SUPPLEMENTAL METHODS

Cell Lines

IL-3 dependent murine Ba/F3 cells transfected with the p210 BCR-ABL1 gene as well as human chronic myeloid leukemia cell lines K562, LAMA84, KYO1 and KCL-22 were obtained from the American Type Culture Collection and grown in the recommended culture medium, RPMI 1640 media supplemented with 10% FBS, penicillin, streptomycin and 1% L-glutamine at 37°C and 5% CO_2. Asciminib resistant cell lines were cultured as aforementioned with inclusion of escalating concentrations of asciminib as indicated. All human cell lines were authenticated by evaluation of short tandem repeats (STR) at 24 loci with the GenePrint 24 System (Promega). Sequencing was performed at the DNA Sequencing Core Facility, University of Utah.

Inhibitors

Asciminib was provided by Novartis Pharmaceuticals (Basel, Switzerland). Imatinib, nilotinib, dasatinib, ponatinib, cyclosporin A, indomethacin, valspodar, probenecid, elacridar and Ko143 were purchased from Selleck Chemicals (Houston, TX, USA). All inhibitors were resuspended in dimethyl sulfoxide at a concentration of 10.0 mM and stored at -20°C. Dilutions of 10.0 mM stock solutions to working concentrations preceded each experiment.

Asciminib-Resistant Cell Line Generation

Generation of asciminib resistant cell lines was performed by increasing the concentration of asciminib in steps of approximately one to two fold from 8.0 nM (beginning concentration) to the indicated final concentration (Fig. S1c, S2d, S3d, S4b, S5c). Asciminib concentration was raised in intermediate asciminib-resistant cell lines only after one week of ≥ 85% cell viability. Cell viability was measured by flow cytometry using the Guava ViaCount assay.

Cell Proliferation Assay

Cells were seeded in 96-well plates at 2×10^3 cells/well in 100 µL culture media with serial dilutions of inhibitor for 72 hr at 37°C. Inhibitor ranges: 0-5,000 nM for parental cell lines and 0-10,000 nM for asciminib resistant

cell lines. Reduced proliferation (expressed as percent of untreated) was determined using a colorimetric methanethiosulfonate viability assay (CellTiter 96 Aqueous One Solution; Promega). Reported IC_{50} values are the mean of three independent experiments done in quadruplicate. For experiments to assess the effect of escalating concentrations of efflux pump inhibitors to restore asciminib sensitivity to final asciminib resistant cell lines, each efflux pump inhibitor was tested over the range of 0-10,000 nM in the presence of the indicated maintenance concentration of asciminib. For experiments to assess the effect of including the ABCG2 efflux pump inhibitor, K0143 was added to a final concentration of 100 nM.

Immunoblot Analysis

Cells were collected for each treatment condition, lysed in RIPA buffer supplemented with phenylmethylsulfonyl fluoride for 30 minutes on ice, centrifuged and clarified lysates were then boiled at 95-100 °C for 5 minutes before running the gels. A modification was made for ABCG2 immunoblots: clarified lysates generated as above were heated at 70°C for 10 min. All lysates were subjected to SDS-PAGE and transferred. Antibodies used were from either Santa Cruz Biotechnology (anti-BCR sc-885 and anti-p-ABL Tyr-412 sc-293130), BD Biosciences (anti-STAT5 BD 610191) or Cell Signaling Technology (anti-pSTAT5 CST 9359, anti-β actin CST 3700 as a protein loading control, anti-ABCG2 CST 42078). The fluorescent secondary antibodies used were anti-rabbit-IRDye 800CW, LI-COR catalog 926-32213 (green) and anti-mouse-IRDye 680LT, LI-COR catalog 926-68022 (red). The immunoblot band images were developed with Image Studio version 5.2 application software in Odyssey Infrared Imager.

LC-MS/MS Determination of Intracellular Asciminib Concentration

Experiments were conducted as described in *Cancer Res* 2013; **73:** 3356-3370. At the end of the 6-hour asciminib exposure at 37 °C, cells were washed three times with fresh culture media at room temperature, counted and 1×10^6 cells were collected microcentrifuge tube by centrifugation. Pelleted cells were washed one time with phosphate-buffered saline, centrifuged and then supernatant was discarded. Cells were resuspended in 200 µL hypotonic lysis buffer [5mM Tris, 5mM EDTA, 5mM EGTA in ddH_2O; pH 7.0], gently sheared through a 27-gauge needle by repeated drawing up and down, incubated on ice for 15 min, centrifuged at 12,000 × g at 4 °C, clarified lysates isolated and stored at -80 °C before analysis.

qPCR Analysis of Efflux Pump Expression Levels

mRNA isolated from asciminib-sensitive and -resistant cells was obtained using the RNeasy Mini Kit (Qiagen). cDNA was synthesized with iScript Mastermix (BioRad). To determine ABCG2 expression, Real-time PCR was performed using the following primers for ABCG2: 5'-GGTGCCATTTACTTTGGGC-3' (forward) and 5'-ACAAAGAGTTCCACGGCTGA-3' (reverse). The primers for the housekeeping gene β-glucuronidase (GUS) were: 5'-GAAAATATGTGGTTGGAGAGCTCATT-3' (forward) and 5'-CCGAGTGAAGATCCCCTTTTTA-3' (reverse). Data was analyzed using Bio-Rad CFX Manager Software (Bio-Rad). All other primers were as published in *Leukemia Research* 2015; **39**: 696–701.

BCR-ABL1 Sequencing

Isolation of mRNA from was completed using the RNeasy® Kit (Qiagen) from 5 million cells cultured for a minimum of two days in vitro. Reverse-transcriptase was performed using iScript cDNA synthesis kit (BioRad). Mutations were detected using both next-generation sequencing (NGS; as described in *Ann Hematol* 2016; **95:** 201-210) and Sanger sequencing (as described in *Cancer Cell* 2014; **26:** 428-442) on *BCR-ABL1* transcripts; native *ABL* was excluded by amplifying from *BCR* exon 13 to exon 10 of *ABL*. Sequences were analyzed using Geneious® version 7.1.9.