PRN1371, an irreversible, covalent inhibitor of FGFR1-4 exhibits sustained pathway inhibition in cancer cell lines

#2091

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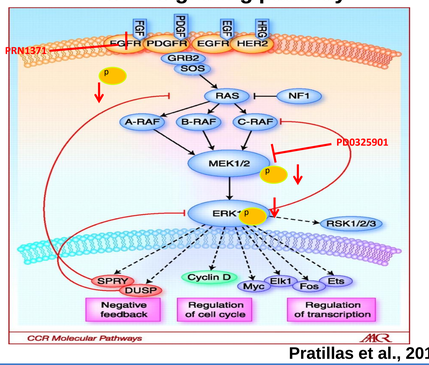
Introduction

Multiple human cancers harbor alterations in FGFRs that drive tumor growth, including mutations, translocations and amplifications. PRN1371 is an irreversible, covalent, FGFR1-4 inhibitor that exhibits highly selective and sustained inhibition of FGFR which extends well beyond circulating drug concentrations. As PRN1371 exhibits a covalent mechanism of action, the duration of inhibition of the FGFR signaling pathway is dependent on protein turnover of FGFR. Different FGFR alterations may exhibit different rates of protein turnover. This may impact dosing regiment of PRN1371 in the clinic. Thus, we set out to investigate duration of pathway inhibition across cancer cell lines of various lineages harboring different FGFR alterations.

FGFR signaling pathway



Pratilla et al., 2010

Materials and Methods

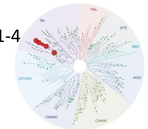
- Three cancer cell lines harboring different FGFR alterations were assessed for pERK EC_{50} of PRN1371 and PD0325901(Pfizer) using alpha-screen assay.
- Cancer cell lines were treated with increasing concentrations of PRN1371 *in vitro* for 1 hour, before compound was washed out.
- Doses for treatment period of the washout experiments were selected based on pERK EC50 and included 10x, 100x and 1,000x EC_{50} pERK (saturating dose).
- Cells were maintained media containing 10% FBS. For washout experiments, RT4 cells were incubated overnight in low-serum conditions (media containing 1% FBS). Cells were stimulated with bFGF at 50 ng/ml in final 20 min of either 1 hr treatment or washout period before protein lysates were harvested and pERK probed using western blot analysis.

Results

PRN1371 is highly potent and selective FGFR inhibitor

Assay	PRN1371
Biochemical IC50 (nM)	
FGFR1	< 1
FGFR2	1
FGFR3	4
FGFR4	20
VEGFR	>500
250 kinase panel (# of kinases)	
90% inh @ 1 μM	5
50% inh @ 1 μM	14

FGFR1-4



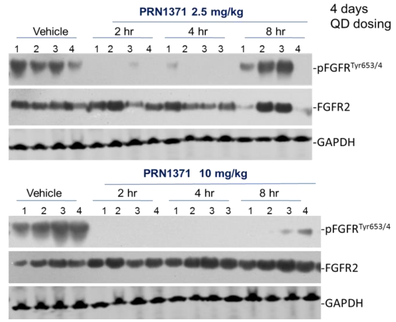
Kinase selectivity profile of PRN1371 screened against 250 kinases. Dots represent individual kinase with > 90% inhibition at 1 μM.

PRN1371 maintains high potency against FGFR alterations

Assay	PRN1371	BGJ398	AZD4547
Transfected Ba/F3 IC50 (nM)			
FGFR2 WT	< 1	7.5	11
FGFR2 (K660E)	1	14	9.8
FGFR2 (K660N)	< 1	6.9	11
FGFR2 (N550K)	4	87	121
FGFR3 WT	2	7.4	37
FGFR3 (K650M)	3	45	133
Cell proliferation IC50 (nM)			
SNU16 (FGFR2 amp)	3	5	7
RT4 (FGFR3:TACC3)	4	184	230
RT112 (FGFR3:TACC3)	4	19	36
AN3CA (FGFR2 mut)	43.3	n/a	n/a
Hep3B (FGFR4; FGF19)	6	96	116
OPM2(FGFR3 transl)	14	n/a	n/a

Cellular activity was assessed in range of BaF/3 cells transfected with FGFR or cancer cell lines exhibiting FGFR alterations.

Sustained pFGFR inhibition in SNU16-tumor bearing mice despite rapid clearance



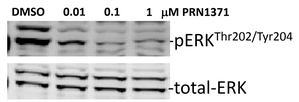
Time pt (hr)	Average plasma conc (ng/ml) Dose: 2.5 mg/kg	Average plasma conc (ng/ml) Dose: 10 mg/kg
2	8.1	68
4	BLQ	2.5
8	BLQ	BLQ
12	BLQ	BLQ

BLQ: Below the Limit of Quantitation

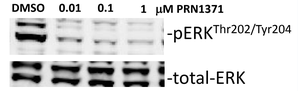
Comparable pERK EC_{50} observed across different cancer cell lines

FGFR alteration	Lineage	Cell line	PRN1371 pERK EC50 (nM)	PD0325901 pERK EC50 (nM)
FGFR3-TACC3	Urinary tract	RT4	0.64	0.41
FGFR1 CN 10	Lung	DMS114	0.91	0.70
FGFR2 K310R, N549K, N550K	Endometrial	AN3CA	0.76	0.70

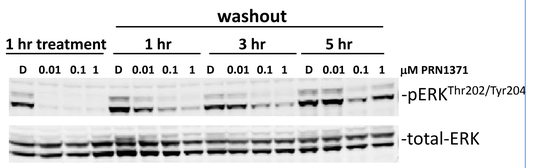
RT4



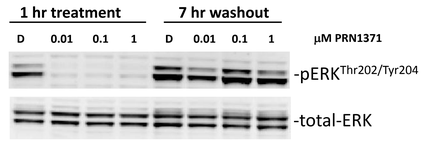
DMS114



pERK rebounds 5 hrs post washout in RT4 cells



Full rebound of pERK 7 hrs post washout in RT4 cells



D: DMSO

In vivo studies confirm that BID > QD for inhibiting tumor growth in xenograft models

Tumor	Doses of PRN1371	Tumor growth inhibition (TGI%)
SNU16	10 mg/kg QD	39%
	5 mg/kg BID	50%
	10 mg/kg BID	68%
RT4	10 mg/kg QD	29%
	2.5 mg/kg BID	39%
	5 mg/kg BID	47%

Conclusions

- PRN1371 is a potent, highly selective irreversible FGFR1-4 inhibitor exhibiting sustained inhibition of FGFR signaling.
- Sustained pFGFR inhibition observed in SNU16-tumor bearing mice despite rapid clearance of PRN1371.
- Time and concentration-dependent rebound of pERK detected in RT4 cells harboring FGFR3:TACC3 fusion.
- Preclinical washout data and *in vivo* xenograft data suggests that different dosing regimens of PRN1371 could be considered based on the protein turnover of different FGFR alterations.
- Phase I clinical trial of PRN1371 for the treatment of solid tumors is ongoing (NCT02608125).