma (Kageyama et al., 2017; Ozaki et al., 2016).

Irrespective of the location of injection, disease progression (e.g., tumor growth and/or metastatic dissemination) can be studied in real time using non-invasive imaging methods such as bioluminescence imaging (Barisione et al., 2015; Surriga et al., 2013). For this technique, the injected cells have been transduced to stably express a luciferase reporter. When the graft-bearing mice are injected with luciferin, the tumor cells emit light that can be detected by an optical imaging instrument such as Perkin Elmer’s In Vivo Imaging System (IVIS). The intensity of the signal has been demonstrated to be a

| Table 2 | Human uveal melanoma cell lines derived from (a) primary tumors used in mouse xenograft experiments (b) metastases used in mouse xenograft experiments |
|-----------------|---------------------------------------------------------------|
| **(a) Cell line (mutations)** | **Source** | **Original publication (Laboratory of origin)** | **Inoculation method** | **Metastasis** | **References** |
| Mel92.1 (GNAQ^Q209L; EIF1AX^G6D) | Primary tumor | De Waard-Siebinga et al. (1995) (Martine Jager) | Subcutaneous | Not reported | Faiao-Flores et al. (2019); Forsberg et al. (2019); Kines et al. (2018); Chen et al. (2017, 2014); Ambrosini, Sawle, Musi, and Schwartz (2015); Ambrosini, Musi, Ho, Stanchina, and Schwartz, (2013); Musi, Ambrosini, Stanchina, and Schwartz, (2014); Surriga et al. (2013); Ho et al. (2012); Samadi et al. (2012); Landreville et al. (2012) |
| Mel202 (GNAQ^Q209L; SF3B1^R625G) | Primary tumor | Ksander, Rubsamen, Olsen, Cousins, and Streilein (1991) (J. Wayne Streilein) | Subcutaneous | Not reported | Ma and Niederkorn (1998), Ma, Luyten, Luider, Jager, and Niederkorn (1996) |
| Mel270 (GNAQ^Q209P) | Primary tumor | Verbik, Murray, Tran, and Ksander (1997) (Bruce Ksander) | Suprachoroidal | Liver | Niederkorn, Mellon, Pidherney, Mayhew, and Anand (1993) |
| MP41 (GNA11^Q209L) | PDX from a primary tumor | Amirouchene-Angelozzi et al. (2014) (Sergio Roman-Roman) | Tail vein | Liver | Faiao-Flores et al. (2019) |
| T105 and T142 (GNA11^Q209L) | Primary tumors | Mouriaux et al. (2016) (Sylvain Guérin) | Subcutaneous | Not reported | Mouriaux et al. (2016) |
| UMT2 (GNA11^Q209L) | Primary tumors | Suesskind et al. (2013) (Sigrid Henke-Fahle) | Suprachoroidal | None | Süßkind, Hurst, Rohrbach, and Schnichels (2017) |

(Continues)
suitable surrogate for tumor size and thus enables dynamic evaluation of the effects of different experimental conditions on tumor progression (Cosette et al., 2016; Poeschinger, Renner, Weber, & Scheuer, 2013).

The syngeneic cutaneous melanoma mouse model has been used for decades in uveal melanoma research. In this system, cutaneous melanoma cells are implanted in mice of the same genetic background as the mice from which the line was derived. Although the cell lines used are not uveal in origin, this system allows for the investigation of intraocular growth and metastasis of melanoma cells, as many of these lines metastasize to the liver (Table 1). This mimics the behavior of uveal melanoma in humans and allows for the study of the full metastatic process, including local invasion, intravasation, survival in the blood, extravasation, and growth in distant organs. The ability to examine the interaction between tumor and host cells as the cancer progresses in an immunocompetent animal is arguably the greatest strength of this model. Additionally, recipient mice may be genetically altered in order to study specific contributions of the host in melanoma progression (Lattier, Yang, Crawford, & Grossniklaus, 2013; Stei, Loeffler, Kurts, et al., 2016).

The most widely used syngeneic model is the inoculation of C57BL/6 mice with the B16LS9 cell line, a derivative of the B16 cutaneous melanoma line that was enriched for hepatic metastatic propensity through serial in vivo passaging (Rusciano, Lorenzoni, & Burger, 1994). This cell line metastasizes to the liver from the eye, and its use has led to valuable insights into the behavior of metastatic melanoma. For instance, this model was used to show that natural killer cells and pigment-derived epithelial factor play distinct roles in counteracting intrahepatic growth of melanoma cells (Jones, Yang, Zhang, Morales-Tirado, & Grossniklaus, 2019). Although B16LS9 cutaneous melanoma cells were used, the histological growth patterns of the hepatic metastases in the mouse model were similar to those observed in the livers of patients with metastatic uveal melanoma (Grossniklaus et al., 2016).

The primary disadvantage of the syngeneic model is that available mouse melanoma cell lines are of cutaneous origin, so the mutations and other molecular drivers of these cells differ from those found in human uveal melanoma. Therefore, their behavior, especially their response to therapy, may differ from what is observed in patients. Interestingly, there are a few syngeneic models that do carry canonical uveal melanoma mutations. Immortalized mouse melanocytes transduced with driver mutations found in patients undergo oncogenic transformation and are capable of producing tumors and even metastases (Moore et al., 2016; Van Raamsdonk et al., 2010). Additionally, the HCmel12 mouse cutaneous melanoma cell line has been reported to carry a GNA11<sup>Q209L</sup> variant (Schrage et al., 2015). Further details on other mutations in this cell line would allow for a more complete assessment of its suitability as a model for uveal melanoma. In the future, if mouse uveal melanoma cell lines could be derived from the genetically engineered mouse models discussed below, they would be powerful tools for syngeneic models. This strategy would allow for the controlled manipulation and study of bona fide uveal melanoma in an immunocompetent host.
XENOGRAFT MOUSE MODELS OF UVEAL MELANOMA

Xenograft models are another widely used approach. As the name implies, cells or tumors from a foreign source are grafted into mice. Most commonly, human uveal melanoma cell lines are used. The primary advantage of these models is that the cells are derived from patients. As such, they largely retain molecular features of the original tumor (Amirouchene-Angelozzi et al., 2014; Griewank et al., 2012; Jager, Magnier, Ksander, & Dubovy, 2016). This technique is therefore well-suited for studying tumor signaling and response to treatment. Many recent publications detailing new potential treatments for uveal melanoma utilize xenograft models (Table 2a,b). Another advantage of xenografts is reproducibility from mouse to mouse (Gould, Junitilla, & de Sauvage, 2015). Many human uveal melanoma cell lines have been described, although some are not commercially available. Frequently used cell lines with validated uveal melanoma mutations are included in Table 2a,b. Many of these xenograft models are useful for studying metastasis, as they produce tumors in organs such as the liver and lungs. It is also worth noting that some cell lines were derived from human uveal melanoma metastases. These are especially applicable for studying tumor growth in visceral organs such as the liver.

Authentication of uveal melanoma cell lines for use in xenograft models is critical. Some lines historically thought to be uveal melanoma have been found to harbor BRAF*V600E* mutations and are now recognized as being of cutaneous origin (Griewank et al., 2012; Yu et al., 2015). Furthermore, several of these were found by short tandem repeat (STR) analysis to be the same cell line (Folberg et al., 2008; Yu et al., 2015). Validation of uveal melanoma cell lines (including species confirmation, STR analysis, and pathogen detection) by individual laboratories is strongly encouraged. However, even after careful molecular characterization of any cancer cell line, the ability of the cells to faithfully recapitulate the behavior of their parental tumors has been questioned due to changes in molecular features that can result from culturing them in vitro (Ben-David et al., 2018; Gillet, Varma, & Gottesman, 2013; Goodspeed, Heiser, Gray, & Costello, 2016). An example of this is that the karyotypes, including the status of chromosome 3, of several of the older cell lines differ from those of the patients’ original tumors (Jager et al., 2016). Additionally, it has been demonstrated that the gene expression profiles of uveal melanoma cell lines in culture diverge from their source tumors even after short-term passaging (Mouriaux et al., 2016). One way to avoid these problems is to implant human tumor specimens directly into mice; this is the basis of patient-derived xenografts.

Patient-derived xenograft (PDX) models are relatively new in the uveal melanoma field but have demonstrated considerable translational potential. The research group led by Didier Decaudin has been the most successful and prolific in generating PDX models of uveal melanoma (Table 3). They implant fresh primary and metastatic tumor specimens in the interscapular fat pad of severe combined immunodeficient (SCID) mice and achieve an engraftment rate of 28% (Némati et al., 2010). Importantly, the tumors that grow in these mice maintain mutations, chromosomal imbalances, and histopathological features of the tumors from which they were derived (Carita, Nemati, & Decaudin, 2015). These PDX models have also been used for the derivation of new cell lines with clinically relevant features such as loss of BAP1 expression (Amirouchene-Angelozzi et al., 2014). They have also been effective for assessing the efficacy of novel combination therapies to treat uveal melanoma (Amirouchene-Angelozzi et al., 2016; Carita et al., 2016).

Another exciting recent development has been the generation of PDX models from hepatic uveal melanoma metastases (Kageyama et al., 2017). In these models, tumor specimens obtained after surgery or biopsy were surgically implanted into the livers of NOD SCID gamma mice. The authors achieved an 83% engraftment rate and found that the histology, genetics, and proteomics of the implanted tumors resembled corresponding features of patient metastases. Tumors could also be monitored by CT imaging. PDX models such as these hold promise for preclinical evaluation of experimental therapeutic compounds and the realization of personalized medicine.

Like all models, xenografts have disadvantages. The chief among these is the necessity of using immunocompromised mice. This can partially be avoided by taking advantage of the immune-privileged nature of the anterior chamber of the eye (Niederkorn, 2012). However, this approach can only be used to study the primary tumor, and the majority of grafts spontaneously regress (Sutmuller et al., 2000). In this new era of immunotherapy, the inability to study the interplay between the tumor and host immune system, especially in sites of metastasis, is a major limitation. In uveal melanoma, this is somewhat tempered by the low response rate of patients to PD-1 and/or CTLA4 inhibition (Algazi et al., 2016; Carvajal et al., 2017). However, other immunomodulatory pathways and cell types have been implicated in this cancer and are being actively investigated (Dougall, Kurtulus, Smyth, & Anderson, 2017; Robertson et al., 2017; Yang et al., 2016). Mice with humanized immune systems would be ideal recipients for xenograft models of all tumor types. Efforts to create such mice are ongoing but are complicated by, among other issues, graft-versus-host disease and interspecies differences in cytokine specificity (Allen et al., 2019; Wege, 2018). Other criticisms of xenografts, particularly PDX models, include their high cost, low engraftment rate, and low throughput (Siolas & Hannon, 2013). These are valid concerns, and the actual utility of these models in informing the treatment of patients with uveal melanoma will become more apparent in coming years.

Another approach to avoiding artifacts induced by two-dimensional cell culturing is the use of three-dimensional (3D) culture systems. Such “tumor organoid” models now exist for several cancers, including those arising in the colon, breast, and pancreas (Drost & Clevers, 2018; Yang, Sun, Liu, & Mao, 2018). 3D cultures derived from patient tumor specimens can be grafted into mice (patient-derived organoid xenografts) and faithfully match the molecular phenotypes and even treatment responses of the source tumors (Sachs et al., 2018; Vlachogiannis et al., 2018). Some even allow for the study of the tumor microenvironment, as they incorporate stromal cells such as cancer-associated fibroblasts and lymphocytes (Neal et al., 2018). In the uveal melanoma literature,
there have been a few reports of 3D cultures in which cells form tumourspheres (Angi, Versluis, & Kalirai, 2015; Lapadula et al., 2019; Vali-Nagy et al., 2018). Further work is needed to determine the feasibility of generating such cultures from patient tumors and whether these 3D cell models better reflect the biology of their parental tumors. If so, they may serve as superior tools for both in vitro assays and xenograft models.

6 | GENETICALLY ENGINEERED MOUSE MODELS (GEMMS) OF UVEAL MELANOMA

The third class of mouse models of uveal melanoma encompasses mice that have been genetically engineered to produce tumors. The primary advantage of these models is that they make it possible to study autochthonous tumorigenesis in an immunocompetent host. In particular, the contribution of specific genetic alterations to oncogenic signaling and disease progression can be assessed (Kersten, de Visser, van Miltenburg, & Jonkers, 2017; Zitvogel, Pitt, Daillere, Smyth, & Kroemer, 2016).

Older models include transgenic mice in which pigment cell-specific promoters of genes such as Tyrosinase drive expression of the SV40 large T antigen or HRAS, although some of these tumors originate from the retinal pigment epithelium rather than the uvea (Kramer, Powell, Wilson, Salvatore, & Grossniklaus, 1998; Syed et al., 1998; Tolleson et al., 2005). In the Tg(GM3) model, the Dopachrome tautomerase (Dct) promoter controls expression of the metabotropic glutamate receptor to produce both uveal melanoma and cutaneous melanoma (Schiffner et al., 2014). RET-driven GEMMs develop melanocytic neoplasms throughout the body, including in the uveal tract (Eyles et al., 2010; Kato et al., 1998). The major weakness of all of these models is that they are driven by molecular changes not observed in patients with uveal melanoma; this limits their clinical applicability.

In the years since the discovery of GNAQ and GNA11 as the main oncogenic drivers of uveal melanoma, three genetically engineered mouse models using these genes have been published (Table 4). In the first, a Tet-on system was used to induce GNAQQ209L expression in mice deficient for p16 Ink4a and p19 Ink4b (Feng et al., 2014). Although over half of the mice developed melanocytic cutaneous lesions by 9 months, there was no report of uveal melanoma. Despite this, cutaneous tumors in this model demonstrated YAP activation downstream of oncogenic GNAQ. Another seminal paper published simultaneously reached the same conclusion and demonstrated in vivo efficacy of a YAP inhibitor using a xenograft model of uveal melanoma (Yu et al., 2014).

In a different model, the expression of GNAQQ209L in a fox-stop-lox conditional knock-in allele inserted at the Rosa26 locus produced uveal melanoma in 3 months with 100% penetrance (Huang, Urtatiz, & Van Raamsdonk, 2015). Furthermore, it appears that cells from these tumors intravasate into blood vessels and metastasize to the lungs. Mice also developed dermal melanomas and melanocytic neoplasms at other sites, including the leptomeninges and inner ear. This model uses Mitf-cre to initiate oncogene expression. Lastly, another model in which a similar conditional knock-in allele encoding GNA11Q209L is activated by the inducible Tyrosinase-creERT2 produced a comparable phenotype, albeit at a later timepoint (Moore et al., 2018). When Bap1 deletion was combined with GNA11Q209L expression, uveal melanomas were unexpectedly smaller. However, skin melanoma burden increased, as did cellular proliferation of these tumors. Comparative genomics from this model identified RasGRP3 as a critical signaling node upstream of MAPK pathway activation, a finding that had been independently reported by another group that used orthogonal methods (Chen et al., 2017).

These models have shed light on key features of uveal melanomagenesis. First, they demonstrate that GNAQ and GNA11 are potent oncogenes. The deletion of tumor suppressors was not required to form uveal melanoma; indeed, in the second model, the expression of the human GNAQ transgene was only 3.3% of that of the murine wild-type allele as measured by RT-PCR of primary melanocyte cultures from the affected mice (Huang et al., 2015). Second, they illuminate differences between the pigment cell-specific promoters used in induction. The constitutive expression of Mitf-cre beginning at E15.5 likely explains the earlier onset of tumor formation in the GNAQQ209L model as compared to the GNA11Q209L model in which Tyr-creERT2 is induced in 4-week-old mice (Huang et al., 2015; Moore et al., 2018). Interestingly, induction of Tyr-creERT in 8-week-old mice in the GNAQQ209L model did not produce overt uveal melanoma. Whether this is simply due to the differences in induction (mouse age and type of inducible Cre recombinase) or the result of differing potencies of the oncogenic drivers remains to be explored. Finally, these GEMMs illustrate which populations of melanocytes are susceptible to oncogenic transformation by these mutations and downstream activated pathways.

Like other models, these GEMMs are not without their disadvantages. Disease progression is considerably slower than in syngeneic or xenograft models due to the time required for tumor initiation. A problem specific to the GNAQQ209L model is the microphthalmia caused by the Mitf-cre allele (Alizadeh, Fitch, Niswender, McKnight, & Barsh, 2008). Additionally, inserting the oncogenes in the Rosa26 locus is somewhat artificial. A model in which an activatable allele is targeted to the endogenous mouse Gnaq or Gna11 locus might better model physiologic expression of these genes. This approach has been successful in generating valuable BratKO GEMMs of cutaneous melanoma (Dankort et al., 2007; Mercer et al., 2005).

A serious obstacle encountered in the above uveal melanoma GEMMs is the induction of transgene expression in melanocytes throughout the entire body. This complicates the models in numerous ways. First, melanocytic neoplasms in other organs may cause pathology, such as the ataxic phenotype caused by melanocytosis of the vestibular system. Second, mice sometimes have to be euthanized before the ocular tumor can be fully studied because of rapid growth of melanomas arising from the dermis. Third, the study of metastasis is difficult because of the number of primary tumors, including some that develop in vital organs such as the heart (Huang et al., 2015; Moore et al., 2018).
An exciting recent publication describes a new method to overcome these issues by utilizing adeno-associated viral delivery of Cre recombinase to the uveal tract (Li et al., 2019). In this model, the suprachoroidal injection of an AAV5-CMV-Cre vector produced ocular melanocytic tumors in adult mice carrying conditional null alleles of the Hippo kinases Lats1 and Lats2, which normally function to suppress YAP/TAZ signaling. Furthermore, a similar vector in which Cre expression is under the control of the pigment cell-specific tyrosinase-related protein 2 (Trp2) promoter produced a comparable phenotype. Importantly, cells of these tumors were positive for melanoma markers Melan-A/Mart1 and HMB45 but negative for RPE65. This indicates that they arose from uveal melanocytes and not cells of the retinal pigment epithelium. Remarkably, the authors found that activation of the YAP pathway alone was both necessary and sufficient for initiation of uveal melanoma. Activation of the MAPK pathway using an inducible Kras G12D allele was not sufficient for tumor formation but did accelerate tumor growth and mortality in the Lats double knockout mice. They explored this intriguing synergy between MAPK and Hippo signaling and discovered an interactive transcriptional network in which AP1 factors amplify the oncogenic output of YAP/TEAD in uveal melanoma. The use of this AAV-Cre system represents a significant improvement upon the aforementioned Cre driver mouse strains in that it limits oncogenic transformation to melanocytes within the uveal tract of adult mice. This is a powerful new

### TABLE 3  Patient-derived mouse xenograft models of uveal melanoma

| PDX model | Source | Original Publication (Laboratory of origin) | Inoculation site | References |
|-----------|--------|---------------------------------------------|------------------|------------|
| 6 cases successfully grafted 3 times | Liver metastases | Kageyama et al. (2017) (Takami Sato) | Liver | Kageyama et al. (2017) |
| MP34, MP38, MP41, MP42, MP46, MP47, MP55, MP71, MP77, and MP80 | Primary tumors | Némati et al. (2010) (Didier Decaudin) | Interscapular fat pad | Carita et al. (2016); Amirouchene-Angelozzi et al. (2016, 2014); Némati et al. (2014, 2010); Madic et al. (2012) |
| MM33 | Subcutis metastasis | | | |
| MM26, MM28, MM52, MM66, and MM74 | Liver metastases | Heegaard, Spang-Thomsen, and Prause (2003) (Jan Ulrik Prause) | Subcutaneous | Heegaard et al. (2003) |
| ØPI-204 | Primary tumor | | | |

### TABLE 4  Genetically engineered mouse models of uveal melanoma

| Model genotype | Induction | Phenotype | Original Publication (Laboratory of origin) |
|----------------|-----------|-----------|---------------------------------------------|
| Dct-rtTA/++; tet-HA-GNAQQ209L/+; p16p19INK1 | 5- to 6-week-old mice; doxycycline in food | >50% of mice developed cutaneous melanoma; no report of lesions in the uveal tract | Feng et al. (2014) (J. Silvio Gutkind) |
| Rosa26-floxed stop-GNAQQ209L/+; Mitf-cre/+ | Embryonic (E15.5) activation by constitutive Cre driver | Skin hyperpigmentation overt uveal melanoma and occasional dermal melanoma at 3 months in 15/15 mice; melanocytic neoplasia of the leptomeninges, hardarian gland, cochlea, and vestibular system; putative metastases in the lungs at 3 months in 18/19 mice | Huang et al. (2015) (Catherine Van Raamsdonk) |
| Rosa26-floxed stop-GNAQQ209L/+; Tyrosinase-creER/+ | 8-week-old mice; daily IP injection of tamoxifen and tail dip in 4-HT for 5 days | Skin hyperpigmentation; melanocytic hyperplasia of the uveal tract (but not overt melanoma) in 3/3 mice | |
| R26-LSL-GNA11Q209L/+; Tyrosinase-creERT2/+ | 4-week-old mice; single IP injection of tamoxifen | Skin hyperpigmentation; overt uveal and dermal melanoma at 6 months in 50% of mice; melanocytic neoplasia of the leptomeninges, third ventricle, hardarian gland, and heart; putative metastases in axillary lymph nodes and lungs at 3-6 months in 100% of mice | Moore et al. (2018) (Yu Chen) |
| R26-LSL-GNA11Q209L/+; BAP1lox/lox; Tyrosinase-creERT2/+ | Compared to above: increased dermal melanoma burden and proliferative index, no change in number or size of uveal melanoma tumors or lung lesions | |
| AAV5-CMV-Cre or AAV5-Trp2-GFP-Cre; Lats1f/f, Lats2f/f | 2- to 4-month-old mice; suprachoroidal injection of AAV | Eye bulging at 2 months and uveal melanoma formation at 6 months in 12/14 and 8/10 mice, respectively | Li et al. (2019) (Junhao Mao) |
| AAV5-Trp2-GFP-Cre; Lats1f/f; Lats2f/f; LSL-KrasG12D | Compared to above: larger uveal melanoma tumors in 7/7 mice and reduced survival (<4 months) | | |
too that could be used in conjunction with both existing and new alleles to generate genetic mouse models that would enable the study of the entire disease process of uveal melanoma in vivo.

In addition to this AAV approach, the RCAS-TVA system might achieve similar results. This method has been used to generate numerous GEMMs of cutaneous melanoma (Cho et al., 2015; Kircher et al., 2019; VanBrocklin, Robinson, Lastwika, Khoury, & Holmen, 2010). RCAS subgroup A is an avian retrovirus capable of infecting cells that express the TVA receptor. Dopachrome tautomerase-TVA transgenic mice express this receptor in pigment-producing cells, and this strain could be crossed with one of the conditional knock-in alleles described above. Intraocular injection of RCAS virus that encodes for Cre would then activate oncogene expression in melanocytes of the eye. An advantage of this model over the AAV approach is targeted delivery to cells of interest such that there is no requirement for inclusion of a pigment cell-specific promoter within the virus. This provides more room for genes of interest, which can be linked with Cre within the same viral vector to enable delivery to the same cells. Additionally, high titer RCAS are easily produced in vitro using the chicken fibroblast DF-1 cell line (Fisher et al., 1999), and there is no need for helper virus in these cells. Retroviruses also permit long-term expression of genes due to genome integration, though this requires that the cells are dividing.

Despite these advancements in genetic models of uveal melanoma, the inherent differences in tumor biology between mice and humans cannot be ignored. Putative metastases that have been observed in published genetic models occur in the lungs, not the liver (Huang et al., 2015; Moore et al., 2018). Additionally, the loss of Bap1 did not enhance the aggressiveness of uveal melanomas; in fact, the ocular phenotype was weaker, and there was no increase in size or incidence of lung lesions compared with mice expressing GNA11 Q209L alone (Moore et al., 2018). The basis for these differences is not understood and merits further investigation. It must also be acknowledged that the chromosomal abnormalities and epigenetic modifications observed in patients with uveal melanoma are nearly impossible to model in a mouse. Thus, while these GEMMs, as well as improved models, will continue to provide valuable insights into the progression of uveal melanoma in vivo, it is unlikely that any one model will fully recapitulate the human disease in all of its intricacies.

7 | CONCLUSIONS

In summary, although there is no perfect mouse model of uveal melanoma, currently available models have been instrumental in elucidating critical signaling pathways and testing new therapeutic strategies for this cancer. Each type of model has distinctive strengths and weaknesses. Synergistic models are excellent for the investigation of tumor progression in an immunocompetent host but use cutaneous melanoma cell lines. Xenograft models allow for the study of human uveal melanoma cells and tumors in a living organism, but this does not include the immune response because recipient mice must be severely immunocompromised. Genetically engineered models allow for studies of autochthonous uveal melanoma formation and dissemination, yet tumors in these mice differ from those in patients in terms of molecular complexity and metastatic behavior. Investigators should leverage the models best suited to address their specific scientific questions. Future model development should aim to overcome current limitations and further enable efforts to investigate uveal melanoma biology and develop therapies most likely to succeed in patients afflicted with this cancer.

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CONFLICT OF INTERESTS

The authors declare no conflicts of interest.

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