SUPPLEMENTAL APPENDIX

A Phase 1 Trial of Itacitinib, a Selective JAK1 Inhibitor, in Patients With Acute Graft-Versus-Host Disease

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Supplemental Methods

Assessments

Responses were assessed in accordance with standardized response criteria by the treating physician and reviewed and confirmed by the study center principal investigator. Duration of response was determined based on progression, as defined per Center for International Blood and Marrow Transplant Research (CIBMTR) criteria, as requirement of additional systemic therapy (including corticosteroid doses in excess of those used on Day 1) or death. In accordance with CIBMTR criteria, graft-versus-host disease (GVHD) flares were not taken into consideration of response. Dose-limiting toxicities were defined as any of the following up to and including Day 28: grade ≥3 (per Common Terminology Criteria for Adverse Events v4.03 criteria) nonhematologic treatment-related toxicity; clinically significant grade ≥3 clinical chemistry abnormality; grade 4 neutropenia for >7 days or a ≥90% decrease in absolute neutrophil count from baseline related to study treatment; platelet count $<10 \times 10^9$/L that did not recover after 2 weeks without transfusion support and with evidence of hypocellularity; and secondary graft failure (>95% recipient cells any time after engraftment with no signs of relapse, or retransplantation because of secondary neutropenia [$<0.5 \times 10^9$/L] and/or thrombocytopenia [$<30 \times 10^9$/L]).

Plasma samples were analyzed for itacitinib using a validated liquid chromatography–tandem mass spectrometry assay (Incyte Corporation, Wilmington, DE). PK parameters were calculated using standard noncompartmental (model-independent) analysis (WinNonlin® version 8.0, Certara, Princeton, NJ).

Biomarker Analysis

For the multiplex assay, test cartridges were primed with samples from each patient to test each analyte in a separate channel using their respective antibodies. Standard curves were provided by the manufacturer. Replicate values within 10% CV of the mean were included.

Statistical Analysis

Patients were excluded from the steady state pharmacokinetic (PK) analysis if sampling time points were missing, if they missed the last dose before PK assessment, or if the time of last dose was not available. For other analyses, cumulative incidence functions of nonrelapse-related mortality (NRM) and relapse mortality were estimated using Gray's method; patients with relapse-related mortality were treated as competing risks, and those without observed mortality were censored at their last date known to be alive. Cumulative incidence rates of NRM, relapse-related mortality, and primary disease relapse rate at 6 months were estimated with 90% CI. Incidence of secondary graft failure, acute graft-versus-host disease flares through Day 100 (defined as progression after initial complete response or partial response that required re-escalation of corticosteroid or other topical/systemic therapy), and chronic graft-versus-host disease (GVHD) at 6 and 12 months were determined. Cumulative corticosteroid dose on Days 28, 56, 100, and 180; frequency of GVHD stages by organ on Days 28, 56, and 100; and immunophenotyping were calculated with summary statistics. GraphPad Prism (San Diego, CA) was used for statistical analysis of the biomarker

data; statistical differences between groups were determined using an unpaired *t* test and one-way analysis of variance ($P<0.05$).

Supplemental Results

Efficacy

At a median (range) follow-up time of 105.0 (29–827) days for the 200-mg group and 136.5 (61–841) days for the 300-mg group, median duration of response was not reached for either group (200-mg group 95% CI lower limit, 63 days; 300-mg group 95% CI lower limit, 71 days).

Pharmacokinetics

The geometric mean AUC_{0-T} was higher in patients receiving potent CYP3A4 inhibitors versus those not receiving CYP3A4 inhibitors or taking a mild inhibitor for both 200-mg (1.7-fold; 6250 nM•h vs 3720 nM•h, respectively) and 300-mg groups (2.9-fold; 6720 nM•h vs 2320 nM•h, respectively).

Two patients who had stage 4 lower gastrointestinal (GI) GVHD had an area under the curve (AUC_{0-T}) of 2540 nM·h and 18,450 nM·h. This AUC_{0-T} of 18,450 nM·h was approximately 4-fold higher than the geometric mean exposure for all other patients (4108 nM·h); this patient was receiving concomitant CYP3A4 inhibitors fluconazole and posaconazole. Itacitinib exposures were similar between patients with or without upper GI tract GVHD involvement (**Supplemental Figure 5**). The range of maximum concentration (231–1280 nM) and AUC_{0-T} (4880–9380 nM·h) for patients with liver GVHD was similar to the geometric mean itacitinib exposure in all other patients (**Supplemental Figure 6**).

Exposures for patients stratified by the presence or absence of potent CYP3A4 inhibitors were similar, with a great degree of overlap between patients with and without the various types of organ involvement (**Supplemental Table 6**). Patients receiving potent CYP3A4 inhibitors showed an approximate 2-fold increase in itacitinib exposure compared with patients who were not receiving CYP3A4 inhibitors.

Biomarker Analysis

Mean (SEM) levels of suppression of tumorigenicity 2 (ST2) were significantly decreased at Day 56 (159.8 [66.3] vs 363.2 [54.1] ng/mL; *P*=0.026; n=11) and Day 100 (80.3 [35.8] vs 363.2 [54.1] ng/mL; *P*=0.010; n=6) among responders versus baseline levels, while no significant changes were observed in the other biomarkers examined for either group (**Supplemental Table 7**). Nonresponders generally had higher levels of ST2, tumor necrosis factor receptor 1 (TNFR1), and regenerating islet-derived protein 3 alpha precursor (REG3A) compared with responders at most time points, but the only on-treatment values of significance were at Day 7 for TNFR1 (6.8 [2.1] vs 2.2 [0.2] ng/mL; *P*=0.001), REG3A (39.5 [19.6] vs 11.1 [3.1]; *P*=0.025) and ST2 (1385 [1011] vs 263.5 [32.3] ng/mL; *P*=0.051) where the difference bordered on significance. Only a single patient from the nonresponder group was evaluable for biomarker analysis on Days 56 and 100.

Supplemental Tables

Supplemental Table 1. Response definitions

Response	Definition
CR	• CIBMTR score of 0 in all evaluable organs with no intervening additional therapy for an earlier progression, PR, or NR
VGPR	• Skin: no rash, or residual erythematous rash involving <25% of the body surface, without bullae (residual faint erythema and hyperpigmentation excluded) • Liver: total serum bilirubin concentration <2 mg/dL or <25% of baseline at enrollment • GI tract: tolerating food or enteral feeding, predominantly formed stools, no overt gastrointestinal bleeding or abdominal cramping, no more than occasional nausea or vomiting
PR	• Improvement in ≥1 organ involved with GVHD symptoms without progression in others, with no intervening additional therapy for an earlier progression, PR, or NR
PD	• Deterioration in ≥1 organ without any improvement in others
MR	• Improvement in ≥1 organ with deterioration in another organ manifesting symptoms of GVHD or development of symptoms of GVHD in a new organ
NR	• Absence of any improvement or progression as defined or need of secondary therapy (including need to re-escalate steroid dose to ≥2.5 mg/kg/d of prednisone or methylprednisolone equivalent of 2 mg/kg/d)

CR, complete response; MR, mixed response; NR, no response; PD, progressive disease; PR, partial response; and VGPR, very good partial response.

Supplemental Table 2. Patient disposition

	Itacitinib		
	200 mg n=14	300 mg n=16	Total N=30
Treated, n (%)	14 (100)	15 (93.8)	29 (96.7)
Treatment duration, median (range), d*	76.0 (5–291)	61.0 (5–817)	70.0 (5–817)
Discontinued treatment, n (%)	14 (100)	16 (100)	30 (100)
Adverse event	8 (57.1)	7 (43.8)[†]	15 (50.0)
Physician decision	3 (21.4)[‡]	5 (31.3)[§]	8 (26.7)
Progressive disease	1 (7.1)	3 (18.8)	4 (13.3)
Death	2 (14.3)	1 (6.3)	3 (10.0)[¶]
Discontinued from study, n (%)	14 (100)	16 (100)	30 (100)
Death	9 (64.3)	8 (50.0)[†]	17 (56.7)
Study terminated by sponsor[‖]	3 (21.4)	7 (43.8)	10 (33.3)
Withdrawal by patient	2 (14.3)	0	2 (6.7)
Other	0	1 (6.3)	1 (3.3)
Dose interruption due to adverse events, n (%)*	8 (57.1)	11 (73.3)	19 (65.5)
Dose reduction due to adverse events, n (%)*	1 (7.1)	3 (20.0)	4 (13.8)

* Data are for 29 patients in total because 1 patient in the 300-mg dose group did not receive study drug.
[†] One patient randomized to itacitinib 300 mg experienced an adverse event prior to any study treatment and did not receive itacitinib (listed as discontinued treatment owing to adverse event) and later died (listed as discontinued from study owing to death); this patient was not included in safety and efficacy analyses.
[‡] Reasons include tapering of GVHD medications, not responding to study drug, and progressive disease (n=1, each).
[§] Reasons include GVHD symptoms resolved, GVHD skin symptoms worsened, adequate response and patient moved out of state, tapered off study medication, and improved GVHD and pancytopenia (n=1, each).
[¶] Primary causes of death included sepsis (n=2) and multi-organ failure (n=1).
[‖] Patients had completed treatment and survival follow-up assessments.

Supplemental Table 3. Itacitinib-related TEAEs and infections occurring in >1 patient

	Itacitinib			
	200 mg, n=14		300 mg, n=15	
Itacitinib-related TEAE, n (%)	Grade 3/4	All grades	Grade 3/4	All grades
Nonhematologic				
Sepsis	1 (7.1)	1 (7.1)	2 (13.3)	2 (13.3)
Increased ALT	0	1 (7.1)	0	1 (6.7)
Increased blood creatinine	1 (7.1)	1 (7.1)	0	1 (6.7)
CMV viremia	0	0	1 (6.7)	2 (13.3)
Fatigue	0	0	0	2 (13.3)
Peripheral edema	0	0	0	2 (13.3)
Hematologic				
Anemia	2 (14.3)	2 (14.3)	3 (20.0)	4 (26.7)
Decreased platelet count	2 (14.3)	2 (14.3)	3 (20.0)	4 (26.7)
Thrombocytopenia	1 (7.1)	2 (14.3)	2 (13.3)	3 (20.0)
Decreased neutrophil count	0	1 (7.1)	2 (13.3)	2 (13.3)
Neutropenia	0	1 (7.1)	1 (6.7)	2 (13.3)
Pancytopenia	2 (14.3)	2 (14.3)	0	0
Decreased WBC count	0	0	1 (6.7)	2 (13.3)

ALT indicates alanine aminotransferase; CMV, cytomegalovirus; TEAE, treatment-emergent adverse event; and WBC, white blood cell.

Supplemental Table 4. Causes of death on study

Itacitinib dose	Primary cause of death	Study day	Relation to study treatment
200 mg	Malignancy relapse	57	NA
200 mg	AE (multi-organ failure)	10	None
200 mg	AE (GVHD, GI bleeding, suspected infection)	88	Related to corticosteroids
200 mg	AE (acute hypoxemic respiratory failure)	114	None
200 mg	Malignancy relapse	498	NA
200 mg	Infection (fungal pneumonia)	192	
200 mg	AE (sepsis)	16	Related to corticosteroids
200 mg	AE (acute respiratory distress syndrome and hematochezia)	92	None
200 mg	Sepsis	283	NA
300 mg	Cardiac arrest, sepsis	209	NA
300 mg	AE (multi-organ failure)	11	None
300 mg	AE (multi-organ failure)	35	None
300 mg	AE (sepsis)	6	None
300 mg	Hepatorenal syndrome	115	NA
300 mg	AE (acute respiratory distress syndrome)	103	None
300 mg	AE* (gastrointestinal bleed)	123	None

NA indicates not applicable.

* Adverse event was not considered treatment emergent as it occurred >30 days after the last dose of study drug.

Supplemental Table 5. Overall response by organ involvement at enrollment

	Patients with treatment-naive aGVHD (n=12)*			
Response, n (%)	**Skin, n=4**	**Liver, n=0**	**Upper GI, n=5**	**Lower GI, n=6**
CR	4 (100.0)	0	4 (80.0)	4 (66.7)
VGPR	0	0	0	0
PR	0	0	0	0
Overall response	4 (100.0)	0	4 (80.0)	4 (66.7)
	Patients with steroid-refractory aGVHD (n=17)*			
Response, n (%)	**Skin, n=9**	**Liver, n=5**	**Upper GI, n=4**	**Lower GI, n=11**
CR	1 (11.1)	0	1 (25.0)	2 (18.2)
VGPR	1 (11.1)	0	0	0
PR	5 (55.6)	1 (20.0)	1 (25.0)	5 (45.5)
Overall response	7 (77.8)	1 (20.0)	2 (50.0)	7 (63.6)

* Each patient could have >1 organ involved at enrollment.

Supplemental Table 6. Itacitinib plasma exposures for patients with and withoutGVHD organ involvement

	AUC_{0-T}, nM·h					
	Upper GI		Lower GI		Liver	
	No	Yes	No	Yes	No	Yes
No or mild CYP3A4 inhibitors	3320±1180 (n=8)	4110±3850 (n=4)	3120±1590 (n=6)	4040±2850 (n=6)	3460±2320 (n=11)	4880 (n=1)
Potent CYP3A4 inhibitors	8150±4900 (n=9)	7070±6210 (n=3)	7100±4650 (n=4)	8270±5390 (n=8)	7780±5800 (n=9)	8200±1080 (n=3)

Data are shown as mean ± SD.

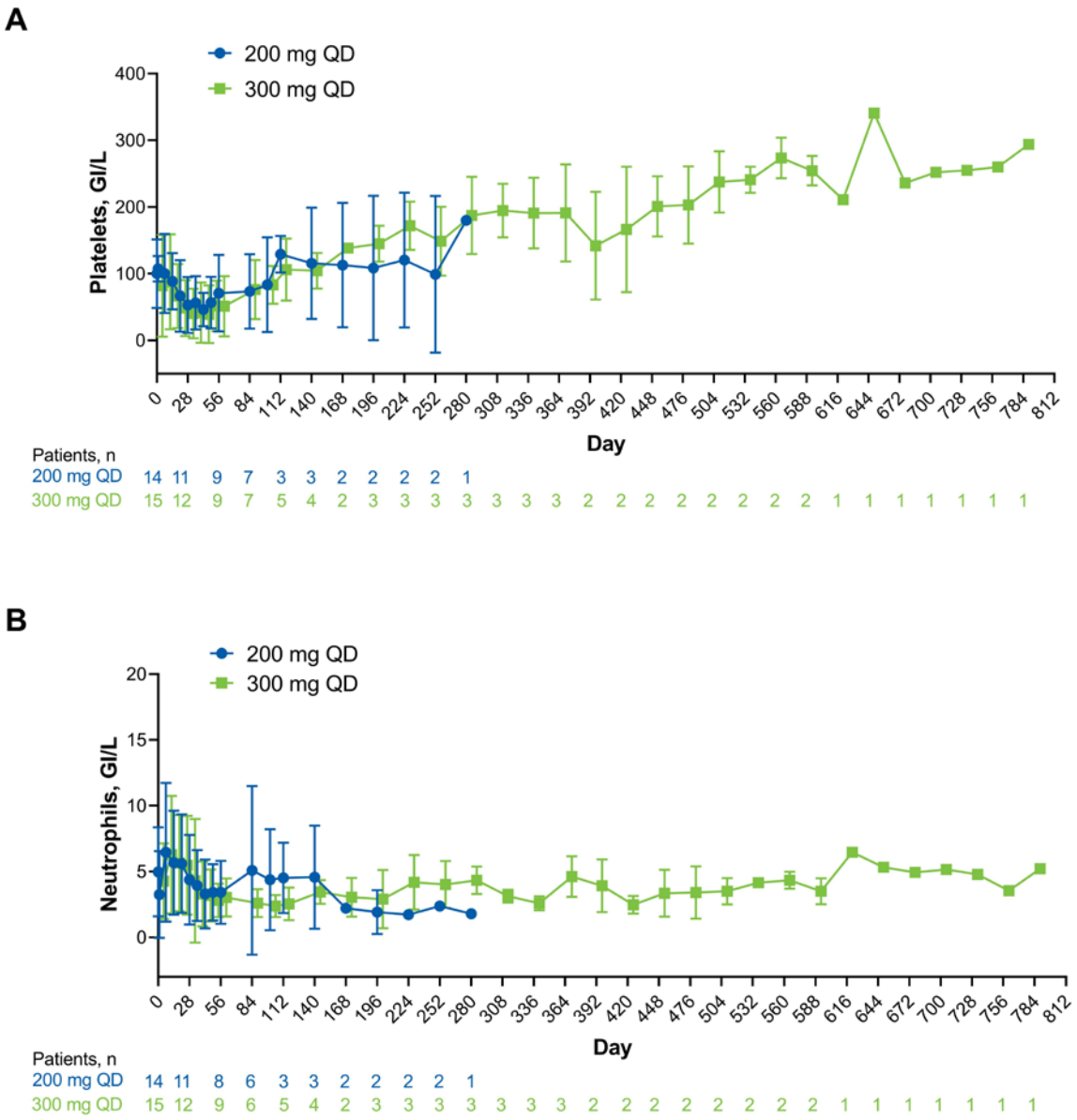
Supplemental Table 7. Longitudinal biomarker analysis in responders and nonresponders

Biomarker	Responders			Nonresponders			Responder vs nonresponder
	N	Mean, ng/mL (SEM)	Adjusted *P* value (vs baseline)	N	Mean, ng/mL (SEM)	Adjusted *P* value (vs baseline)	Adjusted *P* value
TNFR1							
Baseline	19	2.6 (0.3)	NA	8	3.8 (0.6)	NA	0.037*
Day 7	19	2.2 (0.2)	0.788	6	6.8 (2.1)	0.328	0.001*
Day 14	19	2.1 (0.2)	0.434	3	2.2 (0.6)	0.913	0.839
Day 28	19	2.1 (0.2)	0.575	2	1.8 (0.4)	0.891	0.593
Day 56	11	2.2 (0.3)	0.754	1	1.2 (NA)	NA	NA
Day 100	6	2.2 (0.5)	0.929	1	1.9 (NA)	NA	NA
REG3A							
Baseline	19	17.4 (6.0)	NA	8	85.5 (51.4)	NA	0.055
Day 7	19	11.1 (3.1)	0.751	6	39.5 (19.6)	0.875	0.025*
Day 14	19	14.0 (4.8)	0.974	3	17.9 (14.1)	0.796	0.773
Day 28	19	16.6 (4.7)	>0.999	2	6.3 (3.8)	0.795	0.493
Day 56	11	14.2 (2.3)	0.989	1	1.9 (NA)	NA	NA
Day 100	6	11.8 (1.6)	0.956	1	4.3 (NA)	NA	NA
ST2							
Baseline	19	363.2 (54.1)	NA	8	540.8 (182.3)	NA	0.225
Day 7	19	263.5 (32.3)	0.359	6	1385 (1011)	0.736	0.051
Day 14	19	292.6 (45.6)	0.677	3	194.2 (9.7)	0.996	0.411
Day 28	19	283.2 (41.2)	0.568	2	244.5 (78.8)	0.999	0.771
Day 56	11	159.8 (66.3)	0.026*	1	79.3 (NA)	NA	NA
Day 100	6	80.3 (35.8)	0.010*	1	185.8 (NA)	NA	NA
Trappin-2 (elafin)							
Baseline	18	56.1 (35.3)	NA	8	12.0 (3.1)	NA	0.418
Day 7	19	11.9 (3.1)	0.180	6	26.1 (15.4)	0.672	0.168
Day 14	19	8.3 (1.2)	0.128	3	4.8 (0.9)	0.985	0.281
Day 28	19	10.8 (1.3)	0.162	2	4.6 (1.4)	0.992	0.140
Day 56	11	18.7 (7.3)	0.459	1	2.7 (NA)	NA	NA
Day 100	6	17.3 (2.7)	0.621	1	6.2 (NA)	NA	NA

NA indicates not applicable.
*Change from baseline or between response cohorts is statistically significant (P<0.05).

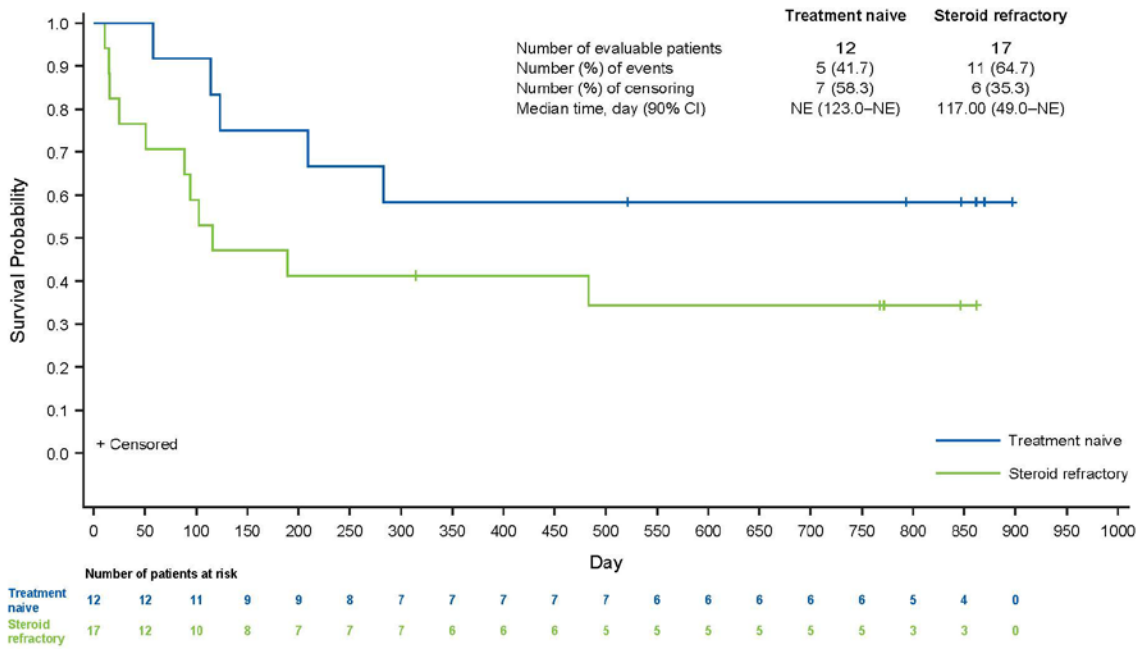
Supplemental Figures

Supplemental Figure 1. Hematologic parameters



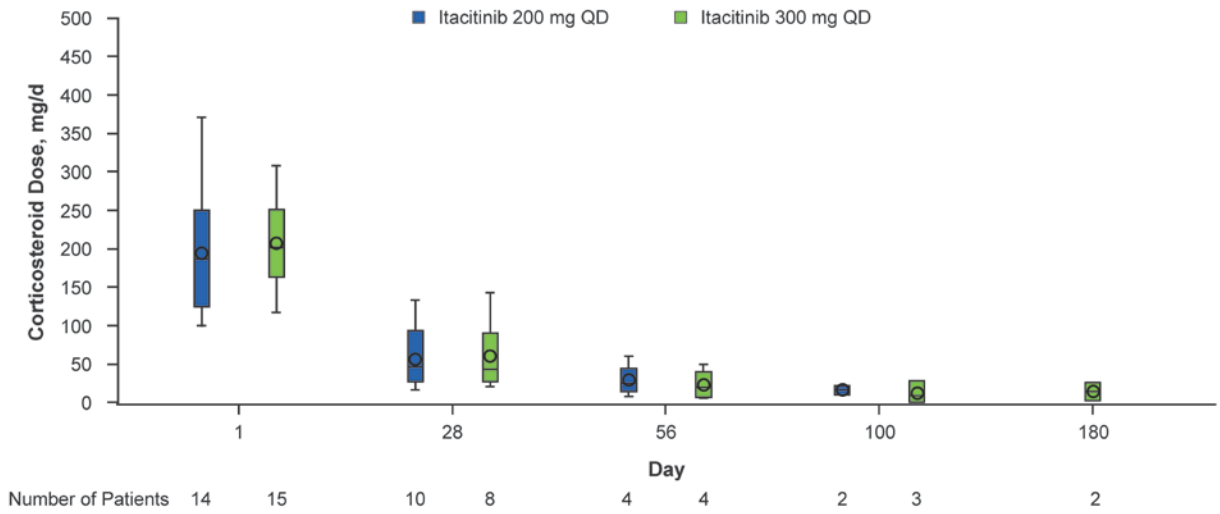
(A) Mean platelet counts and (B) mean neutrophil counts while on treatment over time. Error bars indicate SD. QD indicates once daily.

Supplemental Figure 2. Overall survival



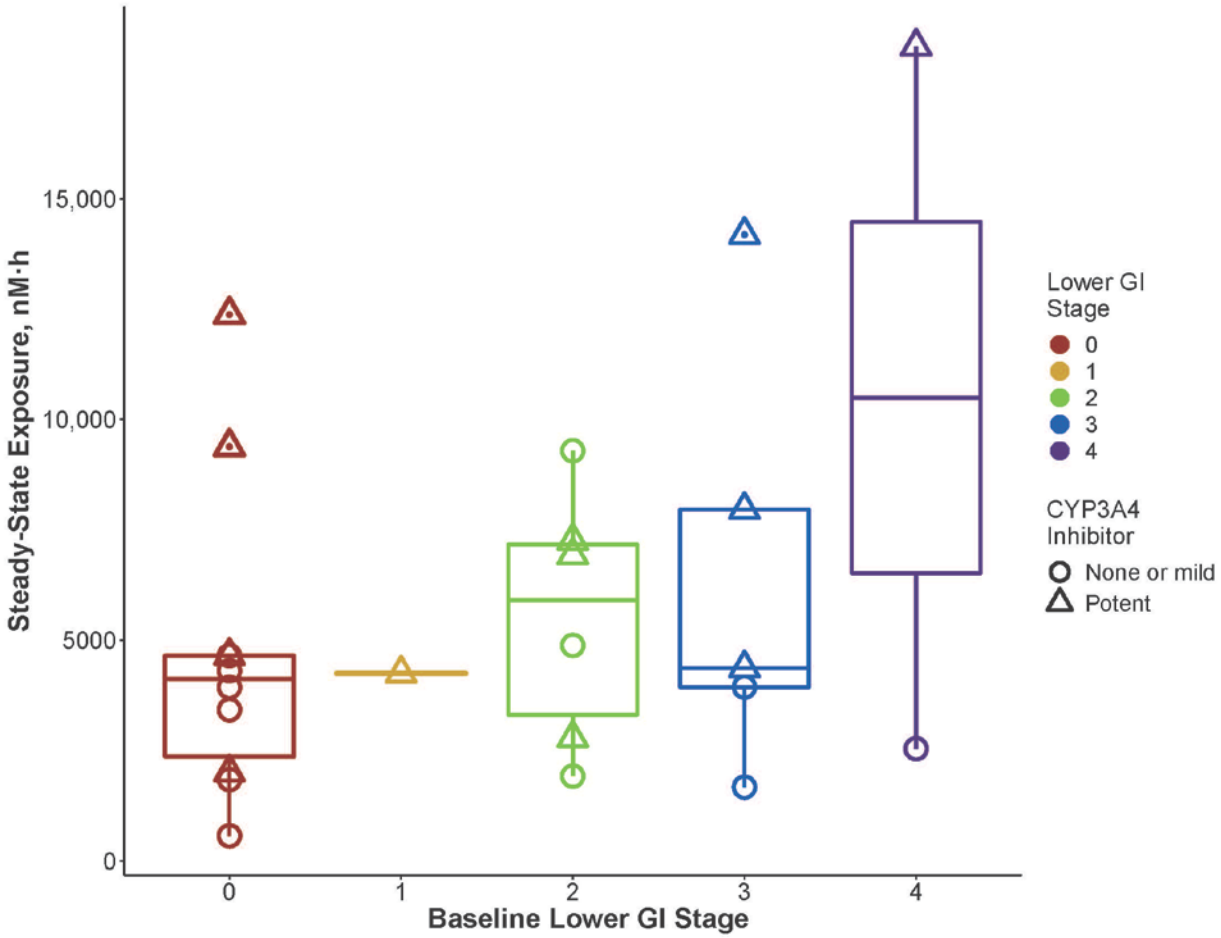
Median overall survival was not reached (lower 90% CI limit, 123 days) among patients with treatment-naive aGVHD and was 117 (49–not reached) days among patients with steroid-refractory aGVHD.

Supplemental Figure 3. Corticosteroid dose by visit in patients treated with itacitinib 200 mg or 300 mg



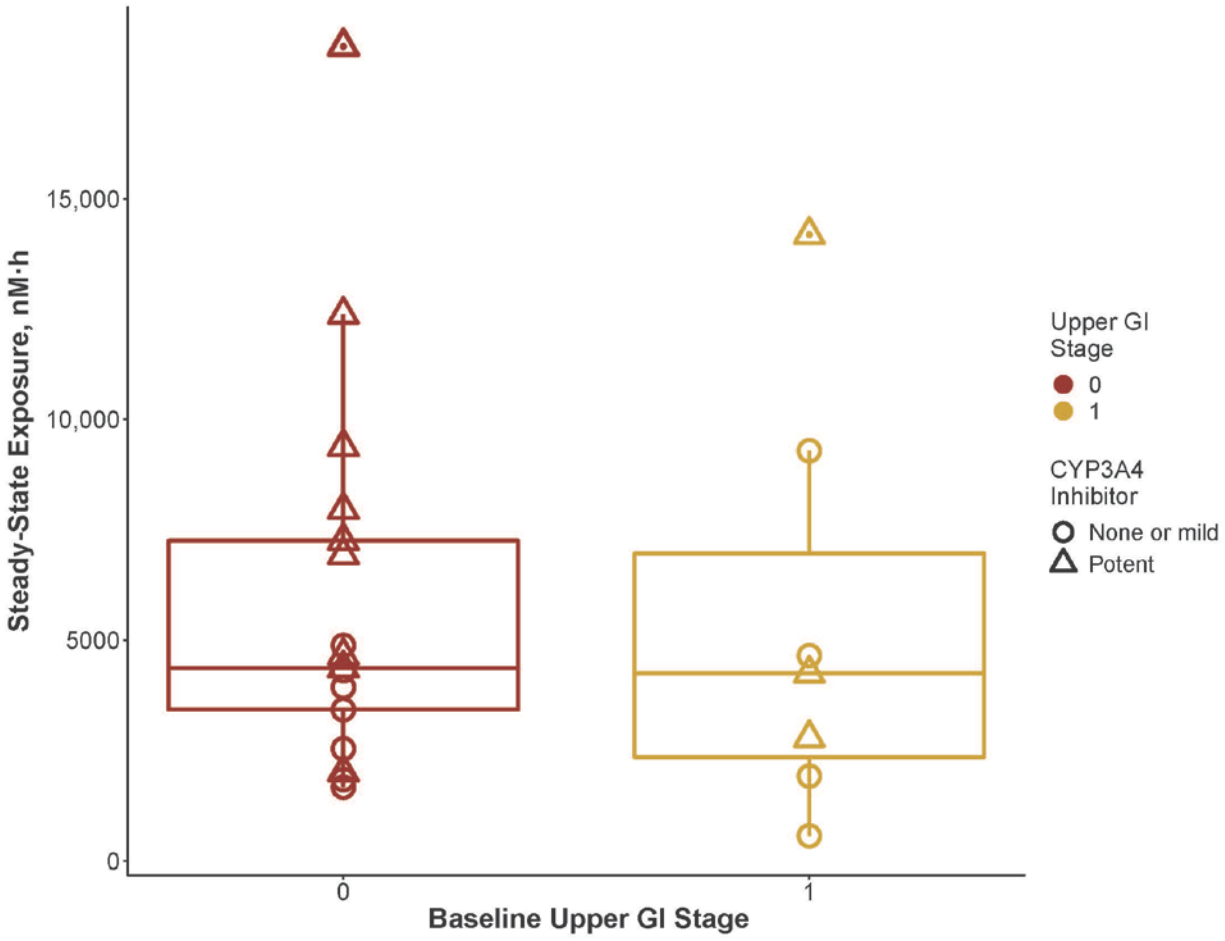
Data shown indicate median (horizontal line), mean (circle), 75th and 25thquartiles (upper and lower box boundaries, respectively), and minimum (lower error bar)/maximum (upper error bar).

Supplemental Figure 4. Itacitinib exposure by baseline lower GI stage



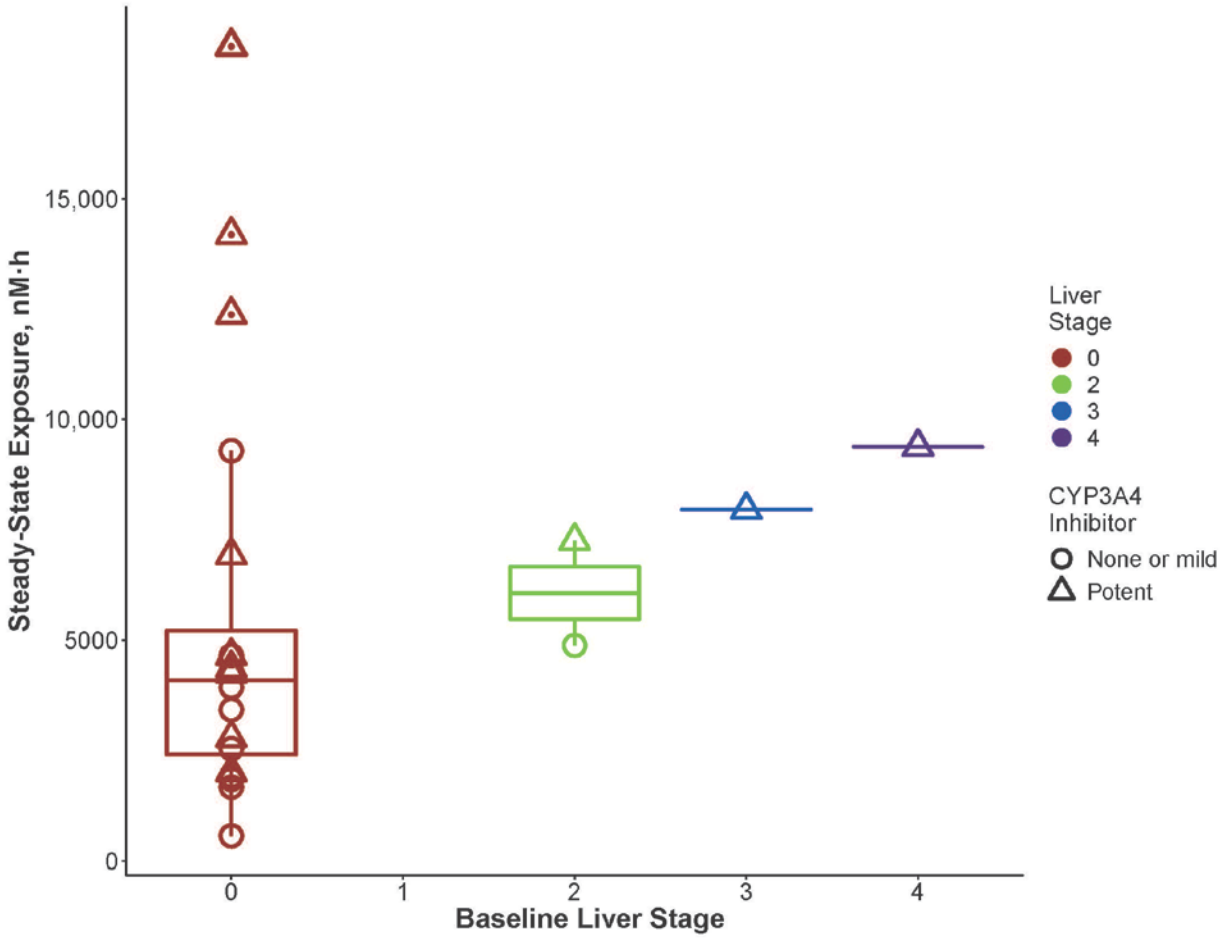
Upper and lower box boundaries represent the 75th and 25th percentile, respectively, with the horizontal line within the box representing the median. The whiskers extend to a maximum of 1.5 × the interquartile range. Values beyond the interquartile range were considered outliers and are indicated by dots in the figure.

Supplemental Figure 5. Itacitinib exposure by baseline upper GI stage



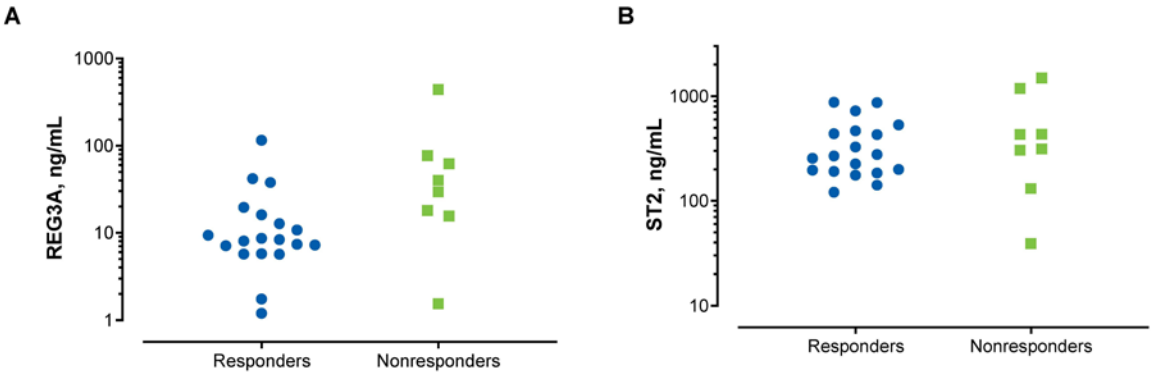
Upper and lower box boundaries represent the 75th and 25th percentile, respectively, with the horizontal line within the box representing the median. The whiskers extend to a maximum of 1.5 × the interquartile range. Values beyond the interquartile range were considered outliers and are indicated by dots in the figure.

Supplemental Figure 6. Itacitinib exposure by baseline liver stage

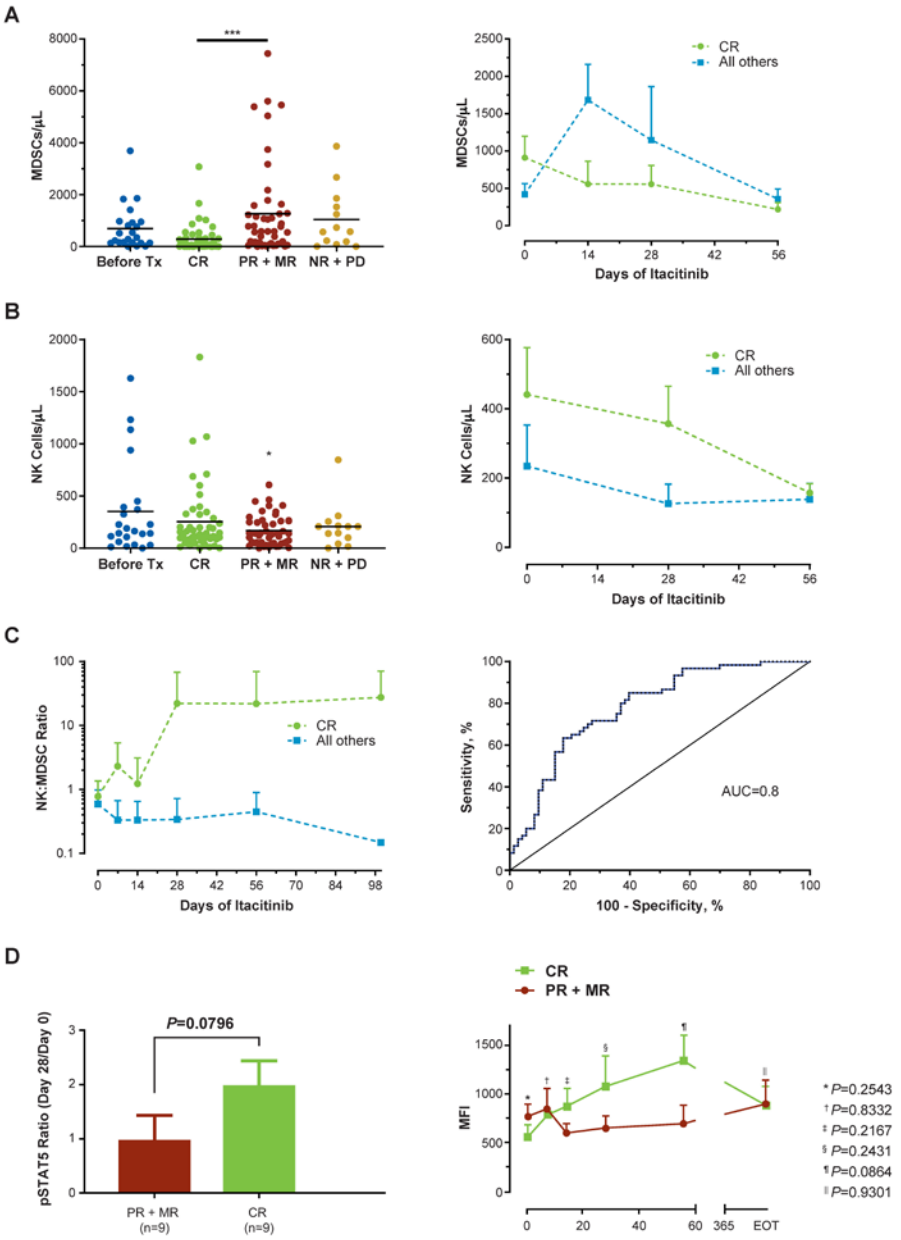


Upper and lower box boundaries represent the 75th and 25th percentile, respectively, with the horizontal line within the box representing the median. The whiskers extend to a maximum of 1.5 × the interquartile range. Values beyond the interquartile range were considered outliers and are indicated by dots in the figure.

Supplemental Figure 7. Baseline levels of (A) REG3A and (B) ST2 in responders and nonresponders



Supplemental Figure 8. Correlates with CR to itacitinib treatment and STAT phosphorylation among responders



Peripheral blood samples from serial time points from baseline through Day 180 were analyzed. **(A)** Absolute peripheral blood MDSC numbers following itacitinib treatment through Day 180 by response. **(B)** Absolute NK numbers following itacitinib treatment through Day 180 by response. **(C)** NK:MDSC ratio following itacitinib treatment through Day 100 by response and sensitivity and specificity analysis for NK:MDSC ratio of >0.31. **(D)** STAT5 phosphorylation ratio (Day 28/baseline) following itacitinib treatment by response (n=18 evaluable patients). EOT indicates end of treatment; MDSC, myeloid-derived suppressor cell; MFI, mean fluorescence intensity; NK, natural killer; pSTAT, phosphorylated STAT; STAT, signal transducer and activator of transcription; and Tx, treatment. NR, PD, and PR are explained in Supplemental Table 1.
*P<0.05; ***P<0.001.