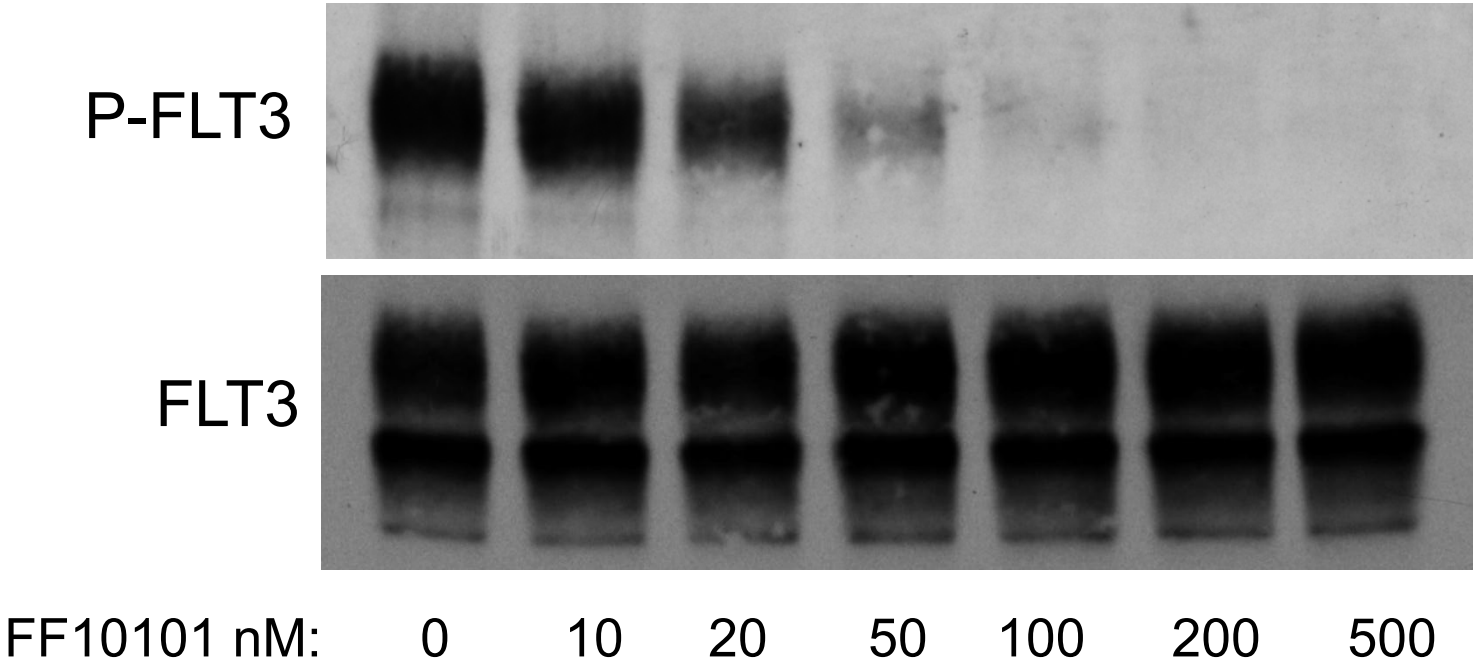
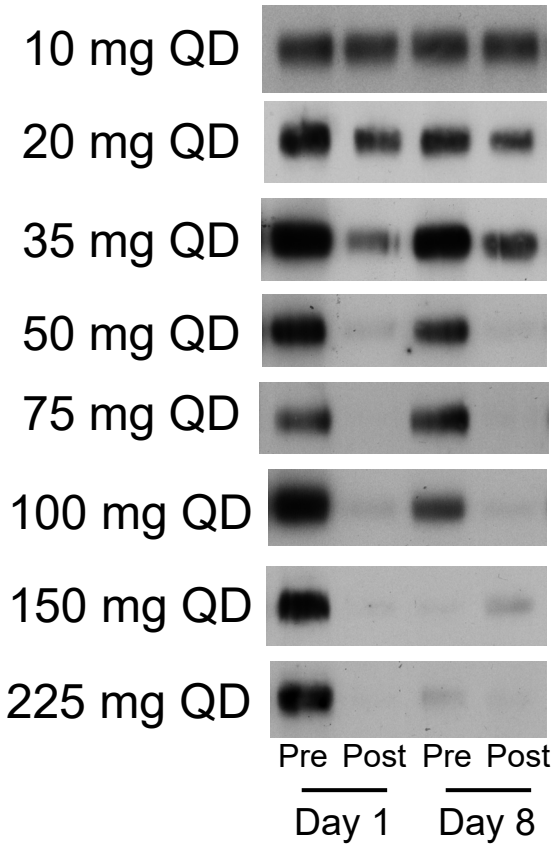
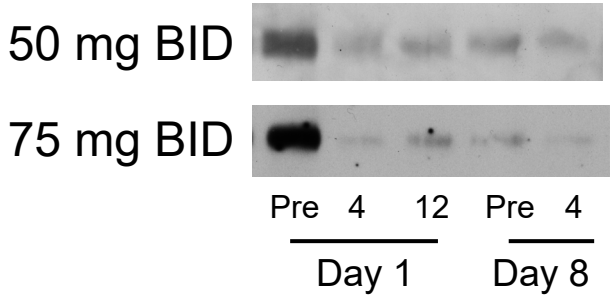



**Supplementary Figure 1. Dose response of FF-10101 against phosphorylated FLT3 in plasma**. FF-10101 (supplied by FujiFilm, Inc., Kanagawa, Japan) was spiked at the indicated concentrations into 100% human plasma. Molm-14 cells (FLT3-ITD-expressing human AML cell line) were incubated for 1 hour and analyzed for P-FLT3 by immunoprecipitation and immunoblotting. Regression analysis (after linear conversion) of the densitometric data resulted in an estimated $IC_{50}$ of 20 nM.

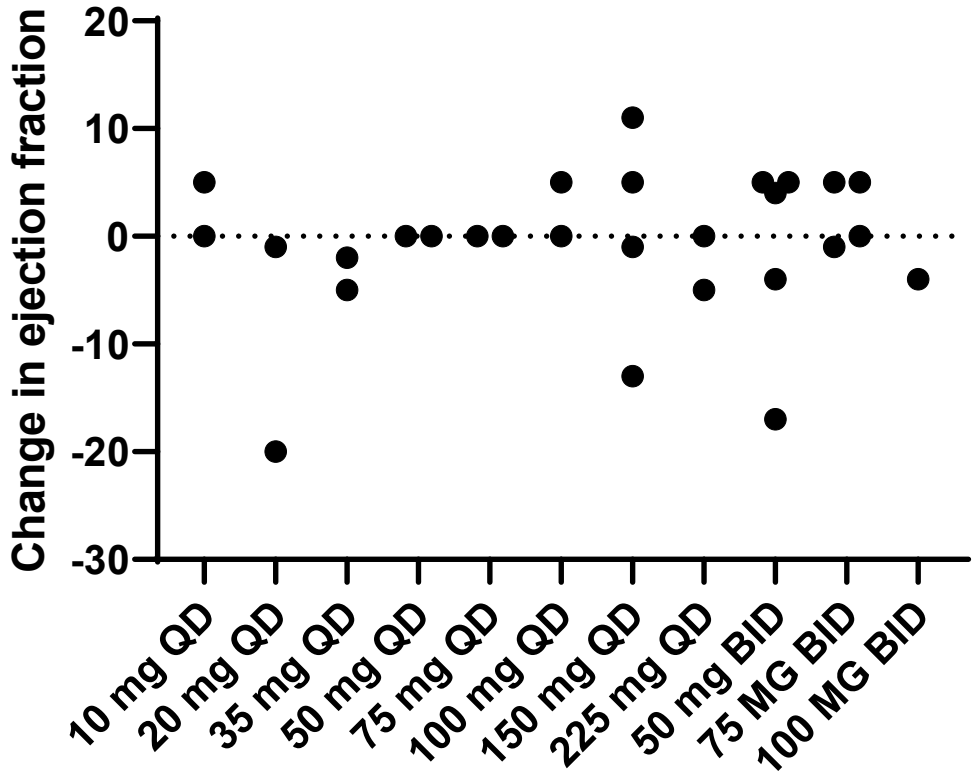
Supplementary Figure 1.



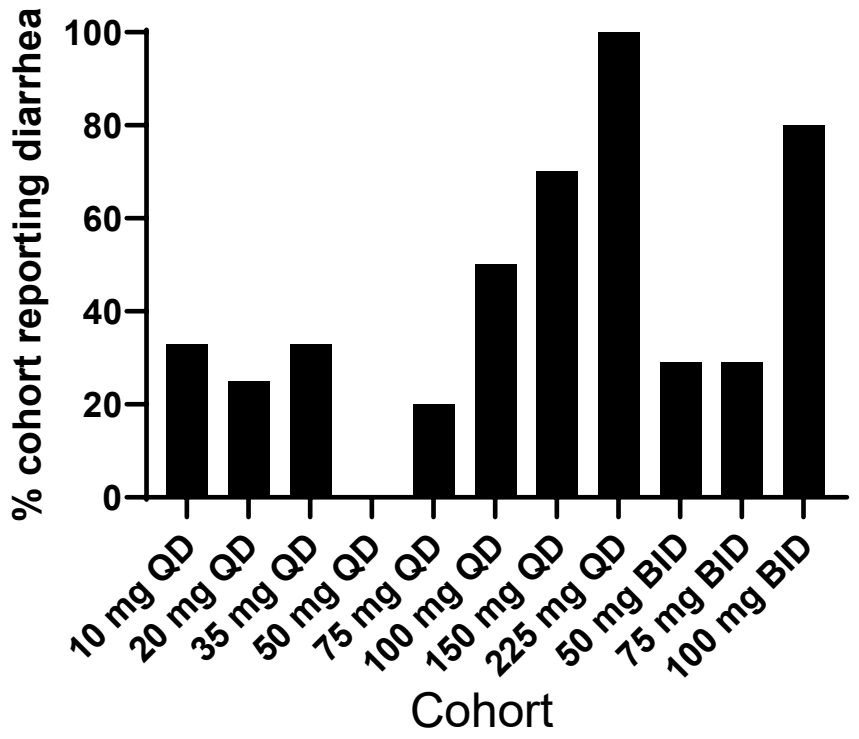




**Supplementary Figure 2. PIA assay results for each dose level.** The PIA assay for FLT3 was performed as described in Methods using plasma collected at designated time points in each cohort. Representative blots for Day 1 and Day 8 of cycle 1 are displayed. "Pre" refers to the sample collected immediately prior to dosing for the day.

Supplementary Figure 2




Supplementary Figure 3




Supplementary Figure 4

**Supplementary Table 1. Incidence of Grade ≥3 Treatment-Emergent Adverse Events (n=54 patients)**

| Preferred Term | Grade 3 / 4 n (%) | Grade 5 n (%) |
|---|---|---|
| Thrombocytopenia | 16 (29.6) | |
| Febrile neutropenia | 13 (24.1) | |
| Anemia | 11 (20.4) | |
| Pneumonia | 8 (14.8) | |
| AST, increased | 7 (13.0) | |
| Leukopenia | 7 (13.0) | |
| Neutropenia | 7 (13.0) | |
| Hypophosphatemia | 6 (11.1) | |
| Creatine phosphokinase, increased | 5 (9.3) | |
| Hypokalemia | 5 (9.3) | |
| ALT, increased | 4 (7.4) | |
| Diarrhea | 4 (7.4) | |
| Differentiation syndrome | 4 (7.4) | |
| Device-related infection | 3 (5.6) | |
| Pneumonia, fungal | 3 (5.6) | |
| Rash | 3 (5.6) | |
| Troponin, increased | 3 (5.6) | |
| Back pain | 2 (3.7) | |
| Delirium | 2 (3.7) | |
| Hypotension | 2 (3.7) | |
| Leukocytosis | 2 (3.7) | |
| Respiratory failure | 2 (3.7) | |
| Sepsis | 2 (3.7) | 3 (5.6) |
| Stomatitis | 2 (3.7) | |
| Subdural hematoma | 2 (3.7) | 1 (1.9) |
| Urinary tract infection | 2 (3.7) | |
| Abdominal pain, upper | 1 (1.9) | |
| Alkaline phosphatase, increased | 1 (1.9) | |
| Cardiac failure | 1 (1.9) | |
| Cardiac failure, congestive | | 1 (1.9) |
| Cardiomyopathy | 1 (1.9) | |
| Cellulitis | 1 (1.9) | |
| Chills | 1 (1.9) | |
| Cholelithiasis | 1 (1.9) | |
| CNS infection, fungal | 1 (1.9) | |
| CNS leukemia | 1 (1.9) | |
| Decreased appetite | 1 (1.9) | |
| Ejection fraction, abnormal | 1 (1.9) | |
| Enterococcal infection | 1 (1.9) | |
| External ear cellulitis | 1 (1.9) | |

| | | |
|---|---|---|
| Facial edema | 1 (1.9) | |
| Fatigue | 1 (1.9) | |
| Gastric hemorrhage | 1 (1.9) | |
| Gastrointestinal hemorrhage | 1 (1.9) | |
| Hemolysis | 1 (1.9) | |
| Hyperglycemia | 1 (1.9) | |
| Hypermagnesemia | 1 (1.9) | |
| Hypertension | 1 (1.9) | |
| Hypertriglyceridemia | 1 (1.9) | |
| Hyperuricemia | 1 (1.9) | |
| Hypoalbuminemia | 1 (1.9) | |
| Hypocalcemia | 1 (1.9) | |
| Hypoxia | 1 (1.9) | |
| Loss of consciousness | 1 (1.9) | |
| Lymphopenia | 1 (1.9) | |
| Mucosal Inflammation | 1 (1.9) | |
| Muscular weakness | 1 (1.9) | |
| Nausea | 1 (1.9) | |
| Nephrolithiasis | 1 (1.9) | |
| Neutrophilia | 1 (1.9) | |
| Osteomyelitis, bacterial | 1 (1.9) | |
| Periorbital edema | 1 (1.9) | |
| Procedural pain | 1 (1.9) | |
| Pulmonary amyloidosis | 1 (1.9) | |
| Pulmonary embolism | | 1 (1.9) |
| Pulmonary hemorrhage | | 1 (1.9) |
| Pulmonary sepsis | | 1 (1.9) |
| Pyrexia | 1 (1.9) | |
| QT prolonged | 1 (1.9) | |
| Skin infection | 1 (1.9) | |
| Squamous cell carcinoma of skin | 1 (1.9) | |
| Staphylococcal infection | 1 (1.9) | |
| Streptococcal bacteremia | 1 (1.9) | |
| Tachycardia | 1 (1.9) | |
| Urine output, decreased | 1 (1.9) | |

**Supplementary Table 2.** Detailed clinical data from responders.

| History | FLT3 | Prior TKI | Dose | Response | Response description |
|---|---|---|---|---|---|
| 56 yo F, diploid karytope, mutations in DNMT3a, Runx1, and a FLT3-ITD mutation. At initial diagnosis treated with 7+3+midostaurin, achieved remission and relapsed after consolidation. Enrolled on FF-10101 at relapse. | ITD | Midostaurin | 75 mg BID | CR | Baseline blasts in marrow 21%. On day 28 of cycle 1, marrow blasts 1%, ANC 1.74, platelets 179K. Off study for allogeneic transplant. |
| 78 yo M diagnosed with CMML initially, treated on protocol with azacitidine + sabatolimab, progressed to AML with a complex karyotype, mutations in TET2, SRSF2, ASXL1, PTPN11. Treated with cytarabine, daunorubicin, and ixazomib on protocol, refractory. | WT | None | 100 mg BID | CRh | Baseline blasts in marrow 18%. At completion of cycle 3, marrow blasts <5%, ANC 0.99, platelets 73K. |
| 78 yo M diagnosed with AML, trisomy 13, mutations in ASXL1, Runx1, and FLT3-ITD. Refractory to 2 cycles azacitidine + venetoclax. | ITD | None | 50 mg BID | CRp | Baseline blasts in marrow 69%. At completion of cycle 2, marrow blasts 3%, ANC 6.0, platelets 14K, but not requiring any transfusions. |
| 84 yo F diagnosed with AML with deletions of 13q and 5q, mutations in TET2, EZH2, Runx1. FLT3-ITD and FLT3-TKD mutations detectable, but below 1%. No response to Aza alone, treated with gilteritinib alone, responded, but progressed after 4 months. FLT3-ITD mutation undetected by conventional PCR at start of FF-10101. | WT | Gilteritinib | 50 mg BID | CRp | Baseline blasts in marrow 30%. After 4 cycles, marrow blasts 1%, ANC 2.44, platelets 13K. |
| 76 yo M diagnosed with AML, diploid karyotype with mutations in DNMT3a and NPM1, and a FLT3-ITD mutation. 7 + 3 + gilteritinib followed by allogeneic transplant. At relapse, karyotype was complex and FLT3-ITD no longer present, but DNMT3a and NPM1 still present. | WT | Gilteritinib | 75 mg BID | PR | Marrow blasts at baseline 31%. After one cycle, marrow blasts at 10%, ANC 2.79, platelets 179K. |

yo = Years old; M = male; F = female; ANC = absolute neutrophil count; K = thousand. QD = once per day. BID = twice daily.