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. Further studies confirmed the agent designated F3 could inhibit the formation of a complex between RAS and its effector RAF. Moreover, it showed selectivity for the inhibition of RAS driven tumor cell systems.

Iterative Medicinal Chemistry followed by 3D/2D growth assays have resulted in the identification of enhanced activity variants of the original compound. Using orthologous assays, we have shown they directly bind mutant K-RAS. The newest variants exhibit sub-micromolar Kds for certain RAS mutant forms. The agents inhibit all three main RAS signaling pathways (RAF/MAPK, PI3K/AKT, RALGDS/RAL) in transient experiments.

NMR analysis of one of the agents in complex with K-RAS12D has now been performed. The results 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. 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 immune checkpoint inhibitor on the growth of a RAS driven syngeneic pancreatic cancer cell line.

Development of the inhibitor continues using iterative Medicinal Chemistry and 3D growth inhibition. Newer derivatives have been identified with enhanced binding activity towards the K-RAS12D isoform.

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

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. Therefore, combination therapy approaches may be the most effective approach going forward.

Results

Development of a RAS inhibitor

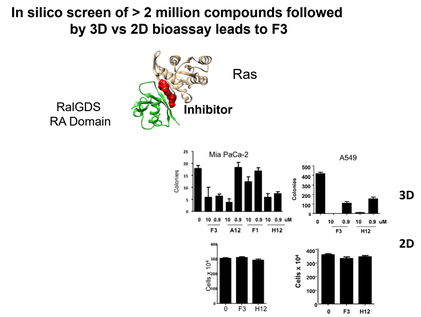

Figure 1. Identification of a RAS inhibitor

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 anti-RAS blocks the RAS/RAF interaction and preferentially inhibits RAS transformation

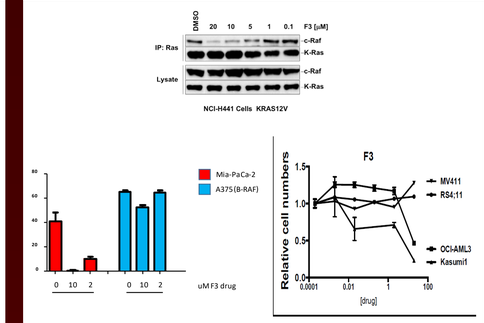

Figure 2. Compound F3 suppresses the RAS/RAF interaction and preferentially inhibits RAS driven tumor cells:

Top: KRAS12V containing NCI-H441 cells 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.
Bottom: F3 sensitivity of mutant RAS cells (MiaPaca-2) was compared to mutant B-RAF (A375) cells (Left). RAS driven AML cell lines were compared to MLL fusion driven AML cell lines. (bottom right). In each, a RAS preference was observed.

Compound F3-8-60 is an enhanced activity derivative that binds K-RAS

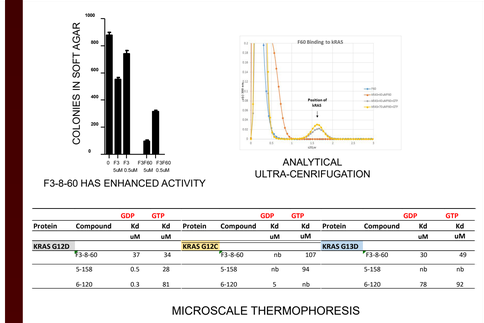

Figure 3. F3 derivative F3-8-60 exhibits enhanced biological activity and directly binds KRAS.

We have used Medicinal Chemistry to develop enhanced activity derivatives of F3. Compound F3-8-60 is more active against Panc1 cells (KRAS12D) in soft agar assays.
Top Right: Analytical Ultracentrifugation shows direct binding to KRAS12V recombinant protein.
Bottom panel: Microscale Thermophoresis has been used to measure Kd values for F3-8-60 and multiple newer derivatives against common K-RAS mutants.

F3-8-60 Blocks RAS Signaling

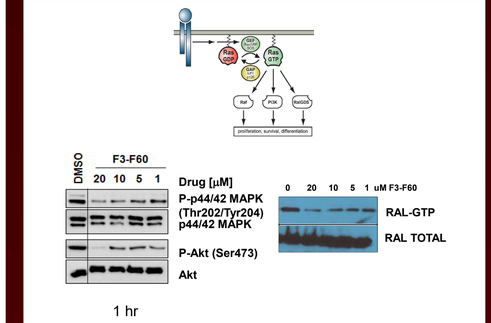

Figure 4. F3-8-60 suppresses RAS mitogenic signaling pathways in Panc1 cells (KRAS12D).

Left Lower: 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.
Right: The RALGDS/RAL pathway was measured using a RAL-GTP pull down kit. RAL activation was inhibited.

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

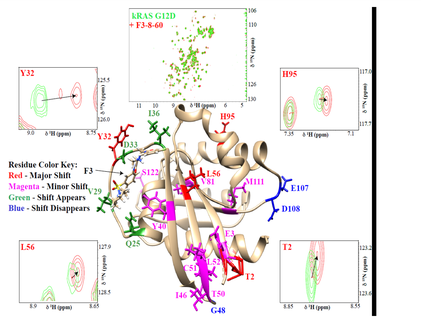

Figure 5. NMR studies demonstrate F3-8-60 binds K-RaS12D and promotes changes in effector domain structure.

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 D33 and I36 appeared, indicating that these normally flexible positions have been stabilized.

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

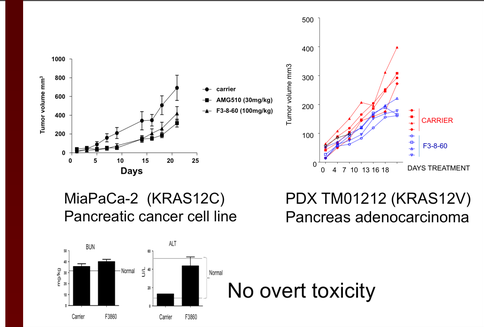

Figure 6. F3-8-60 is active in vivo against pancreatic Cancer Models.

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.
Right: A Pancreatic adenocarcinoma pdx carrying a K-RAS12V mutation was used in similar experiments. Again, tumor growth was reduced.

F3-8-60 enhances the activity of an Immune Checkpoint inhibitor

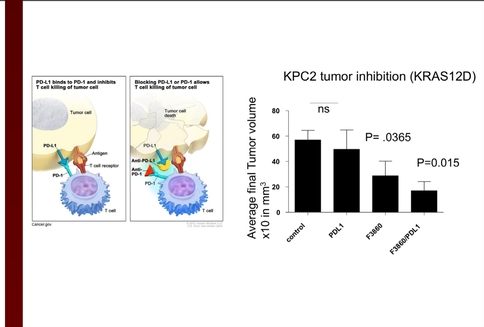

Figure 7. F3-8-60 enhances the effect of Immune Checkpoint inhibitors.

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.
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.

CONCLUSIONS

1. **We have identified a series of direct Novel RAS inhibitors.**
2. **Variant F3-860 is active in vivo against pancreatic tumor cell lines and primary tumors.**
3. **It exhibits no overt toxicity.**
4. **It enhances the activity of Immune Checkpoint Inhibitors against pancreatic cancer.**