Methods

Dose-Escalation Committee

A dose-escalation committee was responsible for the review of safety data at specified time points, to assess whether an escalation or reduction in dose should be administered in the subsequent cohort and to determine when the maximum-tolerated dose had been attained.

Voting members of the dose-escalation committee include representatives from Astellas Pharma, Inc. and the study site principal investigators (or designated sub-investigator). If appropriate, an expert consultant(s) could be invited to review safety data; consultants were not considered part of the dose-escalation committee and did not take part in escalation decision making. Astellas and the site representatives were responsible for evaluating and/or acting on the recommendations of the dose-escalation committee. Non-voting members of the dose-escalation committee could include members of the Astellas study team representing clinical science, biostatistics, global clinical pharmacology lead, data management, product safety and pharmacovigilance.

Inclusion Criteria

All patients must have had serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) lab values ≤2.5 × institutional upper limit normal (ULN) as well as a total serum bilirubin ≤1.5 × institutional ULN, serum creatinine ≤1.5 × institutional ULN or an estimated glomerular filtration rate (eGFR) of >50 ml/min as calculated by the Modification of Diet in Renal Disease (MDRD) equation. Female patients had to have been of non-childbearing potential (eg, postmenopausal or surgically sterile) or must have had a negative pregnancy test at the screening visit and must use two forms of birth control throughout the study period. Male patients were not allowed to donate sperm during the study.

Exclusion Criteria

Patients were not eligible for the study if they had been diagnosed with acute promyelocytic leukemia or BCR-ABL-positive leukemia, had clinically active central nervous system leukemia, or if they had any active malignant tumors other than AML or myelodysplastic syndrome. Furthermore, patients who presented with non-hematologic toxicities of Grade ≥2 from prior AML treatment at screening were excluded. Other exclusion criteria were prior hematopoietic stem cell transplantation (HSCT) within 2 months of Cycle 1 Day 1, persistent non-hematologic toxicities of Grade ≥2 related to the transplant, graft-versus-host disease that required treatment, New York Heart Association class 3 or 4 congestive heart failure, long QT syndrome, or a mean QTcF >450 ms at screening.

Criteria for Resuming Treatment after Hematopoietic Stem Cell Transplantation

Patients who achieved a CRc or PR were permitted to undergo HSCT while remaining in the study and resume treatment with gilteritinib after transplantation under the following conditions: a lapse of 30 to 60-days post HSCT;

evidence of successful engraftment as demonstrated by an absolute neutrophil count ≥500/mm^3 and platelets ≥20,000/mm^3 without transfusions; no Grade ≥2 acute graft-versus-host disease; subject was in CRc.

Assessments

Safety and Tolerability

Physical examinations and blood samples for clinical laboratory assessments were collected on Day –2; Day –1; Cycle 1 Days 1, 4, 8, 15, and 22; Cycle 2 Days 1 and 15; and Day 1 for all subsequent cycles. The 12-lead ECG assessments occurred at screening and then prior to dose on Day –2; Day –1; and Cycle 1 Days 1, 8, 15, and 22; additional ECG tests were conducted on Day –2 and on Cycle 1 Day 15 at 2 h, 4 h, 6 h, and 24 h post dose. However, if a >30 ms increase in QTcF was observed from Day 1 to Day 8, an additional ECG evaluation was performed on Cycle 1 Day 9. Recordings were performed in triplicate at each time point and transmitted electronically for central reading.

Pharmacokinetic and Pharmacodynamic Assessments

For single-dose evaluation, gilteritinib was orally administered on Day –2 and blood samples were drawn on Day –2 prior to dose (0 h) and at 30 min, 1 h, 2 h, 4 h, 6 h, 24 h, and 48 h post dose. Once-daily administration of gilteritinib began following a 48 h observation period after the initial dose (Cycle 1 Day 1). Multiple-dose PK was investigated on Day 15; blood samples were drawn on Cycle 1 Day 15 prior to dose (0 h) and at 30 min, 1 h, 4 h, 6 h, and 24 h post dose. Plasma samples were analyzed for gilteritinib concentrations using a validated liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) method.

The percentage inhibition of phosphorylation of FLT3, AXL, and S6 was assessed in whole-blood samples (4 mL) that were collected during the dose-escalation and -expansion phases. For the dose-escalation phase, blood samples for the plasma inhibitory activity assay of FLT3 were collected on Days −1 and −2; Cycle 1 Days 1, 8±1, 15, and 16; and Cycle 2 Day 1±3. Blood samples at Day −2 and Cycle 1 Day 15 were collected pre-dose (0.5 h before drug administration) and post-dose at 2±10 minutes, 6±20 minutes, and 24 h±90 minutes (the 24-h sample was collected on Day −1 and before the next dose of gilteritinib on Day 16). For the dose-expansion phase, blood samples for PIA were obtained pre-dose (0.5 h before drug administration) and post-dose (2 h after drug administration) on Days 1, 8±1, 15±1 of Cycle 1 and on Day 1±3 of Cycle 2.

Tables

Table S1. Definitions for antileukemic responses

Clinical response term	Definition
Complete remission	Patients must have bone marrow regenerating normal hematopoietic cells and achieve a morphologic leukemia-free state. They must have an absolute neutrophil count >1 × 10^9/L, a platelet count ≥100 × 10^9/L, and normal marrow differential with <5% blasts. Patients will be independent of red blood cell and platelet transfusion (defined as 1 week without red blood cell transfusion and 1 week without platelet transfusion). There should be no evidence of extramedullary leukemia.
Complete remission with incomplete platelet recovery (CRp)	Response meets all of the criteria for CR except that the platelet count is <100 × 10^9/L (Sievers EL, Larson RA, Stadtmauer EA, et al. J Clin Oncol 2001;19:3244-54).
Complete remission with incomplete hematological recovery (CRi)	Response meets all of the criteria for CR except subject experiences residual neutropenia (<1 × 10^9/L) with or without complete platelet recovery. Transfusion independence is not required.
Partial remission	Evidence of peripheral recovery with no (or only a few regenerating) circulating blasts and a reduction in marrow blasts to 5–25%, with a reduction of ≥50% from baseline pretreatment level.
Relapse	Relapse after CR, CRp, or CRi is defined as the reappearance of leukemic blasts in the peripheral blood or ≥5% of blasts in the bone marrow aspirate not attributable to any other cause, or reappearance or new appearance of extramedullary leukemia. Relapse after PR is similarly defined with the reappearance of significant numbers of peripheral blasts and an increase in the percentage of blasts in the bone marrow aspirate to >25% not attributable to any other cause, or reappearance or new appearance of extramedullary leukemia.

Table S2. List of Recruitment Sites, Study Investigators, and Number Subjects

Principal Investigator	Site Name	Total Enrolled (N=265)[a]
Alexander Perl	University of Pennsylvania-Abramson	36
Ellen Ritchie	Weill Cornell Medical College	30
Catherine Smith	University of California- San Francisco	23
Richard Larson	University of Chicago Medical Center	20
Jessica Altman	Northwestern University Medical Center	18
Eunice Wang	Roswell Park Cancer Institute	16
Mark Levis	Johns Hopkins University	15
Maria Baer	University of Maryland Medical Center	15
Giovanni Martinelli	Azienda Ospedaliero- Universitaria Policlinico S. Orsola Malpighi	12
Jorge Cortes	MD Anderson Cancer Center	11
David Claxton	Penn State Milton S. Hershey Medical Center	9
Mark Litzow	Mayo Clinic - Rochester	8
Stephen Strickland	Vanderbilt University Medical Center	7
Celalettin Ustun	University of Minnesota	6
Gary Schiller	UCLA Medical Center	6
Robert Stuart	Medical Center for the University of South Carolina	5
Ellin Berman	Memorial Sloan-Kettering Cancer Center	4
Jeanne Palmer	Mayo Clinic	4
Claudia Baldus	Charite Universitaetsmedizin Berlin	3
Christoph Rollig	University Hospital Carl Gustav Carus at the Technical University of Dresden	3
James McCloskey	Hackensack University Medical Center	3
Harry Erba	University of Alabama at Birmingham	3
Alexander Spira	Virginia Cancer Specialists	3[b]
Andreas Neubauer	Universitätsklinikum Giessen und Marburg GmbH	2
Joseph Jurcic	Columbia University Medical Center	1
Anjali Advani	The Cleveland Clinic	1
Margaret O'Donnell	City of Hope National Medical Center	1

[a]Of the 265 subjects enrolled in the study, 8 were randomized but did not receive study medication and 5 were subjects who were re-enrolled under different patient IDs.
[b]One subject was originally enrolled at University of Pennsylvania-Abramson but transferred to Virginia Cancer Specialists

Table S3. Any Treatment-emergent Adverse Event Grade 3, 4, or 5 Occurring in <10% of the Safety Population by System Organ Class

	Grade 3, n (%)	Grade 4, n (%)	Grade 5, n (%)
Blood and lymphatic system disorders			
Hemolytic anemia	1 (0.4%)	0	0
Leukocytosis	9 (4%)	2 (1%)	0
Leukopenia	2 (1%)	3 (1%)	0
Neutropenia	2 (1%)	18 (7%)	1 (0.4%)
Pancytopenia	0	2 (1%)	0
Cardiac disorders			
Acute myocardial infarction	2 (1%)	0	0
Arrhythmia	1 (0.4%)	0	0
Atrial fibrillation	5 (2%)	0	0
Atrial thrombosis	1 (0.4%)	0	0
Atrioventricular block second degree	1 (0.4%)	0	0
Cardiac arrest	0	1 (0.4%)	2 (1%)
Cardiac failure congestive	2 (1%)	0	0
Myocarditis	2 (1%)	0	0
Pericardial effusion	2 (1%)	0	0
Pericarditis	1 (0.4%)	0	0
Sinus tachycardia	1 (0.4%)	0	0
Supraventricular tachycardia	2 (1%)	1 (0.4%)	0
Ventricular fibrillation	0	0	1 (0.4%)
Ventricular tachycardia	0	0	1 (0.4%)
Eye disorders			
Conjunctival edema	1 (0.4%)	0	0
Conjunctivitis	1 (0.4%)	0	0
Periorbital edema	1 (0.4%)	0	0
Retinal vascular thrombosis	0	1 (0.4%)	0
Gastrointestinal disorders			
Abdominal distension	1 (0.4%)	0	0
Abdominal pain	3 (1%)	0	0
Anal fistula	1 (0.4%)	0	0
Ascites	1 (0.4%)	0	0
Caecitis	2 (1%)	0	0
Colitis	1 (0.4%)	0	1 (0.4%)
Duodenal perforation	1 (0.4%)	0	0
Dysphagia	2 (1%)	0	0
Enteritis	1 (0.4%)	0	0
Enterocolitis	1 (0.4%)	0	0
Gastrointestinal hemorrhage	4 (2%)	1 (0.4%)	0

	Grade 3, n (%)	Grade 4, n (%)	Grade 5, n (%)
Gastrointestinal ulcer	1 (0.4%)	0	0
Hematemesis	1 (0.4%)	0	0
Hematochezia	2 (1%)	0	0
Intestinal obstruction	1 (0.4%)	0	0
Intestinal perforation	1 (0.4%)	0	0
Large intestinal ulcer	1 (0.4%)	0	0
Lower gastrointestinal hemorrhage	2 (1%)	0	0
Malabsorption	0	1 (0.4%)	0
Neutropenic colitis	1 (0.4%)	0	1 (0.4%)
Esophagitis	1 (0.4%)	0	0
Pancreatitis	1 (0.4%)	0	0
Rectal hemorrhage	1 (0.4%)	0	0
Rectal tenesmus	1 (0.4%)	0	0
Small intestinal hemorrhage	1 (0.4%)	0	0
Small intestinal obstruction	3 (1%)	0	0
Swollen tongue	1 (0.4%)	0	0
Upper gastrointestinal hemorrhage	1 (0.4%)	0	0
General disorders and administration site conditions			
Death[a]	0	0	4 (2%)
Generalized edema	1 (0.4%)	0	0
Mucosal inflammation	5 (2%)	2 (1%)	0
Multi-organ failure	0	0	7 (3%)
Edema	2 (1%)	0	0
Pain	3 (1%)	0	0
Hepatobiliary disorders			
Cholecystitis	1 (0.4%)	0	0
Hepatic lesion	1 (0.4%)	0	0
Hyperbilirubinemia	3 (1%)	1 (0.4%)	0
Immune system disorders			
Acute graft versus host disease in intestine	1 (0.4%)	0	0
Acute graft versus host disease in skin	2 (1%)	0	0
Anaphylactic reaction	0	1 (0.4%)	0
Graft versus host disease	2 (1%)	0	0
Infections and infestations			
Abscess limb	1 (0.4%)	0	0

	Grade 3, n (%)	Grade 4, n (%)	Grade 5, n (%)
Arthritis bacterial	1 (0.4%)	0	0
Bacteremia	13 (5%)	3 (1%)	1 (0.4%)
Bronchopneumonia	1 (0.4%)	0	0
Bronchopulmonary aspergillosis	2 (1%)	0	1 (0.4%)
Catheter site cellulitis	1 (0.4%)	0	0
Catheter site infection	1 (0.4%)	0	0
Cellulitis	7 (3%)	0	1 (0.4%)
Clostridial infection	2 (1%)	0	0
Clostridium bacteremia	2 (1%)	0	0
Clostridium difficile colitis	5 (2%)	0	0
Clostridium difficile infection	4 (2%)	0	0
Corona virus infection	1 (0.4%)	0	0
Cytomegalovirus colitis	1 (0.4%)	0	0
Device related infection	4 (2%)	0	0
Diverticulitis	1 (0.4%)	0	0
Viral encephalitis	1 (0.4%)	0	0
Enterobacter sepsis	0	1 (0.4%)	0
Enterococcal infection[b]	6 (2%)	1 (0.4%)	0
Epiglottitis	0	1 (0.4%)	0
Escherichia bacteremia	1 (0.4%)	0	0
Escherichia infection	1 (0.4%)	0	0
Escherichia sepsis	0	1 (0.4%)	0
Escherichia urinary tract infection	2 (1%)	0	0
Fungaemia	1 (0.4%)	0	0
Viral gastroenteritis	1 (0.4%)	0	0
Genital infection bacterial	1 (0.4%)	0	0
Hepatic infection	0	0	1 (0.4%)
Infection	1 (0.4%)	1 (0.4%)	0
Influenza	1 (0.4%)	0	0
Klebsiella bacteremia	2 (1%)	0	0
Klebsiella sepsis	1 (0.4%)	0	0
Fungal laryngitis	1 (0.4%)	0	0
Liver abscess	1 (0.4%)	0	0
Lobar pneumonia	1 (0.4%)	1 (0.4%)	0
Lower respiratory tract infection fungal	1 (0.4%)	0	0

	Grade 3, n (%)	Grade 4, n (%)	Grade 5, n (%)
Lung infection	7 (3%)	0	1 (0.4%)
Neutropenic infection	1 (0.4%)	0	0
Oral infection	1 (0.4%)	0	0
Osteomyelitis	1 (0.4%)	0	0
Parainfluenzae virus infection	1 (0.4%)	0	0
Periodontitis	1 (0.4%)	0	0
Periorbital infection	1 (0.4%)	0	0
Pharyngitis	1 (0.4%)	0	0
Pneumonia fungal	11 (4%)	0	0
Pneumonia haemophilus	1 (0.4%)	0	0
Pneumonia parainfluenza viral	1 (0.4%)	0	0
Post-procedural cellulitis	1 (0.4%)	0	0
Pseudomonas infection	1 (0.4%)	0	0
Respiratory syncytial virus infection	1 (0.4%)	0	0
Respiratory tract infection fungal	1 (0.4%)	0	0
Septic shock	0	2 (1%)	4 (2%)
Sinusitis	4 (2%)	0	0
Skin infection	5 (2%)	0	0
Soft tissue infection	1 (0.4%)	1 (0.4%)	0
Staphylococcal bacteremia	2 (1%)	1 (0.4%)	1 (0.4%)
Staphylococcal infection	1 (0.4%)	0	0
Staphylococcal sepsis	0	0	2 (1%)
Streptococcal bacteremia	3 (1%)	0	0
Streptococcal sepsis	2 (1%)	0	0
Systemic candida	1 (0.4%)	0	0
Systemic mycosis	1 (0.4%)	0	0
Tooth infection	2 (1%)	0	0
Toxic shock syndrome	1 (0.4%)	0	0
Upper respiratory tract infection	2 (1%)	0	0
Urinary tract infection[c]	17 (7%)	0	0
Urosepsis	1 (0.4%)	1 (0.4%)	0
Injury, poisoning and procedural complications			
Brain herniation	1 (0.4%)	0	0
Facial bones fracture	1 (0.4%)	0	0
Hip fracture	1 (0.4%)	0	0

	Grade 3, n (%)	Grade 4, n (%)	Grade 5, n (%)
Pelvic fracture	1 (0.4%)	0	0
Post-procedural hemorrhage	1 (0.4%)	0	0
Road traffic accident	0	1 (0.4%)	0
Spinal fracture	1 (0.4%)	0	0
Splenic hematoma	1 (0.4%)	0	0
Subdural hematoma	4 (2%)	2 (1%)	0
Subdural hemorrhage	0	1 (0.4%)	0
Tendon rupture	1 (0.4%)	0	0
Wound complication	1 (0.4%)	0	0
Investigations			
Activated partial thromboplastin time prolonged	1 (0.4%)	0	0
Blood bilirubin increased	6 (2%)	1 (0.4%)	0
Blood creatinine phosphokinase increased	7 (3%)	4 (2%)	0
Blood creatinine increased	4 (2%)	0	0
Blood lactate dehydrogenase increased	2 (1%)	0	0
Blood urea increased	1 (0.4%)	0	0
Ejection fraction decreased	3 (1%)	1 (0.4%)	0
Electrocardiogram QT prolonged	8 (3%)	0	0
Gamma-glutamyltransferase increased	1 (0.4%)	0	0
International normalized ratio increased	1 (0.4%)	0	0
Lipase increased	1 (0.4%)	0	0
Liver function test abnormal	4 (2%)	0	0
Lymphocyte count decreased	1 (0.4%)	1 (0.4%)	0
Neutrophil count decreased	7 (3%)	14 (6%)	0
Transaminases increased	6 (2%)	0	0
Troponin T increased	1 (0.4%)	0	0
Weight increased	3 (1%)	0	0
White blood cell count decreased	6 (2%)	9 (4%)	0
White blood cell count increased	0	1 (0.4%)	0
Metabolism and nutrition disorders			
Acidosis	1 (0.4%)	0	0
Dehydration	3 (1%)	0	0
Diabetic ketoacidosis	0	0	1 (0.4%)
Failure to thrive	2 (1%)	0	0
Hyperglycemia	8 (3%)	1 (0.4%)	0

	Grade 3, n (%)	Grade 4, n (%)	Grade 5, n (%)
Hyperkaliemia	3 (1%)	1 (0.4%)	0
Hyperuricemia	5 (2%)	1 (0.4%)	0
Hypophosphatemia	14 (6%)	3 (1%)	0
Hypovolemia	1 (0.4%)	0	0
Metabolic acidosis	1 (0.4%)	0	0
Tumor lysis syndrome	2 (1%)	0	0
Musculoskeletal and connective tissue disorders			
Back Pain	5 (2%)	0	0
Bone pain	1 (0.4%)	0	0
Muscular weakness	5 (2%)	0	0
Myalgia	2 (1%)	0	0
Myopathy	1 (0.4%)	0	0
Myositis	1 (0.4%)	0	0
Neck pain	1 (0.4%)	0	0
Osteonecrosis	1 (0.4%)	0	0
Pain in extremity	2 (1%)	0	0
Rhabdomyolysis	1 (0.4%)	0	0
Neoplasms benign, malignant and unspecified (including cysts and polyps)			
Leukemic infiltration brain	1 (0.4%)	0	0
Squamous cell carcinoma	1 (0.4%)	0	0
Nervous system disorders			
Cerebral ischaemia	0	0	1 (0.4%)
Cerebrovascular accident	1 (0.4%)	0	0
Convulsion	2 (1%)	0	0
Dysesthesia	1 (0.4%)	0	0
Encephalopathy	2 (1%)	0	0
Hemorrhage intracranial	0	0	3 (1%)
Hemiparesis	1 (0.4%)	0	0
Loss of consciousness	0	0	1 (0.4)
Neuralgia	1 (0.4%)	0	0
Paraesthesia	1 (0.4%)	0	0
Posterior reversible encephalopathy syndrome	1 (0.4%)	1 (0.4%)	0
Presyncope	2 (1%)	0	0
Sciatica	1 (0.4%)	0	0
Somnolence	1 (0.4%)	0	0

	Grade 3, n (%)	Grade 4, n (%)	Grade 5, n (%)
Syncope	13 (5%)	0	0
Unresponsive to stimuli	0	1 (0.4%)	0
Psychiatric disorders			
Agitation	1 (0.4%)	0	0
Confusional state	1 (0.4%)	2 (1%)	0
Insomnia	1 (0.4%)	0	0
Mental status changes	3 (1%)	0	0
Renal and urinary disorders			
Renal disorder	1 (0.4%)	0	0
Renal failure	1 (0.4%)	1 (0.4%)	2 (1%)
Renal tubular necrosis	1 (0.4%)	1 (0.4%)	0
Urinary incontinence	1 (0.4%)	0	0
Reproductive system and breast disorders			
Pelvic pain	1 (0.4%)	0	0
Scrotal edema	1 (0.4%)	0	0
Vaginal hemorrhage	1 (0.4%)	0	0
Respiratory, thoracic and mediastinal disorders			
Acute promyelocytic leukemia differentiation syndrome	1 (0.4%)	0	0
Acute respiratory distress syndrome	0	1 (0.4%)	0
Acute respiratory failure	0	1 (0.4%)	1 (0.4%)
Aspiration	2 (1%)	0	0
Emphysema	1 (0.4%)	0	0
Hemoptysis	0	0	1 (0.4%)
Hypercapnia	1 (0.4%)	0	0
Laryngeal mass	1 (0.4%)	0	0
Lung infiltration	1 (0.4%)	0	0
Pleural effusion	5 (2%)	0	0
Pneumonitis	1 (0.4%)	0	0
Pulmonary embolism	0	1 (0.4%)	1 (0.4%)
Pulmonary hemorrhage	0	1 (0.4%)	0
Pulmonary edema	1 (0.4%)	0	0
Respiratory distress	3 (1.2%)	0	0
Respiratory failure	2 (1%)	5 (2%)	7 (3%)
Retinoic acid syndrome	1 (0.4%)	0	0
Skin and subcutaneous tissue disorders			

	Grade 3, n (%)	Grade 4, n (%)	Grade 5, n (%)
Acute febrile neutrophilic dermatosis	3 (1%)	0	0
Angioedema	1 (0.4%)	2 (1%)	0
Decubitus ulcer	1 (0.4%)	0	0
Hyperhidrosis	1 (0.4%)	0	0
Pruritus	2 (1%)	0	0
Rash	1 (0.4%)	0	0
Rash maculo-papular	1 (0.4%)	0	0
Rash papular	1 (0.4%)	0	0
Skin induration	1 (0.4%)	0	0
Skin lesion	1 (0.4%)	0	0
Vascular disorders			
Embolism	0	1 (0.4%)	0
Hematoma	2 (1%)	0	0
Hemorrhage	1 (0.4%)	0	0
Orthostatic hypotension	3 (1%)	0	0
Phlebitis	0	1 (0.4%)	0
[a] Includes sudden death; [b]includes enterococcal bacteraemia, enterococcal infection, and enterocolitis infectious, [c] includes bacterial, enterococcal, and pseudomonal			

Table S4. Exposure to Study Drug

Parameter	20mg (n=16)	40mg (n=16)	80mg (n=24)	120mg (n=70)	200mg (n=103)	300`mg (n=20)	450mg (n=3)	Total (N=252)
Median duration of exposure, days (range)[a]	50·5 (3–93)	40 (4–154)	67 (7–515)	88·5 (8–518)	71 (3–377)	32 (7–100)	46 (42–72)	69 (3–518)
Median cumulative dose, mg (range)[b]	1420 (60–3000)	2060 (160–10600)	5920 (560–41200)	11720 (960–84720)	14600 (600–75400)	9600 (2100–30000)	16800 (7585·5–28950)	9950 (60–84720)
Dosing change, n (%)[c]								
Increase	9 (56%)	9 (56%)	12 (50%)	36 (51%)	21 (40%)	0	0	87 (35%)
Decrease	0	0	1 (4)	7 (10%)	13 (13%)	1 (5%)	3 (100%)	25 (10%)
Interruption	2 (13%)	3 (19%)	8 (33%)	26 (37%)	43 (42%)	4 (20%)	2 (67%)	88 (35%)

[a]Duration was defined as (the date of last dosing) – (the date of first dosing) + 1 – number of days without drug administration in between.
[b]Defined as sum of [(stop date of study drug – start date of study drug +1) × dose level] from study dosing data.
[c]Subjects with increased dose level higher than the initial dose level were included in dose increases; subjects with decreased dose level lower than the initial dose level were included in the dose decreases.

Table S5. Reasons for Treatment Discontinuation

Gilteritinib dose	20 mg (n=16)	40 mg (n=18)	80 mg (n=24)	120 mg (n=73)	200 mg (n=110)	300 mg (n=20)	450 mg (n=4)	Total (N=265)
Total number of discontinuations, n (%)	16 (100%)	18 (100%)	23 (96%)	65 (89%)	88 (80%)	20 (100%)	4 (100%)	234 (88%)
Study completion, n (%)	0	0	1 (4%)	8 (11%)	22 (20%)	0	0	31 (12%)
Reasons for treatment discontinuation, n (%)								
Progressive disease	7 (44%)	5 (28%)	6 (25%)	24 (33%)	24 (22%)	8 (40%)	1 (25%)	75 (28%)
Lack of efficacy	4 (25%)	5 (28%)	4 (17%)	17 (23%)	8 (7%)	5 (25%)	1 (25%)	44 (17%)
Adverse event	0	2 (11%)	2 (8%)	5 (7%)	22 (20%)	3 (15%)	0	34 (13%)
Death	2 (13%)	2 (11%)	5 (21%)	2 (3%)	15 (14%)	2 (10%)	1 (25%)	29 (11%)
Other	3 (19%)	2 (11%)	4 (17%)	9 (12%)	5 (5%)	2 (10%)	0	25 (9%)
Withdrawal by subject	0	0	2 (8%)	5 (7%)	10 (9%)	0	0	17 (6%)
Never received drug	0	2 (11%)	0	2 (3%)	3 (3%)	0	1 (25%)	8 (3%)
Lost to follow-up	0	0	0	1 (1%)	1 (1%)	0	0	2 (1%)

Table S6. Treatment-related Adverse Events Leading to Gilteritinib Discontinuation

	Total (N=252)
Blood creatinine phosphokinase increased	3 (1.2%)
Asthenia	1 (0.4%)
Decreased appetite	1 (0.4%)
Decreased white blood cell count	1 (0.4%)
Diarrhea	1 (0.4%)
Disseminated intravascular coagulation	1 (0.4%)
Ejection fraction decreased	1 (0.4%)
Fatigue	1 (0.4%)
Gastrointestinal hemorrhage	1 (0.4%)
Hemoptysis	1 (0.4%)
Hypotension	1 (0.4%)
Increased blood bilirubin	1 (0.4%)
Intracranial hemorrhage	1 (0.4%)
Malabsorption	1 (0.4%)
Myalgia	1 (0.4%)
Myocarditis	1 (0.4%)
Myositis	1 (0.4%)
Nausea	1 (0.4%)
Neutropenia	1 (0.4%)
Paraesthesia	1 (0.4%)
Pleural effusion	1 (0.4%)
Posterior reversible encephalopathy syndrome	1 (0.4%)
Pruritus	1 (0.4%)
Pulmonary embolism	1 (0.4%)
Pyoderma	1 (0.4%)
Renal tubular necrosis	1 (0.4%)
Septic shock	1 (0.4%)
Toxic shock syndrome	1 (0.4%)
Transaminases increased	1 (0.4%)
Ventricular fibrillation	1 (0.4%)
Vomiting	1 (0.4%)

Table S7. Pharmacokinetic Parameters of Gilteritinib with Multiple Dosing (Cycle 1 Day 15)

Parameter	20 mg (n=4)	40 mg (n=3)	80 mg (n=3)	120 mg (n=3)	200 mg (n=2)	300 mg (n=3)	450 mg (n=1)
C_{max} (ng/mL)	45·6 (30·5–137)	106 (76·7–140)	396 (217–516)	282 (248–593)	1462 (886–2038)	1257 (1036–2282)	1528
C_{min} (ng/mL)	25·9 (13·6–75·5)	73·2 (46·3–100)	223 (118–323)	227 (161–416)	1166 (578–1754)	1085 (855–2074)	1172
$AUC_{0–24}$ (ng·h/mL)	917* (540–2440)	2482† (2458–2505)	6234 (4108–10,532)	6180 (4171–10,477)	31,428 (16,288–46,568)	28,711 (22,282–42,022)	34,768
T_{max} (h)	4·00 (4·00–6·00)	3·87 (0·50–6·00)	4·33 (4·00–4·42)	2·17 (1·95–5·75)	6·03 (6·00–6·07)	6·05 (4·08–6·07)	5·93
$t_{1/2}$ (h)	54·5* (49·4–82·6)	152† (60·5–243)	91·0 (60·0–107)	44·9 (27·5–65·1)	142 (98·4–185)	159 (80·7–187)	NC
R_{ac}	3·8* (3·5–5·48)	9·64† (4·16–15·1)	5·99 (4·13–6·97)	3·23 (2·20–4·44)	9·04 (6·43–11·7)	10·0 (5·37–11·7)	NC

Data are presented as median (min–max).
* n=3 for AUC_{0-24}, $t_{1/2}$, and R_{ac}.
† n=2 for AUC_{0-24}, $t_{1/2}$, and R_{ac}.
$AUC_{0–24}$, area under the concentration–time curve over 24 hours; C_{max}, maximum plasma concentration; NC, not calculated due to insufficient data; R_{ac}, accumulation ratio; $t_{1/2}$, elimination ratio (calculated from R_{ac}); T_{max}, time to maximum plasma concentration.

Table S8. Clinical Response to Gilteritinib (20–450 mg) by Mutation Status in FAS Population

Clinical response	FLT3-mutant-positive[a]							
	20 mg (n=14)	40 mg (n=8)	80 mg (n=12)	120 mg (n=56)	200 mg (n=89)	300 mg (n=10)	450 mg (n=2)	Total (N=191)
CR	0	0	2 (17%)	7 (13%)	8 (9%)	1 (10%)	0	18 (9%)
CRp	0	0	0	2 (4%)	7 (8%)	1 (10%)	0	10 (5%)
CRi,	1 (7%)	0	3 (25%)	17 (30%)	20 (22%)	1 (10%)	0	42 (22%)
PR	1 (7%)	3 (38%)	3 (25%)	5 (9%)	7 (8%)	3 (30%)	1 (50%)	23 (12%)
CRc	**1 (7%)**	**0**	**5 (42%)**	**26 (46%)**	**35 (39%)**	**3 (30%)**	**0**	**70 (37%)**
ORR	**2 (14%)**	**3 (38%)**	**8 (67%)**	**31 (55%)**	**42 (47%)**	**6 (60%)**	**1 (50%)**	**93 (49%)**
	Wild-type FLT3							
	20 mg (n=2)	**40 mg (n=8)**	**80 mg (n=12)**	**120 mg (n=14)**	**200 mg (n=11)**	**300 mg (n=10)**	**450 mg (n=1)**	**Total (n=58)**
CR	1 (50%)	0	0	0	0	0	0	1 (2%)
CRp	0	0	0	0	0	0	0	0
CRi	0	0	2 (17%)	1 (7%)	1 (9%)	0	0	4 (7%)
PR	0	0	0	1 (7%)	1 (9%)	0	0	2 (3%)
CRc	**1 (50%)**	**0**	**2 (17%)**	**1 (7%)**	**1 (9%)**	**0**	**0**	**5 (9%)**
ORR	**1 (50%)**	**0**	**2 (17%)**	**2 (14%)**	**2 (18%)**	**0**	**0**	**7 (12%)**

Data are presented as n (%).

[a]Includes all patients with FLT3-ITD, -D835, -ITD-D835, or unknown FLT3 mutation.

CR, complete remission; CRc, composite remission (CR+CRi+CRp); CRi, complete remission with incomplete hematologic recovery; CRp, complete remission with incomplete platelet recovery; FAS, full analysis set; ORR, overall response rate; PR, partial remission.

Table S9. Clinical Response by FLT3 Mutation Type and Prior TKI in FLT3^{mut+} Patients Receiving ≥80 mg Gilteritinib

Clinical response	FLT3^{mut+} Patients Who Received ≥80 mg Gilteritinib				
	FLT3 mutation type			TKI status	
	FLT3-ITD only (n=141)	FLT3-D835 only (n=12)	ITD and D835 (n=13)	Prior TKI (n=45)	TKI naive (n=124)
CR	18 (13%)	0	0	2 (4%)	16 (13%)
CRp	10 (7%)	0	0	2 (4%)	8 (6%)
CRi	33 (23%)	1 (8%)	7 (54%)	10 (22%)	31 (25%)
PR	16 (11%)	1 (8%)	1 (8%)	5 (11%)	14 (11%)
CRc	**61 (43%)**	**1 (8%)**	**7 (5%)**	**14 (31%)**	**55 (44%)**
ORR	**77 (55%)**	**2 (17%)**	**8 (62%)**	**19 (42%)**	**69 (56%)**

CR, complete remission; CRc, composite remission (CR+CRi+CRp); CRi, complete remission with incomplete hematologic recovery; CRp, complete remission with incomplete platelet recovery; FAS, full analysis set; ORR, overall response rate; PR, partial remission.

Figures

Figure S1. DLT Rate Curve (SAF)

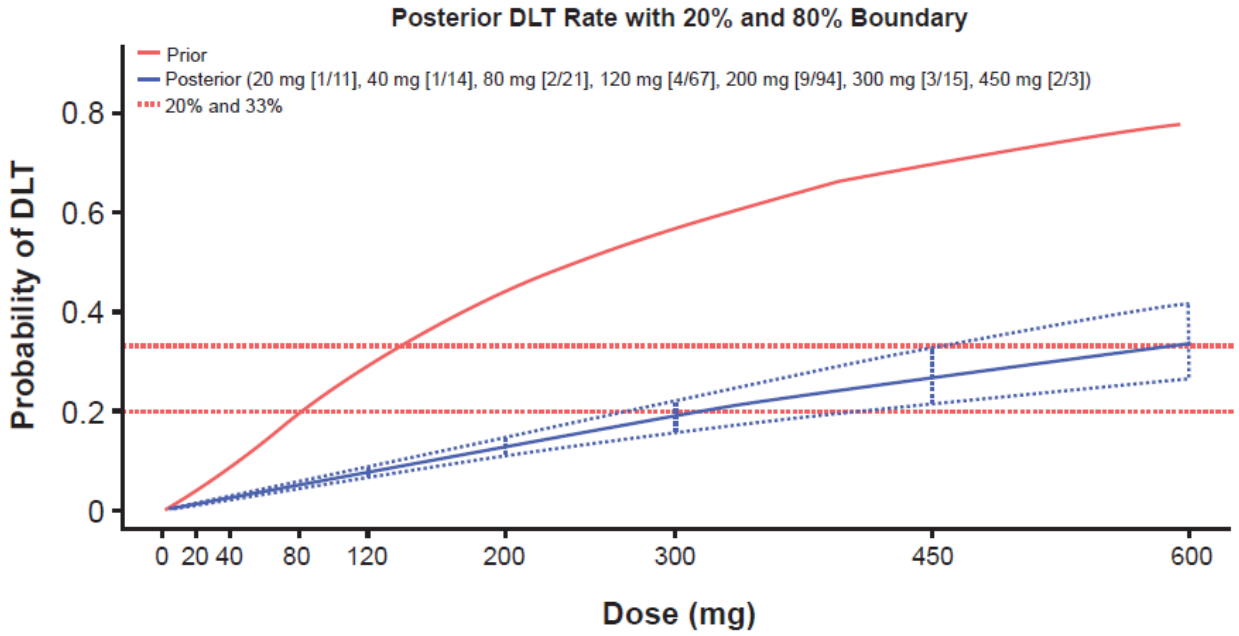
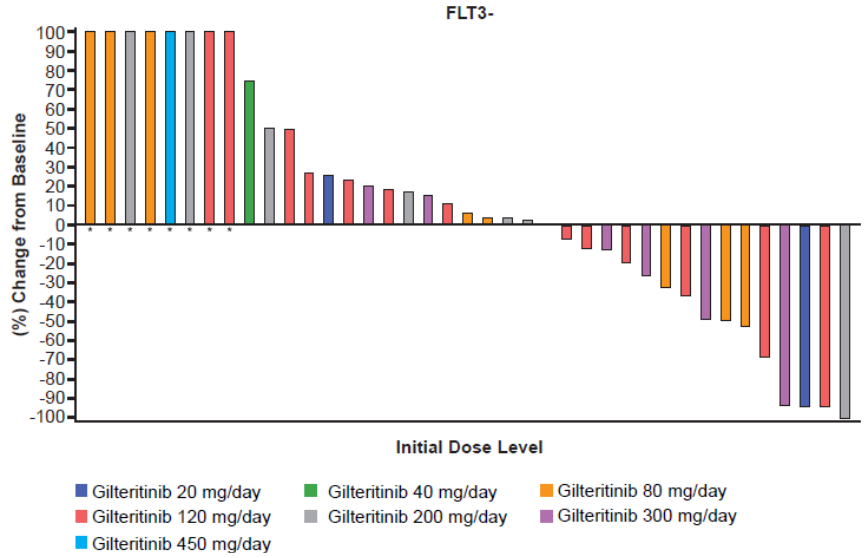
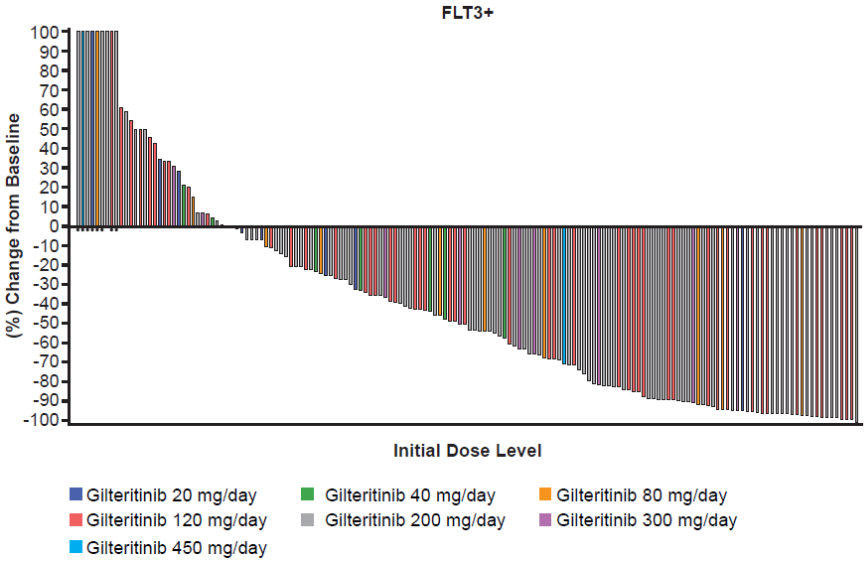


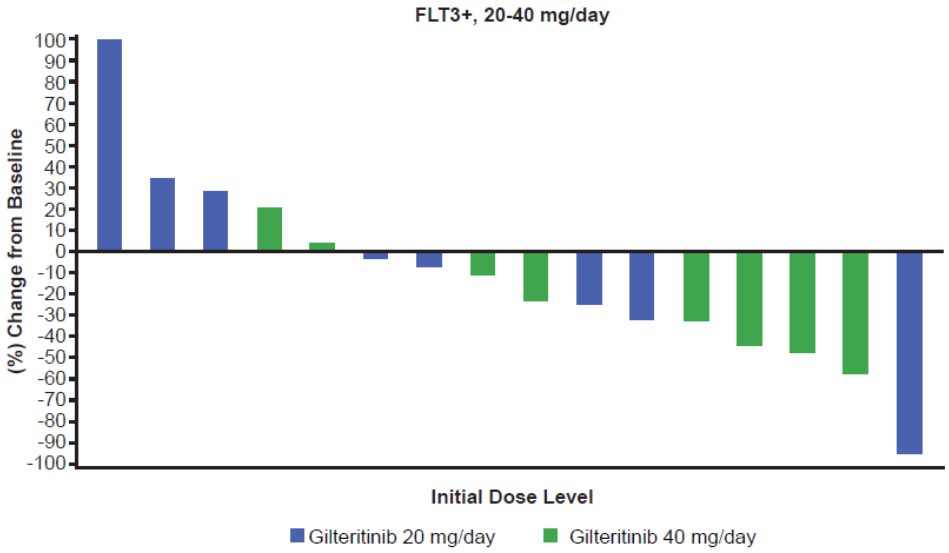
Figure S2. Waterfall Plot of Bone Marrow Myeloblast Reduction

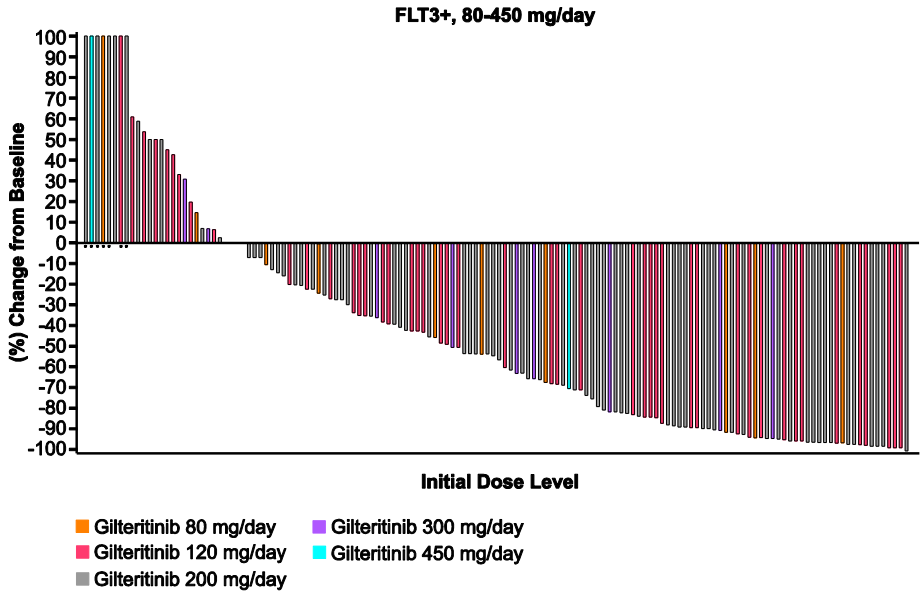
A) Grouping by Local FLT3 Mutation Status (FAS)





B) Grouping by Initial Dose Level (FAS)





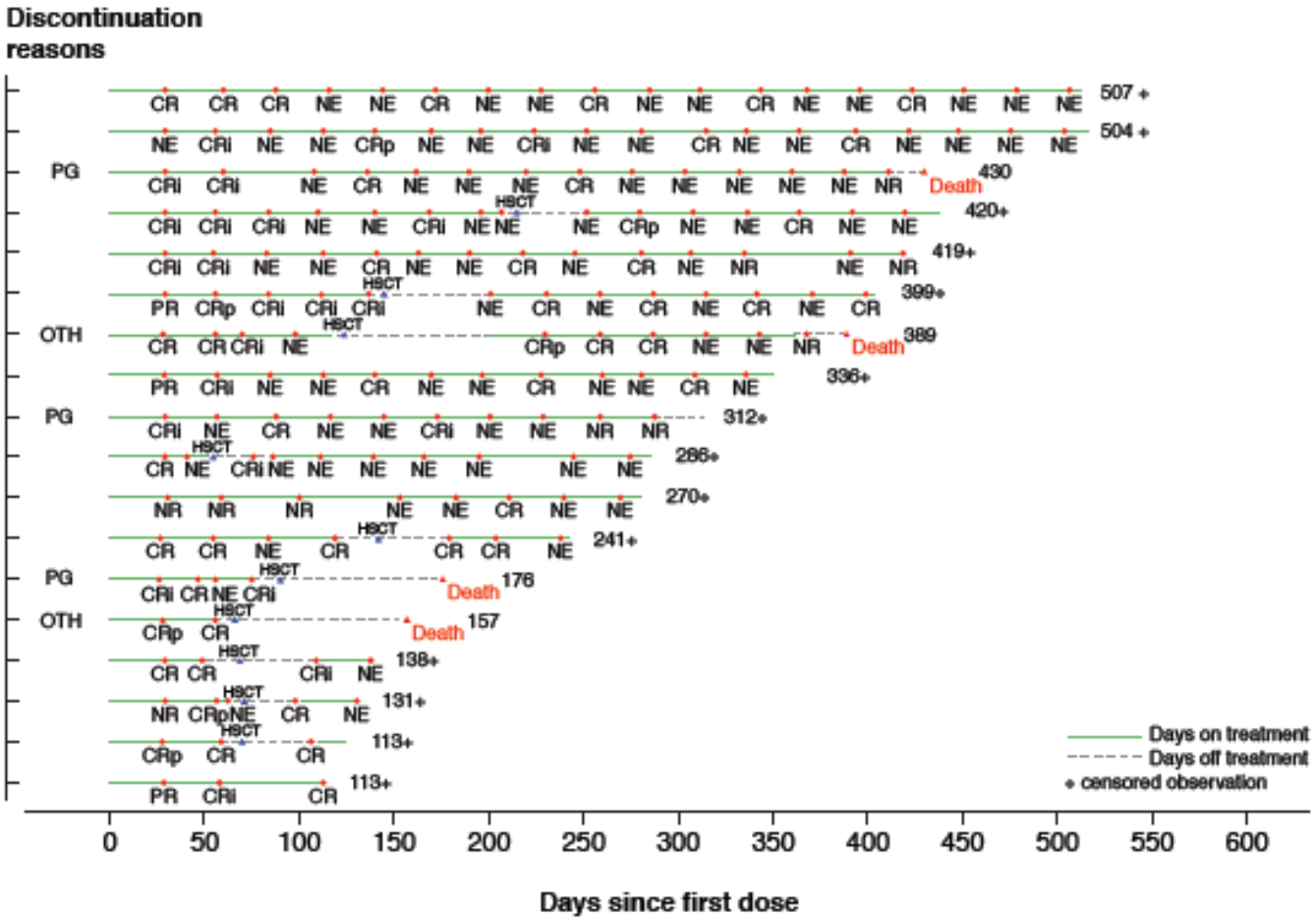
Footnote: Percent myeloblast change from baseline = [(post baseline myeloblast – baseline myeloblast)/baseline myeloblast] × 100%.

At each visit, myeloblast results from central bone marrow aspirate were used; when central aspirate was not available, results from central biopsy were used. When neither central aspirate nor biopsy was available, they were imputed with local values. Maximum change from baseline across all visits for each subject was plotted.

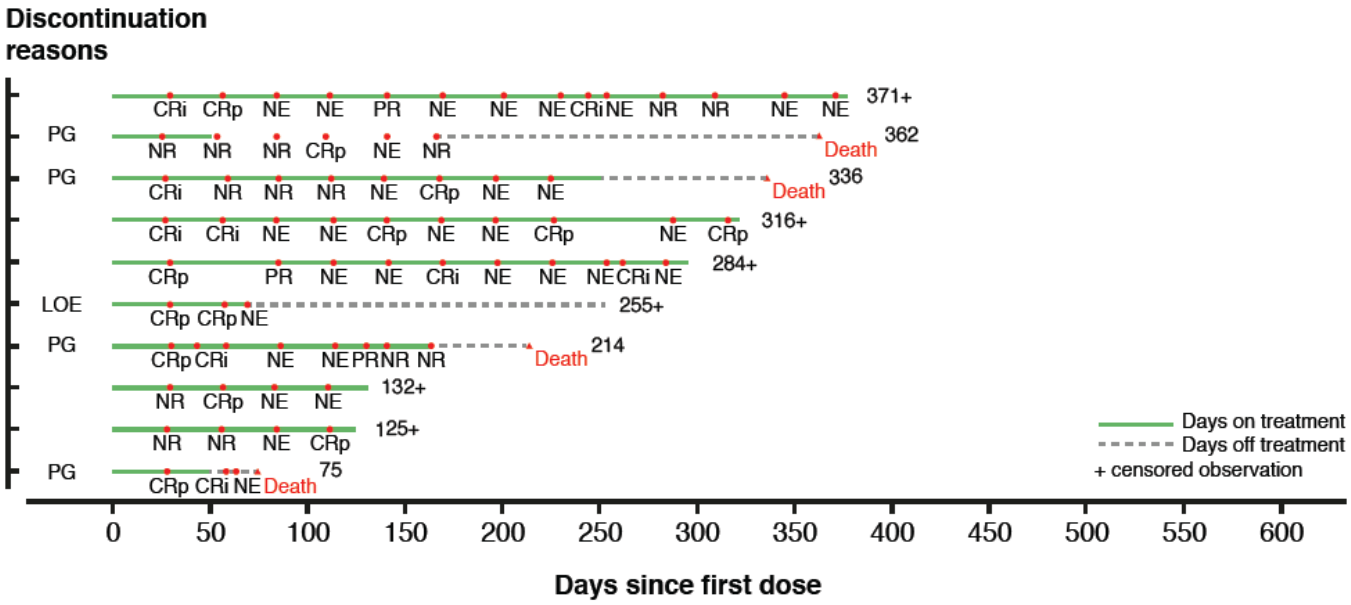
Subjects with percent change >100% were shown as 100%; patients noted with an "*" indicate the subject demonstrated a percent change >100%.

Figure S3. Swimmer Plots of Response and Overall Survival for FLT3^{mut+} Patients Who Received ≥80 mg of Gilteritinib

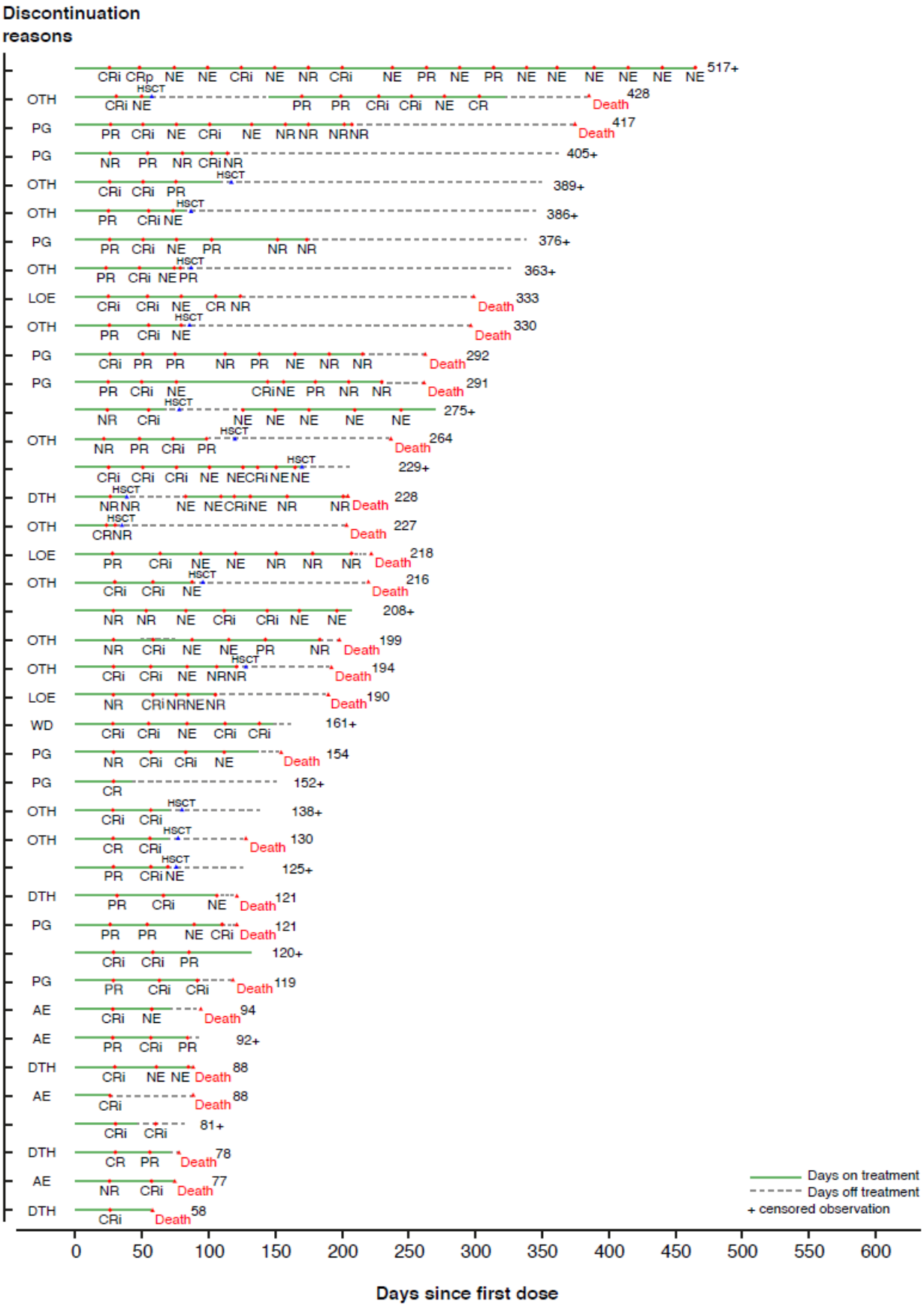
A) Best Response = CR



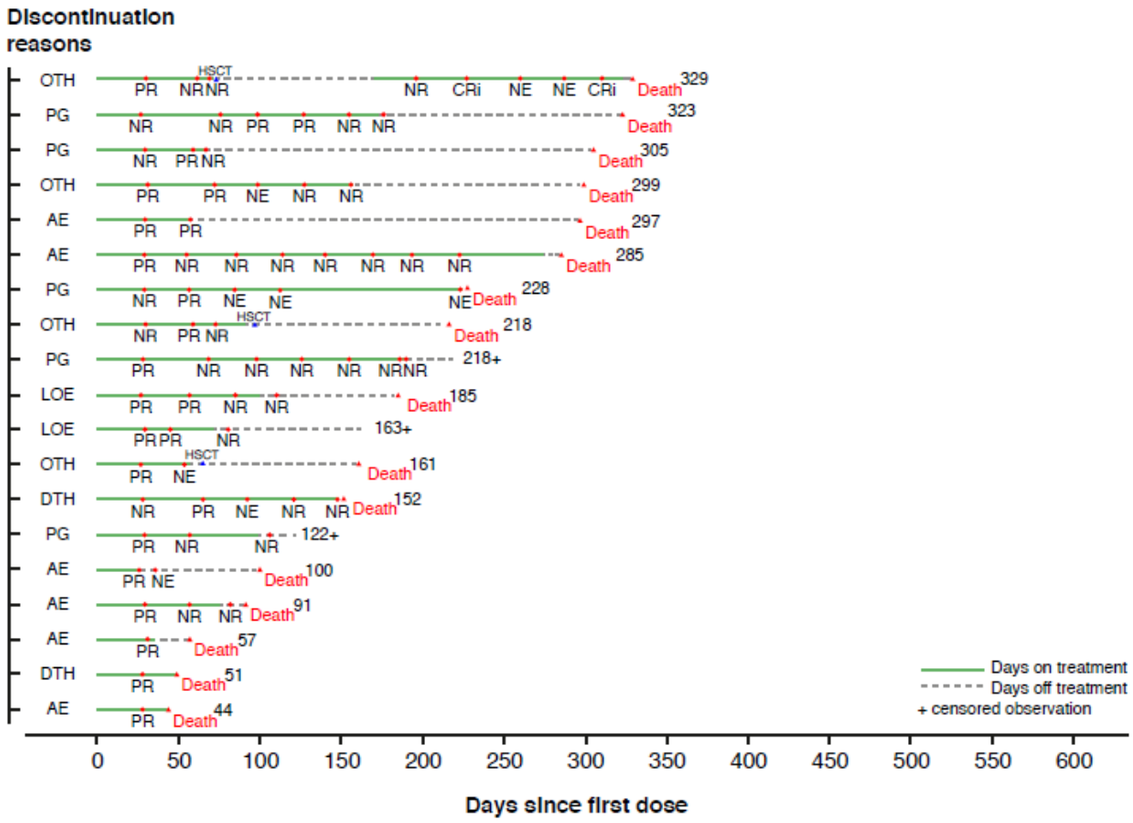
B) Best Response = CRp



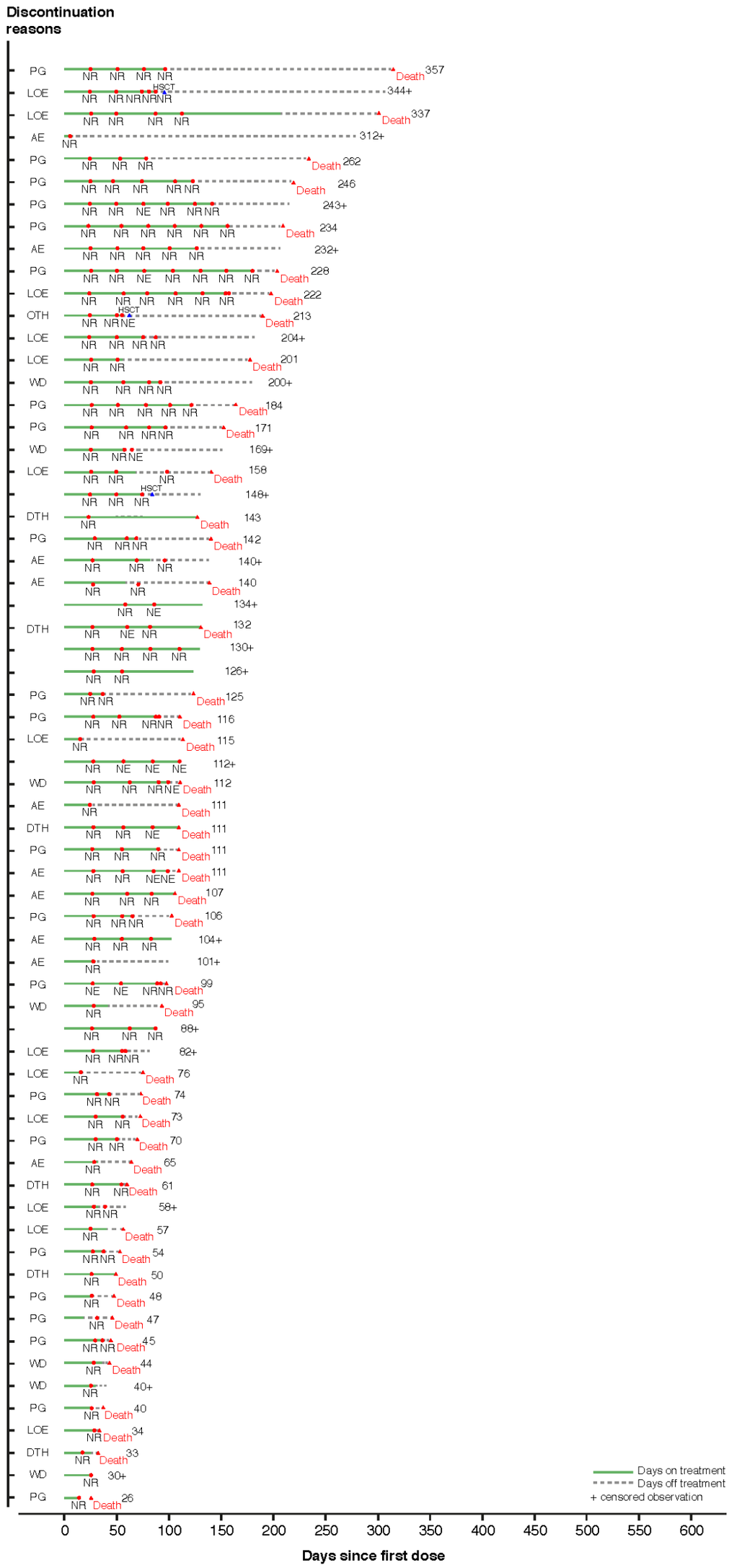
C) Best Response=CRi



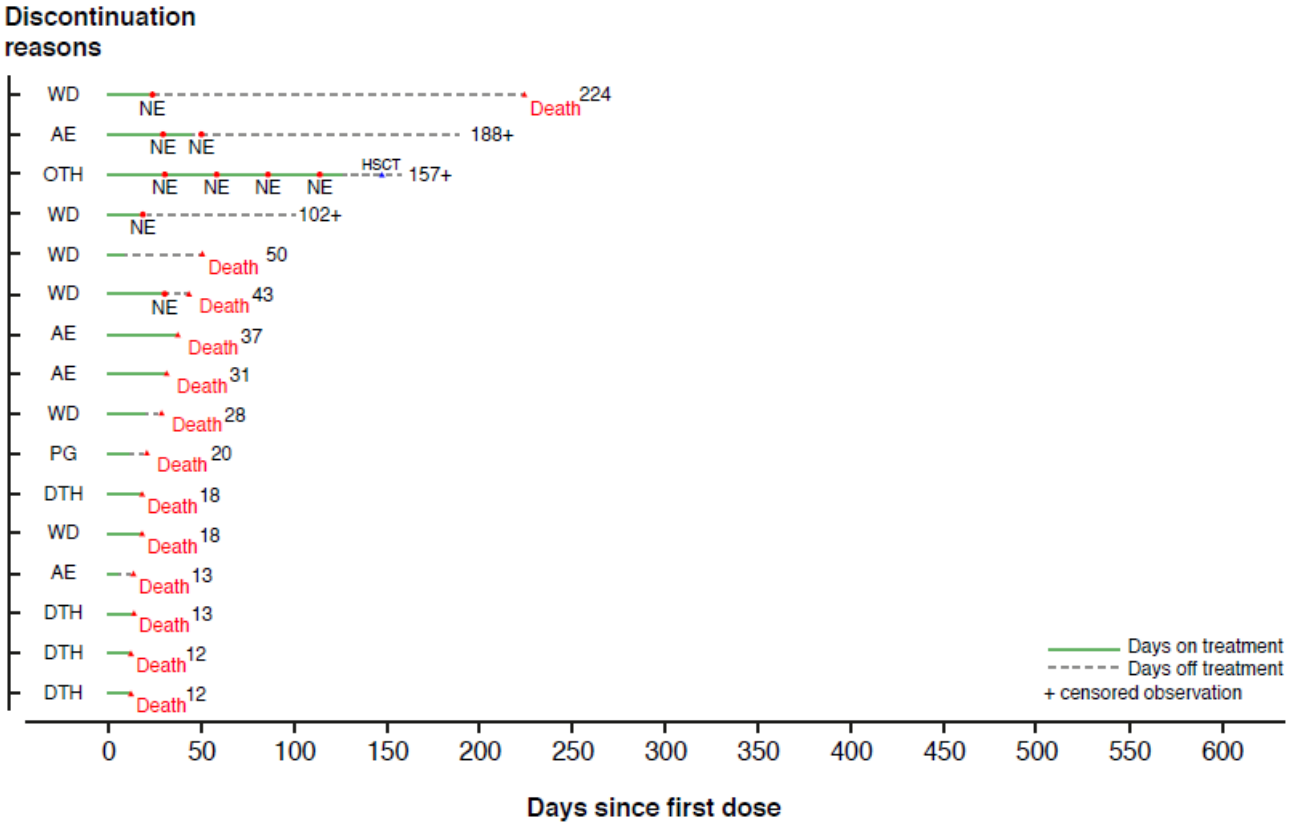
D) Best Response = PR



E) Best Response = No Response



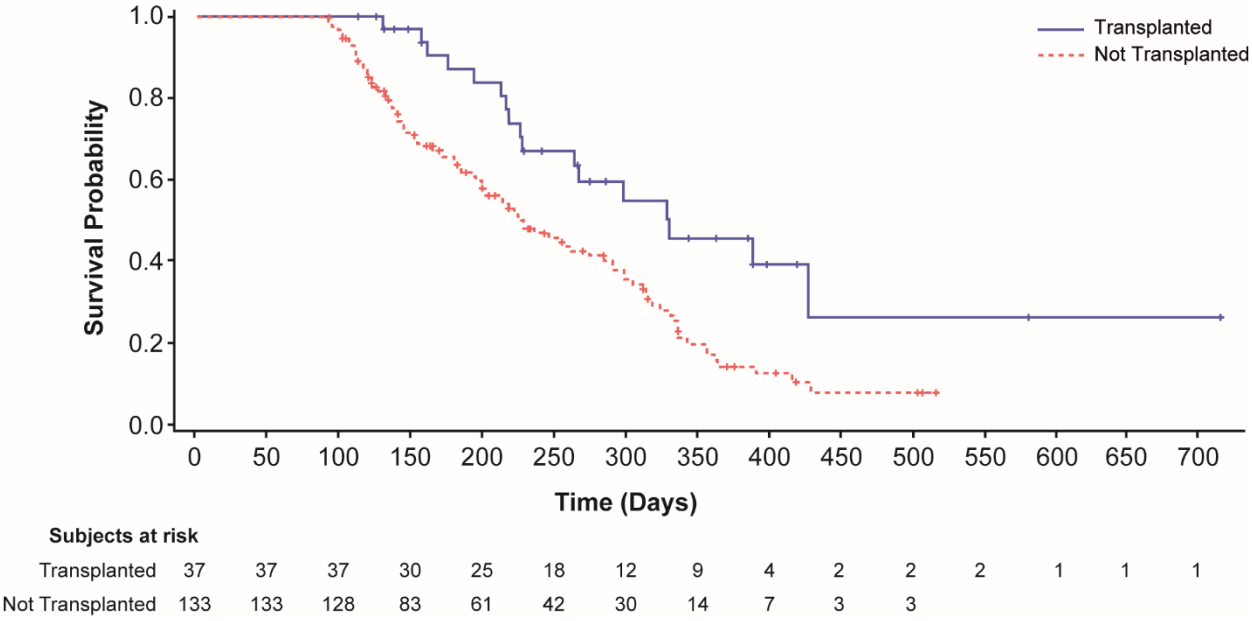
F) Best Response: Not Evaluable



Footnote: AE, adverse event; CR, complete remission; CRi, complete remission with incomplete hematologic recovery; CRp, complete remission with incomplete platelet recovery; DTH, death; HSCT, hematologic stem cell transplantation; LOE, lack of efficacy; NE, not evaluable; NR, nonresponse; OTH, other; PG, progressive disease; PR, partial remission; WD, withdrawal by subject.

The red circles (●) indicate bone marrow biopsies. The horizontal lines that do not end with a red triangle (▲) indicating "Death" represent patients who remained alive at the end of the study. The blue triangles (▲) represent HSCT. The protocol required bone marrow biopsies to be performed at screening and at every two cycles of gilteritinib therapy for patients who did not achieve CRc, and every three cycles for patients who achieved CRc. As shown, patients had up to 18 bone marrow biopsies performed over a 2-year period.

Figure S4. Overall Survival of Patients Who Received Transplant[a] Compared with Non-Transplanted Patients By the Landmark Method With Landmark at Day 92*



[a]Includes patients who did not resume treatment with gilteritinib.

*Survival time was set at ≥92 days.