A Novel RAS inhibitor for Pancreatic Cancer

Howard Donninger[1,3], Rachel Ferril[2], Becca vonBaby[2], Joe Burlison[1,3], Mike Sabo[1,3], Tariq Arshad[4], John Trent[1,3] and Geoffrey J. Clark[1,2]

[1]Molecular Targets Group, James Graham Brown Cancer Center, Departments of [2]Pharmacology & Toxicology and [3]Medicine, School of Medicine, University of Louisville, Louisville, KY, USA, [4]Qualigen Therapeutics, San Diego, CA, USA

Introduction

Pancreatic cancer has a dismal survival rate and no good therapeutic options. Of all the types of cancer, it is Pancreatic cancer that is most closely associated with activating mutations in the RAS oncoprotein. Approximately 90% of pancreatic adenocarcinomas carry point mutations activating K-RAS. Ras directed targeted therapy is the obvious approach to enhancing treatment options for the disease. There is now an FDA approved targeted RAS therapeutic specific to the KRAS12C mutant form. However, this specific mutation is uncommon in Pancreatic cancer. Therefore, drugs which act more broadly on RAS are required.

We have used an in silico screening approach to identify compounds from a large drug-like library that had the potential to bind to a groove in the surface of RAS that is present when RAS is in the correct conformation to bind to its effector RALGDS. We then used a semi-high throughput 3D growth inhibition assay on multiple pancreatic cancer cell lines to identify potential "hits". The selected compounds were counter-screened for the 2D growth inhibition and those that preferentially suppressed growth in 3D were considered the most likely RAS specific candidates. This is because RALGDS inactivation impairs 3D but not 2D growth.

Iterative Medicinal Chemistry followed by 3D/2D growth assays have resulted in the identification of enhanced activity variants of the original compound. The F3-8-60 variant suppresses the RAS/RAF interaction and inhibits all three main RAS signaling pathways (RAF/MAPK, PI3K/AKT, RALGDS/RAL) in transient experiments. Comparisons of RAS vs mutant B-RAF driven cells shows the agent is selective for RAS.

NMR analysis of one of the agents in complex with K-RAS12D has now been performed. The results confirm direct target binding and support the model of drug binding in the proximity of the effector loop and changing the structure of the core effector binding domain.

Xenograft studies have shown the agent is active in vivo against multiple models of human pancreatic cancer, including a pdx tumor. Some variants are oral available. Measuring BUN and AST levels in the treated animals after the last drug injection showed no significant toxic effect on Liver or kidney function.

One of the actions of mutant RAS is to promote an immunosuppressive tumor microenvironment, which may contribute to the poor performance of checkpoint inhibitors in pancreatic cancer. We find that the agent appears to enhance the effects of an anti- PD-L1 immune checkpoint inhibitor on the growth of a RAS driven syngeneic pancreatic cancer cell line (KPC2).

As the agent appears to bind to a separate domain of RAS to that of AMG510 or MRTX1133, we wondered if it might cooperate with these approved or in trials anti-RAS agents. It appears that this may be the case.

Development of the inhibitor continues and recent derivatives exhibit sub-uM affinity binding activity towards G12D and G12C mutant form of K-RAS.

Funding: NIH 5U01HL127518-03, Kentucky Lung Cancer Research Program (GJC), Qualigen Therapeutics, Inc.

Results

Development of a RAS inhibitor

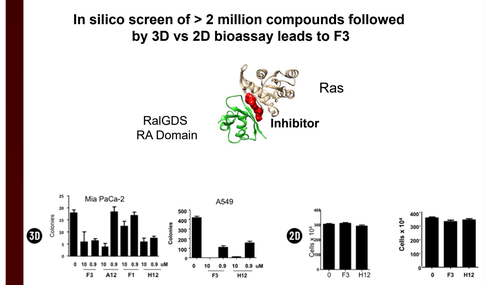


Figure 1. Identification of a RAS inhibitor

Using the known crystal structure of RAS in complex with its effector RALGDS, we used in silico screening of a drug-like compound library to predict compounds which might bind to RAS and block its effector interactions. In silico positives were then screened in vitro in 3D soft agar culture assays against RAS driven tumor cell lines to identify "hits". Positive compounds were counter screened against cells grown in 2D. Compounds that were positive in 3D but negative in 2D were selected. Compound F3 was considered the best.

F3-8-60 inhibits the RAS/RAF interaction and blocks RAS Signaling

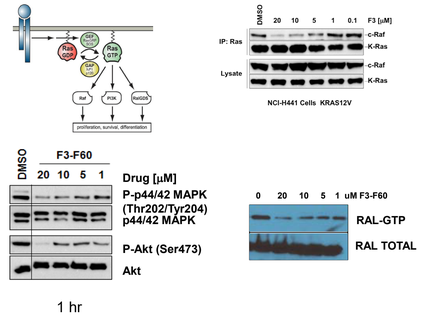


Figure 2. F3 derivative F3-8-60 suppresses the RAS/RAF interaction and preferentially inhibits RAS driven tumor cells:

Top: NCI-H441 cells (K12V) were treated with drug overnight and then immunoprecipitated for RAS with a pan-RAS antibody. The Complex was then probed for c-RAF. A dose dependent inhibition of complex formation was observed. **Lower Left:** Panc1 pancreatic cancer cells (KRAS12D) were treated with F3-8-60 for 1 hour, lysed and subjected to Western analysis to measure levels of active MAPK pathway (phospho ERK) and PI3K pathway (Phospho AKT) using total ERK and AKT protein levels as controls. Both pathways are inhibited. **Lower Right:** The RALGDS/RAL pathway was measured using a RAL-GTP pull down kit. RAL activation was inhibited.

F3-8-60 Blocks RAS but not RAF Signaling and transformation

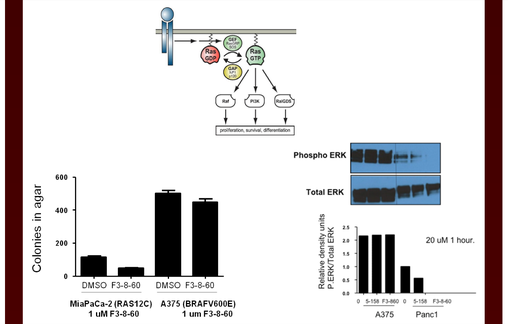


Figure 3. F3-8-60 suppresses RAS mitogenic signaling pathways in Panc1 cells (KRAS12D).

Comparisons of the effect of the agent on mutant B-RAF driven tumor cells relative to mutant RAS were performed. The agent preferential inhibited Ras driven 3D growth and RAS driven MAPK signaling. **Left panel:** Soft agar assays of Panc1 (RAS12D) and A375 (BRaf V600E) showed the RAS cells were more sensitive. **Right:** Phospo ERK analyses showed the agent blocked RAS but not BRAF driven MAPK signaling.

NMR demonstrates F3-8-60 Binds K-RAS 12D and induces changes in effector loop

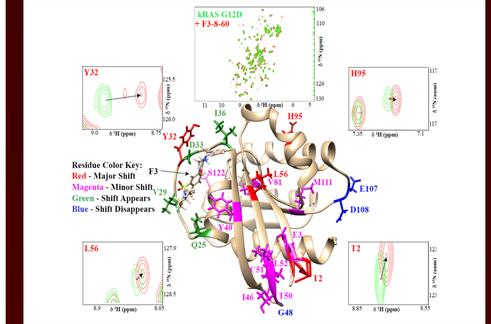


Figure 4. NMR studies demonstrate F3-8-60 binds K-RaS12D and promotes changes in effector domain structure.

The agent was designed to bind to a groove close to the effector loop. NMR analysis of KRAS12D in complex with F3-8-60 was performed. A major shift in RAS residue Y32 as the start of the core effector domain was observed. Peaks for effector loop residues D33 and I36 appeared, indicating that these normally flexible positions have been stabilized. An apparent kd of ~ 1uM was calculated.

F3-8-60 is active in vivo against pancreatic cancer xenograft models.

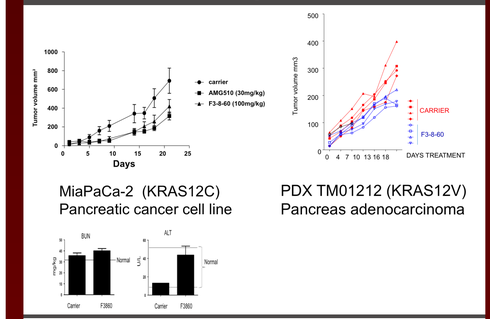


Figure 5. F3-8-60 is active in vivo against pancreatic Cancer Models.

Left: MiaPaca-2 pancreatic cancer cells (KRAS12C) were inoculated into the flanks of NSG mice. When tumors reached 50-100 mm3 they were randomly assigned to an experimental group. N = at least 6. AMG-510 (covalent G12C inhibitor) was used as a comparator. Drugs were administered orally. Rate of tumor growth was reduced. Indicators of Liver and Kidney function remained within normal parameters (Lower panel). **Right:** A Pancreatic adenocarcinoma pdx carrying a K-RAS12V mutation was used in similar experiments. Again, tumor growth was reduced.

F3- compounds co-operate with Checkpoint Inhibitors and other RAS inhibitors

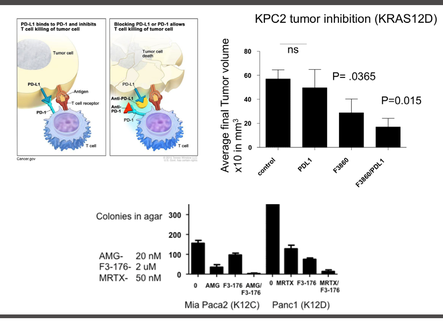
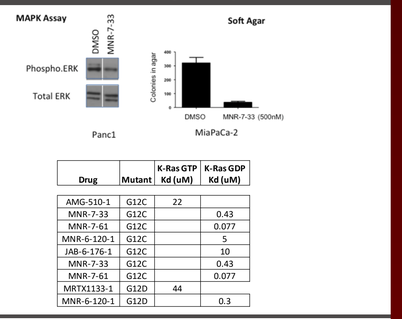


Figure 6. F3-8-60 enhances the effect of Immune Checkpoint inhibitors and established RAS inhibitors.

Top Left: cartoon of principle of immune checkpoint therapy. Antibodies that block the PD-1/PD-L1 interaction prevent the tumor from suppressing T-cell function. **Top Right:** KPC2 cells, derived from a pancreatic tumor of a KRAS12D/p53 mutant transgenic mouse were implanted sub-cutaneously into C57BL6 immune competent mice in a syngeneic xenograft. 72 hours after the cells were injected animals were treated with F3-8-60 and injected with anti-PD-L1 antibody. Antibody was injected once per week. When the control tumors began to approach end point size the experiment was terminated and average tumor volume calculated. The tumors treated with both agents showed the least growth. **Bottom:** Soft agar assays were performed with an F3 variant and AMG510 (K12C specific) and MRTX1133 (K12D specific) alone and in combination. Greater than additive suppression of tumor cell colony growth was observed.

Latest iterations exhibit enhanced KRAS Binding activity



Drug	Mutant	K-Ras GTP Kd (uM)	K-Ras GDP Kd (uM)
AMG-510-1	G12C	22	
MNR-7-33	G12C		0.43
MNR-7-61	G12C		0.077
MNR-6-120-1	G12C		5
JAB-6-176-1	G12C		10
MNR-7-33	G12C		0.43
MNR-7-61	G12C		0.077
MRTX1133-1	G12D	44	
MNR-6-120-1	G12D		0.3

Figure 7. F3 derivative F3-8-60 exhibits enhanced biological activity and directly binds KRAS.

We have used Medicinal Chemistry to develop enhanced activity derivatives of F3. We have now generated almost 300 variants of the parental compound using an iterative process of SAR exploration by bio-screening. Latest variants exhibit sub-uM binding affinities to KRAS.

CONCLUSIONS

1. **We have identified a series of direct novel RAS inhibitors, which may have a unique mode of interaction.**
2. **They are active in vivo against RAS driven tumor systems, including pancreatic pdx.**
3. **They co-operate with immune checkpoint inhibitors in vivo to suppress tumor growth.**
4. **They co-operate with RAS12C and RAS12D specific agents that are approved or in clinical trials.**

Discussion

Recent advances in anti-RAS therapy have given rise to great excitement as the prospect of effective targeted RAS therapy finally becomes a reality. Our latest variants exhibit sub-micromolar RAS binding characteristics and are in the process of in vivo examination. Due to the unstable genetic nature of most tumors, it seems likely that resistance to single agents will evolve as a serious problem. We have observed cooperation between our RAS inhibitor and immune checkpoint therapy. We have also observed cooperative effects with the KRAS12C specific agent AMG-510 and the KRAS12D specific agent MRTX1133. Therefore, combination therapy approaches may be the most effective approach going forward.