Supplementary Materials

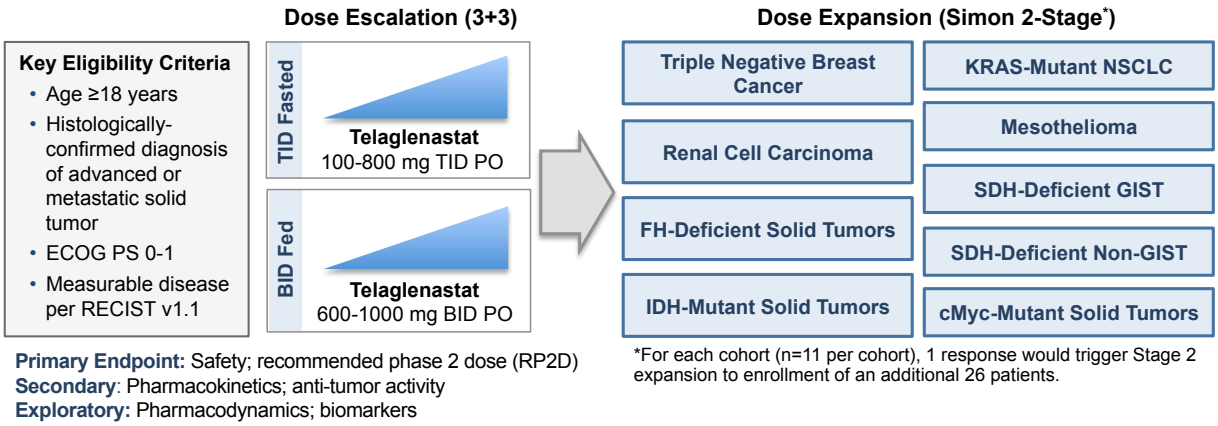

Figure S1. Study Design. Phase 1 open-label dose escalation and dose expansion trial in patients with advanced or metastatic solid tumors. After identification of the RP2D from the dose expansion part of the study, patients were enrolled in 9 dose expansion cohorts, using a Simon 2-Stage design. BID, twice daily; FH, fumarate hydratase; GIST, gastrointestinal stromal tumor; IDH, isocitrate dehydrogenase 1/2; NSCLC, non-small cell lung cancer; PO, per oral; RP2D, recommended phase 2 dose; TID, three times daily; SDH, succinate dehydrogenase.

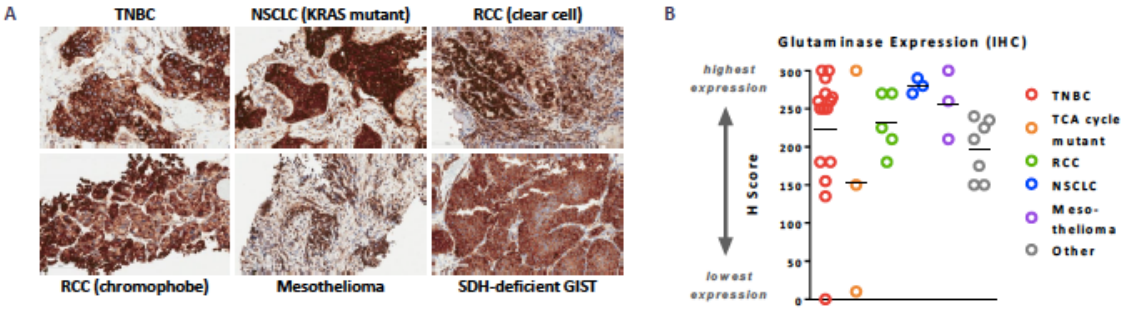

Figure S2. High levels of glutaminase expression in on-study or archival tumor samples

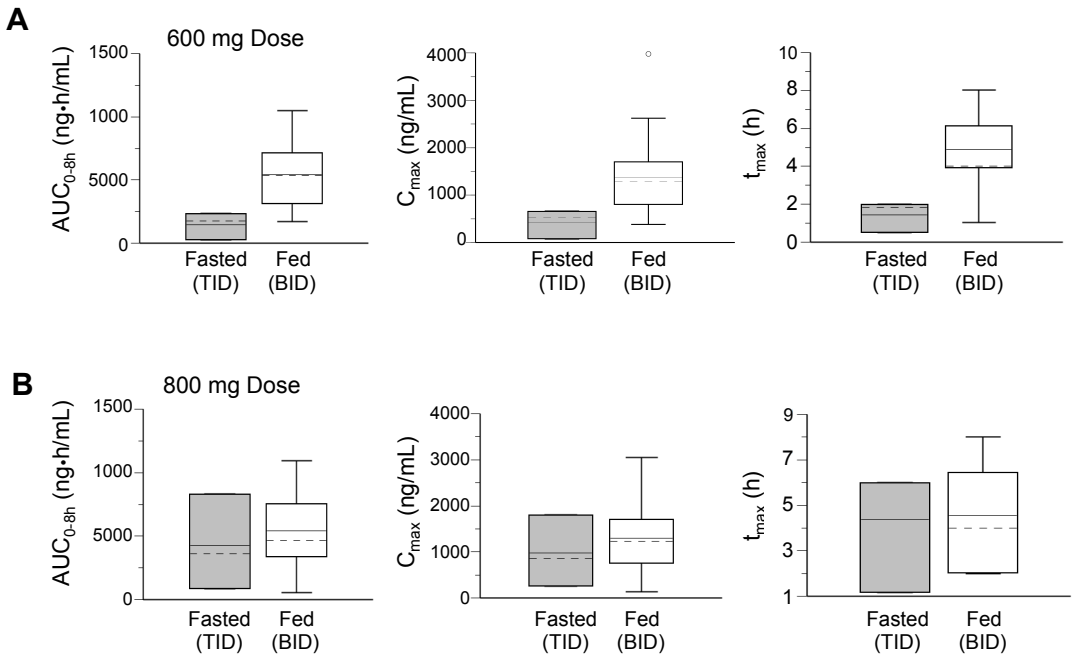

Figure S3. Telaglenastat Pharmacokinetics. $AUC_{0\text{-}8h}$, C_{max}, and T_{max} following single dose of (A) 600 or (B) 800 mg telaglenastat under fasted condition (in TID cohort) or fed condition (in BID cohort). Dashed line, median; solid line, arithmetic mean; ends of box, 25th and 75th percentiles. Whiskers show the lowest data value within 1.5 interquartile range (IQR) of the lower quartile and highest value within 1.5 IQR of upper quartile. Data values outside the whiskers are considered outliers.

Supplementary Methods

Full Eligibility Criteria

Eligible patients had to be ≥18 years of age, have ECOG performance status 0-1, measurable disease per RECIST v1.1 criteria,(32) and adequate organ function, with histologically-confirmed diagnosis of locally-advanced, inoperable, metastatic and/or treatment-refractory solid tumors for whom there are no available therapies that will confer clinical benefit adequate organ function (total bilirubin and ALT <1.5X the upper limit of normal; estimated or calculated creatinine clearance >50 mL/min according to the Cockcroft-Gault formula; absolute neutrophil count ≥1500/mm^3, hemoglobin ≥ 9.0 g/dL, and platelet count ≥ 100,000/mm^3).

Patients were not eligible if they were receiving concomitant warfarin, had any other current or previous malignancy within the past 3 years, or prior cytotoxic chemotherapy, tyrosine kinase inhibitor (or other targeted anti-cancer agent), radiation therapy, or hormone therapy within 14 days or 5 half-lives (whichever is longer) prior to the first day of treatment or immunotherapy, biologic agent, systemic steroid therapy, or unapproved investigational agent within 21 days prior to the first day of treatment. Patients were also excluded if they had unstable or inadequate cardiac function, past small bowel resection or gastric bypass surgery, major surgery within 28 days, or untreated brain metastases or CNS disease.

Study Treatment and Dose-limiting Toxicities

For dose escalation, patients were enrolled in a traditional 3+3 design. A minimum of 3 patients was assigned to each dose level. If 1 of 3 patients experienced a dose-limiting toxicity (DLT), the dose level would be expanded to include an additional 3 patients. With approval of the medical monitor, up to 4 more patients could be enrolled at any dose level that met the criteria for tolerability, as long as they had biopsy-accessible tumors and consented to 1 pre- and 1 post-treatment biopsy.

A third cohort of patients receiving TID fed dosing was planned but later omitted due to increased absorption of telaglenastat with food intake. The TID-fasted cohort had a starting dose of 100 mg, which was determined based on preclinical toxicology studies in rats and monkeys. The BID-fed cohort had a starting dose of 600 mg BID, with first dose taken with breakfast and the second dose in the evening with dinner. Alternative dose escalation schedules and dose reductions were also used in cases of prespecified adverse events (AEs).

For dose expansion, after identification of a RP2D, patients were enrolled into the single agent telaglenastat dose expansion part of the study, with disease-specific cohorts of at least 11 patients each with triple-negative breast cancer (TNBC), NSCLC (adenocarcinoma only), RCC, mesothelioma, fumarate hydratase (FH)-deficient tumors, succinate dehydrogenase (SDH)-deficient gastrointestinal stromal tumors (GIST), SDH-deficient non-GIST, non-glioma tumors with mutations in isocitrate dehydrogenase 1 or 2 (IDH1; IDH2), and tumors harbouring unequivocal amplifications (e.g., >5-fold) in the c-Myc gene. Most patients in the dose expansion cohorts received telaglenastat 600 mg BID, with the exception of one subcohort of RCC patients who received telaglenastat 800 mg BID. The dose expansion part of the trial used a Simon 2-Stage optimal design, in which 1 response per 11 response-evaluable

patients would trigger the expansion of each cohort to an additional 26 patients (37 response-evaluable patients total).

Telagleanastat was administered on a flat dosing basis, not adjusted for body weight or body surface area. Telaglenastat capsules were administered on 21-day cycles until disease progression, occurrence of any toxicity that, in the opinion of the investigator, warranted discontinuation or withdrawal for any reason.

Dose reductions were permitted during Cycle 1 only if a patient experienced a DLT. After Cycle 1, dose reductions or interruptions for AEs could take place at any time at the discretion of the investigator and medical monitor. Patients who experienced a DLT were not required to discontinue from the study as long as the toxicity had returned to grade ≤1 or baseline within 14 days, upon which patients could restart treatment at 1 dose level lower. Patients who did not recover within 14 days were withdrawn from the study. For non-hematologic or hematologic toxicities (other than changes in liver function tests), patients were to hold study drug and given supportive care for grade ≥2 toxicities (for baseline grade 2, worsening to grade 3). For grade 2 toxicities, patients could restart treatment at the same dose level upon resolution to grade ≤1 or baseline. For grades 3 or 4, patients were to reduce to the next lower dose level upon resolution to grade ≤1 or baseline. In liver function tests, grade 1 increases in ALT, AST, or bilirubin did not warrant dosing adjustments. For grade 2, telaglenastat was to be reduced to the next lower dose level. For grade ≥3, telaglenastat was to be held until resolution to grade ≤1, then resumed at the next lower dose level.

Endpoint Evaluations

The recommended phase 2 dose (RP2D) was selected based on the maximum tolerated dose (MTD), which was defined as the highest dose level with either no DLTs reported in 3 DLT-evaluable patients, or no more than 1 DLT reported in 6 DLT evaluable patients. In the event that MTD was not established, other considerations included: (1) proportionality of dose-exposure relationship; (2) magnitude of systemic inhibition of glutaminase in platelets (goal of achieving ≥90% inhibition at C_{min}); (3) overall safety and efficacy observations from this and concurrent clinical trials.

Secondary objectives included PK and anti-tumor activity (RECIST v1.1) of single agent telaglenastat. PK samples were collected on Cycle 1, Days 1 and 15 (and on Cycle 2 Day 1 for TID-fasted patients) at predose and postdose at 0.5, 1, 2, 4, 6, and 8 hours.

Exploratory analyses of biomarker and pharmacodynamics were conducted with patients who had biopsy-accessible lesions who agreed to submit tumor tissue had tumor biopsies at screening/baseline and during Cycle 2 Day 1. Samples were analysed for expression levels of genes considered possibly related to glutamine utilization in tumors and for immunohistochemical analysis of glutaminase and glutamine synthetase. Plasma samples were collected on day 1 (any cycle) and at end of treatment or disease progression for evaluation of biomarkers of inflammation and alterations in metabolism. Whole blood samples were collected from consenting patients at screening or scheduled visits for genetic sequencing and exploratory biomarker analysis.