Supplemental Appendix

Ruxolitinib for the treatment of steroid-refractory acute GVHD (REACH1): a multicenter, open-label, phase 2 trial

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Supplemental methods

Secondary/exploratory trial endpoints

Additional secondary endpoints included overall response rate (ORR) at any time, nonrelapse mortality (NRM; proportion of patients who died from causes other than malignancy relapse, with relapse as a competing risk), relapse rate (proportion of patients with relapse of underlying malignancy), overall survival (time from first ruxolitinib treatment [time 0] to death from any cause), incidence of chronic graft-versus-host disease (cGVHD), clinical safety data, and pharmacokinetics. Plasma samples for pharmacokinetics were analyzed using a validated liquid chromatographic tandem mass spectrometry assay. Exploratory endpoints included assessment of average corticosteroid dose by study visit and biomarker assessments.

Description and timing of assessments

Acute graft-versus-host disease (aGVHD) grading was determined by the investigator per Mount Sinai Acute GVHD International Consortium (MAGIC) guidelines[1] on a weekly basis for the first 8 weeks and every 28 days thereafter, as well as at scheduled study visits on Days 100, 180, and 365 and at the end of treatment. Response was assessed according to the Center for International Blood and Marrow Transplant Research modifications to the International Bone Marrow Transplant Registry response index.[2,3] Patients who withdrew from treatment for reasons other than graft-versus-host disease (GVHD) progression were assessed for GVHD status every 28 days. Duration of response was assessed after all patients either completed Day 84 and Day 180 study visits or discontinued from the study. Patients were

assessed for signs and symptoms of cGVHD on Days 100, 180, and 365 and at end of treatment per National Institutes of Health consensus guidelines.[4] Adverse events were assessed according to National Cancer Institute Common Terminology Criteria for Adverse Events v4.03 from the time of consent through 30 days after the last ruxolitinib dose. Pharmacokinetic samples were collected on Days 1, 7, and 14 predose (trough) and 1, 2, and 4 to 8 hours postdose.

Plasma collection for correlative biomarkers

Whole blood was collected in heparinized collection tubes at designated time points. Plasma was isolated after centrifugation of whole blood at 1500 × g for 15 minutes at room temperature, frozen immediately on dry ice or between −20°C and −70°C, and then shipped to Incyte for analysis. All patients consented to the blood collection. A total of 350 plasma samples were collected from 69 patients for proteomic analysis, and 68 patients who had a defined response at Day 28 were included in the biomarker analysis.

Protein biomarker assessment

Circulating levels of regenerating islet-derived protein 3 alpha (REG3A), suppression of tumorigenicity 2 (ST2), tumor necrosis factor receptor 1 (TNFR1), and Trappin-2/Elafin were measured using SimplePlex (ProteinSimple, San Jose, CA, USA) multiplex platform based on the manufacturers' instructions.

The proximity extension assay platform by OLINK Proteomics (Watertown, MA, USA) was used to conduct high content (>1000 proteins) multiplex proteomic analysis in participant plasma samples. In this assay, a pair of oligonucleotide-labelled antibodies,

Proseek probes, is allowed to pairwise bind to the target protein present in the sample in a homogeneous assay. When the 2 Proseek probes are in close proximity, a new polymerase chain reaction (PCR) target sequence is formed by a proximity-dependent DNA polymerization event. The resulting sequence is subsequently detected and quantified using standard real-time PCR. Data are presented as normalized protein expression in log2 scale.

Statistical analysis

Supportive analyses of the primary endpoint included assessment of Day 28 ORR by aGVHD grade at enrollment; steroid-refractory status; average reported daily dose of ruxolitinib from Day 1 to Day 28; and baseline characteristics, including age, sex, race, and GVHD organ involvement at enrollment. Post hoc model-based analysis of Day 28 response using logistic regression assessed HLA matching, aGVHD grade at enrollment, and steroid-refractory status as covariates.

For the biomarker analyses, statistical differences in protein expression between groups were determined using unpaired *t* tests, paired *t* tests (baseline vs Day 28), and ordinary one-way analysis of variance. Significance was conferred at $P<0.05$ and absolute fold-change in broad protein expression was calculated as Day 28/baseline >1.5 (ie, >1.5 or <0.66) within each group. False discovery rate *P* values were calculated across strata.

Supplemental results

Post hoc survival analyses

In post hoc model-based analysis of NRM using the Fine and Gray model, HLA matching, aGVHD grade at enrollment, steroid-refractory status, and duration of prior corticosteroid exposure were assessed as covariates. Acute GVHD grade III/IV (hazard ratio [HR], 0.252 [95% CI, 0.11–0.58]; *P*=0.0013) and longer prior corticosteroid exposure (HR, 1.01 [95% CI, 1.01–1.02]; *P*=0.0001) were significantly associated with increased NRM. Additional NRM analyses were conducted with response status added to the model, with adjustments for HLA matching, aGVHD grade at enrollment, and corticosteroid exposure. In this model, response status was an independent predictor of NRM (HR, 0.442 [95% CI, 0.29–0.69]; *P*=0.0003).

Pharmacokinetics

Except for use of moderate or potent cytochrome P450 3A4 inhibitors, no concomitant medications explored were identified as significant predictors of interindividual variability in pharmacokinetic parameters. Likewise, no laboratory indices of kidney and liver function (except for liver involvement on apparent oral clearance variability) were predictive of variability. When compared with data from a myelofibrosis (MF) population, absorption was slightly quicker for patients with MF than for patients with aGVHD (approximately 0.5–1 vs 1–2 hours, respectively) and for patients who did not have upper or lower gastrointestinal (GI) involvement (grade 0 or 1).

Protein biomarker assessments

Supplemental Table 4 illustrates the average individual concentrations of ST2, REG3A, TNFR1, and Trappin-2/Elafin for each patient within each cohort between responders (complete response, very good partial response, partial response) and nonresponders (mixed response, progressive disease/death). Significantly elevated levels of ST2 (P=0.0001) and TNFR1 (P=0.012) were observed in nonresponders versus responders at baseline. No significant differences were observed in the baseline levels of REG3A or Trappin-2/Elafin between nonresponders and responders. Longitudinally, levels of ST2 decreased over time with treatment (Supplemental Figure 3A). Although baseline levels of ST2 were significantly different between responders and nonresponders, there were no apparent differences in the degree of reduction in ST2 levels between the cohorts. Analysis of REG3A levels in responders and nonresponders over the various visits demonstrated a rapid decrease by Day 14, with minimal differences observed between the groups in response to treatment (Supplemental Figure 3B). A similar decrease in Trappin-2/Elafin levels in responders and nonresponders over the various visits was observed in both groups in response to treatment (Supplemental Figure 3C). TNFR1 protein expression did not significantly change over time in response to treatment (Supplemental Figure 3D).

Broad proteomic analysis was conducted on 36 responders and 6 nonresponders. Among responders, 60 proteins were upregulated, and 19 were downregulated (Supplemental Tables 5 and 6). Proteins significantly modulated in responders are illustrated in a heatmap (Supplemental Figure 4) using R v3.5.0 “heatmaply” package.[5] Proteins that were significantly modulated in the responder

population were analyzed further using ingenuity pathway analysis (Qiagen, Inc., Germantown, MD, USA). Analysis determined an association between the expression pattern in the patients who received ruxolitinib and the hematopoiesis and interleukin-17–mediated inflammation pathways.

Supplemental tables

Supplemental Table 1. REACH1 inclusion and exclusion criteria

Inclusion criteria

• Male or female, aged ≥12 y
• Has undergone first allo-HSCT from any donor source (matched unrelated donor, sibling, haploidentical) using bone marrow, peripheral blood stem cells, or cord blood for hematologic malignancies; recipients of nonmyeloablative and myeloablative transplants are eligible
• Clinically suspected grades II–IV aGVHD per MAGIC guidelines, occurring after allo-HSCT with any conditioning regimen and any anti-GVHD prophylactic program. Biopsies should be obtained to pathologically confirm acute GVHD; in cases where a biopsy is negative, is unable to be obtained, or is clinically contraindicated, clinical suspicion of aGVHD by the treating physician is sufficient
• Patients with steroid-refractory aGVHD, defined as any of the following:
  ○ Patients with progressive GVHD (ie, increase in stage in any organ system or any new organ involvement) after 3 d of primary treatment with methylprednisolone ≥2 mg/kg/d (or equivalent)
  ○ Patients with GVHD that has not improved (ie, decrease in stage in at least 1 involved organ system) after 7 d of primary treatment with methylprednisolone ≥2 mg/kg/d (or equivalent)
  ○ Patients who previously began corticosteroid therapy at a lower dose (≥1 mg/kg/d methylprednisolone) for treatment of skin GVHD or skin GVHD accompanied by upper GI GVHD but develop new GVHD in another organ system
  ○ Patients who cannot tolerate a corticosteroid taper, that is, begin corticosteroids at 2.0 mg/kg/d, demonstrate response, but progress before a 50% decrease from the initial starting dose of corticosteroids is achieved
• Evidence of myeloid engraftment (eg, absolute neutrophil count ≥0.5 × 10^9/L for 3 consecutive days if ablative therapy was previously used)
• Willingness to avoid pregnancy or fathering children based on 1 of the following criteria:
  ○ Women of nonchildbearing potential (ie, surgically sterile with a hysterectomy and/or bilateral oophorectomy or ≥12 mo of amenorrhea)
  ○ Women of childbearing potential who have a negative serum pregnancy test at screening and who agree to take appropriate precautions to avoid pregnancy from screening through safety follow-up
  ○ Men who agree to take appropriate precautions to avoid fathering children from screening through safety follow-up
• Written informed consent and/or assent from the patient, parent, or guardian
• Willingness to comply with all study visits and procedures

Exclusion criteria
• Has received more than 1 allo-HSCT • Has received more than 1 systemic treatment in addition to corticosteroids for acute GVHD

- Presence of GVHD overlap syndrome per NIH guidelines
- Has had a splenectomy
- Presence of an active uncontrolled infection defined as hemodynamic instability attributable to sepsis or new symptoms, worsening physical signs, or radiographic findings attributable to infection
- Known HIV infection
- Active HBV or HCV infection that requires treatment or at risk for HBV reactivation (defined as hepatitis B surface antigen positive or anti–hepatitis B core antibody positive)
- Serum creatinine ≥2.0 mg/dL or creatinine clearance <40 mL/min as measured or calculated by Cockroft-Gault equation
- Patients with evidence of primary relapsed disease or patients who have been treated for relapse after the allo-HSCT was performed
- Unresolved toxicity or complications (other than aGVHD) due to previous allo-HSCT
- Any corticosteroid therapy for indications other than GVHD at doses of methylprednisolone or equivalent ≥1 mg/kg/d within 7 d of enrollment
- Severe organ dysfunction unrelated to underlying GVHD, including
    - Cholestatic disorders or unresolved veno-occlusive disease of the liver (defined as persistent bilirubin abnormalities not attributable to GVHD and ongoing organ dysfunction)
    - Clinically significant or uncontrolled cardiac disease, including unstable angina, acute myocardial infarction within 6 mo from Day 1 of study drug administration, New York Heart Association class III or IV congestive heart failure, circulatory collapse requiring vasopressor or inotropic support, or arrhythmia that requires therapy
    - Clinically significant respiratory disease that requires mechanical ventilation support or 50% oxygen
- Currently breastfeeding
- Received JAK inhibitor therapy after allo-HSCT for any indication; treatment with a JAK inhibitor before allo-HSCT is permitted
- Treatment with any other investigational agent, device, or procedure, within 21 d (or 5 half-lives, whichever is greater) of enrollment
- Any condition that would, in the investigator’s judgment, interfere with full participation in the study
- Known allergies, hypersensitivity, or intolerance to any of the study medications, excipients, or similar compounds

allo-HSCT, allogeneic hematopoietic stem cell transplantation; HBV, hepatitis B virus; HCV, hepatitis C virus; JAK, Janus kinase; NIH, National Institutes of Health.

Supplemental Table 2. Concomitant anti-infection therapy

Medication	Ruxolitinib (N=71)
Any concomitant anti-infection therapy	71 (100.0)
Antibiotics	27 (38.0)
Nystatin	13 (18.3)
Amphotericin b, liposome	6 (8.5)
Erythromycin	5 (7.0)
Amphotericin b	1 (1.4)
Azithromycin	1 (1.4)
Bacitracin	1 (1.4)
Nystadermal	1 (1.4)
Polymyxin b with trimethoprim	1 (1.4)
Polysporin sterile ophthalmic	1 (1.4)
Rifaximin	1 (1.4)
Tobramycin	1 (1.4)
Vancomycin	1 (1.4)
Combinations of penicillins, including beta-lactamase inhibitors	16 (22.5)
Pip/tazo	13 (18.3)
Duocid	3 (4.2)
Augmentin	2 (2.8)
Amoxicillin with clavulanate potassium	1 (1.4)
Unacid	1 (1.4)
Combinations of sulphonamides and trimethoprim, including derivatives	25 (35.2)
Bactrim	25 (35.2)
First-generation cephalosporins	5 (7.0)
Cefazolin	4 (5.6)
Cefalexin	1 (1.4)
Fluoroquinolones	31 (43.7)
Levofloxacin	19 (26.8)
Ciprofloxacin	15 (21.1)
Moxifloxacin	2 (2.8)
Fourth-generation cephalosporins	29 (40.8)
Cefepime	29 (40.8)
Cefepime hydrochloride	1 (1.4)
Glycopeptide antibacterials	30 (42.3)
Vancomycin	30 (42.3)
Imidazole derivatives	18 (25.4)
Metronidazole	18 (25.4)
Macrolides	13 (18.3)
Azithromycin	12 (16.9)
Erythromycin lactobionate	1 (1.4)

Nucleosides and nucleotides (excluding reverse transcriptase inhibitors)	69 (97.2)
Aciclovir	55 (77.5)
Ganciclovir	21 (29.6)
Valganciclovir	12 (16.9)
Valaciclovir hydrochloride	7 (9.9)
Valganciclovir hydrochloride	6 (8.5)
Cidofovir	3 (4.2)
Ribavirin	2 (2.8)
Famciclovir	1 (1.4)
Valaciclovir	1 (1.4)
Other antivirals	
Letermovir	1 (1.4)
Other antibacterials	11 (15.5)
Daptomycin	8 (11.3)
Linezolid	6 (8.5)
Methenamine	1 (1.4)
Other antimycotics for systemic use	42 (59.2)
Micafungin	31 (43.7)
Caspofungin	9 (12.7)
Micafungin sodium	5 (7.0)
Caspofungin acetate	1 (1.4)
Triazole derivatives	59 (83.1)
Posaconazole	28 (39.4)
Voriconazole	22 (31.0)
Fluconazole	16 (22.5)
Isavuconazonium	11 (15.5)
Isavuconazonium sulfate	6 (8.5)

Data are provided as n (%).

Supplemental Table 3. Efficacy measures (Day 28) and infections stratified by aGVHD grade at enrollment

	Grade II (n=23)	Grade III/IV (n=48)
ORR, n (%)	19 (82.6)	20 (41.7)
CR	11 (47.8)	8 (16.7)
VGPR	4 (17.4)	3 (6.3)
PR	4 (17.4)	9 (18.8)
DOR, median (95% CI), mo		
CR/VGPR	n=15	n=11
	NE (29.0–NE)	345.0 (106.0–NE)
PR	n=4	n=9
	NE (NE–NE)	159.0 (29.0–NE)
OS, median (95% CI), mo		
CR/VGPR	n=15	n=11
	NE (4.1–NE)	NE (5.3–NE)
PR	n=4	n=9
	NE (NE–NE)	5.4 (1.4–NE)
OS, 6-mo probability (95%CI)		
CR/VGPR	n=15	n=11
	73.3 (43.6–89.1)	81.8 (44.7–95.1)
PR	n=4	n=9
	100.0 (100.0–100.0)	45.7 (11.0–75.7)
OS, 12-mo probability (95% CI)		
CR/VGPR	n=15	n=11
	65.2 (35.1–83.9)	81.8 (44.7–95.1)
PR	n=4	n=9
	100.0 (100.0–100.0)	30.5 (4.5–63.4)
NRM, n (%)		
CR/VGPR	4 (26.7)	1 (9.1)
PR	—	5 (55.6)
NRM, 6-mo cumulative incidence rate (95% CI)		

CR/VGPR	20.0 (4.9–42.4)	9.1 (0.5–33.3)
PR	-	54.3 (15.5–81.9)
NRM, 12-mo cumulative incidence rate (95% CI)		
CR/VGPR	28.1 (8.5–52.0)	9.1 (0.5–33.3)
PR	—	69.5 (23.8–91.2)
Prior corticosteroid use, median (range), d	17.0 (6–285)	12.0 (3–224)
ORR in patients with skin involvement at baseline, n (%)	n=17	n=19
	15 (88.2)	7 (36.8)
CR	7 (41.2)	2 (10.5)
VGPR	4 (23.5)	2 (10.5)
PR	4 (23.5)	3 (15.8)
ORR in patients with no skin involvement at baseline, n (%)	n=6	n=29
	4 (66.7)	13 (44.8)
CR	4 (66.7)	6 (20.7)
VGPR	0	1 (3.4)
PR	0	6 (20.7)
Patients with grade 4 infections	n=3	n=16
Response status, n (%)		
CR	2 (66.7)	1 (6.3)
VGPR	1 (33.3)	1 (6.3)
PR	0	2 (12.5)
Other response (including no response)	0	12 (75.0)
Organ involvement at baseline, n (%)		
Skin	3 (100.0)	6 (37.5)
Non-skin	0	10 (62.5)

CR indicates complete response; DOR, duration of response; NE, not evaluable; OS, overall survival; PR, partial response; VGPR, very good partial response.

Supplemental Table 4. ST2, REG3A, TNFR1, and trappin-2/elafin levels at baseline

Protein	Mean ± SEM (range), ng/mL	Responders mean ± SEM, ng/mL	Nonresponders mean ± SEM, ng/mL	*P*
ST2	413.8±41.5 (55.0–2151.5)	282.8±23.9	590.0±81.8	0.0001
REG3A	25.1±4.3 (0.9–235.5)	23.3±4.8	27.6±7.9	0.62
TNFR1	3.6±0.3 (1.0–18.4)	2.9±0.2	4.4±0.6	0.012
Trappin2/elafin	55.2±17.5 (2.4–1037.7)	55.6±26.5	54.8±20.7	0.98

SEM, standard error of the mean.

Supplemental Table 5. Proteins significantly upregulated in responders (n=36)

Assay	Protein	Uniprot ID	Raw *P* value	FDR *P* value	Fold-change*
EPO	Erythropoietin	P01588	<0.001	<0.001	4.26
THPO	Thrombopoietin	P40225	<0.001	<0.001	2.78
GCG	Glucagon	P01275	<0.001	0.001	2.75
FGF-21	Fibroblast growth factor 21	Q9NSA1	<0.001	0.004	2.73
Flt3L	Fms-related tyrosine kinase 3 ligand	P49771	<0.001	<0.001	2.52
FGF-21	Fibroblast growth factor 21	Q9NSA1	0.001	0.005	2.51
FGF-21	Fibroblast growth factor 21	Q9NSA1	<0.001	0.003	2.37
CCL28	C-C motif chemokine ligand 28	Q9NRJ3	0.002	0.016	2.20
FABP4	Fatty acid binding protein 4	P15090	<0.001	<0.001	2.15
FAM3B	Family with sequence similarity 3 member B	P58499	<0.001	<0.001	2.12
IL6	Interleukin 6	P05231	0.001	0.010	2.12
FABP2	Fatty acid binding protein 2	P12104	0.004	0.026	2.08
IL6	Interleukin 6	P05231	0.001	0.009	2.08
IL6	Interleukin 6	P05231	0.001	0.011	2.06
IL6	Interleukin 6	P05231	0.003	0.018	2.00
CCL25	C-C motif chemokine ligand 25	O15444	<0.001	<0.001	1.99
VWC2	Von Willebrand factor C domain containing 2	Q2TAL6	<0.001	<0.001	1.98
FAM19A5	Family with sequence similarity 19 member A5	Q7Z5A7	<0.001	<0.001	1.90
PLIN1	Perilipin 1	O60240	<0.001	0.001	1.88
MCP-1	C-C motif chemokine ligand 2	P13500	<0.001	<0.001	1.85
NPPC	Natriuretic peptide C	P23582	<0.001	<0.001	1.84
SCF	Kit ligand	P21583	<0.001	<0.001	1.84
SCF	Kit ligand	P21583	<0.001	<0.001	1.83
FBP1	Fructose-biphosphatase 1	P09467	0.006	0.037	1.83
SCF	Kit ligand	P21583	<0.001	<0.001	1.79

SMOC2	SPARC related modular calcium-binding protein 2	Q9H3U7	<0.001	<0.001	1.79
TSHB	Thyroid stimulating hormone beta	P01222	0.001	0.011	1.75
MCP-1	C-C motif chemokine ligand 2	P13500	<0.001	<0.001	1.73
hK14	Kallikrein 14	Q9P0G3	<0.001	<0.001	1.72
IL8	Interleukin 8	P10145	0.001	0.009	1.72
CD8A	CD8a molecule	P01732	0.005	0.030	1.68
PPY	Pancreatic prohormone	P01298	<0.001	0.001	1.67
CPA2	Carboxypeptidase A2	P48052	0.017	0.077	1.66
CHRDL2	Chordin-like protein 2	Q6WN34	<0.001	<0.001	1.65
BMP-6	Bone morphogenetic protein 6	P22004	<0.001	<0.001	1.65
FAM3C	Family with sequence similarity 3 member C	Q92520	<0.001	<0.001	1.64
TNNI3†	Troponin I3, cardiac type	P19429	0.031	0.12	1.62
MK	Midkine (neurite growth-promoting factor 2)	P21741	0.012	0.059	1.62
ADAM-TS 15	ADAM metallopeptidase with thrombospondin type 1 motif 15	Q8TE58	<0.001	<0.001	1.61
MMP12	Matrix metallopeptidase 12	P39900	<0.001	0.002	1.60
CEACAM5	Carcinoembryonic antigen related cell adhesion molecule 5	P06731	<0.001	<0.001	1.60
CXCL16	C-X-C motif chemokine ligand 16	Q9H2A7	<0.001	<0.001	1.59
hK11	Kallikrein 11	Q9UBX7	<0.001	<0.001	1.58
SLITRK2	SLIT and NTRK-like family member 2	Q9H156	<0.001	<0.001	1.57
GHRL†	Ghrelin and obestatin prepropeptide	Q9UBU3	<0.001	0.004	1.56
KAZALD1	Kazal-type serine peptidase inhibitor domain 1	Q96I82	<0.001	<0.001	1.56
Notch 3	Neurogenic locus notch homolog protein 3	Q9UM47	<0.001	<0.001	1.54
CLMP	CXADR-like membrane protein	Q9H6B4	<0.001	<0.001	1.54
TFPI-2	Tissue factor pathway inhibitor 2	P48307	0.004	0.025	1.54
GDF-2	Growth differentiation factor 2	Q9UK05	<0.001	<0.001	1.53
GAL	Galanin propeptides	P22466	0.002	0.012	1.53
Ep-CAM	Epithelial cell adhesion molecule	P16422	<0.001	0.002	1.53
IGFBP-1	Insulin-like growth factor binding protein 1	P08833	0.034	0.13	1.53
GDF-8	Growth differentiation factor 8	O14793	<0.001	0.002	1.53

CTRC	Chymotrypsin C	Q99895	0.009	0.046	1.52
LEP	Leptin	P41159	0.004	0.023	1.52
GDF-15	Growth differentiation factor 15	Q99988	<0.001	0.001	1.52
ROBO2	Roundabout homolog 2	Q9HCK4	<0.001	<0.001	1.51
FR-alpha	Folate receptor alpha	P15328	<0.001	<0.001	1.51
KIM1	Hepatitis A virus cellular receptor 1	Q96D42	<0.001	0.001	1.50

Broad proteomic analysis used 12 panels containing approximately 90 proteins on each panel. Select protein assays may appear on more than 1 panel for analysis; therefore, the protein assay may have more than 1 measurement.
FDR, false discovery rate; LOD, limit of detection.
* Fold-change denotes change from baseline (Day 28/baseline). Values above 1 indicate upregulation.
† Indicates that >20% of values were below LOD and replaced with 0.5 × LOD in the analysis.

Supplemental Table 6. Proteins significantly downregulated in responders (n=36)

Assay	Protein	Uniprot ID	Raw *P* value	FDR *P* value	Fold-change*
TNC	Tenascin C	P24821	0.001	0.009	0.66
CD69	CD69 molecule	Q07108	0.005	0.032	0.66
ZBTB16	Zinc finger and BTB domain–containing protein 16	Q05516	0.001	0.005	0.66
CXCL11	C-X-C motif chemokine ligand 11	O14625	0.013	0.064	0.66
TANK†	TRAF family member-associated NFκB activator	Q92844	0.002	0.014	0.66
CD2AP	CD2-associated protein	Q9Y5K6	0.005	0.032	0.66
LAIR-2	Leukocyte-associated immunoglobulin-like receptor 2	Q6ISS4	<0.001	0.001	0.65
ST1A1	Sulfotransferase family 1A member 1	P50225	0.006	0.036	0.65
STK4	Serine/threonine kinase 4	Q13043	0.007	0.040	0.65
CXCL9	C-X-C motif chemokine ligand 9	Q07325	0.034	0.13	0.65
GZMB	Granzyme B	P10144	0.027	0.11	0.65
GZMH	Granzyme H	P20718	0.001	0.008	0.64
SIT1	Signalling threshold regulating transmembrane adaptor 1	Q9Y3P8	<0.001	<0.001	0.62
BANK1	B-cell scaffold protein with ankyrin repeats 1	Q8NDB2	0.004	0.026	0.62
CXCL10	C-X-C motif chemokine ligand 10	P02778	0.036	0.13	0.62
RNASE3	Ribonuclease A family member 3	P12724	<0.001	0.002	0.61
ZBTB16	Zinc finger and BTB domain-containing protein 16	Q05516	<0.001	0.002	0.61
PDGF subunit B	Platelet derived growth factor subunit B	P01127	0.036	0.13	0.61
IL2-RA	Interleukin 2 receptor subunit alpha	P01589	0.004	0.026	0.57

Broad proteomic analysis utilized 12 panels that contained approximately 90 proteins on each panel. Select protein assays may appear on more than 1 panel for analysis; therefore, the protein assay may have more than 1 measurement.

* Fold-change denotes change from baseline (Day 28/baseline). Values below 1 indicate downregulation.

† Indicates that >20% of values were below LOD and replaced with 0.5 × LOD in the analysis.

Supplemental Table 7. Treatment-emergent adverse events in ≥10% of patients

	Ruxolitinib (N=71)	
Event	**Any grade**	**Grade 3/4**
Anemia	46 (64.8)	36 (50.7)
Hypokalemia	35 (49.3)	13 (18.3)
Peripheral edema	32 (45.1)	8 (11.3)
Platelet count decreased*	32 (45.1)	28 (39.4)
Neutrophil count decreased	28 (39.4)	23 (32.4)
Muscular weakness	24 (33.8)	8 (11.3)
Dyspnea	23 (32.4)	5 (7.0)
Hypomagnesemia	23 (32.4)	2 (2.8)
Hypocalcemia	22 (31.0)	8 (11.3)
Nausea	22 (31.0)	4 (5.6)
Fatigue	21 (29.6)	10 (14.1)
White blood cell count decreased	21 (29.6)	12 (16.9)
Diarrhea	20 (28.2)	5 (7.0)
Alanine aminotransferase increased	18 (25.4)	4 (5.6)
Hyperglycemia	18 (25.4)	14 (19.7)
Hypophosphatemia	18 (25.4)	11 (15.5)
Vomiting	18 (25.4)	2 (2.8)
Aspartate aminotransferase increased	17 (23.9)	1 (1.4)
Back pain	17 (23.9)	3 (4.2)
Acute kidney injury	16 (22.5)	5 (7.0)
Hypertension	16 (22.5)	9 (12.7)
Abdominal pain	15 (21.1)	5 (7.0)
Headache	15 (21.1)	3 (4.2)
Hypotension	15 (21.1)	10 (14.1)
Pyrexia	15 (21.1)	2 (2.8)
Decreased appetite	14 (19.7)	6 (8.5)
Fall	14 (19.7)	2 (2.8)
Hyponatremia	14 (19.7)	10 (14.1)
Sinus tachycardia	14 (19.7)	0
Thrombocytopenia*	14 (19.7)	10 (14.1)
Cough	13 (18.3)	0
Hypoalbuminemia	13 (18.3)	10 (14.1)
Arthralgia	12 (16.9)	1 (1.4)
Blood creatinine increased	12 (16.9)	0
Hematuria	12 (16.9)	0
Abdominal distension	11 (15.5)	2 (2.8)
Blood bilirubin increased	11 (15.5)	7 (9.9)
Constipation	11 (15.5)	0

Depression	11 (15.5)	0
Dry mouth	11 (15.5)	0
Flatulence	11 (15.5)	0
Lower GI hemorrhage	11 (15.5)	6 (8.5)
Pain in extremity	11 (15.5)	1 (1.4)
Anxiety	10 (14.1)	1 (1.4)
Confusional state	10 (14.1)	3 (4.2)
Dizziness	10 (14.1)	0
Hypoglycemia	10 (14.1)	1 (1.4)
Insomnia	10 (14.1)	0
Respiratory failure	10 (14.1)	10 (14.1)
Cytomegalovirus infection	9 (12.7)	4 (5.6)
Dry eye	9 (12.7)	0
Hypertriglyceridemia	9 (12.7)	2 (2.8)
Pollakiuria	9 (12.7)	0
Rash maculopapular	9 (12.7)	2 (2.8)
Sepsis	9 (12.7)	8 (11.3)
Epistaxis	8 (11.3)	1 (1.4)
Lymphocyte count decreased	8 (11.3)	8 (11.3)
Pleural effusion	8 (11.3)	1 (1.4)

Data are presented as n (%). The data cutoff was July 2, 2018. Multiple occurrences of the same adverse event in 1 patient were counted only once per preferred term at the highest grade.
* The terms *platelet count decreased* and *thrombocytopenia* were used by investigators when reporting adverse events and therefore were recorded as separate terms in the safety database.

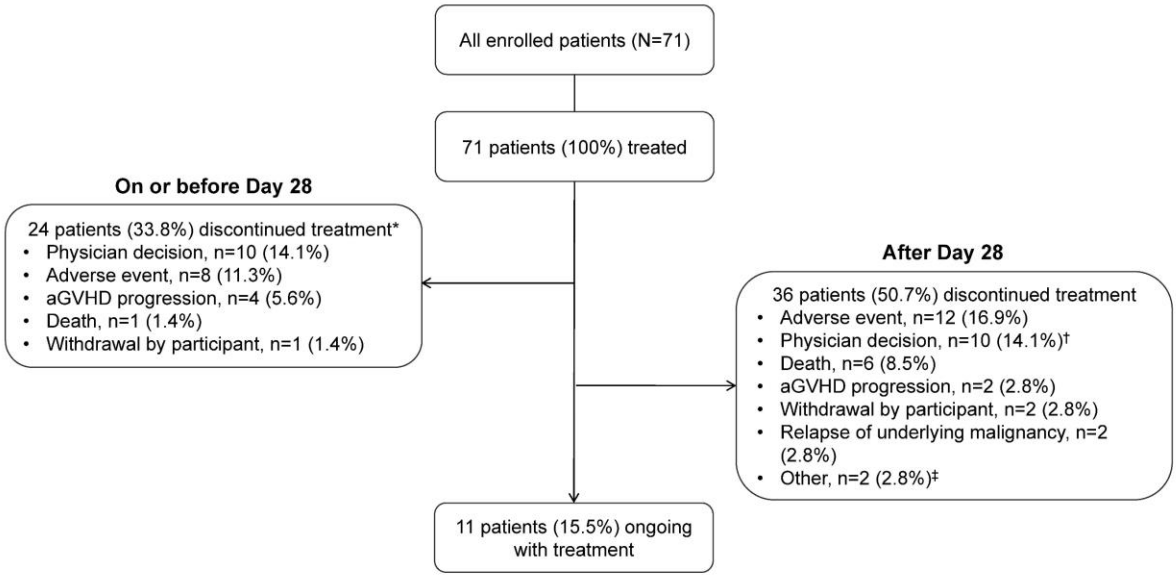
Supplemental Table 8. Summary of CMV events

	Before enrollment			Treatment-emergent		
CMV status	**CMV viremia**	**CMV infection**	**No CMV viremia or infection**	**CMV viremia**	**CMV infection**	**No CMV viremia or infection**
D–/R– (n=23)	0	0	23 (100.0)	0	0	23 (100.0)
D–/R+ (n=16)	2 (12.5)	2 (12.5)	12 (75.0)	1 (6.3)	0	15 (93.8)
D+/R+ (n=24)	5 (20.8)	5 (20.8)	14 (58.3)	3 (12.5)	8 (33.3)	13 (54.2)
D+/R– (n=7)	0	0	7 (100.0)	0	2 (28.6)	5 (71.4)
Any/unknown (n=1)	1 (100.0)	0	0	0	0	1 (100.0)

Data are presented as n (%).
CMV, cytomegalovirus; D, donor; R, recipient.

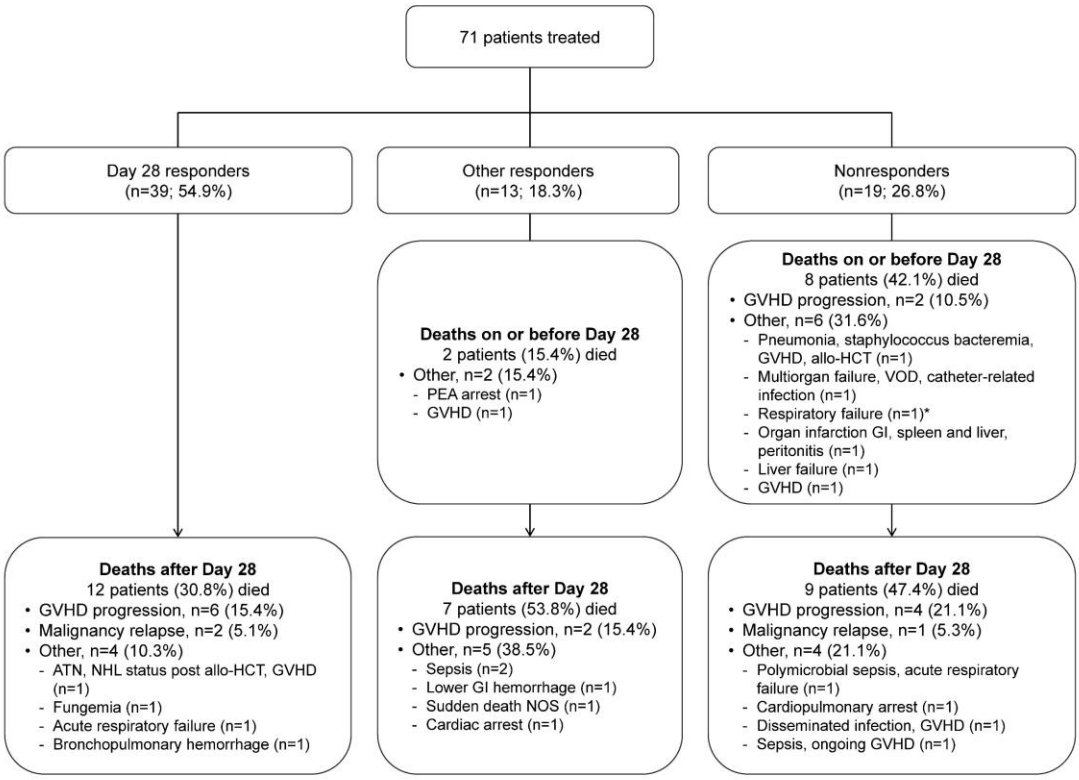
Supplemental figures

Supplemental Figure 1. Summary of patient disposition



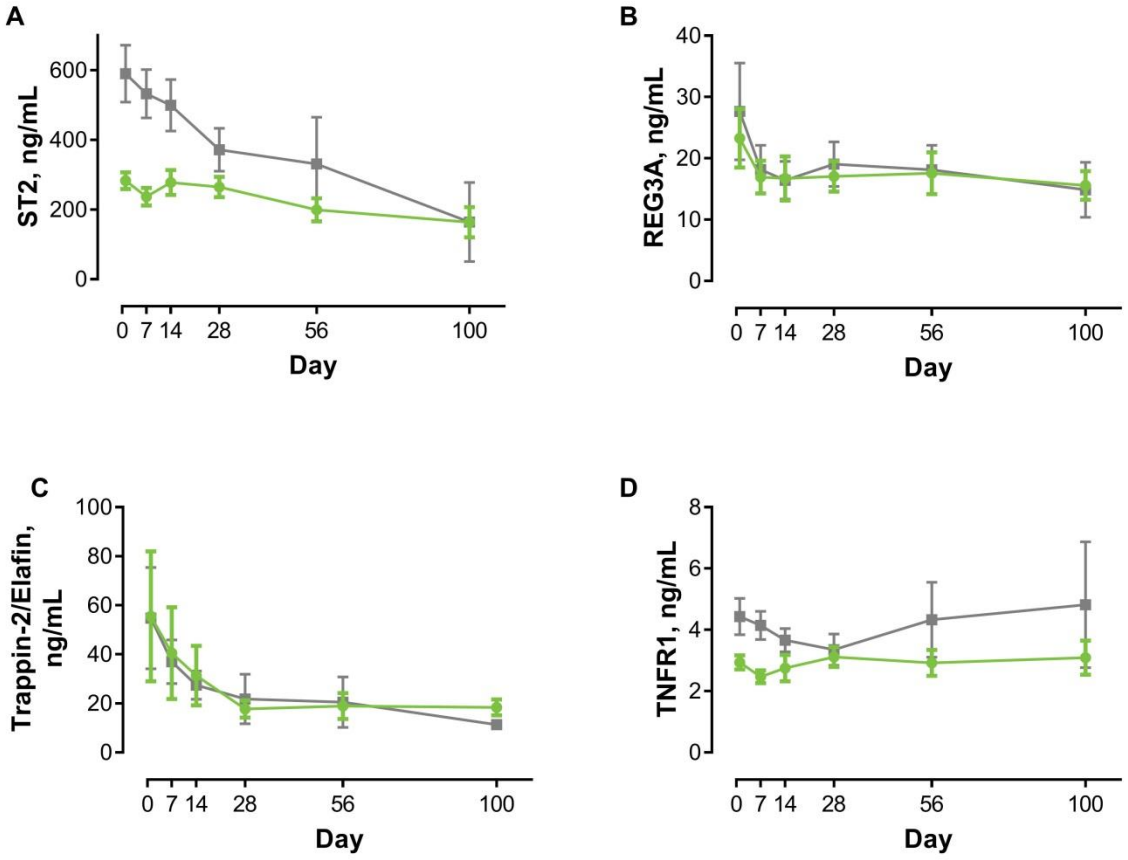
The data cutoff was July 2, 2018.
* One patient (1.4%) discontinued study treatment on Day 28 (adverse event).
† Includes 4 patients who discontinued because of clinical improvement.
‡ Other reasons for treatment discontinuation included transfer of care to an out-of-state facility and need for additional GVHD treatment (n=1 each).

Supplemental Figure 2. Mortality by response status



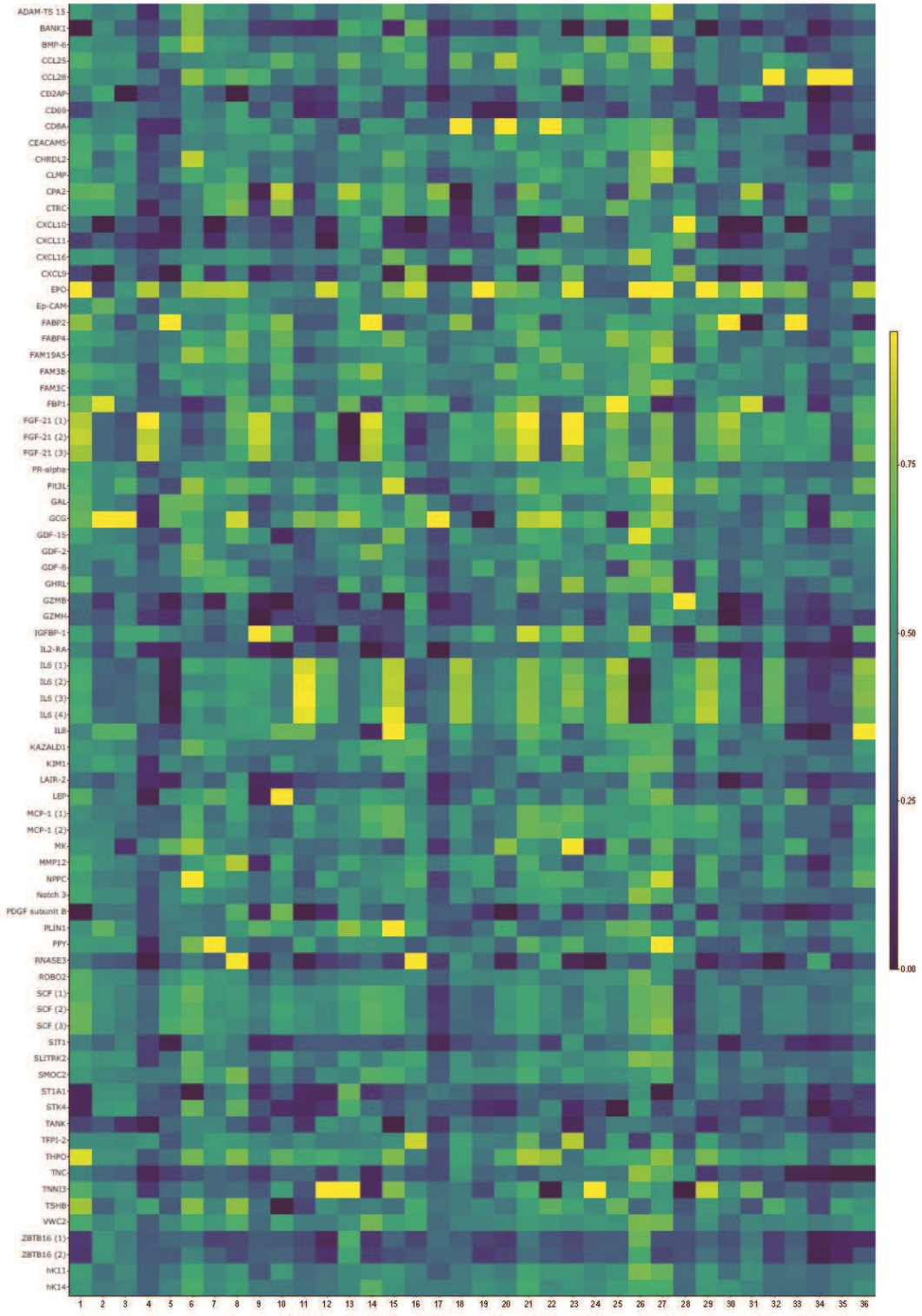
The data cutoff was July 2, 2018.
ATN, acute tubular necrosis; NHL, non-Hodgkin lymphoma; NOS, not otherwise specified; PEA, pulseless electrical activity; VOD, veno-occlusive disease.
* Death occurred on Day 28.

Supplemental Figure 3. Longitudinal ST2, REG3A, TNFR1, and Trappin-2/-Elafin expression



Mean concentrations of (A) ST2, (B) REG3A, (C) Trappin-2/Elafin, and (D) TNFR1 (± SEM) were plotted for each clinical response group including responders (complete response, very good partial response, partial response; green circles) and nonresponders (mixed response, progressive disease/death; grey squares) at designated visits.

Supplemental Figure 4. Heatmap of significantly modulated proteins in the responder cohort (n=36)



Broad proteomic analysis was conducted on 1012 proteins in circulation at baseline and Day 28. Significance was conferred at *P*<0.05 and absolute fold-change calculated as Day 28/baseline >1.5 (ie, >1.5 or <0.66) within each

group. False discovery rate *P* values were calculated across strata. A total of 60 proteins were upregulated, and 19 were downregulated in responders. Ingenuity pathway analysis identified hematopoiesis and interleukin-17–mediated inflammation as key pathways modulated in responders.

Supplemental references

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5. Galili T, O'Callaghan A, Sidi J, Sievert C. Heatmaply: an R package for creating interactive cluster heatmaps for online publishing. *Bioinformatics*. 2018;34(9):1600-1602.