

Selective Inhibition of FGFR4 by INCB062079 Is Efficacious in Models of FGF19- and FGFR4-Dependent Cancers

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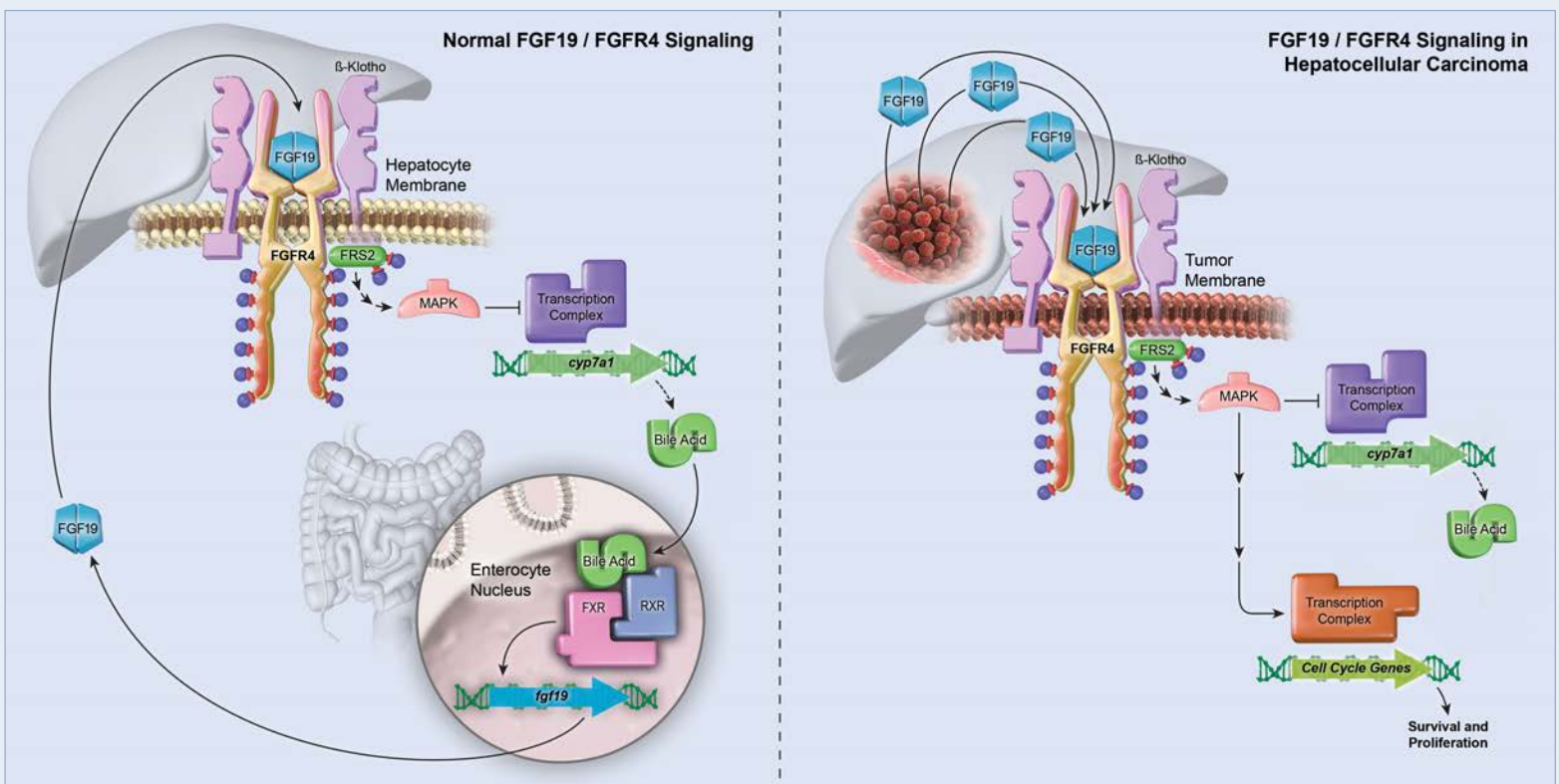


Abstract

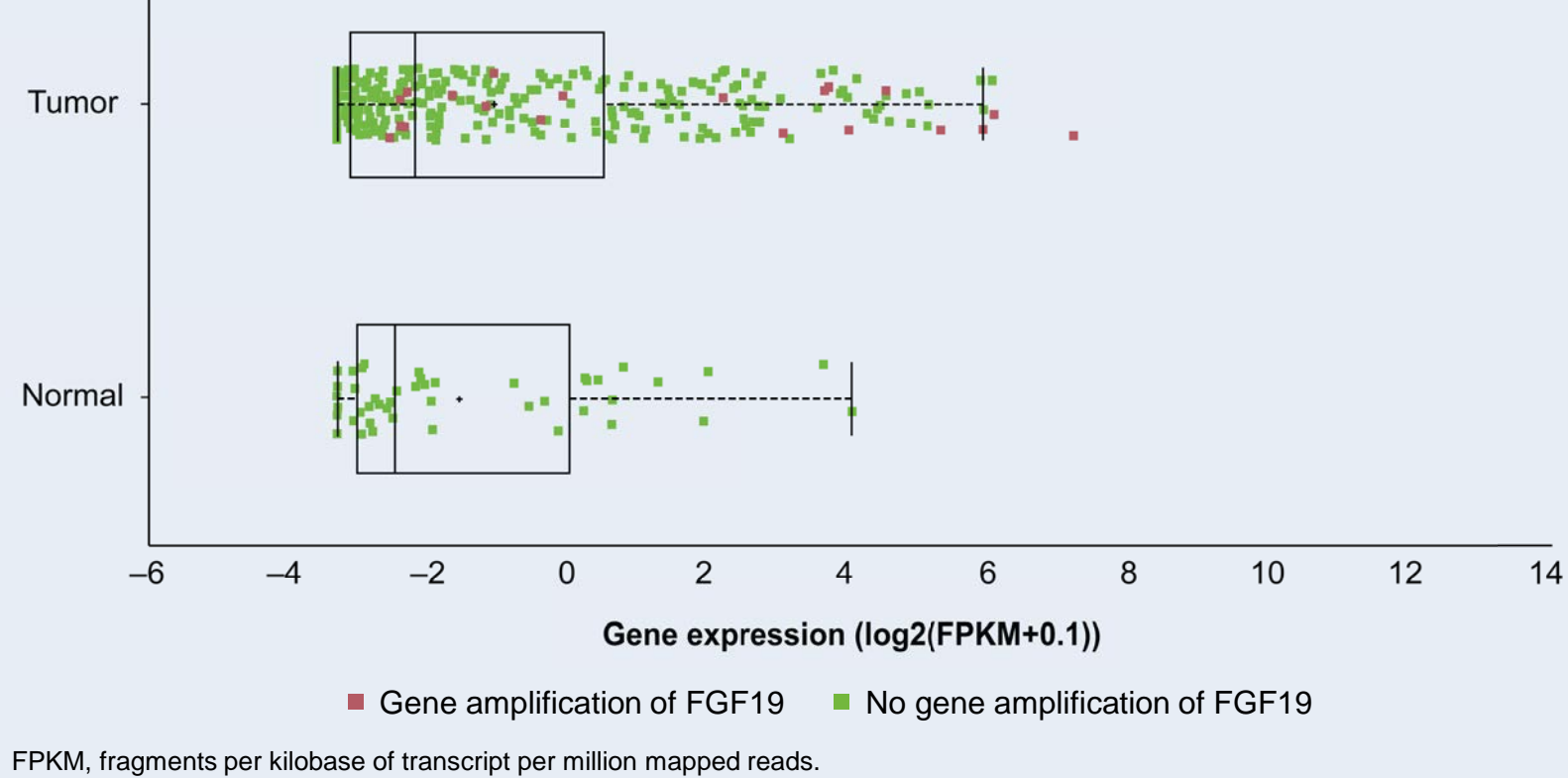
Aberrant signaling through Fibroblast Growth Factor Receptors (FGFR) has been reported in multiple types of human cancers. FGFR4 signaling contributes to the development and progression of subsets of cancer: in approximately 10 percent of hepatocellular carcinoma (HCC), genetic amplification of FGF19, encoding an endocrine FGF ligand that activates FGFR4-KLB receptors, has been reported. In models with this alteration, FGF19-FGFR4 signaling is oncogenic and antagonism of the FGF19-FGFR4 axis has been shown to be efficacious suggesting that selective targeting of FGFR4 may be an effective strategy for malignancies with FGFR4 activation.

We describe the preclinical characterization of INCB062079 a potent and selective inhibitor of the FGFR4 kinase. In biochemical assays INCB062079 inhibited FGFR4 with low nM potency and exhibited at least 250-fold selectivity against other FGFR kinases and greater than 800-fold selectivity against a large kinase panel. This selectivity derives from the ability of INCB062079 to bind irreversibly to Cys552, a residue within the active site of FGFR4 that is non-conserved among other FGFR receptors. Covalent binding of INCB062079 to Cys552 was demonstrated using a LC/MS/MS-based proteomic analysis that confirmed specificity for the target Cys. In assays using HCC cells with autocrine production of FGF19, INCB062079 inhibited the autophosphorylation of FGFR4 and blocked signal transduction by FGFR4 to downstream markers of pathway activation. Cancer cell lines that have amplification and expression of FGF19 are uniquely sensitive to growth inhibition by INCB062079 (EC_{50} less than 200 nM) compared with HCC cell lines or normal cells without FGF19-FGFR4 dependence (EC_{50} > 5000 nM) confirming selectivity for FGFR4. *In vivo*, oral administration of INCB062079 inhibited the growth and induced significant regressions of subcutaneous xenograft tumors dependent upon FGFR4 activity at doses that were well-tolerated (10–30 mg/kg BID) and did not result in a significant increase in serum phosphate levels which is observed with FGFR1/2/3 inhibition. Suppression of tumor growth correlated with pharmacodynamic inhibition of FGFR4 signaling. Collectively, these preclinical studies demonstrate that INCB062079 potently and selectively inhibits models of FGF19-FGFR4-dependent cancers *in vitro* and *in vivo*, supporting clinical evaluation in patients harboring oncogenic FGFR4 activation.

A FGF19-FGFR4 Signaling



B Analysis of FGF19 Expression in Normal Liver Versus HCC Tumors From The Cancer Genome Atlas

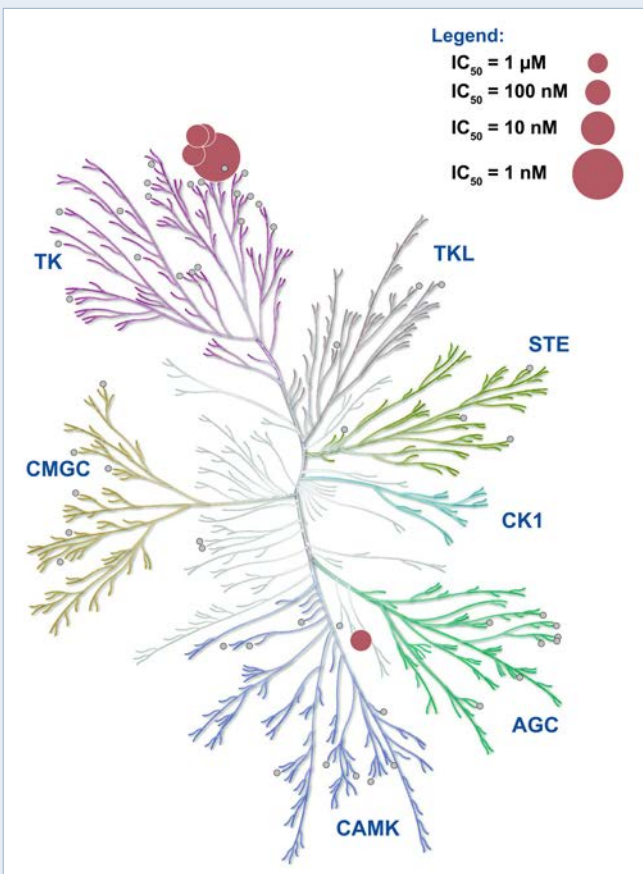


INCB062079 Is Selective for FGFR4

A Biochemical Activity

Enzyme	IC ₅₀ (mean, nM)
FGFR4	1.2
FGFR1	>300
FGFR2	>300
FGFR3	>300

B Selectivity of INCB062079



C Cellular Activity

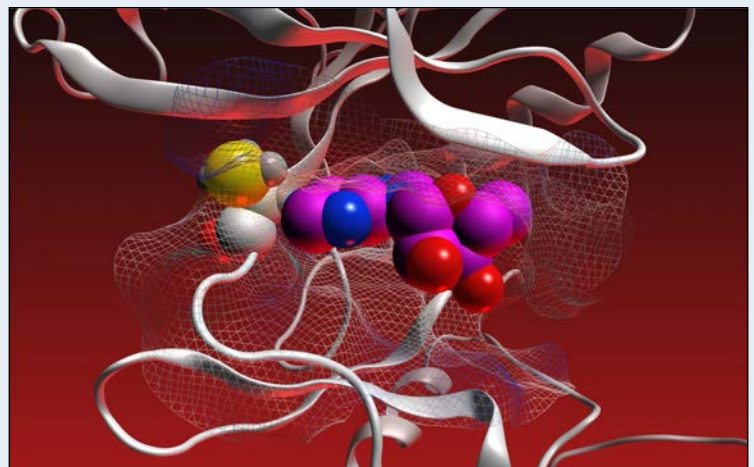
Cell Line	GI ₅₀ (mean, nM)
Ba/F3-TEL-FGFR4	3
Ba/F3-TEL-FGFR1	539
Ba/F3-TEL-FGFR3	1059

A. Inhibition of the kinase activity of recombinant FGFR enzymes by INCB062079.

B. Kinase profiling – IC_{50} values of INCB062079 for 59 kinases indicated by size of spots. Small gray circles indicate tested kinases with IC_{50} > 1200 nM (1000-fold greater than FGFR4 IC_{50}).

C. Growth inhibitory 50 (GI_{50}) values for INCB062079 determined by CellTiter-Glo® assays (Promega, Madison, WI, USA) against Ba/F3 cell lines expressing TEL-FGFR kinase domain fusions.

A FGFR4 Active Site ATP and the Cys552 Position



E Truncated FGFR4 Active Site Blast Alignment Highlighting Target Cysteine

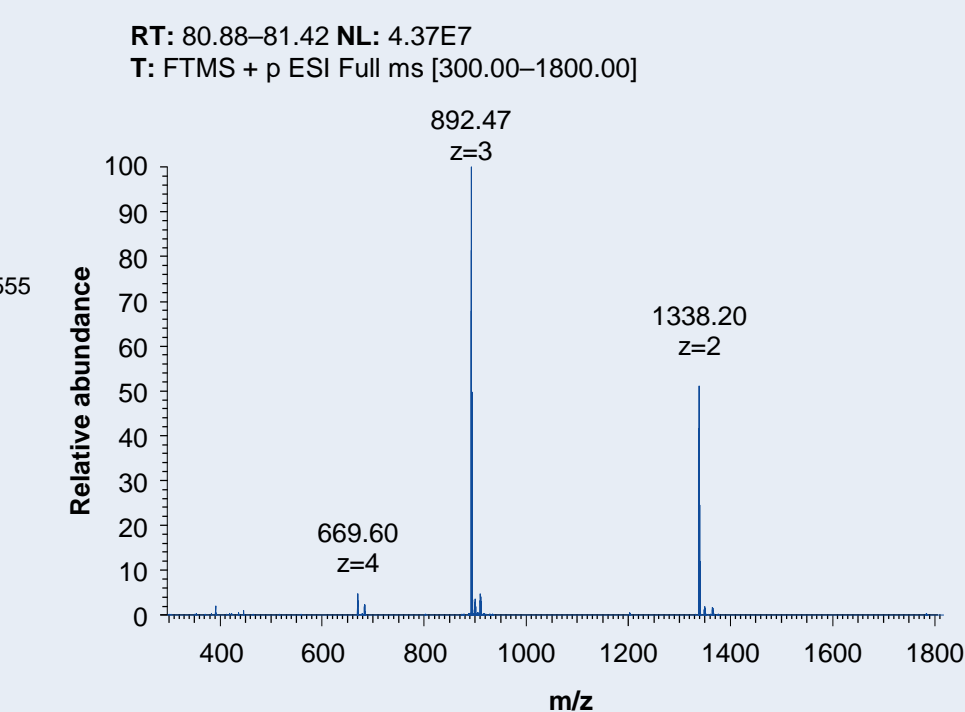
Sequence	Cys552
FGFR4 N I I N L L G V C T Q E G P L Y V I V E C A A K	C
FGFR3 N I I N L L G A C T Q G P L Y V L V E Y A A K	A
FGFR2 N I I N L L G A C T Q D G P L Y V I V E Y A S K	A
FGFR1 N I I N L L G A C T Q D G P L Y V I V E Y A S K	A

B Cysteine-Containing FGFR Peptides Monitored

Cys Position	Peptide	Monoisotopic Mass*	Charge	RT (min)
C477	LVLGKPLGEGQFGQVVR	1828.00	2, 3, 4	44.9
C540, C552	NIINLLGVCTQEGPLYVIVECAAK	2673.39	2, 3, 4	81.2
C540, C552	NIINLLGVCTQEGPLYVIVECAAK Modified by INCB062079	proprietary	2, 3, 4	91.2
C592	SSEGPLSFFVLVSCAYQVAR	2166.08	2, 3	65.8
C713	MDRPPCPPELYGLMR	1830.86	2, 3, 4, 5	37.9
C724	ECWHAAPSRQPTFK	1713.8	2, 3, 4	20.8
C774	LTFGPYSPSGGDASSTCSSS DSVFSDPLPLGSSSPFGSGVQT	4409.95	3, 4	73.3

* Cysteines alkylated with IAM.

F MS Data: Full Scan Mass Spectra



G MS Data: CID Fragment Ion Spectra

