Novel RAS inhibitors for NF1 disease

Howard Donninger [1,3], Rachel Ferril,[2] Destine Ede[2], Desmond R. Harrell-Stewart[1,2], Joe Burlison [1,3], John Trent PhD[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

Introduction

Neurofibromatosis type 1 is a genetic disease that results from either heritable or spontaneous autosomal dominant mutations in the NF1 gene. Neurofibromatosis type 1 individuals frequently suffer benign tumors known as Plexiform Neurofibromas which develop from cranial and peripheral nerve sheaths. Plexiform Neurofibromas have the potential to develop into malignant peripheral nerve sheath tumor (MPNST). MPNST exhibit a low overall 5 year survival rate of less then 40%, and there is no effective treatment or cure.

The NF1 gene encodes the protein Neurofibromin. Neurofibromin is a negative regulator (a GAP) for the notorious RAS oncoprotein. Although RAS is frequently activated by mutation in many cancers, this is not the case with NF1 disease. Here, the wild type RAS protein is stabilized in the active configuration due to the loss of NF1 function (Figure 1). This is a transforming event that drives the disease. Currently, there are no targeted inhibitors of wild type RAS that are effective in the clinic.

In an attempt to combat the problem of a lack of a therapeutic treatment for Neurofibromatosis Type 1, and indeed, RAS driven cancer in general, we have performed *in silico* screening of two million compounds followed by bioassay to identify a small molecule, referred to as F3, that binds and inhibits active RAS by blocking its ability to interact with its effectors. We have subsequently used a medicinal chemistry approach to identify more effective derivatives of F3. Our current lead is designated F3-8-60, which exhibits enhanced anti-RAS biological activity in vitro and enhanced RAS binding.

In vivo, F3-8-60 inhibits the metastasis of an MPNST cell line and suppresses the growth of MPNST pdx. We observe no toxicity associated with the drug. We are currently working towards studies to allow an IND filing. We propose this approach may lead to novel therapeutics for NF1 disease.

Results

Development of a RAS inhibitor for NF1 disease

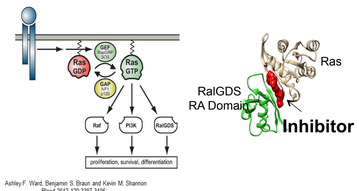


Figure 1. Development of an inhibitory strategy for NF1 disease.
LEFT: Ras activity is controlled by positive and negative regulators. NF1 is negative regulator, so inactivation of NF1 by mutation removes a "brake" on RAS resulting in an elevation of RAS activity.
RIGHT: using the known crystal structure of RAS in complex with its effector RALGDS, we used *in silico* screening to predict compounds which might bind to RAS and block its effector interactions. In silico positives were then screened in vitro in 3D culture assays to identify "hits".

F3 inhibits MPNST soft agar growth

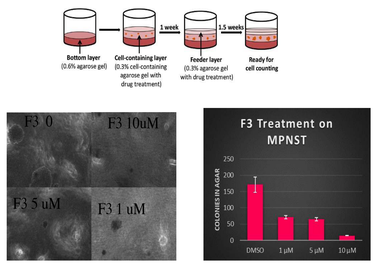


Figure 2. Anti-RAS compound F3 potently suppresses 3D growth of MPNST cells.
MPNST cells (S462.TY) were plated in soft agar in the presence or absence of test drug. Colony formation was scored after 2 weeks. Compound F3 scored as one of the most positive. An IC50 of ~ 1uM could be obtained.

F3 compounds block the RAS/RAF interaction

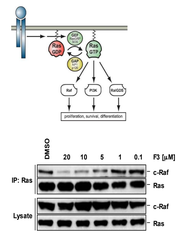
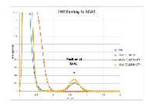


Figure 3. Inhibition of RAS effector interactions and signaling by compound F3 and F3 derivatives.
LEFT: Endogenous, mutant K-RAS was immunoprecipitated (IP) from MiaPaCa-2 cells in the presence or absence of various concentrations of F3. The complexes were immunoblotted (IB) for RAS and c-RAF to determine the effects on RAS/RAF binding.

Compound F3-8-60 exhibits enhanced RAS binding



Analytical Ultracentrifugation

F3
KRAS GTPγ Kd 17uM
KRAS GDP Kd 23uM

F3-8-60
KRAS GTPγ Kd ~ 2-15uM
KRAS GDP Kd ~ 29-116uM

Microscale thermophoresis
KRAS His-tagged protein from Abcam (ab156968) with the Nanotemper His tag dye.

Figure 4. Compound F3 and enhanced activity derivative F3-8-60 directly bind to K-RAS.
Activated K-RAS protein was prepared and and used in direct compound binding assays.
LEFT: Analytical Ultracentrifugation (AUC) was used to confirm co-sedimentation of F3-8-60 with GTP-loaded mutant K-RAS protein.
RIGHT: Mircoscale Thermophoresis was performed in order to obtain kd values for F3 and F3-8-60.

F3 derivatives block RAS signaling in MPNST cells

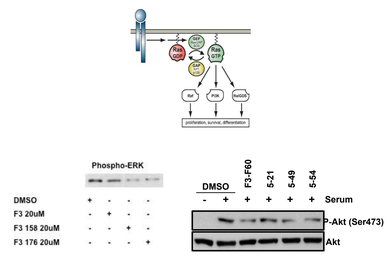


Figure 5. F3 class compounds block RAS signaling.
Top panel: cartoon of RAS signaling pathways.
Left Panel: F3 and early stage derivatives inhibit the levels of phospho-ERK- a measure of MAPK pathway activation in MPNST cells (S46.2TY).
Right Panel: F3-8-60 and several later derivatives suppress the PI-3 kinase pathway in serum stimulated S46.2TY MPNST cells.

F3-8-60 suppresses Metastasis of MPNST cell line (STS26.T)

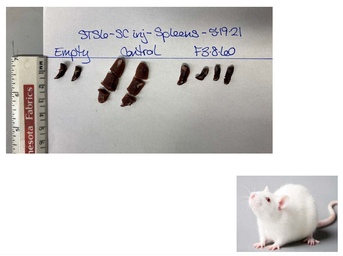


Figure 6. F3-8-60 Suppresses MPNST metastasis.
STS26.T cells (MPNST) were injected into the flank of NRG mice. Surface tumors were not observed, but potent metastasis to the Liver and spleen were apparent. Animals treated with 1mg/kg F3-8-60 three times a week by ip injection exhibited normal sized spleens. Control treated mice did not lose weight and liver /kidney enzymes were normal

F3-8-60 anti-RAS suppresses MPNST pdx

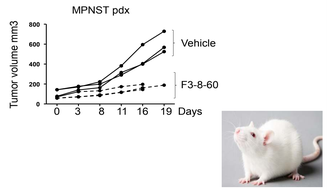


Figure 7. F3-8-60 suppresses MPNST pdx growth To date, the best model for human tumor drug response is the Primary tumor graft system. Here, an MPNST primary tumor (gracious gift, Johns Hopkins NF1 Biospecimen repository) was grafted into a cohort of NSG mice. Graftees were randomized into control and experimental pools. When a tumor graft reached 50-100 mm3, it was treated with carrier or F3-8-60 at 1mg/kg three times per week by ip injection for three weeks.

CONCLUSIONS

1. **We have identified a series of direct Novel RAS inhibitors.**
2. **The current lead compound is active in vivo against MPNST tumor systems, including primary tumor grafts.**
3. **They exhibit no apparent in vivo toxicity.**
4. **These agents may be developed into novel targeted therapy for NF1 disease.**

Discussion

NF1 disease is largely caused by deregulation of RAS due to loss of function of NF1. We have been working on developing a small molecule that binds the wild type form of RAS and blocks its ability to interact with its effectors. Our current lead, designated F3-8-60, binds to K-RAS in the active conformation with a kd of between 2-15 uM. It suppresses RAS signaling in MPNST cell lines and is active in vitro and in vivo. Moreover, it is active against MPNST pdx, suggesting there is real clinical potential in these compounds. We are continuing to optimize the drug via Medicinal chemistry and iterative screening. ADME/PK studies are ongoing. As at least some of the cognitive issues associated with NF1 patients also appear to be due to aberrant RAS activity, and as our compounds can pass the blood brain barrier, we also hypothesize they may have utility in treating neurological defects caused by excess RAS activity.

Funding: NIH 1P20 RR18733 (GJC), KLCRP (GJC), NIH 1U01HL127518-01 (GJC), NIH R25-134283 (DE), CDMRP NF 180094 (GJC), Qualigen Therapeutics Inc. (San Diego CA) (GJC).