Novel transgenic mouse model of tGLI1 for *in vivo* studies of breast cancer and glioblastoma

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INTRODUCTION

Truncated glioma-associated oncogene homolog 1 (**tGLI1**) was first identified in 2009 as a gain-of-function variant of the GLI1 transcription factor. tGLI1 lacks exon 3 and part of exon 4, but retains all functional GLI1 domains to promote expression of GLI1 target genes. Since its discovery, we have shown that tGLI1 expression is unique to tumor tissue of breast and brain cancers. In glioblastoma (GBM), we show that tGLI1 is more potent in driving an aggressive phenotype compared to GLI1 and that tGLI1-positive GBM xenografts have increased growth rate, vascularity, tubule formation by surrounding vascular endothelial cells, and induces expression of target genes such as Heparanase, *VEGF-A, VEGF-C,* and *TEM7*. Most recently, we found that tGLI1 is enriched in mesenchymal GBM and drives formation of glioma stem cells. In breast cancer, we have shown that tGLI1 drives breast cancer brain metastasis (BCBM) and supports breast cancer stem cell radioresistance by promoting breast cancer cell motility, invasion, anchorage-independent growth, and VEGF-A secretion by breast cancer cells. As with GBM, tGLI1 promotes breast cancer stem cells by upregulating stemness genes such as *Nanog, Sox2, CD44,* and *Oct4*, to activate astrocytes to render the brain more susceptible to BCBM.

To study the role of tGLI1 in preclinical models of GBM and breast cancer, we generated the **first** conditional tGLI1 knock-in (KI) mouse model. Cre-mediated recombination will induce expression of human *tGLI1* transgene, allowing for its transcription, while concomitantly stopping transcription of mouse *Gli1* gene We will cross our tGLI1-KI mice with GFAP-cre or MMTV-cre to induce tGLI1 expression in astrocytes or mammary epithelial cells, respectively. Our novel transgenic tGLI1-KI mouse is the first to model the oncogenic impact of tGLI1 in vivo. We anticipate our tGLI1-KI mouse model to expand our understanding of tGLI1-driven tumorigenesis and support therapeutic development in GBM and breast cancer.

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RATIONALE

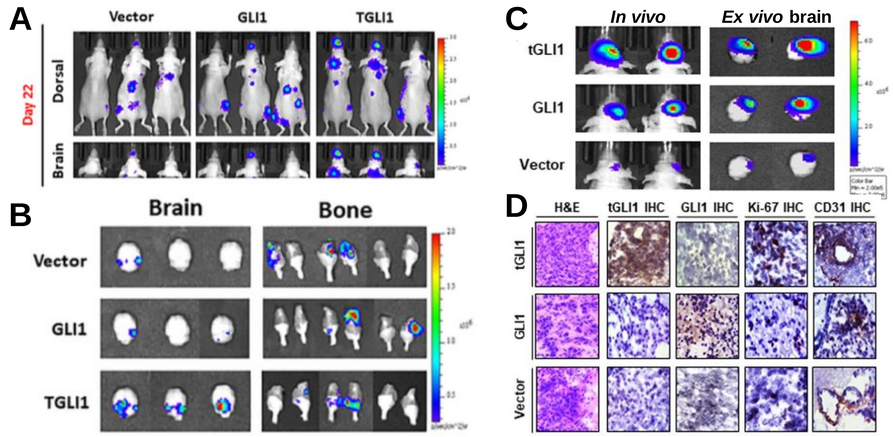

Figure 1. tGLI1 expression increases BCBM and bone metastasis of breast cancer and promotes GBM tumorigenesis. (A, B) tGLI1-overexpression induces BCBM and bone metastasis of MDA-MB-231 cells in an intracardiac injection metastasis model at Day 22. **(C)** tGLI1-positive GBM cells establish larger tumors in orthotopic GBM xenograft models. **(D)** tGLI1-positive GBM cells have increased cell proliferation (Ki-67) and microvessel density (CD31) as detected by IHC.

METHOD

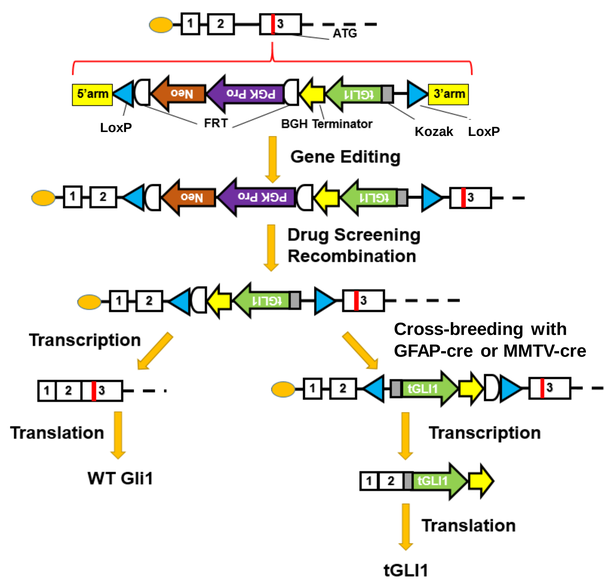

Figure 2. Overall scheme for generating transgenic tGLI1 conditional knock-in mice. tGLI1 transgenic mice have the human *tGli1* transgene in the inverse orientation, leaving only wild-type mouse *GLI1* gene translated. Cross-breeding with a tissue-specific cre recombinase mouse, such as GFAP-cre, recombines mouse *GLI1* and re-orients the human *tGLI1* transgene, allowing for its transcription. Cre-positive tGLI1 mice have early termination of mouse *GLI1* gene, expressing only human *tGLI1* in cre-positive mice.

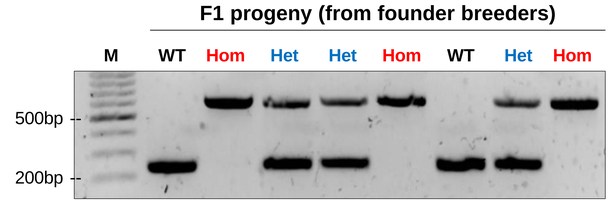

Figure 3. tGLI1 transgenic mice are generated with expected Mendelian probability. Breeding of heterozygous tGLI1 founder animals yields predicted genotypes near the expected Mendelian probabilities (F1 progeny). Generation of F2 progeny was accomplished by breeding homozygous F1 progeny, yielding 100% homozygous F2 pups. **WT**: 243bp; homozygous floxed (**Hom**): 606bp; heterozygous floxed (**Het**): 243bp/606bp; M: DNA marker).

FUTURE DIRECTION

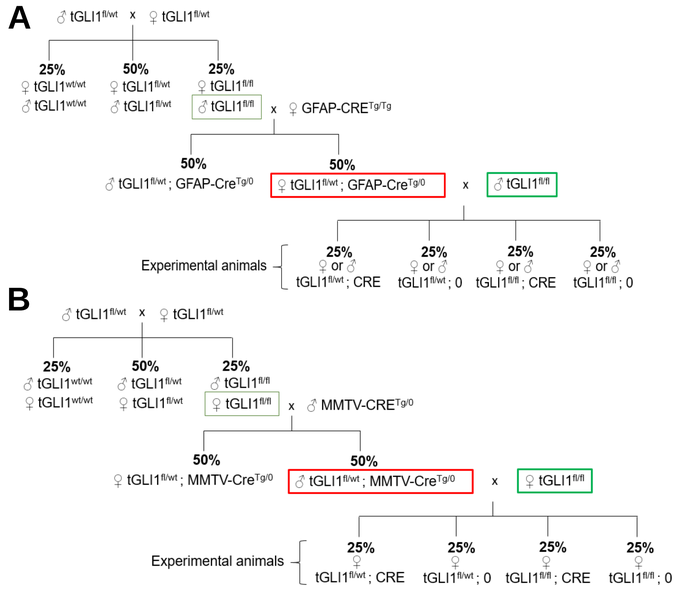

Figure 4. Proposed breeding scheme to generate transgenic mice with conditional knock-in of tGLI1 upon crossbreeding with tissue-specific cre mouse model. (A) To induce tGLI1 expression in the brain, tGLI1 mice will be crossed with GFAP-cre mice. Progeny from this scheme will have tGLI1-KI in astrocytes, modeling what we have observed in human GBM. We have already begun establishing animals with homozygous or heterozygous *tGLI1* knock-in in the brain using this scheme. **(B)** tGLI1 mice will also be crossed with the mammary-specific cre recombinase (MMTV-cre) to induce *tGLI1* knock-in within the mammary glands. Progeny from this cross can be heterozygous or homozygous for tGLI1, with or without cre recombinase. We anticipate that females with tGLI1-KI will have spontaneous neoplastic growth within their mammary glands. Additionally, we will also cross these mice with spontaneous models of breast cancer (e.g. MMTV-PyMT or MMTV-neu) to generate double-transgenic mice that have spontaneous mammary tumorigenesis with concomitant knock-in of human tGLI1. We expect that these double-transgenic mice will have shorter mammary tumor latency and exhibit faster tumor growth rate along with increased aggressiveness and distant metastasis.