Pan-RAS Inhibitors to Treat Luminal B Breast cancer

UNIVERSITY OF LOUISVILLE SCHOOL OF MEDICINE

Abstract No. B020

Raphael N. Jigo$, Howard Donninger*, Rachel Ferrill$, Becca Von Baby$, Joe Burlison*, John O. Trent*, Rob Monsen*, Michael Sabo*, Tariq Arshad#, Geoffrey J. Clark$

$Department of Pharmacology and Toxicology, *Department of Medicine, Brown Cancer Center. University of Louisville, Louisville, KY, #Qualigen Therapeutics, Inc., Carlsbad, CA.

qualigen THERAPEUTICS

Methodology

In-silico Drug Screening: Computer-based (in silico) drug design was used to identify small molecules with the potential to bind and inhibit RAS.

In vitro Bioassay to detect anti-tumor activity: Mouse (E0771) and human (BT-474) Luminal B breast cancer cell lines were used in 3D growth assays with inhibitor at concentrations of 10, 5, 1, 0.5 and 0.3 mM to test inhibition of tumor growth.

Signaling Assays: Cells were tested with ***F3-8-60*** for 1 hour, lysed and immunoblotted to determine effect on RAS effector signaling proteins. RAL Pulldown Assay was also performed to determine effect on RAS/RAL signaling.

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

Summary of Key Findings

RAS is seldom mutated in breast cancer, but it is often hyperactivated by upregulation of positive regulator activity (such as Her-2) or down-regulation of negative regulator activity (such as NF1 or DAB2IP). These effects are particularly common in Luminal B breast cancer.

We have developed a series of novel direct pan-RAS inhibitors that exhibit a distinct binding mechanism to other currently described RAS inhibitors.

Our RAS inhibitors both suppress the interaction of RAS with its downstream mitogenic effectors and suppress RAS signaling pathways (MAPK and RAL pathways) in Luminal B breast cell model systems. Cells driven by mutant B-RAF are insensitive.

They inhibit 3D growth at doses that have little effect on normal 2D growth. They are active against *in vivo* xenograft breast tumors and can be oral available. Our latest agents are pan-RAS inhibitors and enhanced binding and in vivo activity against K-RAS.

Support: NIH 5U01HL127518-03 (GJC), Kentucky Lung Cancer Research Program (GJC), CDMRP: BC220575 (GJC), Qualigen Therapeutics, Inc. (Carlsbad).

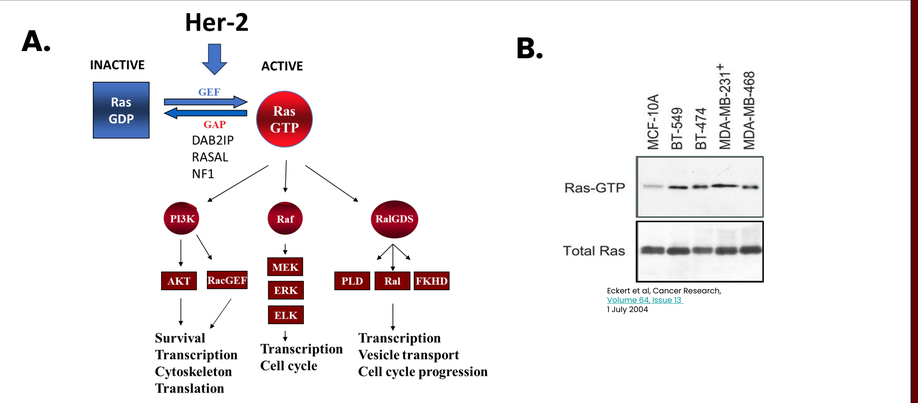
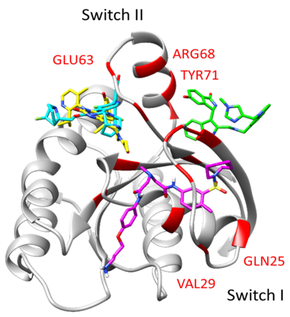


Figure 1. RAS is hyperactivated in many breast cancers due to defective regulation.

A. Schematic of RAS oncoprotein signaling. RAS shuttles between an active (GTP bound) form and an inactive (GDP bound) form under the influences of GEFs (Guanine Nucleoside Exchange factors) and GAPS (GTPase activating proteins). Her-2 promotes activation of RAS via the assembly of a GEF complex at the plasma membrane. Loss of GAPs (NF1, DAB2IP, RASAL) also promotes the activation of RAS.
B. Many breast cancer cell lines exhibit upregulated levels of activated RAS (GTP). MCF-10A is a non-tumorigenic breast cell line.

Binding of RAS inhibitor compounds to K-RAS



F3-8-60 blocks the association of RAS with its effector RAF in Luminal B breast cells

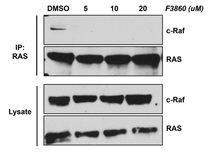
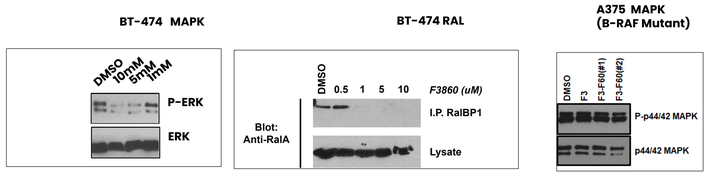


Figure 2. We have identified a series of direct RAS inhibitors that block RAS function by preventing RAS from associating with its effectors.

TOP: RAS (G12D) overlaid with F3-8-60 (magenta), MRTX-1133 (cyan), BI-2852 (green), and AMG510 (yellow). Residues colored red show significant shifts by NMR when titrated with our RAS inhibitor.

Bottom: Endogenous RAF co-immunoprecipitated with RAS in Luminal B cells (BT-474) in the presence or absence of anti-RAS drug F3-8-60.

Signaling



Growth in Soft Agar

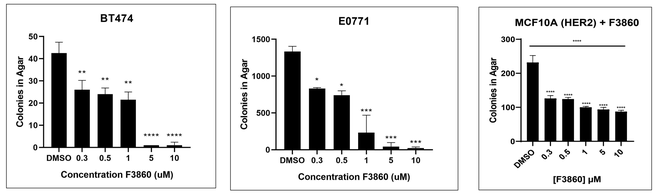
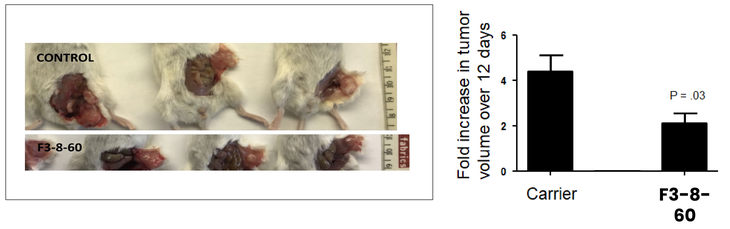


Figure 3. F3-8-60 anti-RAS suppresses signaling and 3D growth in Luminal B systems.

TOP panels: Treatment of BT474 cells for 1 hour with different concentrations of F3-8-60 suppresses basal levels of phospho ERK (MAPK pathway) and RAL GTP (RALGDS pathway). A375 cells are a Melanoma cell line driven by a mutant B-RAF and as such do not exhibit MAPK suppression.

Bottom: Luminal B cell lines BT474 and E0771 show suppression of tumor colony formation in 3D soft agar assays at sub-uM levels of F3-8-60. MCF10A cells transfected with mutant Her2 also show loss of colony formation activity.

F3-8-60 suppresses Luminal B tumor development Breast tumors in mice (10 mg/kg 5 days on/2 days off)



New inhibitor variants exhibit enhanced RAS binding activity and enhanced anti-tumor effects in vivo

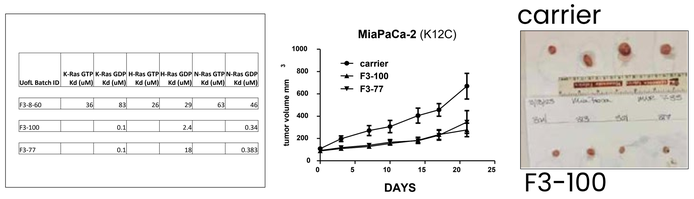


Figure 4. F3-8-60 anti-RAS suppresses orthotopic Luminal B tumor growth and we have now developed enhanced activity variants.

TOP: F3-8-60 treatment of BT-474 Luminal B breast tumor cells engrafted into the mammary fat pad shows a statistically significant decrease in average tumor size.

Bottom panels: Binding affinities F3-8-60 to RAS proteins and newer variants with their in vivo activity against a mutant RAS driven pancreatic tumor cell line (30mg/kg ip).