A Novel RAS inhibitor for Luminal B and Triple Negative Breast Cancers

Raphael N. Jigo[1], Howard Donninger[2], Rachel Ferril[1], Becca vonBaby[1], Joe Burlison[2], John Trent[2], Tariq Arshad[3], Mike Sabo[2], and Geoffrey J. Clark[1]

[1]Department of Pharmacology & Toxicology, [2]Department of Medicine, James Graham Brown Cancer Center, University of Louisville, Louisville, KY, USA, [3]Qualigen Therapeutics, San Diego, CA, USA

UNIVERSITY OF LOUISVILLE

qualigen THERAPEUTICS

Introduction

Breast cancer accounts for about 2 million new cancer cases annually and is the main cause of cancer death in women globally. Characterization of molecular breast cancer subtypes based on mRNA gene expression has significantly aided in care and treatment of breast cancer patients; however, the Luminal B and Triple Negative Breast Cancer (TNBC) subtypes still lack effective treatment options to date. Luminal B tumors account for 10 – 20% of breast cancer cases in the US and are typically estrogen receptor (ER) and human epidermal growth factor 2 (HER2) positive. These tumors do not respond well to endocrine therapy, clinically present as being highly metastatic, and possess a high proliferative index. TNBC, on the other hand, accounts for 20% of cases and is characterized by the absence of ER, HER2, and PR. It is one of the most aggressive and treatment-resistant subtypes and shares similar prognoses and survival rates as Luminal B tumors.

Historically, the RAS oncoprotein has not been implicated in breast cancer despite bearing oncogenic mutations in 30% of cancers. Recent findings, however, have reported about 62% of Luminal B tumors to express decreased levels of tumor suppressors DAB2IP and RASAL2, as well as decreased NF1 expression in TNBC. These tumor suppressors normally function to negatively regulate RAS; hence, loss of their expression would cause RAS hyperactivation without RAS mutation. We hypothesize, therefore, that Luminal B and TNBC tumors are sensitive to the interdiction of wild type RAS.

Methods

In-silico Drug Screening: Computer-based drug design was used to identify small molecules with the potential to bind and inhibit RAS. Binding was confirmed by AUC and NMR.

In vitro Bioassay to detect anti-tumor activity: To test effects on anchorage-independent growth, murine (E0771) and human (BT474) Luminal B breast cancer cell lines were used in 3D growth assays with inhibitor at concentrations of 10, 5, 1, 0.5 and 0.3mM. Human MDA-MB-231 TNBC cells were treated at 2.5, and 0.5mM.

Signaling Assays: Cells were treated with F3860 for 1 hour, lysed and immunoblotted to determine effect on RAS effector signaling proteins. RAL Pulldown Assay was performed to determine effect on RAS/RAL signaling.

In-vivo assay: NSG mice were injected in the mammary fat pad with BT474 cells, then treated with inhibitor to determine effects on tumor growth.

Results

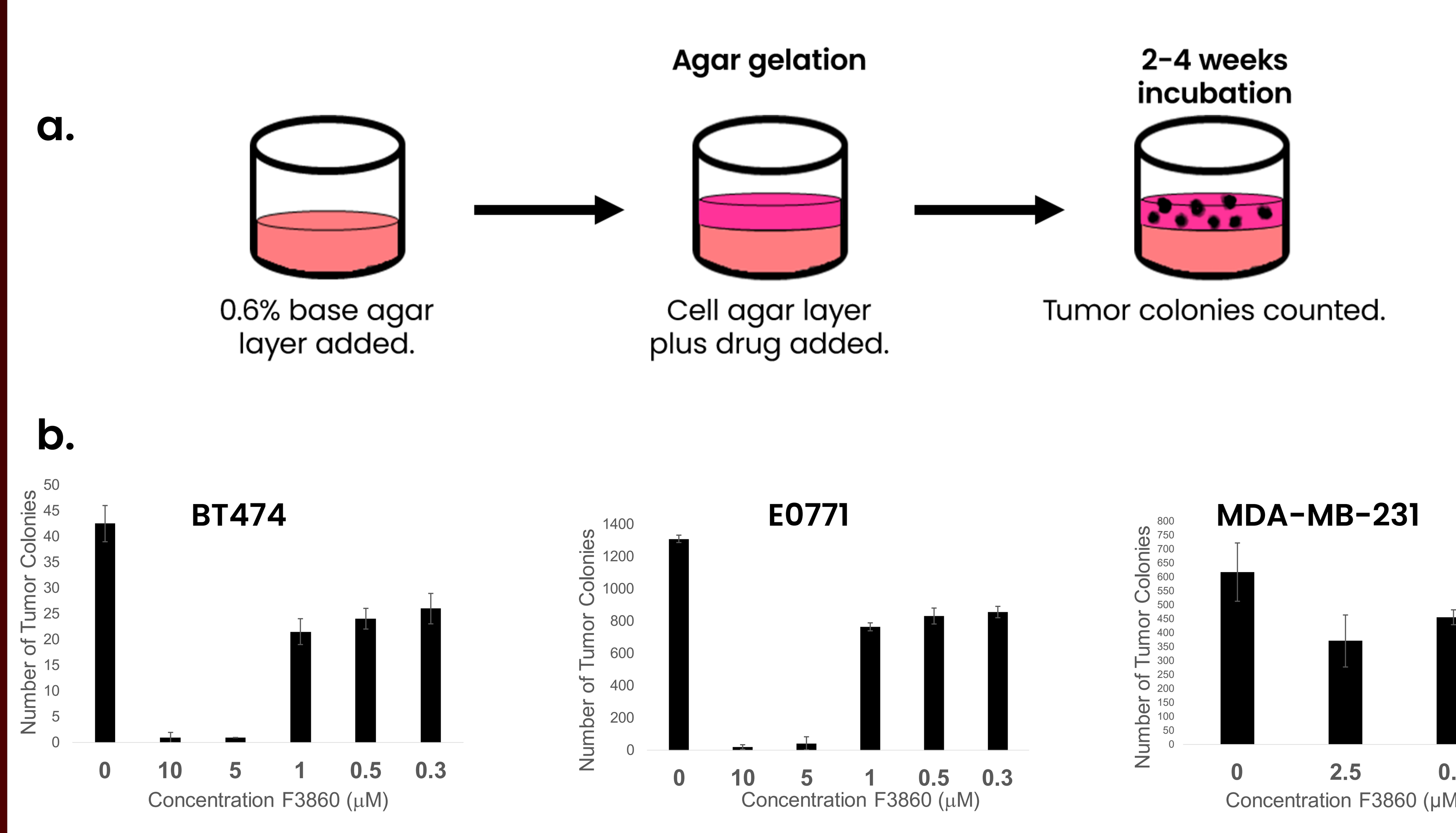
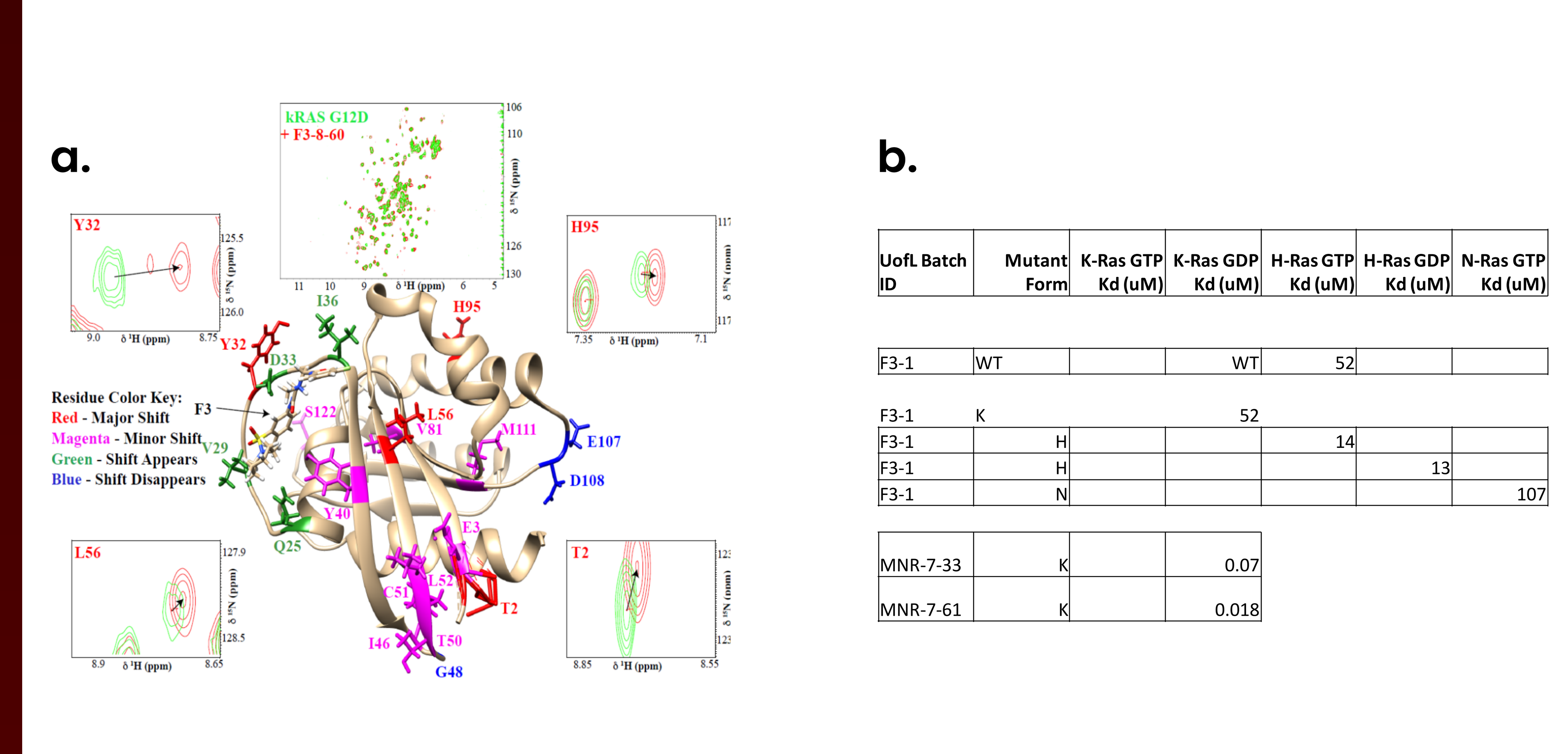


Figure 1. Inhibition of 3D growth of Luminal B and TNBC cell lines.



UofL Batch ID	Mutant Form	K-Ras GTP Kd (uM)	K-Ras GDP Kd (uM)	H-Ras GTP Kd (uM)	H-Ras GDP Kd (uM)	N-Ras GTP Kd (uM)
F3-1	WT		WT	52		
F3-1	K		52			
F3-1	H			14		
F3-1	H				13	
F3-1	N					107
MNR-7-33	K		0.07			
MNR-7-61	K		0.018			

Figure 3. Ras binding of the compounds.

a. Nuclear Magnetic Resonance (NMR) confirmed binding of the compound to RAS provokes a disruption in the the effector binding domain of KRas G12D. Apparent kd is low uM.

b. More recent variants developed through medicinal chemistry exhibit significantly higher affinity to wild type RAS in Microscale Thermophoresis assays.

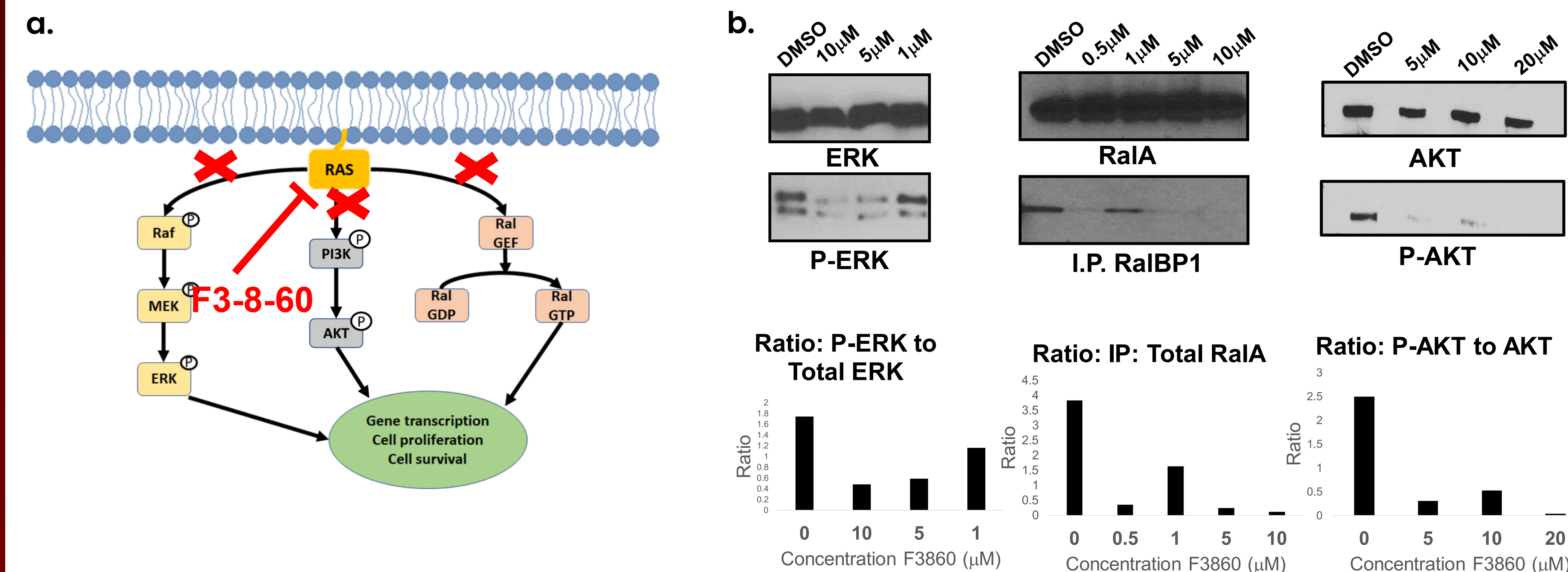


Figure 2. Inhibition of RAS signaling pathways.

a. RAS signals through distinct effector pathways.

b. F3860 inhibits MAPK (left) and RAS/RAL signaling (middle) in BT474 Luminal B cells, and AKT signaling (right) in MDA-MB-231 Triple Negative cells.

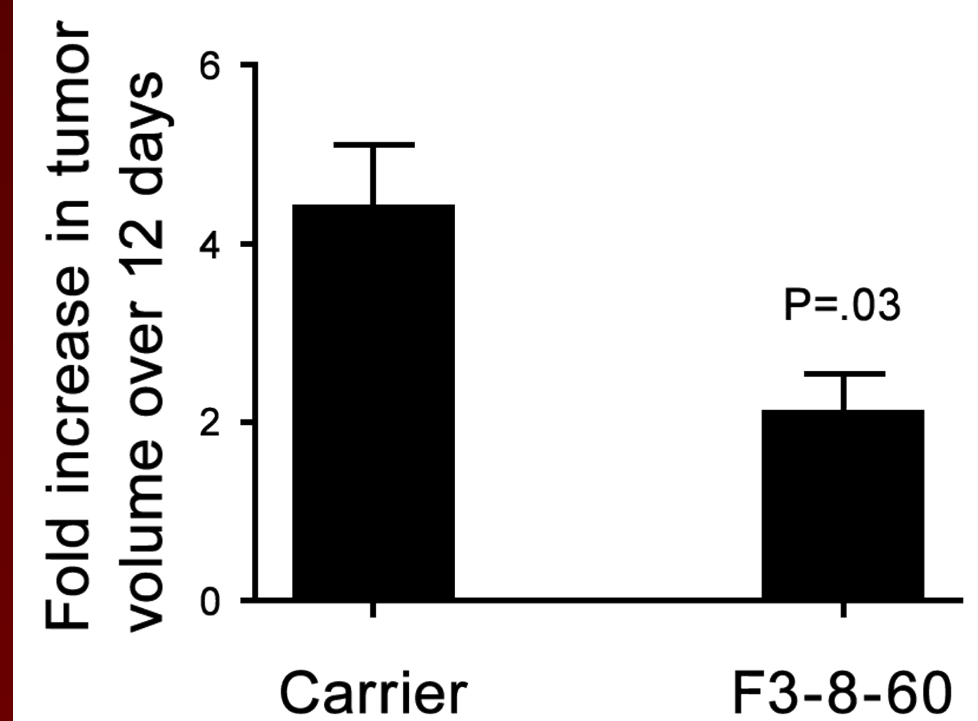


Figure 4. ***F3860*** **inhibits Luminal B tumor growth in vivo.**

NSG immunodeficient mice were orthotopically grafted with BT474 cells and treated over a 12-day period by IP injection. *F3860* effectively suppresses tumor growth rate. No apparent toxicities were seen with treatment.

Discussion

We have found that novel RAS inhibitor F3860 inhibits tumorigenic phenotypes in vitro and in vivo in model systems of Luminal B and Triple Negative breast cancers. It also specifically suppresses key RAS molecular signaling activities at nanomolar levels in Luminal B cells, and at micromolar levels in TNBC cells. Medicinal Chemistry to generate enhanced binding activity has been successful. The assay of these agents is ongoing.

Support: CDMRP BC220575, Qualigen Therapeutics, Inc.