First-in-human phase 1 trial evaluating safety, pharmacokinetics, and pharmacodynamics of NLRP3 inflammasome inhibitor, GDC-2394, in healthy volunteers

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SUPPLEMENTARY INFORMATION

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SUPPLEMENTARY METHODS

Randomization and blinding

The lead site pharmacist assigned eligible participants a unique identification number from the master randomization list, which ensured that the eligible participants received either GDC-2394 or placebo in either a 1:1 or 5:1 ratio in the SAD stage to allow for sentinel dosing, a 3:1 ratio in the MAD stage, and a 4:1 ratio in the FE stage.

Both participants and investigative site staff were blinded to treatment assignments, except for the unblinded pharmacist. The sponsor biostatistician was unblinded to the study treatment assignments, whereas other members on the safety monitoring committee (SMC) may have been unblinded for decision-making purposes. PK samples were collected from placebo-treated subjects to maintain blinding, but were not analyzed, and laboratory staff performing PK analyses were unblinded to treatment assignments to identify appropriate samples for analysis.

Screening, treatment, and follow-up periods

In the SAD stage, screening occurred in the 28 days before dosing, with admission to the CRU on Day –2. Participants stayed in the CRU for 72 h after dosing on Day 1 to monitor adverse events (AEs) and returned for a follow-up visit on Day 15.

In the MAD stage, screening occurred in the 28 days before dosing, with admission to the CRU on Day –2. Participants stayed in the CRU for the 7-day treatment period and received oral doses of GDC-2394 or placebo BID on Days 1 through 6 followed by a single oral dose on the morning of Day 7. Participants remained in the CRU for 72 h after the final dose and were then monitored during the 28-day follow-up period.

In the FE stage, in Period 1 (fasted period), admission to the CRU occurred on Day –2, dosing occurred on Day 1 after an overnight fast, and participants were assessed through Day 4 (washout period). In Period 2 (high-fat period), the same participants were readmitted to the CRU, received study drug after a high-fat breakfast on Day 1, were assessed through Day 4, discharged, and then followed through Day 15.

In the DDI stage, participants remained in the CRU from Day –1 through Day 11 (24 h after the final dose of GDC-2394), with a final follow-up on Day 38.

Fasting rules

Participants in the SAD stage and in the fasted period of the FE stage were required to fast for at least 8 hours before and for at least 4 hours after dosing. Subjects in the high-fat period of the FE stage were required to fast for at least 8 hours before having the pre-dose breakfast. In the MAD stage, for the morning dosing, participants were required to fast overnight for a minimum of 8 hours prior to dosing, and for a minimum of 4 hours postdose on PK-intensive days (Days 1 and 7) or for at least 1 hour postdose on other treatment days; for the evening dosing, participants were required to fast for a minimum of 4 hours predose and a minimum of 1 hour postdose. In the DDI stage, administration of GDC-2394 on Days 3–10 followed the same

fasting rules as in the MAD stage, and administration of midazolam on Days 1 and 10 followed the same fasting rules as in the SAD stage. For all stages, water was restricted for an hour before and after dosing with ~240 mL of water.

Dose-limiting adverse event (DLAE) definition and dose-escalation stopping criteria
An adverse event (AE) was considered a suspected DLAE if the AE met all of the following criteria:

- Occurred after initiation of study drug (GDC-2394 or placebo)
- Was moderate, severe, or potentially life threatening (i.e., Grades 2, 3, or 4 on the DAIDS toxicity grading scale)
- Was clinically significant as determined by the investigator or the safety monitoring committee (SMC)
- Was assessed as related to study drug by the investigator or the SMC

Any one of the following was a suspected DLAE if it was assessed as related to study drug by the investigator or the SMC:

- Symptomatic bradycardia or hypotension
- Grade ≥2 (≥10%) methemoglobinemia via venous blood co-oximetry or Grade 1 (5% to <10%) methemoglobinemia with symptoms (e.g., headache, shortness of breath, nausea, tachycardia, fatigue or lethargy, confusion or stupor, or loss of consciousness), regardless of severity, that did not have a clear alternative explanation
- Unexplained hemoglobin decline from baseline ≥3 g/dL or hemoglobin decline from baseline ≥2 g/dL with evidence of hemolysis
- Grade ≥2 elevation in ALT or AST (≥2.5x upper limit of normal [ULN])

Dose escalation within the SAD or MAD stage was suspended if any of the following stopping criteria were met:

- Three or more subjects within a cohort experienced confirmed DLAEs
- Two or more subjects within a cohort experienced confirmed DLAEs classified in the same primary system organ class and/or were considered to be similar types of AEs
- Two or more subjects within a cohort experienced a confirmed severe DLAE, defined as a Grade 3 event
- One subject experienced a confirmed potentially life-threatening DLAE, defined as a Grade 4 event
- A clinically significant group mean decrease in heart rate
- Other findings (any cohort, including food effect or DDI) that, at the discretion of the SMC or the investigator, indicate that dose escalation should be suspended

In the event that dose escalation was suspended, a cumulative review of all available safety data was conducted. PK data was analyzed and reviewed in cases where exposure information was required to make an adequate safety assessment and would impact dose-escalation decisions. Continued dosing and dose escalation may have resumed if, upon further review of clinical information by the SMC and investigator, it was jointly decided that the reported safety findings were not causally related to study drug (e.g., clearly related to an alternative cause).

Dose escalation may also have been suspended if a pattern of DLAEs emerged across cohorts that suggested a dose-response relationship in the frequency or severity of AEs.

Dose stopping rules: criteria for individuals

The investigator would have stopped study drug dosing for an individual (any cohort) for any one of the following reasons:

- Confirmed DLAE
- Positive pregnancy test result
- Concurrent illness or requirement for prohibited medication
- Upon the subject's request (withdrawal of consent)
- At the discretion of the investigator

The study site informed the sponsor of any individuals who met stopping criteria. For a subject who withdrew because of an active study drug-related AE, every effort was made to ensure the subject completed follow-up procedures.

Dose stopping rules: criteria for cohorts and for the study

The sponsor would have suspended dosing of all subjects within a cohort, as well as any other ongoing cohorts evaluating the same or higher dose levels, if any of the following criteria were met:

- Two or more subjects in a cohort experienced a GDC-2394–related, Grade ≥3 (severe), clinically significant, non-serious adverse reaction that was similar in nature or suggested a similar underlying cause
- A pattern of AEs was apparent that required suspension of further dosing at equivalent or higher exposures, in the opinion of the investigator or SMC
- A clinically significant pattern of toxicity was apparent in multiple subjects even if no individual subject was discontinued due to an AE, in the opinion of the investigator or sponsor

The sponsor would have suspended dosing of all subjects in the study if any of the following criteria were met:

- One or more subjects developed a GDC-2394–related serious AE
- Evidence of liver injury consistent with a potential Hy's Law case
- Sustained (at least two ECG measurements >30 minutes apart) QT interval corrected through use of Fridericia's formula (QTcF) that was >500 ms and >60 ms longer than the baseline value or sustained absolute QTcF that was >515 ms regardless of baseline value
- Episode of torsade de pointes without a clear alternative explanation
- Pattern of AEs that required suspension of further dosing in the study, in the opinion of the investigator or SMC
- Permanent cessation of dosing in two MAD cohorts due to GDC-2394–related safety concerns

The SMC, together with the investigator, would have convened and reviewed details of the case(s) meeting the above-listed cohort or study dosing stopping rules. If the SMC and the

investigator determined that stopping rules had not been met (e.g., a clear alternative cause for the AE had been identified and the event was determined to be unrelated to study drug) or if changes to the study conduct (e.g., inclusion/exclusion criteria or patient monitoring) could be identified that would have adequately protected the safety of remaining participants, then the SMC may have recommended continued dosing, pending approval by the investigator. Otherwise, dosing must have stopped.

Participants: exclusion criteria

Potential participants were excluded if they had received treatment with: an investigational therapy within 90 days or 5 half-lives (whichever was longer), any prescribed or over-the-counter drug or herbal remedies in the 14 days or 5 half-lives (whichever was longer), or a vaccine within 14 days prior to initiation of study drug. Additional exclusion criteria included: history of substance abuse, recent use of tobacco/nicotine products, or regular, frequent alcohol consumption; recent surgical procedure; known glucose-6-phosphate dehydrogenase (G6PD) deficiency or history of hemolysis or hemolytic anemia; positivity for hepatitis C virus antibody, hepatitis B surface antigen, or HIV antibody at screening; tuberculosis infection; malabsorption syndrome or other condition that would interfere with enteral absorption; a recent infection requiring antibiotics or any evidence of a current infection; a recent blood transfusion; a history of malignancy, clinically significant abnormal ECG, ventricular dysrhythmias; history of significant drug and/or food allergies; or any serious medical condition or abnormality in clinical laboratory tests that precluded their safe participation.

Participants were also excluded from the DDI stage if they had any known allergy or condition limiting midazolam administration; history of recent benzodiazepine therapy; consumption of grapefruit, pomegranate, pomelos, tangelos, exotic citrus fruits, oranges, star fruit, or any products containing these fruits within 72 hours of entry into the CRU; or treatment with CYP3A4 inhibitors or inducers within 4 weeks or 5 drug-elimination half-lives before study drug administration.

PK sample collection

Blood samples for PK analysis were collected in K2-EDTA collection tubes (Becton Dickinson, Franklin Lakes, NJ), and plasma was separated by centrifugation and stored frozen at -80°C until analysis. For the SAD stage and each period of the FE stage, blood samples were collected on Day 1 (predose, 15 min, 30 min, 1 h, 2 h, 4 h, 6 h, 8 h and 12 h postdose), Day 2 (24 h postdose), Day 3 (48 h postdose), Day 4 (72 h postdose), and at Day 15 clinic visit. In the MAD stage, sample collection occurred on Day 1 (same timepoints as in the SAD), predose on Days 2, 3 and 5, Day 7 (same timepoints as in the SAD and Day 1), on Day 8 (24 h after final dose), Day 9 (48 h after final dose), Day 10 (72 h postdose), and at the Day 14 clinic visit. In the DDI stage, sample collection for midazolam PK occurred on Day 1 (predose), Day 1 (15 min–20 h postdose); Day 2 (24 h postdose); Day 10 (predose), Day 10 (15 min–20 h postdose); Day 11 (24 h after the final midazolam dose). Additionally, plasma samples were analyzed for GDC-2394 PK in the mornings of Days 7–10 before dosing in the DDI stage to ensure that steady state had been achieved.

Urine samples for PK analysis were kept refrigerated during the collection period. After centrifugation of the collected samples, aliquots of the supernatants were stored frozen at -80°C until analysis. For the SAD stage and each period of the FE stage, urine samples were collected predose and at intervals of 0–12 h, 12–24 h, and 24–48 h after drug administration. For the MAD stage, urine samples were collected before Day 1 dosing and at intervals of 0–12 h and 12–24 h after Day 1 dosing on Days 1 and 2, and before Day 7 dosing and at 0–12 h, 12–24 h, and 24–48 h after Day 7 dosing on Days 7–9.

Pharmacodynamic assessments: sample collection and assay details

Whole blood samples for the SAD cohorts and each period of the FE stages were collected on Day 1 (predose, 1 h, 4 h, and 8 h postdose); Day 2 (24 h postdose), Day 3 (48 h postdose), Day 4 (72 h postdose), and at the Day 15 clinic visit. In the MAD cohorts, sample collection occurred on Day 1 (predose, 1 h, 4 h, 8 h, and 12 h postdose), predose on Days 2, 3, and 5, Day 7 (same as Day 1), Day 8 (24 h after final dose), Day 9 (48 h after final dose), Day 10 (72 h postdose), and at the Day 14 clinic visit. Whole blood was collected into sodium heparin Vacutainer tubes (Becton Dickinson, Franklin Lakes, NJ). Within 4 hours of collection, 935 mL of heparinized blood was aliquoted in duplicate into appropriate wells of a 48-well tissue culture microplate (Cat#3548, Corning, Glendale, AZ) at room temperature. To prime NLRP3, 200 ng/mL LPS from *E.coli* 0111:B4 (Cat#TLRL-3PELPS, InvivoGen, San Diego, CA) was added to each microplate well containing blood; microplates were then placed into a 5% CO_2 incubator at 37°C for 3 hours. Microplates were removed from the incubator, and 1.75 μM ATP (Cat#A7699, Sigma-Aldrich, St. Louis, MO) was added to each well. After an additional hour of incubation, plates were removed and centrifuged at 500 RCF for 10 minutes at room temperature. Plasma (supernatant) was then collected from each well and stored frozen at -80°C until analysis. IL-1β concentrations in the supernatant of unstimulated and stimulated whole blood samples were determined using the Quantikine enzyme-linked immunosorbent assay (Cat.#DLB50, ELISA; Bio-Techne, Minneapolis, MN) according to the manufacturer's protocol, by extrapolating from a standard curve using 4-parameter logistic regression analysis. IL-18 concentrations in a subsequent aliquot of the same plasma supernatant of unstimulated/stimulated samples were determined by ELISA. Briefly, a monoclonal mouse anti-human IL-18 antibody (Cat#D044-3, MBL, Woburn, MA) was immobilized onto 96-well microtiter plates (Cat#439454, Nunc, Waltham, MA) to capture IL-18. Detection was performed with a biotin-labeled, monoclonal rat anti-human IL-18 antibody (Cat#D045-6, MBL, Woburn, MA) and streptavidin-horseradish peroxidase (Cat#RPN4401V, Sigma-Aldrich, St. Louis, MO).

SUPPLEMENTARY RESULTS

Additional safety details for the SAD, MAD, and FE stages

During the SAD stage, 14 of 24 (58.3%) participants receiving single doses of GDC-2394 and 3 of 8 (37.5%) participants receiving placebo had at least one AE (Table 2; Table S5). There was no apparent correlation between AE incidence and dosing level. The most frequently reported AEs by system organ class were gastrointestinal disorders, occurring in 8 of 24 (33.3%) GDC-2394-treated participants and 2 of 8 (25.0%) of placebo-treated participants. The most frequently reported related AEs were nausea (GDC-2394, 3/24 (12.5%); placebo, 1/8 (12.5%)) and constipation (GDC-2394 2/24 (8.3%); placebo, 0%). Most AEs were mild in severity (n=16, 94.1%); 1 (5.9%) participant who received 450-mg GDC-2394 experienced a moderate AE of cyclic vomiting syndrome.

In the MAD stage, 8 of 12 (66.7%) participants receiving GDC-2394 and 2 of 4 (50.0%) participants receiving placebo experienced at least one AE (Table 2). The most frequently reported (≥10%) AEs (Table S6) were upper respiratory tract infection (GDC-2394, 3/12 (25.0%); placebo, 0%), dizziness (GDC-2394, 2/12 (16.7%); placebo, 0%), headache (GDC-2394, 2/12 (16.7%); placebo 0%), and abdominal distension (GDC-2394, 0%; placebo, 2/4 (50.0%)). 5 (31.2%) participants experienced 6 related AEs (GDC-2394, 4/12 (33.3%); placebo, 1/4 (25%)); the most frequently reported related AE was dizziness (GDC-2394, 2/12 (16.7%); placebo, 0%). Most (17/18 or 94.4%) AEs experienced by subjects receiving GDC-2394 in the MAD stage were mild; 1 (5.6%) AE of moderate ear infection was reported by a participant who received 300-mg BID GDC-2394 for 7 days. A severe AE of elevated liver function test on Day 7 was reported for a participant in the placebo group.

In the FE stage, 5 of 10 (50%) participants experienced at least one AE, but no AE was reported by more than one participant (Table 2; Table S7). 1 (10%) participant reported a related AE of insomnia. All AEs were mild.

Supplementary Safety Narratives

Subjects with hepatic abnormalities after receiving GDC-2394 in the DDI portion of the study

Participant 1, a 28-year-old white male, received study medication per protocol. He experienced an alanine aminotransferase (ALT) increase of 89 U/L (normal range 0-40 U/L) on day 9; ALT peaked at 113 U/L on day 13. The elevation of ALT was considered related to study drug. There were no concomitant elevations of aspartate aminotransferase (AST), alkaline phosphatase, or bilirubin, and there were no clinical symptoms or signs associated with the ALT elevation. The event was considered resolved on day 20.

Participant 2, a 62-year-old white male, received study medication per protocol and was discharged from the CRU on day 11. Between days 13–20, he reported fatigue, abdominal distension, eyelid hematoma, and headache. On day 20, laboratory workup showed ALT 351 U/L (normal range 0-40 U/L), AST 145 U/L (normal range 10-50 U/L) total bilirubin 51 µmol/L (normal range 2-20 µmol/L), direct bilirubin 36 µmol/L (normal range 0-5 µmol/L), and alkaline phosphatase 167 U/L (normal range 30-150 U/L). Physical examination over the next several days was remarkable for discoloration of the sclera, consistent with jaundice, and a soft, non-distended abdomen with mild tenderness in the right upper quadrant (RUQ), and he complained of pruritus. The urine was dark, and the urine dipstick test was positive for bilirubin (2+). Cytomegalovirus (CMV) serology (CMV IgG and IgM), Epstein-Barr virus viral capsid antigen (EBV VCA) IgM, hepatitis A IgM, hepatitis B surface antigen, hepatitis B core antibody, and hepatitis C antibody were negative. A liver ultrasound showed mild gallbladder thickening with a small amount of gallbladder sludge, and mildly thickened bile duct walls, but no evidence of obvious obstruction. Based on the timing as well as the absence of plausible alternative explanations, the events were considered DILI, related to GDC-2394. The event appeared to satisfy Hy's Law criteria.[TempleR] On day 35, total bilirubin had peaked at 225 µmol/L although ALT had decreased to 158 U/L. On day 36, he began receiving oral ursodeoxycholic acid which was continued through day 66. Laboratory parameters and clinical status improved during this time and DILI was considered resolved by day 87.

Participant 3, a 21-year-old white male, received study medication per protocol and was discharged from the CRU on day 11. Between days 18–23, he reported epigastric pain, dark urine, yellowing of the sclera and skin and widespread itching. On day 23, physical examination showed jaundice and dark urine, but there was no elicited abdominal tenderness. Laboratory workup showed ALT 335 U/L (normal range 0-40 U/L), AST 147 U/L (normal range 10-50 U/L) total bilirubin 61 µmol/L (normal range 2-20 µmol/L), direct bilirubin 35 µmol/L (normal range 0-5 µmol/L), and alkaline phosphatase 276 U/L (normal range 30-150 U/L). CMV serology (CMV IgG and IgM), EBV serology (EBV VCA IgG, EBV VCA IgM and EBV Epstein-Barr nuclear antigen (EBNA) antibody), hepatitis A IgM, hepatitis B surface antigen, hepatitis B surface antibody, hepatitis B core antibody and hepatitis C antibody were negative. A liver ultrasound was considered normal. By day 27, ALT had peaked at 504 U/L and began to normalize over the subsequent 2 weeks; other laboratories and physical findings also improved during this time. Based on the timing as well as the absence of plausible alternative explanations, the events

were considered DILI, related to GDC-2394. The event appeared to satisfy Hy's Law criteria[TempleR] and was considered resolved by day 60.

SUPPLEMENTARY REFERENCES

1. Temple R. Hy's law: predicting serious hepatotoxicity. *Pharmacoepidemiol Drug Saf*. 2006;15:241-243.

SUPPLEMENTARY TABLES

TABLE S1 Participant demographics and baseline characteristics in the SAD stage

	SAD C1 150 mg (n=6)	SAD C1 Placebo (n=2)	SAD C2 450 mg (n=6)	SAD C2 Placebo (n=2)	SAD C3 900 mg (n=6)	SAD C3 Placebo (n=2)	SAD C4 1800 mg (n=6)	SAD C4 Placebo (n=2)	All Subjects (N=32)
Age (yr), mean (SD)	28.8 (11.8)	43.0 (5.7)	31.0 (9.0)	42.0 (14.1)	28.0 (7.2)	31.5 (13.4)	28.5 (15.5)	32.5 (2.1)	31.1 (10.8)
Sex									
Male	2 (33.3%)	0	1 (16.7%)	1 (50.0%)	1 (16.7%)	1 (50.0%)	1 (16.7%)	1 (50.0%)	8 (25.0%)
Female	4 (66.7%)	2 (100.0%)	5 (83.3%)	1 (50.0%)	5 (83.3%)	1 (50.0%)	5 (83.3%)	1 (50.0%)	24 (75.0%)
Ethnicity									
Hispanic or Latino	0	0	0	0	0	0	0	1 (50.0%)	1 (3.1%)
Not Hispanic or Latino	6 (100.0%)	2 (100.0%)	6 (100.0%)	2 (100.0%)	6 (100.0%)	2 (100.0%)	6 (100.0%)	1 (50.0%)	31 (96.9%)
Race									
Asian	0	0	0	0	0	0	1 (16.7%)	0	1 (3.1%)
Black or African American	0	0	0	0	0	0	1 (16.7%)	0	1 (3.1%)
Native Hawaiian or other Pacific Islander	0	0	1 (16.7%)	0	0	0	0	0	1 (3.1%)
White	6 (100.0%)	2 (100.0%)	5 (83.3%)	2 (100.0%)	6 (100.0%)	2 (100.0%)	4 (66.7%)	2 (100.0%)	29 (90.6%)
Weight (kg), mean (SD)	79.7 (17.0)	68.8 (17.2)	81.2 (12.3)	98.2 (48.7)	75.6 (9.6)	66.2 (14.6)	63.7 (13.5)	80.2 (21.1)	75.9 (17.4)

TABLE S2 Participant demographics and baseline characteristics in the MAD stage

	MAD C1 300 mg BID (n=6)	MAD C1 Placebo (n=2)	MAD C2 900 mg BID (n=6)	MAD C2 Placebo (n=2)	All Subjects (N=16)
Age (y), mean (SD)	34.0 (10.4)	37.5 (17.7)	24.2 (3.5)	27.0 (9.9)	29.9 (9.8)
Sex, n (%)					
Male	5 (83.3%)	1 (50.0%)	1 (16.7%)	0	7 (43.8%)
Female	1 (16.7%)	1 (50.0%)	5 (83.3%)	2 (100.0%)	9 (56.2%)
Ethnicity, n (%)					
Hispanic or Latino	0	0	0	1 (50.0%)	1 (6.2%)
Not Hispanic or Latino	6 (100.0%)	2 (100.0%)	5 (83.3%)	1 (50.0%)	14 (87.5%)
Not Stated	0	0	1 (16.7%)	0	1 (6.2%)
Race, n (%)					
Asian	0	1 (50.0%)	0	0	1 (6.2%)
Black or African American	0	0	1 (16.7%)	0	1 (6.2%)
Native Hawaiian or other Pacific Islander	1 (16.7%)	0	0	0	1 (6.2%)
White	5 (83.3%)	1 (50.0%)	5 (83.3%)	2 (100.0%)	13 (81.2%)
Weight (kg), mean (SD)	82.7 (11.7)	70.8 (39.2)	74.2 (28.8)	50.4 (0.1)	74.0 (23.0)

TABLE S3 Participant demographics and baseline characteristics in the FE stage

	FE 600 mg (n=8)	FE Placebo (n=2)	All Subjects (N=10)
Age (yr), mean (SD)	34.4 (13.5)	49.5 (10.6)	37.4 (13.9)
Sex, n (%)	8	2	10
Male	6 (75.0%)	1 (50.0%)	7 (70.0%)
Female	2 (25.0%)	1 (50.0%)	3 (30.0%)
Ethnicity, n (%)			
Hispanic or Latino	1 (12.5%)	0	1 (10.0%)
Not Hispanic or Latino	7 (87.5%)	2 (100.0%)	9 (90.0%)
Race, n (%)			
Asian	1 (12.5%)	0	1 (10.0%)
Native Hawaiian or other Pacific Islander	1 (12.5%)	0	1 (10.0%)
White	6 (75.0%)	2 (100.0%)	8 (80.0%)
Weight (kg), mean (SD)	81.4 (20.7)	83.3 (11.4)	81.8 (18.7)

TABLE S4 Participant demographics and baseline characteristics in the DDI stage

	DDI 900 mg BID + 10 mg midazolam (n=9)
Age (y), mean (SD)	29.9 (12.3)
Sex, n (%)	
Male	6 (66.7%)
Female	3 (33.3%)
Ethnicity, n (%)	
Not Hispanic or Latino	9 (100.0%)
Race, n (%)	
White	7 (77.8%)
Multiple	1 (11.1%)
Unknown	1 (11.1%)
Weight (kg), mean (SD)	84.6 (19.2)

TABLE S5 Overview of AEs and most frequently reported AEs in the SAD stage

	SAD C1 150 mg (n=6)	SAD C1 Placebo (n=2)	SAD C2 450 mg (n=6)	SAD C2 Placebo (n=2)	SAD C3 900 mg (n=6)	SAD C3 Placebo (n=2)	SAD C4 1800 mg (n=6)	SAD C4 Placebo (n=2)	All GDC-2394 (n=24)	All Placebo (n=8)	All Subjects (N=32)
Total number of subjects with at least one AE	4 (66.7%)	2 (100.0%)	3 (50.0%)	1 (50.0%)	4 (66.7%)	0	3 (50.0%)	0	14 (58.3%)	3 (37.5%)	17 (53.1%)
Total number of AEs	5	4	5	1	8	0	4	0	22	5	27
Total number of deaths	0	0	0	0	0	0	0	0	0	0	0
Total number of subjects withdrawn from study due to an AE	0	0	0	0	0	0	0	0	0	0	0
Serious AE	0	0	0	0	0	0	0	0	0	0	0
Related serious AE	0	0	0	0	0	0	0	0	0	0	0
Related AE	3 (50.0%)	1 (50.0%)	1 (16.7%)	0	0	0	2 (33.3%)	0	6 (25.0%)	1 (12.5%)	7 (21.9%)
Grade 3-5 AE (at greatest intensity)	0	0	0	0	0	0	0	0	0	0	0
AEs (preferred terms) occurring in ≥2 subjects overall											
Nausea	1 (16.7%)	1 (50.0%)	1 (16.7%)	0	1 (16.7%)	0	1 (16.7%)	0	4 (16.7%)	1 (12.5%)	5 (15.6%)
Abdominal pain	0	1 (50.0%)	0	1 (50.0%)	1 (16.7%)	0	0	0	1 (4.2%)	2 (25.%)	3 (9.4%)
Constipation	2 (33.3%)	0	0	0	0	0	0	0	2 (8.3%)	0	2 (6.2%)
Dizziness	0	0	0	0	1 (16.7%)	0	1 (16.7%)	0	2 (8.3%	0	2 (6.2%)
Medical device site erythema	1 (16.7%)	0	0	0	1 (16.7%)	0	0	0	2 (8.3%	0	2 (6.2%)
Upper respiratory tract infection	0	0	1 (16.7%)	0	1 (16.7%)	0	0	0	2 (8.3%	0	2 (6.2%)
Oropharyngeal pain	1 (16.7%)	0	0	0	1 (16.7%)	0	0	0	2 (8.3%	0	2 (6.2%)
Phlebitis	0	0	1 (16.7%)	0	1 (16.7%)	0	0	0	2 (8.3%	0	2 (6.2%)

Investigator text for AEs was coded using MedDRA version 25.0.
Multiple occurrences of the same AE in one individual were counted only once except for 'Total number of AEs' row in which multiple occurrences of the same AE were counted separately. Multiple AEs for one individual that met the specified criteria were counted only once.

TABLE S6 Overview of AEs and most frequently reported AEs in the MAD stage

	MAD C1 300 mg BID (n=6)	MAD C1 PLB (n=2)	MAD C2 900 mg BID (n=6)	MAD C2 PLB (n=2)	All GDC-2394 (n=12)	All Placebo (n=4)	All Subjects (N=16)
Total number of subjects with at least one AE	4 (66.7%)	1 (50.0%)	4 (66.7%)	1 (50.0%)	8 (66.7%)	2 (50.0%)	10 (62.5%)
Total number of AEs	9	4	9	2	18	6	24
Total number of deaths	0	0	0	0	0	0	0
Total number of subjects withdrawn from study due to an AE	0	0	0	0	0	0	0
Serious AE	0	0	0	0	0	0	0
Related serious AE	0	0	0	0	0	0	0
Related AE	1 (16.7%)	0	3 (50.0%)	1 (50.0%)	4 (33.3%)	1 (25.0%)	5 (31.2%)
Grade 3-5 AE (at greatest intensity)	0	1 (50.0%)	0	0	0	1 (25.0%)	1 (6.2%)
AEs (preferred terms) occurring in ≥2 subjects overall							
Dizziness	0	0	2 (33.3%)	0	2 (16.7%)	0	2 (12.5%)
Headache	1 (16.7%)	0	1 (16.7%)	0	2 (16.7%	0	2 (12.5%)
Abdominal distension	0	1 (50.0%)	0	1 (50.0%)	0	2	2 (12.5%)
Upper respiratory tract infection	3 (50.0%)	0	0	0	3 (25.0%)	0	3 (18.8%)

TABLE S7 Overview of AEs in the FE stage

	600 mg (n=8)	Placebo (n=2)	All Subjects (N=10)
Total number of subjects with at least one AE	3 (37.5%)	2 (100.0%)	5 (50.0%)
Total number of AEs	3	3	6
Total number of deaths	0	0	0
Total number of subjects withdrawn from study due to an AE	0	0	0
Serious AE	0	0	0
Related serious AE	0	0	0
Related AE	1 (12.5%)	0	1 (10.0%)
Grade 3-5 AE (at greatest intensity)	0	0	0
AEs (preferred terms)	3	3	6
Folliculitis	1 (12.5%)	0	1 (10.0%)
Rhinitis	0	1 (50.0%)	1 (10.0%)
Skin laceration	1 (12.5%)	0	1 (10.0%)
Insomnia	1 (12.5%)	0	1 (10.0%)
Erythema	0	1 (50.0%)	1 (10.0%)
Phlebitis	0	1 (50.0%)	1 (10.0%)

TABLE S8 Overview of AEs and most frequently reported AEs in the DDI stage

	900 mg BID + 5 mg midazolam (N=9)
Total number of subjects with at least one AE	9 (100.0%)
Total number of AEs	36
Total number of deaths	0
Total number of subjects withdrawn from study due to an AE	0
Serious AE	2 (22.2%)
Related serious AE	2 (22.2%)
Related AE	7 (77.8%)
Grade 3-5 AE (at greatest intensity)	2 (22.2%)
AEs (preferred terms) occurring in ≥2 subjects overall	
Fatigue	3 (33.3%)
Headache	3 (33.3%)
Drug-induced liver injury	2 (22.2%)
Contusion	2 (22.2%)
Rash	2 (22.2%)

TABLE S9 Predose GDC-2394 and indacenyl amine plasma levels in the DDI cohort, Days 7–10

		Analyte	
Visit	**Statistic**	**GDC-2394 (ng/mL)**	**Indacenyl amine (ng/mL)**
D7 predose	Mean (SD)	5780 (2210)	70.0 (19.8)
	Range	3120–8810	34.4–87.4
D8 predose	Mean (SD)	5220 (2530)	84.3 (37.8)
	Range	2740–10400	42.4–147
D9 predose	Mean (SD)	5170 (2000)	84.1 (32.2)
	Range	2690–8540	42.2–153
D10 predose	Mean (SD)	4300 (1380)	66.5 (24.8)
	Range	2600–6600	36.2–102

TABLE S10 Summary of urinary PK parameters for GDC-2394 in the SAD and FE stages

Treatment	Statistic	$Ae_{(0\_48h)}$ (mg)	$Fe_{(0-48h)}$	$CL_{R(0-48h)}$ (mL/hr)
150 mg (n=6)	Mean	24.3	0.162	439
	SD	9.3	0.062	108
	CV%	38.4	38.4	24.7
450 mg (n=6)	Mean	81.0	0.180	604
	SD	9.5	0.021	124
	CV%	11.8	11.8	20.5
900 mg (n=6)	Mean	163	0.181	538
	SD	31.5	0.035	131
	CV%	19.3	19.3	24.3
1800 mg (n=6)	Mean	247	0.137	348
	SD	67.9	0.038	113
	CV%	27.5	27.5	32.5
600 mg (fasted; n=8)	Mean	74.8	0.125	442
	SD	7.7	0.013	61.5
	CV%	10.2	10.2	13.9
600 mg (high-fat; n=8)	Mean	71.4	0.119	499
	SD	7.4	0.012	137
	CV%	10.4	10.4	27.4

$Ae_{(0-48h)}$, amount excreted unchanged in urine from 0–48 h; $CL_{R(0-48h)}$, renal clearance, calculated from $Ae_{(0-48h)}/AUC_{0-48}$; $Fe_{(0-48h)}$, fraction of dose recovered in urine, calculated as $Ae_{(0-48h)}$ divided by the dose

TABLE S11 Summary of urinary PK parameters for GDC-2394 on Day 7 in the MAD stage

Treatment	Statistic	$Ae_{(0\_12h)}$ (mg)	$Fe_{(0-12h)}$	$CL_{R(0-12h)}$ (mL/hr)
300 mg BID (n=6)	Mean	44.5	0.148	486
	SD	5.8	0.019	65.7
	CV%	13.1	13.1	13.5
900 mg BID (n=6)	Mean	111	0.123	340
	SD	53.0	0.059	162
	CV%	47.9	47.9	47.8

$Ae_{(0-12h)}$, amount excreted unchanged in urine from 0-12 h; $CL_{R(0-12h)}$, renal clearance, calculated from $Ae_{(0-12h)}/AUC_{0-12}$; $Fe_{(0-12h)}$, fraction of dose recovered in urine, calculated as $Ae_{(0-12h)}$ divided by the dose

TABLE S12 Indacenyl amine PK parameters after treatment with GDC-2394 in the SAD stage

Treatment	Statistic	$T_{1/2}$ (hr)	C_{max} (ng/mL)	$AUC_{0\text{-last}}$ (hr*ng/mL)	$AUC_{0\text{-inf}}$ (hr*ng/mL)	MR_{Cmax}	$MR_{AUC0\text{-last}}$	$MR_{AUC0\text{-inf}}$
150 mg (n=6)	Mean	35.9	5.8	133	153	0.002	0.006	0.007
	SD	25.6	2.3	61.0	61.4	0.001	0.002	0.002
	CV%	71.3	38.8	45.7	40.1	46.2	34.5	29.3
450 mg (n=6)	Mean	35.9	21.8	455	500	0.003	0.009	0.010
	SD	20.5	13.4	267	286	0.003	0.007	0.008
	CV%	57.1	61.7	58.6	57.1	100.1	76.5	75.7
900 mg (N=6)	Mean	40.0	19.9	931	987	0.001	0.008	0.008
	SD	20.0	5.2	421	395	0.000	0.005	0.005
	CV%	49.9	26.2	45.2	40.0	17.0	63.2	58.8
1800 mg (n=6)	Mean	40.4	68.6	2460	2610	0.002	0.008	0.009
	SD	23.3	28.6	1060	1010	0.000	0.003	0.002
	CV%	57.8	41.7	43.1	38.5	27.7	32.2	27.3

$AUC_{0\text{-inf}}$, area under the concentration-time curve from time 0 to infinity; $AUC_{0\text{-last}}$, AUC from time 0 to last quantifiable concentration; C_{max}, maximum plasma concentration; MR_{AUC}: metabolite:parent AUC ratio; MR_{Cmax}: metabolite:parent Cmax ratio; $T_{1/2}$, terminal half-life;

TABLE S13 Indacenyl amine PK parameters after treatment with GDC-2394 in the MAD stage, Day 7

Treatment	Statistic	$T_{1/2}$ (hr)	C_{max} (ng/mL)	C_{min} (ng/mL)	$AUC_{0\text{-tau}}$ (hr*ng/mL)	AR (AUC)	MR_{Cmax}	$MR_{AUC0\text{-tau}}$
300 mg BID (n=6)	Mean	49.2	19.9	13.9	200	4.3	0.003	0.005
	SD	16.9	4.2	3.1	40.2	0.60	0.001	0.001
	CV%	34.3	21.3	22.0	20.1	13.9	23.9	18.2
900 mg BID (n=6)	Mean	47.3	120	87.1	1190	6.1	0.006	0.009
	SD	21.8	26.2	23.4	247	1.14	0.002	0.002
	CV%	46.1	21.9	26.9	20.9	18.6	29.5	24.6

AR (AUC), accumulation ratio based on AUC; $AUC_{0\text{-tau}}$, AUC from beginning to end of a dosing interval, tau; C_{max}, maximum plasma concentration; C_{min}, minimum plasma concentration; $MR_{AUC0\text{-tau}}$, metabolite:parent $AUC_{0\text{-tau}}$ ratio; MR_{Cmax}, metabolite:parent C_{max} ratio; $T_{1/2}$, terminal half-life

TABLE S14 Percent IL-1β and IL-18 inhibition relative to baseline after LPS/ATP stimulation of whole blood samples in the SAD stage

		GDC-2394				
	Timepoint (h)	**150 mg (n=6)**	**450 mg (n=6)**	**900 mg (n=6)**	**1800 mg (n=6)**	**Placebo (n=8)**
IL-1β inhibition (%), mean (SD)	1	92.5 (14.4)	99.0 (0.5)	99.7 (0.5)	99.9 (0.1)	9.2 (62.5)
	4	99.4 (0.3)	99.0 (0.6)	99.6 (0.5)	99.9 (0.1)	-32.2 (61.4)
	8	97.1 (1.9)	99.0 (0.6)	99.4 (0.4)	99.8 (0.2)	-41.9 (100.2)
	24	70.2 (9.5)	89.2 (4.8)	84.1 (11.0)	98.9 (0.6)	-65.0 (135.7)
	48	52.3 (34.1)	-45.5 (53.8)	48.4 (17.3)	72.1 (30.4)	-44.6 (135.1)
	72	6.3 (36.7)	2.0 (25.0)	27.4 (28.6)	45.9 (22.2)	-13.5 (97.2)
	336	7.9 (36.3)	-11.1 (98.5)	6.0 (41.2)	40.9 (44.5)	31.3 (35.4)
IL-18 inhibition (%),[a] mean (SD)	1	94.1 (13.6)	94.0 (15.3)	99.1 (1.8)	98.2 (4.2)	16.0 (48.2)
	4	99.4 (1.8)	102.0 (30.3)	105.2 (16.5)	99.6 (1.4)	-25.7 (59.1)
	8	94.4 (10.6)	134.3 (85.5)	107.7 (24.9)	99.2 (2.0)	-42.1 (99.7)
	24	78.1 (15.1)	84.6 (25.1)	74.9 (28.2)	101.5 (5.3)	-42.9 (121.8)
	48	53.9 (30.6)	-45.8 (57.4)	50.7 (15.5)	69.6 (30.4)	-45.8 (127.1)
	72	-4.2 (30.5)	17.0 (29.2)	42.1 (20.8)	48.0 (19.4)	0.2 (73.1)
	336	13.7 (40.6)	-48.7 (161.8)	19.4 (39.4)	35.5 (56.5)	28.9 (40.5)

[a]Percent IL-18 inhibition was adjusted for circulating levels of IL-18.

TABLE S15 Percent IL-1β and IL-18 inhibition relative to baseline after LPS/ATP stimulation of whole blood samples during the dosing period in the MAD stage

		GDC-2394		Placebo (n =4)
	Timepoint (h)	**300 mg BID (n=6)**	**900 mg BID (n=6)**	
IL-1β inhibition (%), mean (SD)	1	99.4 (0.5)	99.8 (0.2)	3.2 (15.1)
	4	99.5 (0.2)	99.8 (0.1)	29.2 (41.6)
	8	99.0 (0.5)	99.7 (0.2)	-6.2 (21.4)
	12	95.4 (3.0)	99.5 (0.5)	-39.3 (48.6)
	24	95.7 (3.2)	99.4 (0.4)	-12.7 (9.5)
	48	97.9 (1.5)	99.5 (0.3)	10.5 (33.5)
	96	97.7 (1.2)	99.4 (0.5)	25.4 (24.9)
	144	96.2 (3.1)	99.4 (0.2)	-1.4 (12.9)
	145	99.7 (0.2)	99.9 (0.1)	1.9 (16.2)
	148	99.7 (0.1)	99.9 (0.1)	18.5 (46.7)
	152	98.9 (0.7)	99.8 (0.2)	-1.2 (23.3)
	156	95.7 (4.5)	99.3 (0.8)	-8.7 (40.1)
IL-18 inhibition (%),[a] mean (SD)	1	94.2 (12.3)	99.6 (1.5)	15.7 (17.9)
	4	95.4 (13.2)	100.3 (1.6)	34.8 (35.9)
	8	94.2 (9.4)	100.7 (1.2)	-9.2 (12.9)
	12	87.2 (22.1)	98.1 (1.7)	-52.6 (84.3)
	24	91.5 (3.8)	98.5 (0.4)	-15.6 (39.8)
	48	102.4 (9.2)	99.7 (0.9)	13.5 (23.5)
	96	96.9 (3.5)	99.6 (0.9)	11.7 (43.0)
	144	93.4 (3.1)	99.5 (0.7)	-3.5 (31.9)
	145	93.5 (6.5)	101.6 (2.1)	-1.7 (15.7)
	148	99.6 (4.4)	100.8 (2.0)	29.1 (41.6)
	152	97.8 (1.3)	100.7 (2.4)	-7.1 (22.8)
	156	95.8 (1.6)	99.0 (3.4)	3.4 (25.0)

[a]Percent IL-18 inhibition was adjusted for circulating levels of IL-18.

SUPPLEMENTARY FIGURES

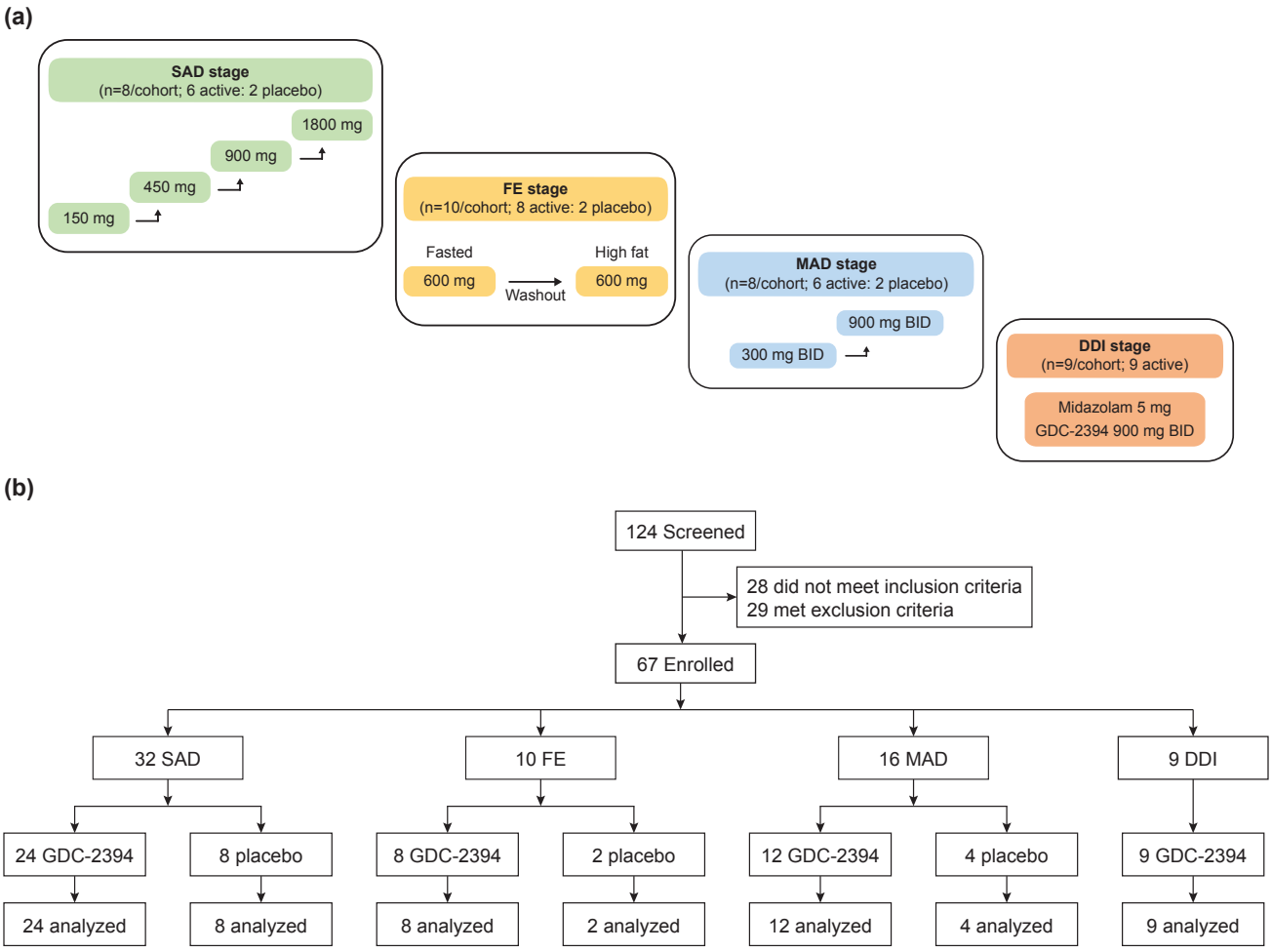

FIGURE S1 (a) Study design and (b) study flow diagram. DDI, drug-drug interaction; FE, food effect; MAD, multiple ascending dose; SAD, single ascending dose

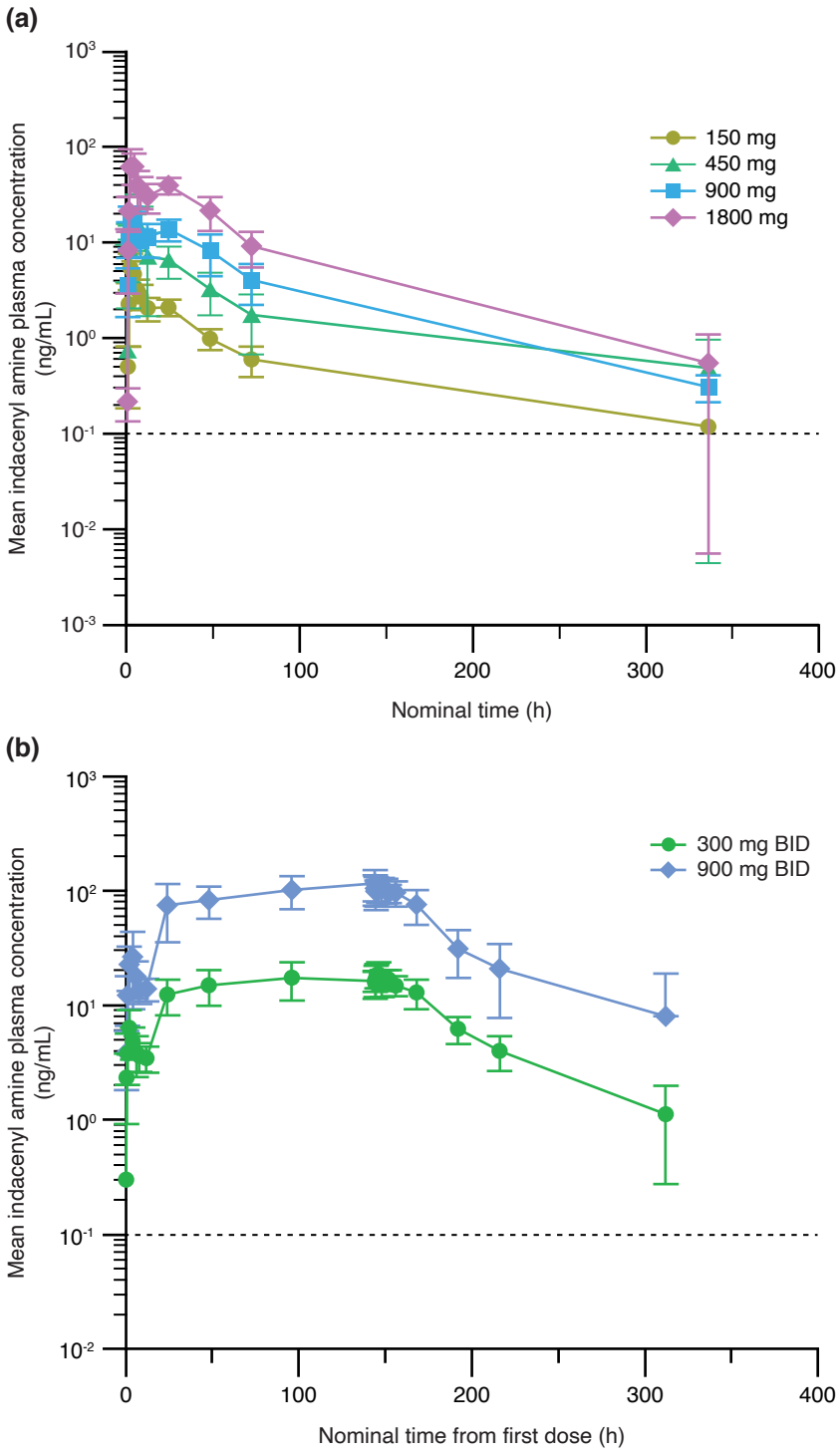

FIGURE S2 Mean plasma concentration-versus-time profile of indacenyl amine in the (a) SAD (150 mg [n=6], 450 mg [n=6], 900 mg [n=6], 1800 mg [n=6]) and (b) MAD stages (300 mg BID [n=6], 900 mg BID [n=6]). Error bars represent the standard deviation. Dashed lines represent LLOQs.