

Development of Inhibitors of the Fibroblast Growth Factor Receptor (FGFR) Kinase Using a Fragment Based Approach.

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INTRODUCTION

- Data in a number of tumour types has implicated Fibroblast Growth Factor (FGF) and Fibroblast Growth Factor receptor (FGFR) signalling as being key to the molecular pathology of cancer.
- In particular the t(4;14) gene translocation in 15% of Multiple Myeloma patients leads to the ectopic expression of FGFR3.
- Multiple lead series of FGFR inhibitors were developed using Astex's fragment based medicinal chemistry approach, Pyramid™, linked to high throughput X-ray Crystallography.
- We describe here the identification and characterisation of these lead molecules.
- Compounds derived from these lead series exhibit potent FGFR inhibitory activity in *in vitro* systems dependent upon FGFR signalling for survival. We also demonstrate *in vivo* activity in several of these cell based systems consistent with an FGFR inhibitory effect.

Pyramid™ Screen

- Full Pyramid™ screen performed vs FGFR1
- >30 X-Ray hits obtained from Pyramid™ screen
- >10 distinct chemotypes discovered and considered in Hit Validation
- Several series chosen at end of hit validation and progressed into Hits-to-Leads (H2L)

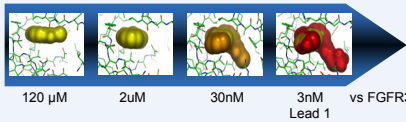


Figure 1: Example of Fragment Evolution

Progress in Hits-to-Leads

- Several series exhibited highly potent FGFR inhibition
- Three series showed 10-100 fold selectivity for FGFR3 over VEGFR2
- Metabolite id used to focus chemistry in removal of PK liability in key series
- Lead 1 identified from Series 1 and further profiling performed.



Series	FGFR3 (IC ₅₀ nM)	VEGFR2 (IC ₅₀ nM)	LP-1 (IC ₅₀ nM)
Series 1	3	110	4.3
Series 2	14	1100	110
Series 3	3.2	160	38

Table 1: Series Status at End of Hits to Leads

- Example compounds from each of 3 series were profiled for their *in vitro* activity against FGFR3 and VEGFR2 in a biochemical assay.

- In addition a FGF-induced pERK ELISA in LP-1 cells, a human, t(4;14) positive multiple myeloma line was performed

RESULTS

Enzyme	Lead 1 (μM)
FGFR3	0.0033
FGFR1	0.013
FGFR2	0.034
FGFR4	0.033
VEGFR1	0.068
VEGFR2	0.11
VEGFR3	58% @ 0.03
FK3	63% @ 0.1
PDGFRβ	0.29
EGFR	>10
>34 kinases	>1

Table 2: *In vitro* selectivity profile of Lead 1

- Lead 1 identified as a potent and selective inhibitor of FGFR1-4
- Compound exhibits reduced activity vs VEGFR2, Flt-3, PDGFRB and EGFR

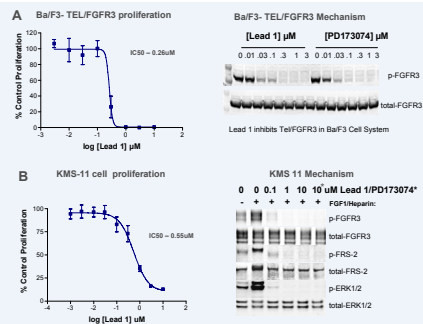


Figure 2: Mechanism of Action of Lead 1 in LP-1 Multiple Myeloma and FGFR3 Ba/F3 Cell Lines

- Ba/F3 cells were engineered to stably express the TEL/FGFR3 fusion protein (A)
- Ba/F3-TEL/FGFR3 (A) or the t(4;14) positive Multiple Myeloma cell line KMS-11 (B) were exposed to Lead 1 for 72h. Cell viability was determined using an Alamar Blue™ assay.

- Antiproliferative IC₅₀s of 0.26 and 0.55 μM respectively were obtained.
- The mechanism of action of these effects were investigated by western blotting.

- Cells were exposed to Lead 1 or the FGFR inhibitor PD173074, as a positive control, for 1h in serum free media.

- FGFR3 autophosphorylation and downstream signalling was inhibited in both cell lines at doses consistent with the antiproliferative activity of Lead 1.

RESULTS

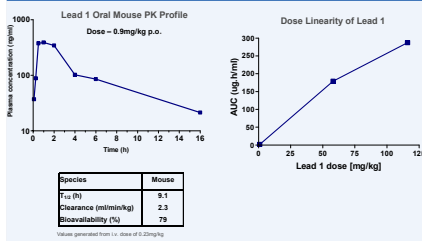


Figure 3: Pharmacokinetic profile of Lead 1 in the mouse

- For oral administration to mice Lead 1 was dosed as a solution in 5:4:1 Water:PEG400:DMA. Plasma and tumour concentrations were then determined by LC-MS/MS.

- Lead 1 has low plasma clearance and a long half life.

- The compound was orally bioavailable in the mouse and exhibited excellent dose linearity with the ability to achieve high exposures.

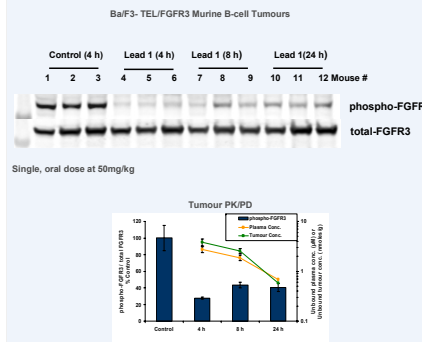
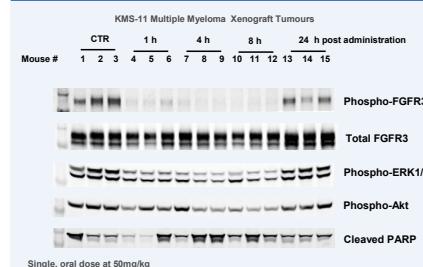


Figure 4: Modulation of Pharmacodynamic Biomarkers in Ba/F3-TEL/FGFR3 Xenograft tumours in nude mice

RESULTS



Single, oral dose at 50mg/kg

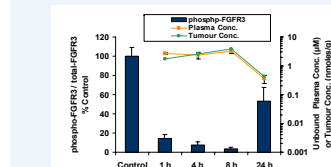


Figure 5: Modulation of Pharmacodynamic Biomarkers in KMS11 Xenograft tumours in NOD/SCID mice

- Ba/F3 TEL-FGFR3 (Fig 4) or KMS-11 (t(4;14) +ve Multiple Myeloma) (Fig 5) tumour-bearing mice were administered a single oral dose of Lead 1 at 50 mg/kg. Plasma samples and tumours were removed at various times following dosing and free levels of Lead 1 determined (based on measured plasma protein binding of 90%). Remaining tumour samples had western blots performed for pFGFR3 and/or downstream markers.

- pFGFR3 is rapidly inhibited following dosing in both models. The signal is beginning to return by 24h.

- In KMS11 tumours similar inhibitory effects were observed downstream in the MAPK and PKB pathways. Apoptosis is also induced within the tumour as indicated by the appearance of cleaved PARP.

- These tumour markers are inhibited in a way consistent with the levels of compound observed within plasma and tumour samples which remain >3 x cell-based proliferation IC₅₀ up to 24h.

- These data support the effectiveness of once daily dosing in an efficacy model

RESULTS

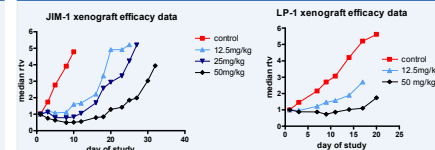


Figure 6A: Lead 1 is efficacious in t(4;14) +ve Multiple Myeloma tumour models

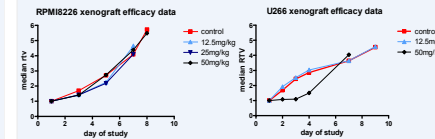


Figure 6B: Lead 1 is non-efficacious in t(4;14) -ve Multiple Myeloma tumour models

- In the studies shown Lead 1 was administered once daily, at the doses indicated, for 21 days or until the study was terminated due to tumour size.
- The volume of the tumour was calculated as an ellipsoidal volume every 2 days.
- Figure 6A shows that the compound exhibits a dose dependent inhibition of the growth of two FGFR3 positive multiple myeloma models, Jim-1 and LP-1.
- No inhibition was observed in t(4;14) negative myeloma models (Fig. 6B) when dosed in the same way

CONCLUSIONS

- A series of potent, selective, orally bioavailable FGFR inhibitors has been identified using Astex's fragment based chemistry approach.
- Examples from this series are active in cell lines that express FGFR3 and have a mechanism of action consistent with FGFR3 inhibition resulting in the antiproliferative effects observed.
- Compounds exhibited total inhibition of xenograft growth in instances where cell lines are dependent upon FGFR for proliferation but were inactive in backgrounds where aberrant FGFR signalling does not play a role.
- The pre-clinical data presented here describes a first in class series of compounds that warrant further evaluation and may offer benefit to patients whose disease is dependent upon aberrant FGFR signalling.

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