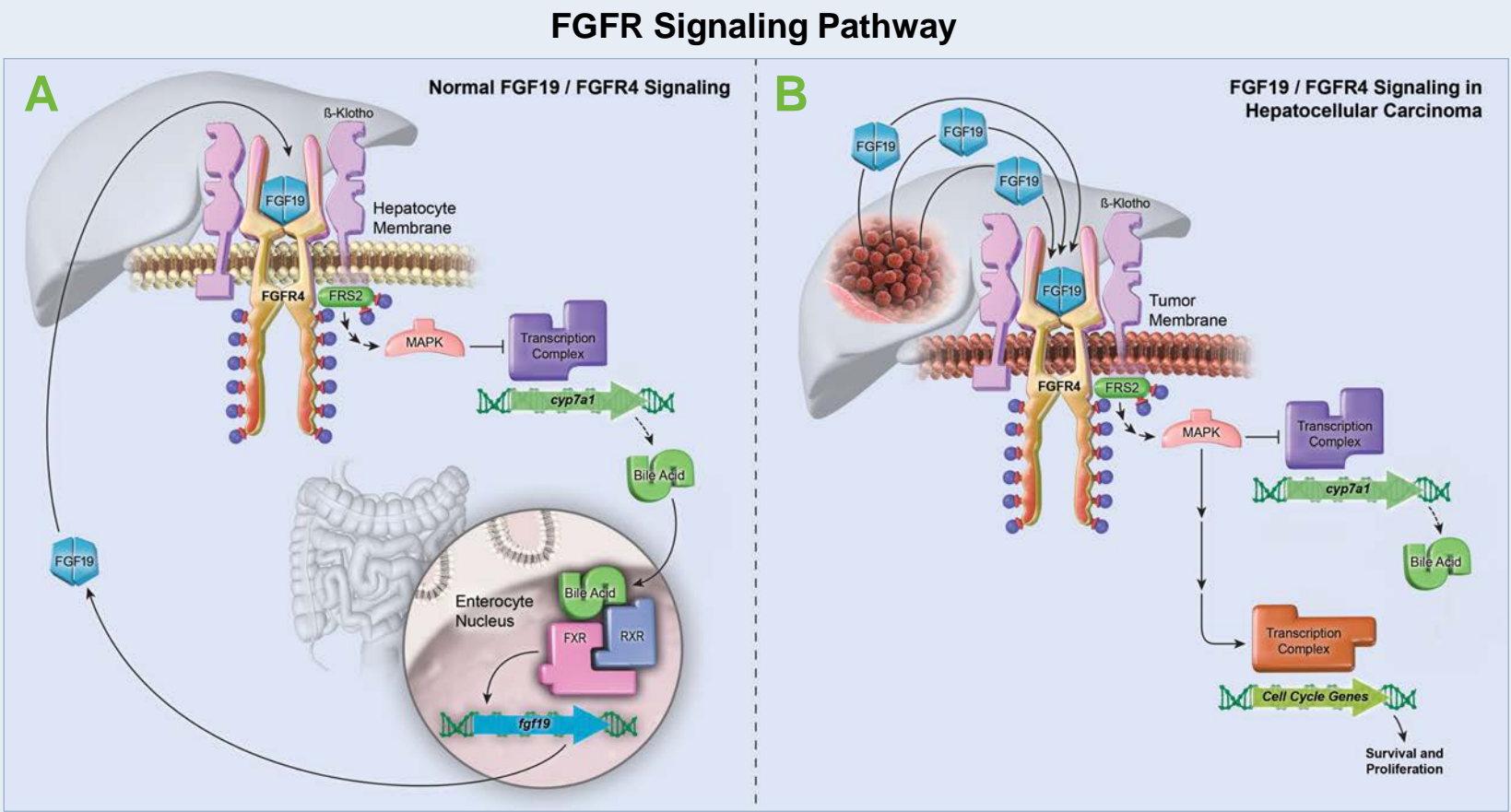




Abstract

Hepatocellular carcinoma (HCC) is the most common form of primary liver cancer with limited treatment options for advanced stage disease. Thus, there is a critical medical need for improved therapies. In approximately 10% of HCC, a focal amplicon at 11q13 harboring FGF19 has been reported. High levels of FGF19 have been shown to drive HCC tumor development and progression in preclinical models, suggesting that selective targeting of FGFR4, a high affinity receptor for FGF19, may be efficacious in these HCC tumors. INCB062079 a potent and selective irreversible inhibitor of FGFR4 (>250-fold vs FGFR1/2/3) suppresses the growth of HCC cell lines driven by amplification and overexpression of FGF19. In subcutaneous xenograft models of HCC, oral dosing of INCB062079 at tolerated doses resulted in dose-dependent inhibition of tumor growth with regressions observed at higher doses consistent with inhibition of FGFR4 signaling in the tumors. In combination with sorafenib, the only approved targeted therapy for HCC, FGFR4 inhibition exhibited additive tumor growth inhibition in the Huh7 model. To assess exposure of INCB062079 in orthotopic tumors after oral dosing, Hep3B tumors were implanted surgically into the liver and their development monitored by analysis of plasma alpha-fetoprotein (AFP). At efficacious doses, INCB062079 strongly suppressed the levels of AFP and FGF19 secreted by the tumors, and their levels correlated well with the reduction in terminal liver tumor mass, suggesting that these may be surrogate markers for response of HCC tumors to INCB062079. In two PDX models of HCC with amplification of FGF19 (4-6 CNV), INCB062079 administration reduced tumor growth by 50–66% at doses that were well tolerated. Additional surrogate markers for FGFR4 inhibition were explored including several parameters related to FGF19 regulation of bile acid metabolism. The mRNA levels of CYP7A1, encoding cholesterol 7 α -hydroxylase, the rate limiting enzyme in bile acid synthesis, were induced in the livers of cynomolgus monkeys upon dosing with INCB062079. Correspondingly there was a dose-dependent increase in fecal bile acids. In summary these data demonstrate that INCB062079 is highly and selectively efficacious in models of HCC with FGF19-FGFR4 oncogene addition and elicits pharmacodynamic responses in primates providing support for the clinical evaluation of INCB062079 in genetically selected liver cancer patients.



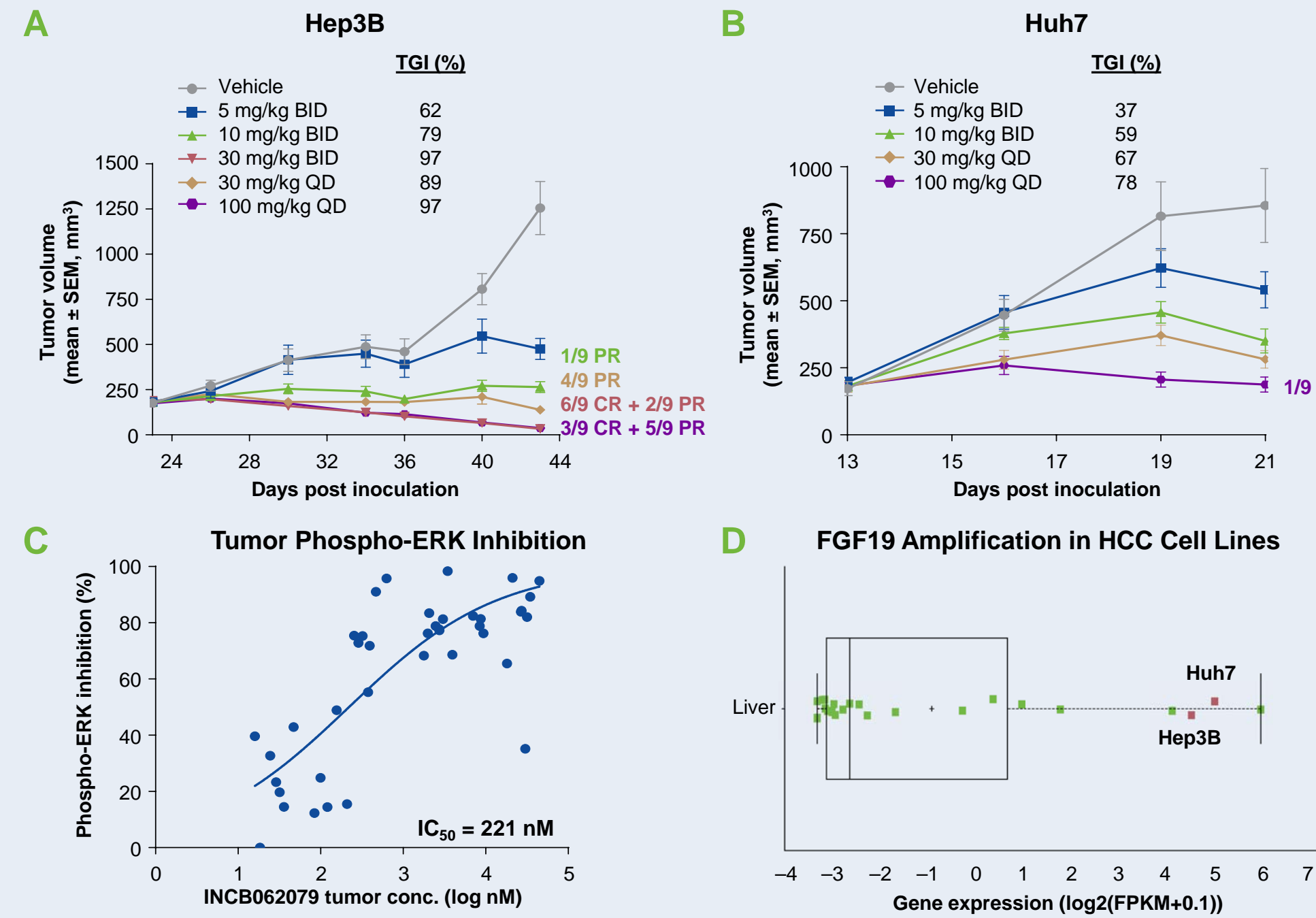
- A. Metabolic function of FGF19-FGFR4:** suppresses post-prandial bile acid (BA) production via regulated expression of Cyp7a1 rate-limiting enzyme in BA synthesis. Levels of FGF19 are regulated through BA-FXR/RXR-mediated feedback mechanism in intestinal enterocytes.
- B. FGFR signaling pathway in tumors:** overexpression of FGF19 in HCC cells causes autocrine activation of FGFR4 signaling through the Ras-MAPK and PI3K-AKT pathways to promote tumor cell growth and survival.

INCB062079 Is Potent and Selective for FGFR4

A Biochemical Activity		B Cellular Activity	
Enzyme	IC ₅₀ (mean, nM)	Cell Line	Mean GI ₅₀ (nM)
FGFR4	1.2	Ba/F2-TEL-FGFR4	3
FGFR1	>300	Ba/F3-TEL-FGFR1	539
FGFR2	>300	Ba/F3-TEL-FGFR3	1059
FGFR3	>300		

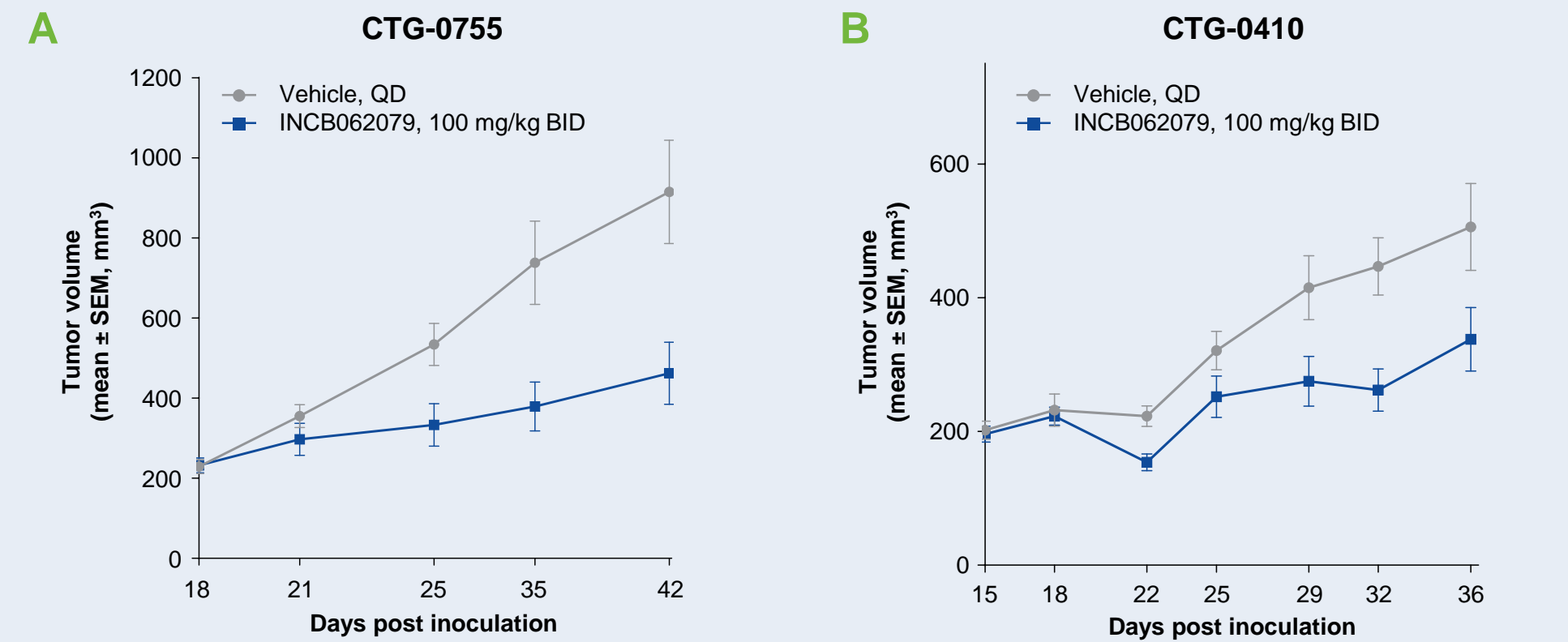
- A.** Inhibitory activity of INCB062079 against recombinant FGFR enzymes.
- B.** Growth inhibitory 50 (GI₅₀) values for INCB062079 assessed by CellTiter-Glo®.

Activity of INCB062079 in Xenograft Models of HCC With FGF19 Amplification and FGFR4 Overexpression



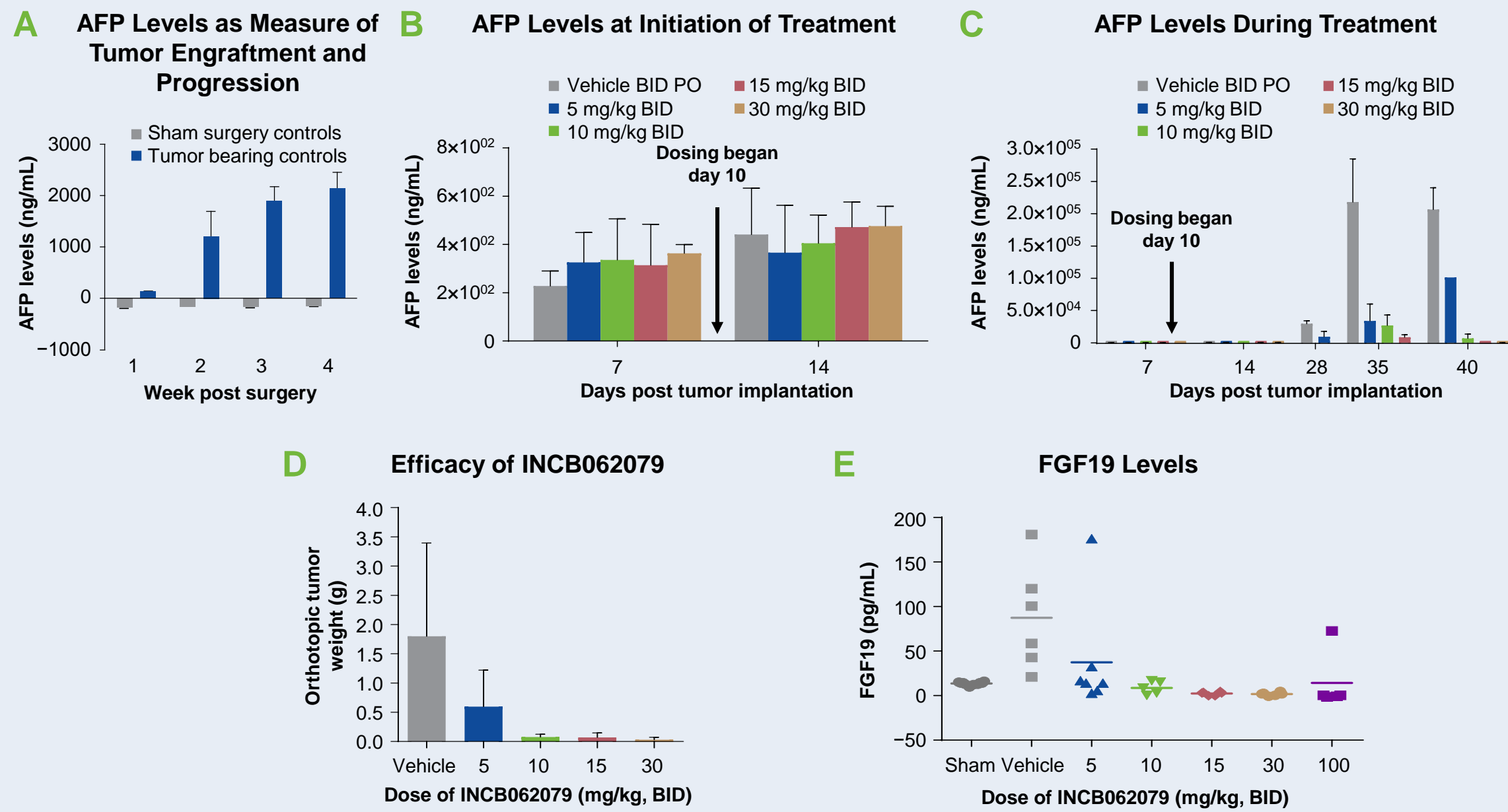
- A. & B.** Female SCID mice were inoculated subcutaneously with 5×10^6 Hep3B or Huh7 cells in matrigel. At tumor volumes of approximately 200 mm³, mice were randomized into treatment groups (n = 9 mice/group) and given INCB062079 or vehicle by oral gavage at the indicated doses for 21 days. All dosing groups received Hydrogel and Dietgel. Significant tumor growth inhibition (TGI) ($P \leq 0.05$), and partial (PR) and complete (CR) regressions, were observed at doses of ≥ 10 mg/kg INCB062079 BID. Doses were well tolerated with transient body weight loss < 10% at nadir.
- C.** Single dose administration of INCB062079 at 0.1, 0.3, 1, 3, 10, 30, or 100 mg/kg given by oral gavage to SCID mice bearing human Hep3B tumor xenografts. Plasma and tumors were harvested at 1 hour post dose for PK/PD analysis. INCB062079 inhibited normalized tumor phospho-ERK1/2 with an IC₅₀ of 221 nM.
- D.** mRNA expression levels for FGF19 in HCC cell lines from the Cancer Cell Line Encyclopedia with expression shown in log2 scale. Huh7 and Hep3B cells highlighted in red express high levels of FGF19 consistent with gene amplification in these cell lines.

Activity of INCB062079 in PDX HCC Models With FGF19 Amplification and FGFR4 Overexpression



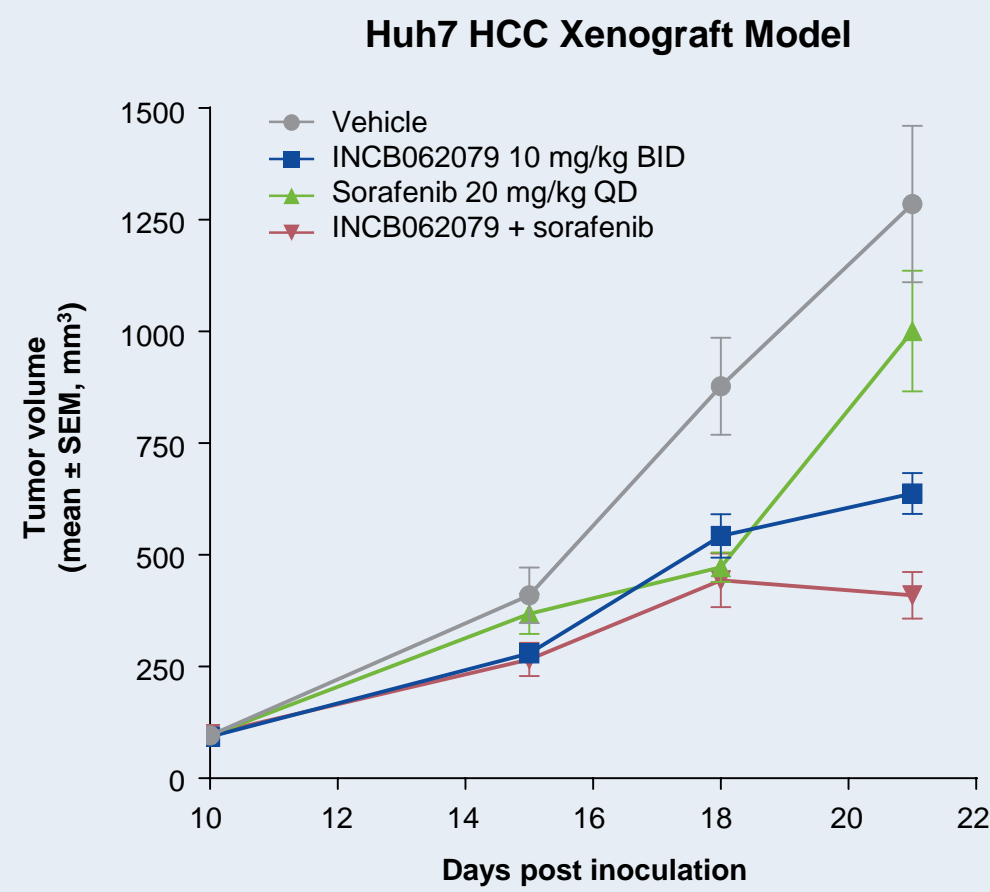
Female nude mice were implanted subcutaneously with tumor fragments from low passage (#2) Champions TumorGraft™ human HCC models CTG-0755 (**A**) or CTG-0410 (**B**). Upon tumors reaching 1–1.5 cm³, harvested tumor fragments were re-implanted subcutaneously. When PDX tumor volumes reached approximately 200 mm³, mice were randomized into treatment groups (n = 9 mice/group) and given INCB062079 or vehicle by oral gavage at 100 mg/kg BID for 14 to 21 days. All dosing groups received Hydrogel and Dietgel. Significant TGI ($P < 0.05$ or greater) was observed in both PDX models. INCB062079 treatment was generally well tolerated with transient body weight loss < 12% at nadir in both models.

INCB062079 Suppresses Tumor Growth and AFP in an Orthotopic Model of Hep3B HCC



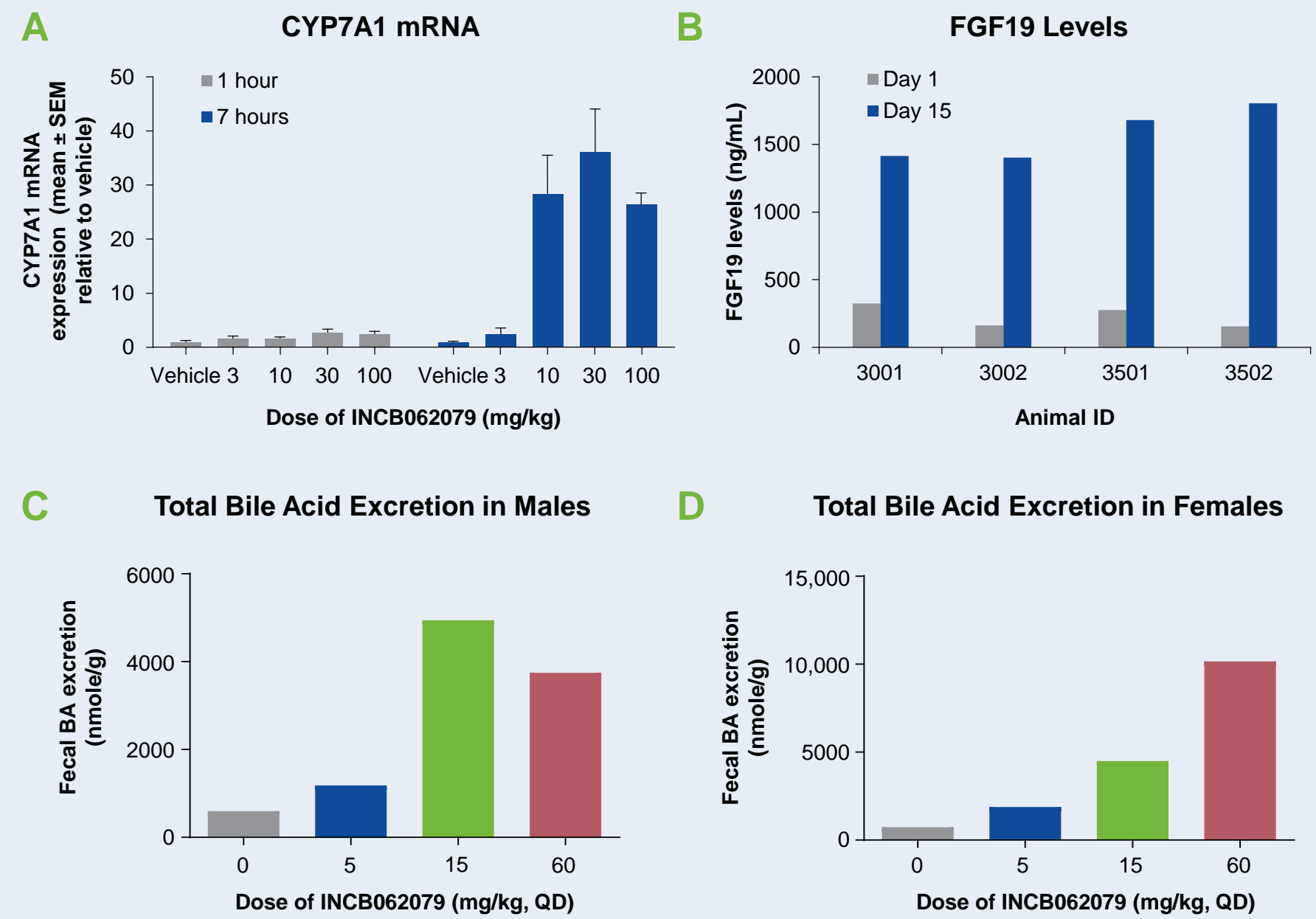
- A.–E.** Hep3B cells (2×10^6) were implanted into the upper left hepatic lobe of female SCID mice to establish orthotopic liver tumors.
- A.** Following surgical implantation, serum levels of AFP were used as a measure of tumor engraftment and as a surrogate marker for monitoring tumor progression.
- B.** Plasma levels of AFP at the initiation of treatment. AFP levels were detectable on day 7, and INCB062079 dosing initiated on day 10.
- C.** Significant reductions in AFP levels ($P < 0.05$) upon treatment with INCB062079 at ≥ 10 mg/kg BID.
- D.** Efficacy of INCB062079 as measured by reduction of terminal tumor mass at day 28, relative to vehicle treated mice. Doses of ≥ 10 mg/kg BID of INCB062079 were highly effective ($P < 0.05$ or greater). Doses of INCB062079 were well tolerated.
- E.** Plasma levels of human FGF19 (ELISA) secreted by Hep3B primary tumors were decreased in all INCB062079 treatment groups at 2 hours post final dose, corresponding to reductions in AFP levels and primary tumor mass.

Efficacy of Combination of INCB062079 With Sorafenib in HCC Xenografts



Female SCID mice were inoculated subcutaneously with 5×10^6 human Huh7 cells in matrigel. When tumors reached 100–150 mm³ volume, mice (n = 9/group) were administered INCB062079 and sorafenib by oral gavage as single agents and in combination on the dosing regimens shown. Significant anti-tumor efficacy ($P < 0.05$ or greater) was observed with INCB062079 alone versus vehicle control, and with the INCB062079 and sorafenib combination versus both vehicle and sorafenib alone, given at an optimal tolerated dose. INCB062079 was well tolerated at all doses (< 10% transient body weight loss), and INCB062079 plus sorafenib combination groups exhibited 12–15% body weight loss but no mortality. All groups received Hydrogel and Dietgel.

Effects of FGFR4 Inhibition on Markers of BA Metabolism



- A.** Relative expression of the CYP7A1 mRNA (encoding cholesterol 7 α -hydroxylase, the rate-limiting enzyme in bile acid synthesis) increased in Hep3bB tumors following dosing with INCB062079, as determined by ddPCR (n = 3 animals/group).
- B.** Plasma levels of FGF19 in cynomolgus monkeys dosed with INCB062079 at baseline (day 1) and for 14 days (day 15).
- C. & D.** Increased total bile acid (BA) excretion in male (n=2) (**C**) and female (n=2) (**D**) cynomolgus monkeys dosed for 14 days with INCB062079.

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 genetic alterations in FGF19-FGFR4 *in vitro*
- Human xenograft and PDX models of HCC with genetic activation of FGFR4 and FGF19 amplification were inhibited by oral administration of INCB062079
- INCB062079 significantly inhibited primary orthotopic HCC growth with concomitant reduction in AFP, a potential surrogate marker of efficacy
- Combination of INCB062079 with sorafenib resulted in enhanced anti-tumor efficacy relative to sorafenib alone in HCC xenografts
- 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.

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