

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

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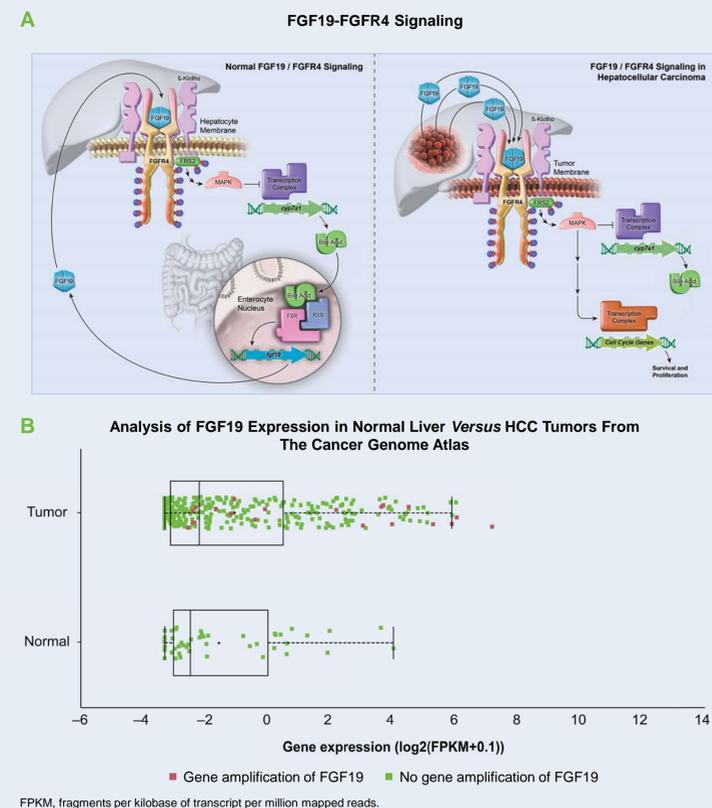
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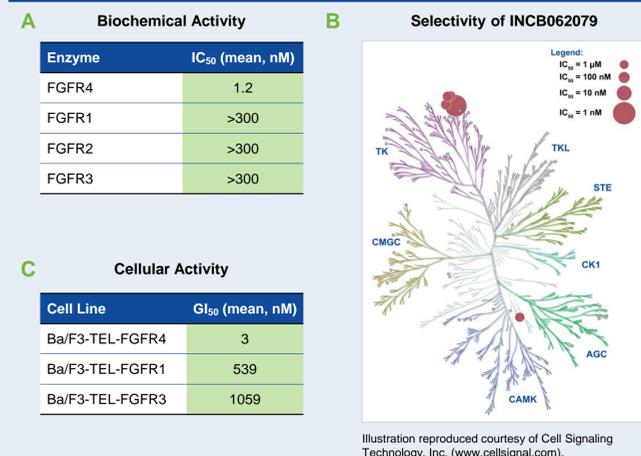
Abstract

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.

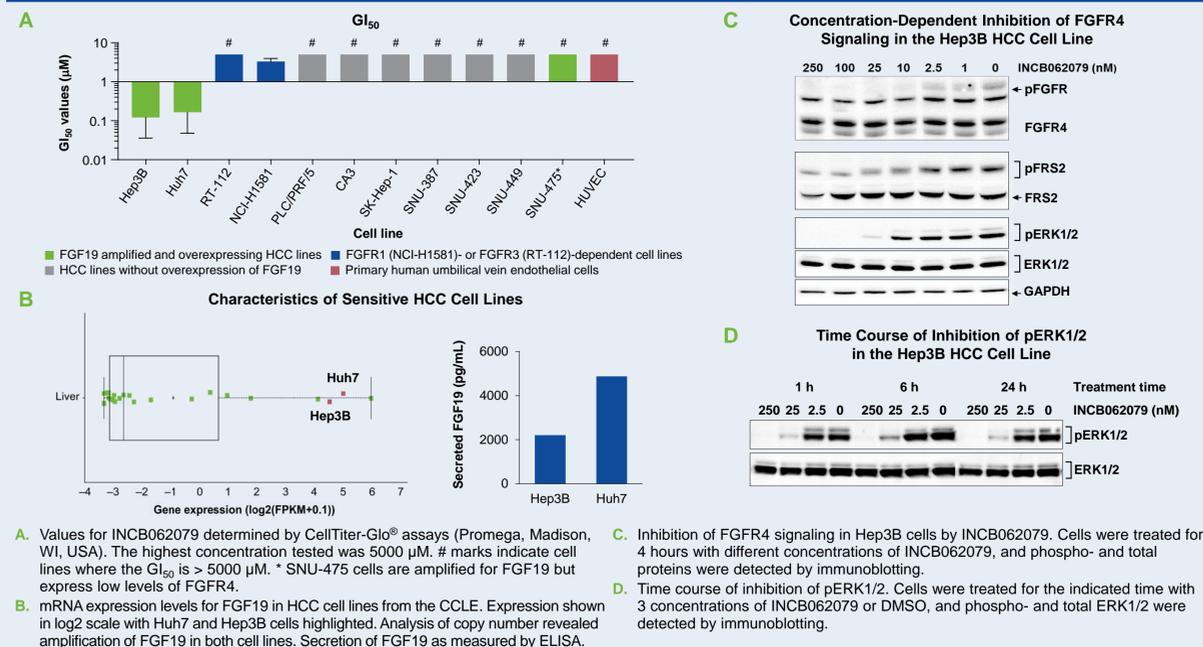


INCB062079 Is Selective for FGFR4



- A.** Inhibition of the kinase activity of recombinant FGFR enzymes by INCB062079.
- B.** Kinase profiling – IC₅₀ values of INCB062079 for 59 kinases indicated by size of spots. Small gray circles indicate tested kinases with IC₅₀ > 1200 nM (1000-fold greater than FGFR4 IC₅₀).
- C.** Growth inhibitory 50 (GI₅₀) values for INCB062079 determined by CellTiter-Glo® assays (Promega, Madison, WI, USA) against Ba/F3 cell lines expressing TEL-FGFR kinase domain fusions.

Cellular Activity of INCB062079 in HCC Cell Lines



Covalent Modification of FGFR4-Cys552 by INCB062079

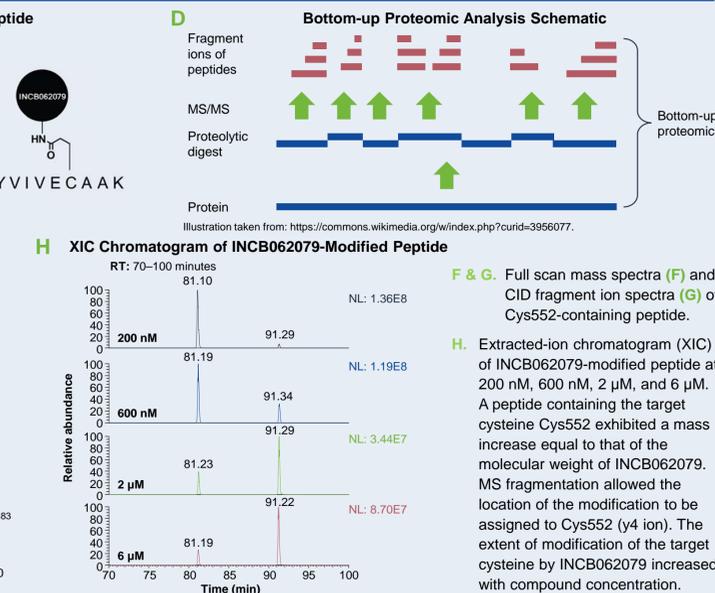
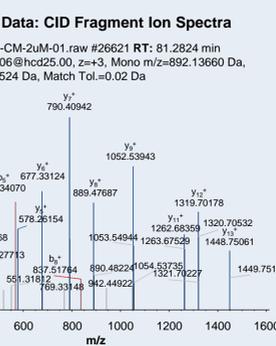
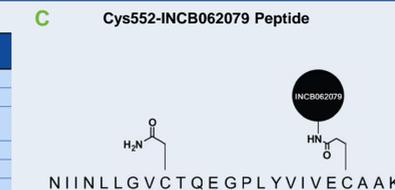
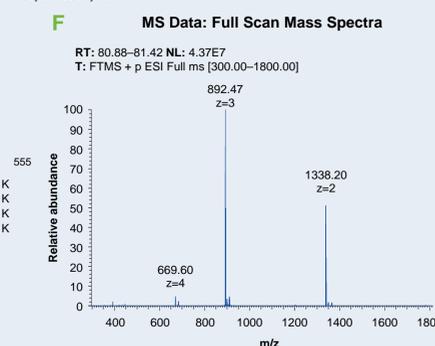
The covalent binding of INCB062079 to FGFR4 was confirmed using bottom-up proteomic analysis



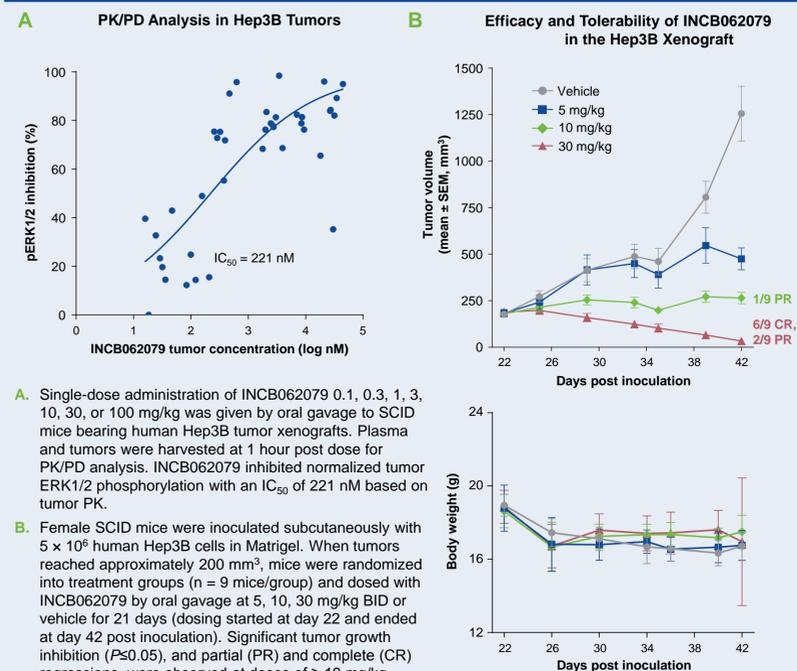
B Cysteine-Containing FGFR Peptides Monitored

Cys Position	Peptide	Monoisotopic Mass*	Charge	RT (min)
C477	LVLGKPLGEGFGQVVR	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	SSEGLPSFPLVSCAYQVAR	2166.08	2, 3	65.8
C713	MDRPPCPPELYGLMR	1830.86	2, 3, 4, 5	37.9
C724	ECWHAAPSQRPTFK	1713.8	2, 3, 4	20.8
C774	LTFGPYSPSGDASSTCSS DSVFSDPLPLGSSFPFGSGVQT	4409.95	3, 4	73.3

* Cysteines alkylated with IAM.



Efficacy in a FGF19-FGFR4-Driven Model of HCC



Conclusions

- INCB062079 is a potent inhibitor of FGFR4 with selectivity against other FGFR and non-FGFR kinases
- The selectivity of INCB062079 derives from direct covalent modification of FGFR4-Cys552 that is not conserved among other FGFR proteins
- INCB062079 blocked signaling from activated FGFR4 and selectively inhibited growth of cell lines with alterations in the FGF19-FGFR4 axis *in vitro*
- Subcutaneous xenograft models of HCC with activation of FGFR4 were dose-dependently inhibited by oral administration of INCB062079 consistent with suppression of pERK1/2 in the tumors
- INCB062079 induced significant tumor regression at tolerated doses when the plasma exposure exceeded the *in vivo* IC₅₀ for approximately 24 hours
- A phase 1 clinical trial of INCB062079 is scheduled to begin in the first half of 2017

Author Disclosures
All Authors: Incyte Corporation: Employment and Stock Ownership.

Acknowledgments
Layout and printing support was provided Evidence Scientific Solutions, Philadelphia, PA, funded by Incyte Corporation.

