Title: First-in-Human Study of INCB062079, a Fibroblast Growth Factor Receptor 4 Inhibitor, in Patients with Advanced Hepatobiliary Cancers and Other Solid Tumors

Journal: Targeted Oncology

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Supplementary Methods

Quantitation of INCB062079 in Human Plasma

INCB062079 calibration standards used for method validation runs were prepared fresh in human plasma on the day of analysis at nominal concentrations of 2, 10, 20, 50, 100, 200, 1000, and 2000 nM. INCB062079 method validation quality control (QC) samples at lower limit of quantification, low, mid, high upper limit of quantification, and over-the-range concentrations (2, 6, 200, 1600, 2000, and 50000 nM, respectively) were prepared prior to the start of validation and frozen and stored at −70°C until analysis. Additional sets of validation QC samples at low and high concentrations were stored at −−70°C and −20°C for long-term frozen sample storage stability evaluation.

Briefly, 50 µL of each plasma sample was placed in a 96-well plate well. Fifty µL of internal standard (dissolved in 50:50 acetonitrile:water) was added to each well, followed by 100 µL 0.1 M $NaHCO_3$. Then, 800 µL of methyl tert-butyl ether (MTBE) was added, and the samples were covered and vortexed. After centrifugation, 400 µL of the MTBE layer for each sample was transferred to a clean 96-well plate. The samples were dried under nitrogen at approximately 50°C. A 200 µL aliquot of reconstitution solution (acetonitrile:water, 50:50, v/v) was added to each sample. The plate was capped, vortexed briefly, then placed in the autosampler tray where 2 µL of the sample was injected into a liquid chromatography–tandem mass spectrometry (LC-MS/MS) for analysis. The LC-MS/MS analysis was carried out with a Sciex API-4000 mass spectrometer coupled with a high-performance liquid chromatography (HPLC) pump and an autosampler (Runs 1–4 and Runs 6–7). Additionally, the method was cross-validated to a Sciex 6500 mass spectrometer coupled with an HPLC pump and an autosampler (Runs 5 and 8). Chromatographic separation was achieved on an ACE Excel C18-AR, 30 × 2.1 mm, 3 µm HPLC column, with isocratic elution. The mass spectrometer was operated in positive ESI mode and the resolution setting used was "unit" for both Q1 and Q3. The multiple reaction monitoring (MRM) transition was m/z 430.1 → 332.0 for INCB062079, and the MRM transition was m/z 437.0 → 336.0 for the internal standard, INCB062079-D7.

Peak-area integrations were performed using Analyst software (version 1.6.2) from AB Sciex, and regressions were carried out in Watson Bioanalytical LIMS (version 7.4.1) from Thermo Fisher Scientific. Concentrations were calculated using duplicate eight-point curves ranging from 2 nM to 2000 nM for INCB062079. Weighted linear regression was used for INCB062079, according to the following formula: $y = mx + b$ (weighting factor = $1/x^2$), where: x = INCB062079 concentration (nM), y = peak-area ratio, m = slope, b = intercept.