

Activity of the Selective FGFR 1, 2, and 3 Inhibitor INCB054828 in Genetically Defined Models of Triple-Negative Breast Cancer

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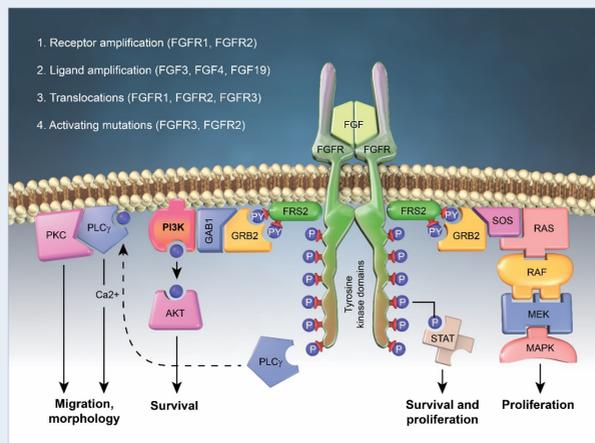


Introduction

Aberrant signaling through Fibroblast Growth Factor Receptors (FGFR) has been reported in multiple types of human cancers. Genomic analysis of triple-negative breast cancer (TNBC) has revealed recurrent genetic alterations in *FGFR1* and *FGFR2* genes.¹ In addition, a rare gene fusion involving *FGFR3* has been identified in a TNBC clinical specimen and cell line.² Furthermore, CAL-51 cells have been demonstrated to be dependent on autocrine FGF2 signaling.³ FGFR proteins contribute to the development of malignancies by promoting tumor cell proliferation, survival, and migration and supporting angiogenesis. Therefore, targeting FGFR kinases may provide therapeutic benefit to patients with cancers that have genetic alterations in genes encoding components of the FGF-FGFR axis. INCB054828 is a potent inhibitor of FGFR1, FGFR2, and FGFR3, has selective pharmacological activity against cancer cells with FGFR alterations, and is currently undergoing phase 2 clinical evaluation in selected patients.⁴

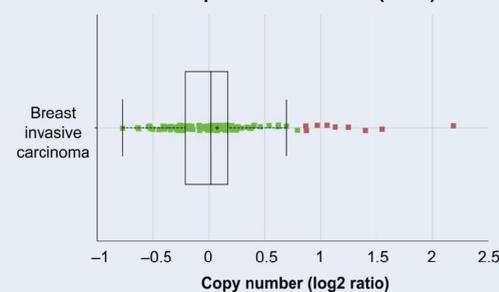
In this study, we evaluated the activity of INCB054828 across a panel of TNBC cell lines. Consistent with previous data, the most potent INCB054828 activity was observed in cell lines that harbored alterations in FGFR genes including amplification of FGFR2 or expression of the TACC3-FGFR3 fusion. Occurrence of these FGFR gene aberrations showed enrichment in the LAR subtype. To confirm this association *in vivo*, 4 PDX models of TNBC were tested: 2 chemo-refractory models with FGFR1 amplification (CNV = 4 and 6) and 2 without any known FGF/FGFR alterations. Both of the models with FGFR1 copy number gain showed a response to INCB054828 as monotherapy with 36% and 78% tumor growth inhibition that was statistically significant versus vehicle control ($P < 0.05$ and $P < 0.001$, respectively). At the maximally efficacious dose of 1 mg/kg daily, neither PDX model lacking FGF/FGFR alteration responded to the treatment. To assess the effect of the microenvironment on drug sensitivity, mouse 4T1 breast cancer cells were orthotopically implanted into the mammary fat pad; under these conditions, 4T1 tumors retained sensitivity to a standard dose of INCB054828. In summary, these results demonstrate that the FGFR1/2/3 inhibitor INCB054828 is highly active against models of TNBC with FGFR gene alterations and confirms the importance of patient stratification strategies for clinical trials with FGFR-targeted therapies.

FGFR Signaling and Alterations in Cancer

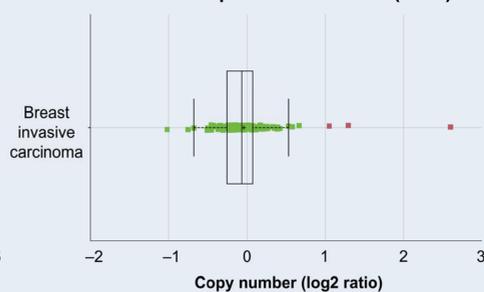


Genetic Alterations in FGFR1 and FGFR2 in TNBC

A FGFR1 Amplification in TNBC (9/116)



B FGFR2 Amplification in TNBC (3/116)



Analysis of FGFR1 (A) and FGFR2 (B) copy number in 116 TNBC samples present in TCGA. Copy number (log₂ ratio) is plotted for TNBC samples. Cases with gene amplification (> 3.5 copies) are highlighted in red. The prevalence of amplification for FGFR1 is 7.8% and for FGFR2 is 2.6%, consistent with prior analyses.^{1,6}

INCB054828 Is a Selective FGFR1/2/3 Inhibitor

A Biochemical Activity

Enzyme	Mean IC ₅₀ ± SD (nM)
FGFR1	0.4 ± 0.04
FGFR2	0.5 ± 0.09
FGFR3	1 ± 0.4
FGFR4	30 ± 16

B Selectivity of INCB054828

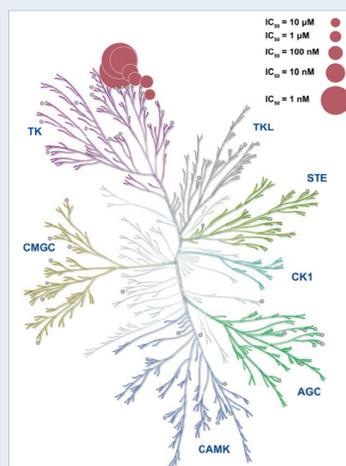


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C Cellular Activity

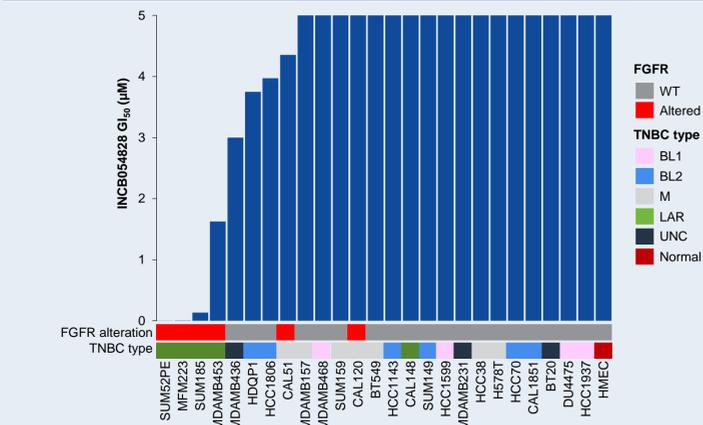
Assay	Cell Model	Mean IC ₅₀ ± SD (nM)
Phospho-FGFR1	Ba/F3-TEL-FGFR1	3 ± 1
Phospho-FGFR3	Ba/F3-TEL-FGFR3	4 ± 2
Phospho-FGFR2	KATOIII	3 ± 2

A. Inhibitory activity of INCB054828 against recombinant FGFR enzymes.

B. Selectivity of INCB054828 against a panel of kinases. Small grey circles indicate tested kinases with IC₅₀ > 10,000 nM.

C. Inhibition of FGFR phosphorylation in cells by INCB054828.

Growth Inhibition of Genetically Defined TNBC Cell Lines by INCB054828



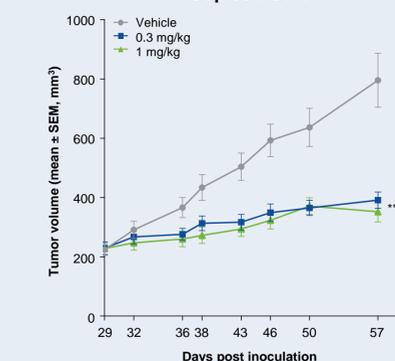
HMEC, human mammary epithelial cells.

Growth inhibitory activity of INCB054828 against a panel of TNBC cell lines. Cells were treated with a concentration range (0.0008–15 µM) of INCB054828, and cellular viability was assayed using the CellTiter-Glo® Reagent (Promega, Madison, WI).

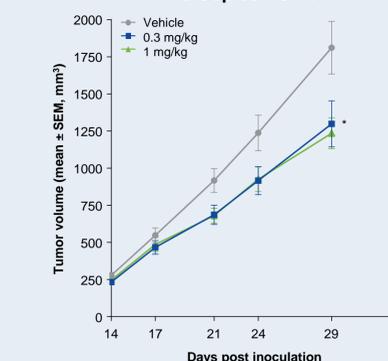
TNBC cell lines were classified according to their molecular subtypes: BL1 (basal-like 1), BL2 (basal-like 2), M (mesenchymal), and LAR (luminal androgen receptor) as described in Lehmann et al.⁵ UNC, unclassified.

Efficacy of INCB054828 in PDX Models of Relapsed and Refractory TNBC

A Model CTG-1019: 4 Copies FGFR1

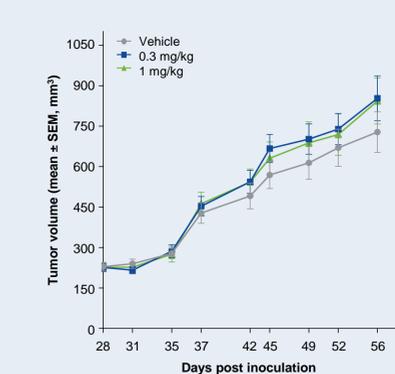


B Model CTG-0437: 6 Copies FGFR1

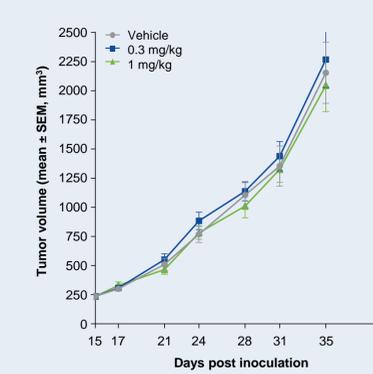


Characteristics	CTG-1019	CTG-0437
Tumor type	Breast	Breast
Tumor status	Metastatic	Metastatic
ER/PR/HER2 status	Triple-negative	Triple-negative
Harvest site	Liver	Chest wall
Histology	Invasive ductal carcinoma	Adenocarcinoma
Tumor grade	Poorly differentiated	Poorly differentiated
Diagnosis	Recurrent	Not available
Treatment history	Pretreated	Not available
Disease stage	IV	III
FGFR alterations (CNV)	FGFR1 (4)	FGFR1 (6)

D Model CTG-0052: No Known FGFR Alteration

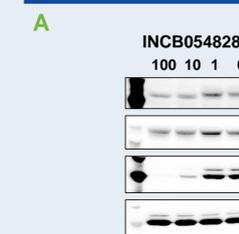


E Model CTG-0869: No Known FGFR Alteration

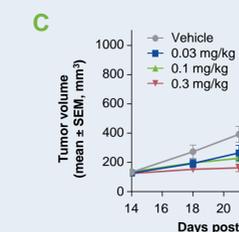
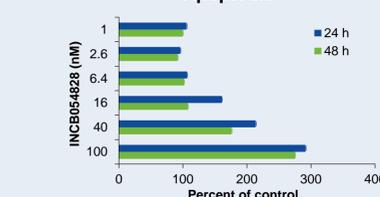


Female nude mice were implanted subcutaneously with tumor fragments from Champions Oncology low passage TumorGraft™ models with FGFR1 amplification (CTG-1019 [4 FGFR1 copies], CTG-0437 [6 FGFR1 copies]) (A–C), or with no known alterations in any of the FGFR genes (CTG-0052 or CTG-0869) (D & E). After tumors reached 1 to 1.5 cm³, they were harvested and tumor fragments re-implanted subcutaneously. Upon tumors reaching 150 to 300 mm³, mice were randomized into treatment groups (n = 12 mice/group) and given either vehicle or INCB054828 at 0.3 mg/kg or 1.0 mg/kg QD by oral gavage for 28 days. In both models with FGFR1 amplification, there was a statistically significant inhibition of tumor growth in the INCB054828 treatment groups compared with the vehicle group (* $P < 0.05$; *** $P < 0.001$, one-way ANOVA followed by Newman–Keuls multiple comparison test). All doses were well tolerated.

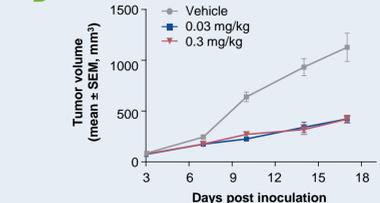
INCB054828 Inhibits Murine 4T1 Breast Cancer Cells *In Vitro* and in Syngeneic Tumor Models



B Apoptosis



D Tumor volume



- INCB054828 suppresses FGFR-dependent signaling in 4T1 murine breast cancer cells.
- Induction of apoptosis as detected by caspase 3/7 activity in 4T1 cells treated with INCB054828 for 24 or 48 hours.
- Dose-dependent suppression by INCB054828 of the murine 4T1 syngeneic model of breast cancer. INCB054828 was administered orally to Balb/c mice bearing subcutaneous 4T1 tumors at 0 (vehicle), 0.03, 0.1, or 0.3 mg/kg QD for 14 days (n = 7–8 mice per group). All doses were well tolerated.
- INCB054828 suppresses growth of 4T1 orthotopic tumors. 4T1 cells were implanted into the mammary fat pad of Balb/c immunocompetent mice. INCB054828 was administered orally at 0 (vehicle), 0.03, or 0.3 mg/kg QD for 14 days (n = 10 mice per group). INCB054828 inhibited growth of 4T1 tumors with similar efficacy independent of the site of implantation.

Conclusions

- The prevalence of genetic amplification for FGFR1 and FGFR2 is 7.8% and 2.6%, respectively, in TNBC
- INCB054828 selectively inhibits the growth of TNBC cell lines harboring genetic alterations in FGFR1, FGFR2, or FGFR3, consistent with the biochemical activity of the compound
- Oral administration of INCB054828 at well-tolerated doses exhibits selective efficacy against PDX models of refractory TNBC with FGFR1 amplification
- INCB054828 is equally efficacious against subcutaneous and orthotopic models of TNBC
- These data support clinical evaluation of INCB054828 in patients with evidence of FGFR activation including subsets of breast cancer
- INCB054828 is currently being investigated in phase 2 clinical trials (see AACR presentation numbers CT111, CT057, CT059, and CT063)

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Author Disclosures

All Authors: Incyte Corporation; Employment and Stock Ownership.

Acknowledgments

Efficacy studies in human TNBC PDX models were conducted by Champions Oncology, Hackensack, NJ, funded by Incyte Corporation. Layout and printing support was provided by Evidence Scientific Solutions, Philadelphia, PA, funded by Incyte Corporation.

