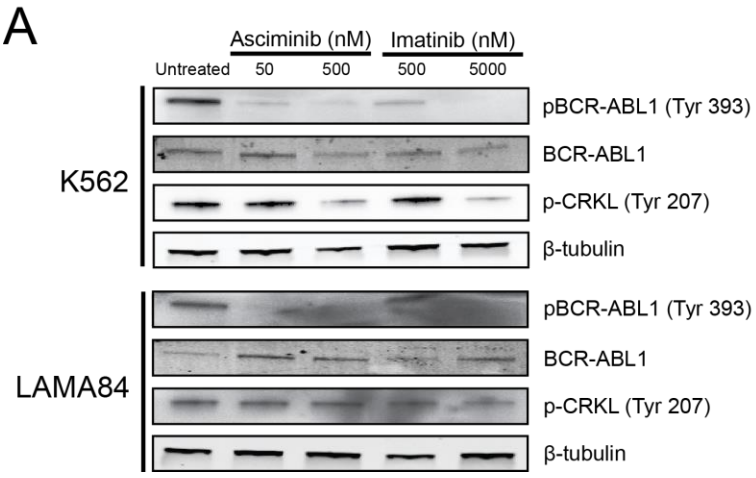

B

	Asciminib IC_{50} (nM)	Imatinib IC_{50} (nM)
Ph⁺ leukemia cell lines		
K562	8.1	188.9
LAMA84	3.2	165.4
Non-Ph⁺ leukemia cell lines		
HL-60	>1000	>4000
U937	>1000	>4000

Figure S1. Immunoblot analysis of BCR-ABL1 signaling and cellular proliferation IC_{50} summary following asciminib treatment in human Ph⁺ and non-Ph⁺ leukemia cell lines, related to Figure 1. (**A**) Effects of asciminib and imatinib on BCR-ABL1 signaling in human Ph⁺ leukemia cell lines by immunoblot. K562 and LAMA84 cells were treated with the indicated concentrations of asciminib or imatinib for 6 h, lysed and subjected to SDS-PAGE, and probed with antibodies for phospho-ABL1, total ABL1, and β-tubulin. (**B**) IC_{50} values for asciminib and imatinib in human leukemia cell lines. Two Ph⁺ (K562, LAMA84) and two non-Ph⁺ (HL-60, U937) cell lines were distributed into 384-well plates in graded concentrations of each inhibitor (asciminib: 0-1000 nM; imatinib: 0-4000 nM), cultured for 72 h, and analyzed by microplate reader using a standard MTS-based colorimetric assay. Response curves were analyzed by non-linear regression and IC_{50} values computed using Graphpad Prism software. Values represent the mean of three independent experiments performed in quadruplicate.

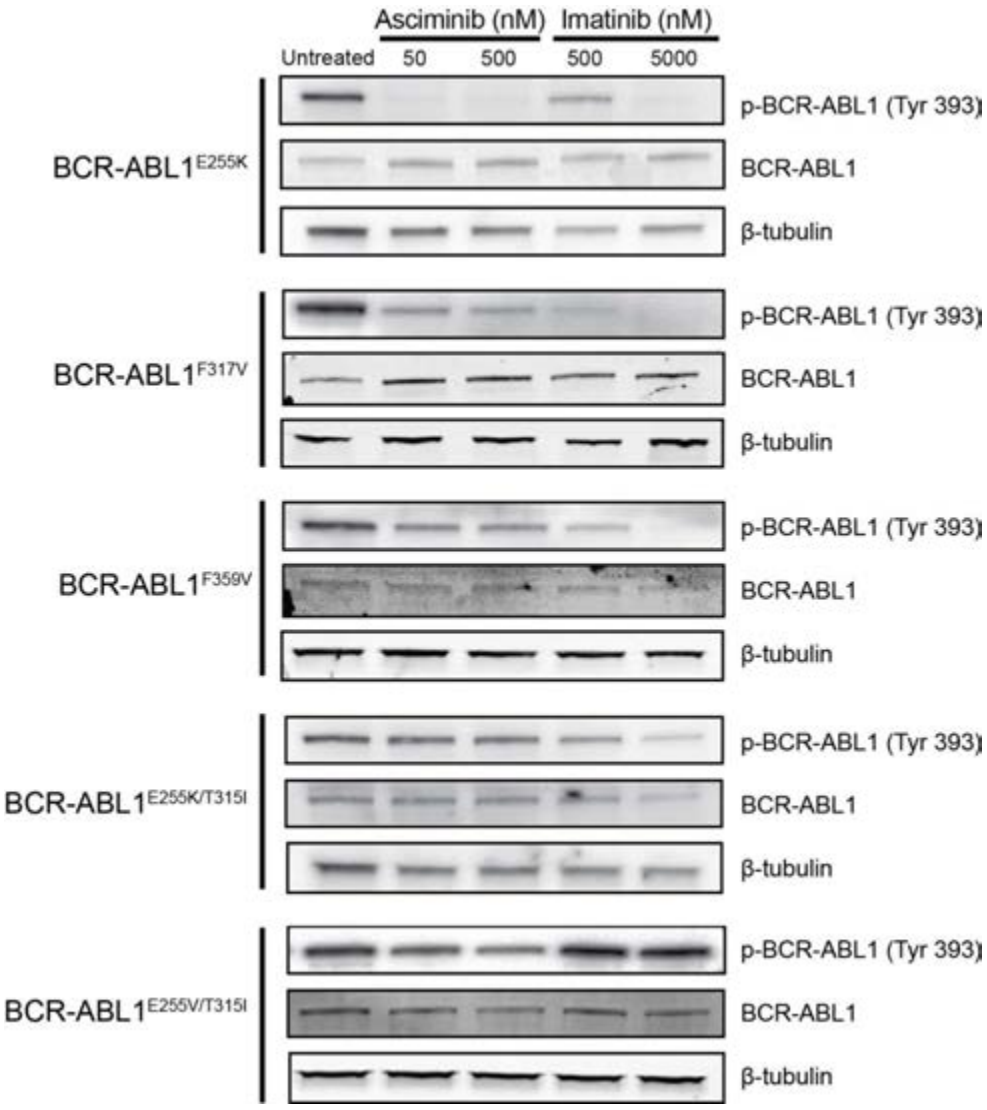

Figure S2. Immunoblot analysis of BCR-ABL1 signaling following asciminib treatment in expanded panel of BCR-ABL1 single and compound mutants, related to Figure 2. Ba/F3 cells expressing the indicated single or compound mutant pSRα BCR-ABL1 constructs were treated with the indicated concentrations of asciminib or imatinib for 6 h, lysed and subjected to SDS-PAGE, and probed with antibodies for phospho-ABL1, total ABL1, or β-tubulin.

Table S1. Cellular proliferation IC_{50} values for asciminib for all Ba/F3 BCR-ABL1 single mutant cell lines, related to Figures 2 and 3.

Cell line	Vector	Asciminib IC_{50}, nM ± SEM
Ba/F3 Parental	---	>10000
Ba/F3 native BCR-ABL1	MIG	3.8 ± 0.5
Ba/F3 BCR-ABL1 G250E	MIG	22.0 ± 1.9
Ba/F3 BCR-ABL1 Y253H	MIG	19.5 ± 10.2
Ba/F3 BCR-ABL1 E255V	MIG	13.3 ± 0.5
Ba/F3 BCR-ABL1 F311I	MIG	142.1 ± 24.1
Ba/F3 BCR-ABL1 T315I	MIG	28.7 ± 4.5
Ba/F3 BCR-ABL1 F317L	MIG	364.6 ± 156.4
Ba/F3 BCR-ABL1 A337V	MIG	>2500
Ba/F3 BCR-ABL1 F359C	MIG	>2500
Ba/F3 BCR-ABL1 F359I	MIG	>2500
Ba/F3 BCR-ABL1 F359V	MIG	>2500
Ba/F3 BCR-ABL1 H396R	MIG	16.0 ± 0.5
Ba/F3 BCR-ABL1 P465S	MIG	>2500
Ba/F3 BCR-ABL1 V468F	MIG	>2500
Ba/F3 native BCR-ABL1	pSRα	4.5 ± 1.6
Ba/F3 BCR-ABL1 G250E	pSRα	5.4 ± 3.7
Ba/F3 BCR-ABL1 Q252H	pSRα	14.9 ± 2.9
Ba/F3 BCR-ABL1 Y253F	pSRα	3.4 ± 3.3
Ba/F3 BCR-ABL1 Y253H	pSRα	11.4 ± 6.1
Ba/F3 BCR-ABL1 E255K	pSRα	9.5 ± 2.5
Ba/F3 BCR-ABL1 E255V	pSRα	3.4 ± 0.8
Ba/F3 BCR-ABL1 T315A	pSRα	4.6 ± 5.9
Ba/F3 BCR-ABL1 T315I	pSRα	137.3 ± 32.2
Ba/F3 BCR-ABL1 F317L	pSRα	17.4 ± 3.9
Ba/F3 BCR-ABL1 F317V	pSRα	113.5 ± 5.4
Ba/F3 BCR-ABL1 A344P	pSRα	2666 ± 3.5
Ba/F3 BCR-ABL1 M351T	pSRα	28.1 ± 20.8
Ba/F3 BCR-ABL1 F359V	pSRα	40.2 ± 1.2
Ba/F3 BCR-ABL1 P465S	pSRα	1448 ± 13.6

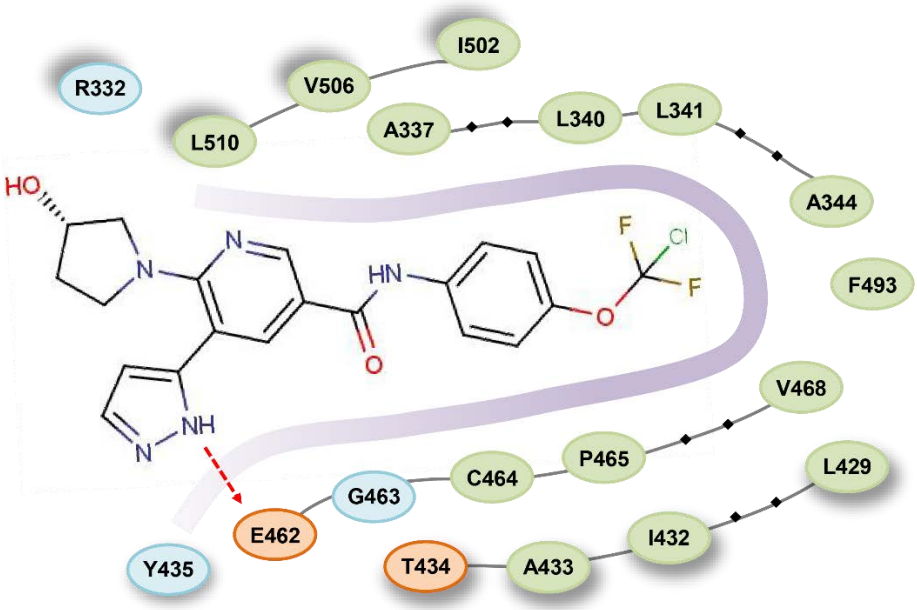

Figure S3. Network of protein residues of the asciminib-binding site, related to Figure 3. Select residues which comprise the myristoyl-binding pocket (stylized by purple line) are indicated according to relative 3D-structural proximity to one another and to the chemical structure of asciminib bound to ABL1 kinase.

Table S2. Summary of cell-based resistance screen for single-agent asciminib starting from Ba/F3 pSRα BCR-ABL1 cells, related to Figure 3.

Treatment condition	Total wells surveyed, n	Wells with outgrowth, n (%)	Clones sequenced, n	Mutant(s) recovered	n	Frequency among all clones (%)	Frequency among mutated clones (%)
Asciminib 25 nM	96	91	36	Native BCR-ABL1	36	100	---
Asciminib 50 nM	96	49	27	Native BCR-ABL1	27	100	---
Asciminib 100 nM	96	38	24	Native BCR-ABL1	24	100	---
Asciminib 200 nM	96	21	21	Native BCR-ABL1	21	100	---
Asciminib 400 nM	96	25	25	Native BCR-ABL1 L1057M Y1064F R1099G	22 1 1 1	88 4 4 4	--- 33.3 33.3 33.3
Asciminib 800 nM	96	31	31	Native BCR-ABL1 Y342C A344P F1066L	27 2 1 1	87.1 6.5 3.2 3.2	--- 50 25 25
Asciminib 1600 nM	96	24	24	Native BCR-ABL1 A344P Y353C P465S G671R	17 2 1 2 2	70.8 8.3 4.2 8.3 8.3	--- 28.6 14.2 28.6 28.6

Table S3. Summary of cell-based resistance screens of asciminib alone or in combination with ponatinib starting from Ba/F3 MIG BCR-ABL1 cells, related to Figure 3.

Treatment condition	Total wells surveyed, n	Wells with outgrowth, n (%)	Clones sequenced, n	Mutant(s) recovered	n	Frequency among all clones (%)	Frequency among mutated clones (%)
Asciminib 200 nM	960	87	87	Native BCR-ABL1	43	49.4	---
				V225G	1	1.1	2.3
				Y226N	1	1.1	2.3
				V228E	2	2.3	4.5
				V228S	1	1.1	2.3
				L284F	2	2.3	4.5
				T315I	2	2.3	4.5
				F317C	1	1.1	2.3
				V338A	1	1.1	2.3
				V338E	1	1.1	2.3
				L340Q	1	1.1	2.3
				L340R	1	1.1	2.3
				Y342C	1	1.1	2.3
				Y342D	1	1.1	2.3
				Y342N	1	1.1	2.3
				A344P	6	6.9	13.6
				L354M	1	1.1	2.3
				L387S	3	3.4	6.8
				G463D	1	1.1	2.3
				C464G	1	1.1	2.3
				C464W	2	2.3	4.5
				V468D	3	3.4	6.8
				L471F	1	1.1	2.3
				F497L	2	2.3	4.5
				E499D	1	1.1	2.3
				I502F	1	1.1	2.3
				I502N	1	1.1	2.3
				S503*	1	1.1	2.3
				V506A	1	1.1	2.3
				L510Q	2	2.3	4.5
Asciminib 10000 nM	96	9	9	Native BCR-ABL1	1	11.1	---
				K294E	1	11.1	12.5
				Y342C	1	11.1	12.5
				A344P	3	33.3	37.5
				A433D	1	11.1	12.5
				F497L	1	11.1	12.5
				S503*	1	11.1	12.5
Ponatinib 10 nM + Asciminib 10 nM	96	96	24	Native BCR-ABL1	20	83.3	---
				D276N/E281K	4	16.7	100.0
Ponatinib 20 nM + Asciminib 20 nM	96	45	45	Native BCR-ABL1	28	62.2	---
				M244I/A288T/D325N	4	8.9	23.5
				D276N / E281K	1	2.2	5.9
				D276N/E281K/E470K	1	2.2	5.9
				T315I	8	17.8	47.1
				T315I / G372E	1	2.2	5.9
				T315I / A424T	2	4.4	11.8
Ponatinib 40 nM + Asciminib 40 nM	192	36	36	Native BCR-ABL1	35	97.2	---
				K263E	1	2.8	100
Ponatinib	192	33	33	Native BCR-ABL1	27	81.8	---

80 nM + Asciminib 80 nM				K263E M351I G442E	2 1 3	6.1 3.0 9.1	33.3 16.7 50.0
Ponatinib 160 nM + Asciminib 160 nM	192	22	22	Native BCR-ABL1 M318I	21 1	95.5 4.5	--- 100

Table S4. Summary of cell-based resistance screen for combinations of asciminib and nilotinib or ponatinib starting from Ba/F3 pSRα BCR-ABL1 cells, related to Figure 3.

Treatment condition	Total wells surveyed, n	Wells with outgrowth, n (%)	Clones sequenced, n	Mutant(s) recovered	n	Frequency among all clones (%)	Frequency among mutated clones (%)
Nilotinib 50 nM + Asciminib 10 nM	96	15	13	Native BCR-ABL1 Q252H E255K T315I L818F	7 2 1 3 1	53.8 15.4 7.7 23.1 7.7	--- 33.3 16.7 42.9 16.7
Nilotinib 50 nM + Asciminib 25 nM	96	4	4	Native BCR-ABL1 T315I	2 2	50 50	--- 100
Nilotinib 50 nM + Asciminib 50 nM	96	6	6	Native BCR-ABL1 T315I	1 5	16.7 83.3	--- 100
Nilotinib 50 nM + Asciminib 100 nM	96	3	3	Native BCR-ABL1 F359I	2 1	66.7 33.3	--- 100
Nilotinib 100 nM + Asciminib 10 nM	96	4	4	Native BCR-ABL1 T315I	3 1	75 25	--- 100
Nilotinib 100 nM + Asciminib 25 nM	96	7	7	Native BCR-ABL1 T315I	2 5	28.6 71.4	--- 100
Nilotinib 100 nM + Asciminib 50 nM	96	2	2	Native BCR-ABL1 T315I	1 1	50 50	--- 100
Nilotinib 100 nM + Asciminib 100 nM	96	2	2	Native BCR-ABL1	2	100	---
Nilotinib 200 nM + Asciminib 10 nM	96	6	5	Native BCR-ABL1 Y253H T315I F359C	1 1 2 1	20 20 40 20	--- 25 50 25
Nilotinib 200 nM + Asciminib 25 nM	96	3	3	T315I	3	100	100
Nilotinib 200 nM + Asciminib 50 nM	96	1	1	Native BCR-ABL1	1	100	---
Nilotinib 200 nM + Asciminib 100 nM	96	1	1	Native BCR-ABL1	1	100	---
Ponatinib 2.5 nM + Asciminib 10 nM	96	4	4	Native BCR-ABL1	4	100	---
Ponatinib 2.5 nM + Asciminib 25 nM	96	1	1	Native BCR-ABL1	1	100	---
Ponatinib 2.5 nM + Asciminib 50 nM	96	2	2	Native BCR-ABL1	2	100	---
Ponatinib 2.5 nM + Asciminib 100 nM	96	1	1	Native BCR-ABL1	1	100	---
Ponatinib 5 nM + Asciminib 10 nM	96	2	2	Native BCR-ABL1	2	100	---
Ponatinib 5 nM + Asciminib 25 nM	96	1	1	Native BCR-ABL1	1	100	---
Ponatinib 5 nM + Asciminib 50 nM	96	3	3	Native BCR-ABL1	3	100	---
Ponatinib 5 nM + Asciminib 100 nM	96	0	0	---	---	---	---
Ponatinib 10 nM + Asciminib 10 nM	96	4	4	Native BCR-ABL1	4	100	---
Ponatinib 10 nM + Asciminib 25 nM	96	1	1	Native BCR-ABL1	1	100	---
Ponatinib 10 nM + Asciminib 50 nM	96	1	1	Native BCR-ABL1	1	100	---

Ponatinib 10 nM + Asciminib 100 nM	96	1	1	Native BCR-ABL1	1	100	---

Table S5. Summary of baseline characteristics of asciminib-treated patients surveyed by BCR-ABL1 deep sequencing, related to Figure 4.

Patient	Previous TKI(s) failed	Disease phase	Baseline BCR-ABL1 sequencing	Baseline BCR-ABL1 transcripts (% I.S.)	Last follow-up BCR-ABL1 sequencing (Day, VAF %)	Last follow-up BCR-ABL1 transcripts (Day, % I.S.)
1	Nilotinib, Dasatinib	CML-CP	F359V (79.1%) T315I (20.6%)	22.6%	Day 673: F359V (98.0%)	Day 673: 24.7%
2	Radotinib, Imatinib, Dasatinib	CML-CP	T315I (29.3%)	81.0%	Day 589: F359I (45.8%) T315I (39.7%) A433D (11.3%) P112S (2.7%)	Day 589: 89.1%
3	Imatinib, Bosutinib	CML-CP	T315I (76.5%) A337T (3.3%) G250E (2.2%) G463D (2.2%)	42.0%	Day 469: F359I (99.0%)	Day 469: 26.0%
4	Imatinib, Nilotinib, Dasatinib, Radotinib	CML-CP	F317L (100%)	87.7%	Day 505: F317L (99.0%)	Day 505: 0.1%
5	Imatinib, Dasatinib, Nilotinib, Ponatinib	CML-CP	E355G (99.6%) T315I (99.4%) E238D (2.1%)	6.3%	Day 224: E355G (99.6%) T315I (99.5%)	Day 224: 31.0%
6	Imatinib, Dasatinib, Nilotinib, Bosutinib	CMP-AP	E279K (42.2%) E238D (2.2%) N297T (2.2%) D233A (2.0%)	82.0%	Day 294: E279K (97.8%) Y353C (3.5%)	Day 294: 73.0%

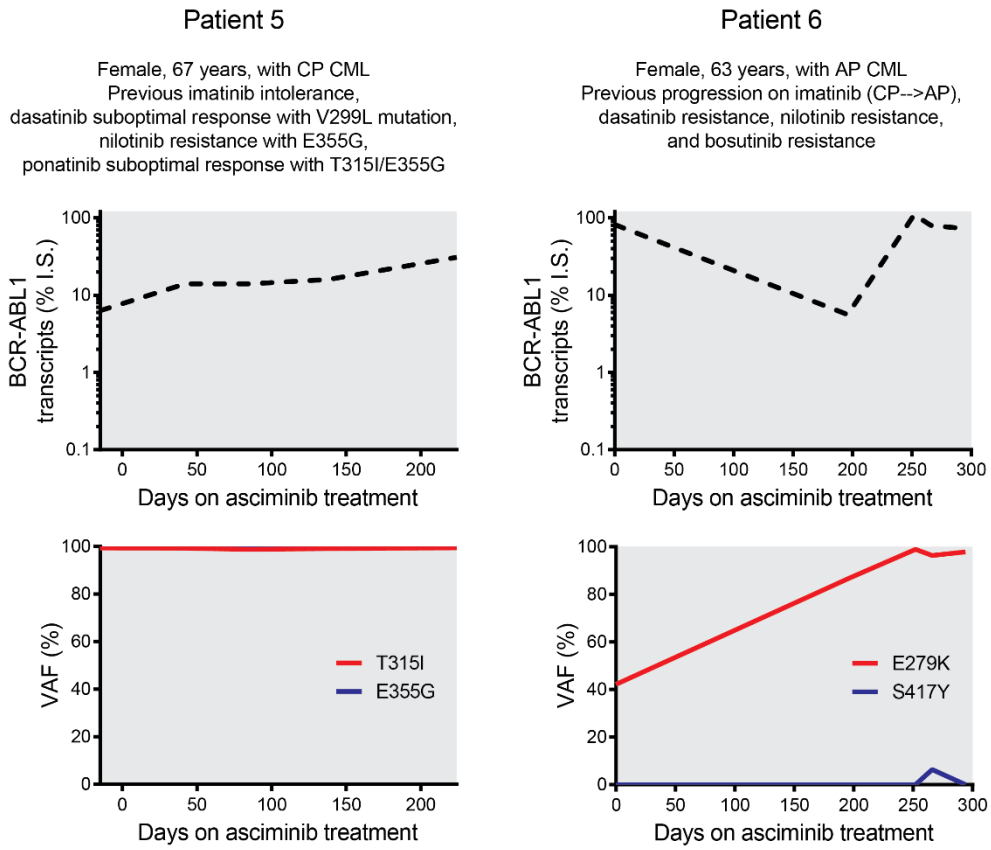

Figure S4. Summary of clinical response and *BCR-ABL1* deep sequencing in two additional patients treated with asciminib, related to Figure 4. Among the six asciminib-treated patients profiled in this study, two (Patients 5 and 6) showed evidence of *BCR-ABL1* mutations that expanded or persisted at high levels at the time of relapse or a suboptimal response. Molecular labs for *BCR-ABL1* transcripts (upper panels) along with matching *BCR-ABL1* mutations identified by NGS-based sequencing (lower panels) are shown at multiple timepoints over the course of treatment.

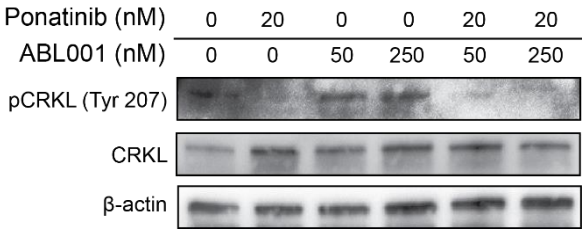

Figure S5. Immunoblot analysis of primary CML patient cells harboring the BCR-ABL1^{F359I} mutant following *ex vivo* treatment with asciminib alone and in combination with ponatinib, related to Figure 4. Primary mononuclear cells from a patient with CML-CP harboring the BCR-ABL1^{F359I} mutant were treated *ex vivo* with ponatinib, asciminib, or the combination at the indicated concentrations overnight, then lysed and analyzed by Western blot for effects on CRKL phosphorylation, as a biomarker for BCR-ABL1 kinase activity.

Table S6. Cellular proliferation IC_{50} values for asciminib for all Ba/F3 BCR-ABL1 compound mutant cell lines, related to Figure 6.

Cell line	Vector	Asciminib IC_{50}, nM
Ba/F3 BCR-ABL1 G250E/T315I	MIG	>2500
Ba/F3 BCR-ABL1 Y253H/T315I	MIG	>2500
Ba/F3 BCR-ABL1 E255V/V299L	MIG	>2500
Ba/F3 BCR-ABL1 E255V/T315I	MIG	>2500
Ba/F3 BCR-ABL1 V299L/F317L	MIG	>2500
Ba/F3 BCR-ABL1 T315I/M351T	MIG	>2500
Ba/F3 BCR-ABL1 T315I/H396R	MIG	>2500
Ba/F3 BCR-ABL1 T315I/E453K	MIG	>2500
Ba/F3 BCR-ABL1 T315L	MIG	>2500
Ba/F3 BCR-ABL1 T315M	MIG	>2500
Ba/F3 BCR-ABL1 F317L/F359V	MIG	>2500
Ba/F3 BCR-ABL1 G250E/T315I	pSRα	>10000
Ba/F3 BCR-ABL1 E255K/T315I	pSRα	>10000
Ba/F3 BCR-ABL1 E255V/T315I	pSRα	>10000

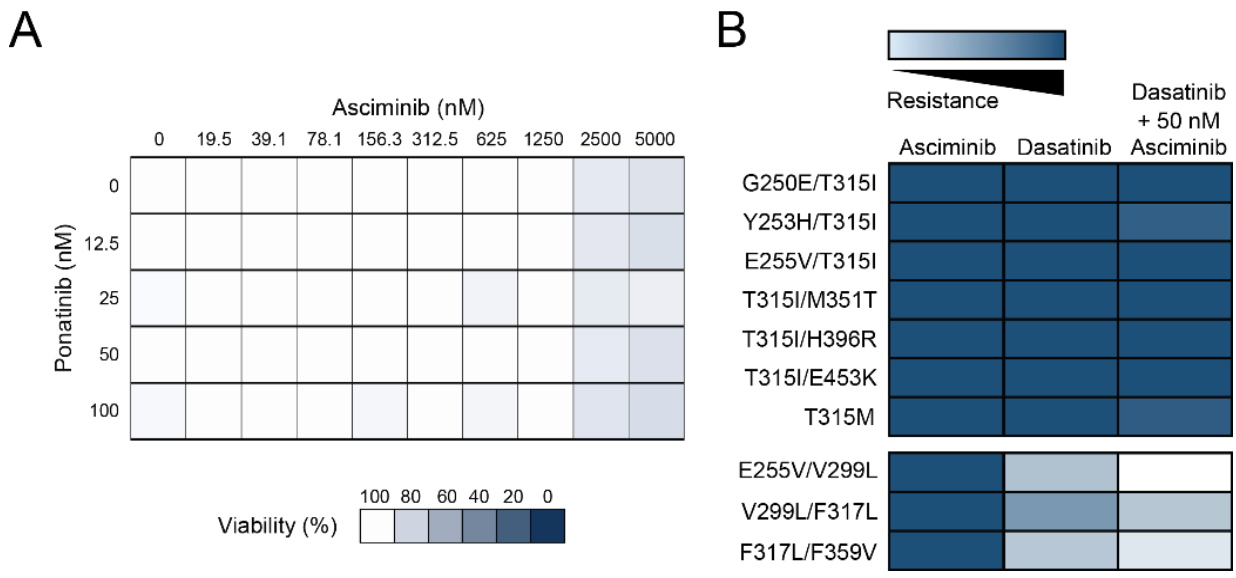

Figure S6. Asciminib plus ponatinib is non-toxic to parental Ba/F3 cells and asciminib plus dasatinib is ineffective against T315I-inclusive BCR-ABL1 compound mutants, related to Figure 6. (A) Ba/F3 parental cells were cultured in complete medium supplemented with WEHI-3B-conditioned medium as a source of IL-3 and distributed into 384-well plates in the presence of the indicated matrix of concentrations of asciminib and ponatinib alone and in combination. After a 72 hr incubation, plates were analyzed by standard MTS-based colorimetric assay. Absorbance was averaged across a replicates, and viability was normalized to untreated wells. **(B)** Ba/F3 cells expressing the indicated MIG BCR-ABL1 compound mutants were distributed into 96-well plates in the presence of graded concentrations of asciminib alone (0-2500 nM), dasatinib alone (0-768 nM), or graded dasatinib combined with 50 nM asciminib. Cells were cultured for 72 h and analyzed by MTS-based colorimetric assay. IC_{50} values were calculated based on non-linear regression using Prism software and displayed as a sensitivity heatmap, wherein a color scale from white to dark blue indicates increased resistance. Data was summarized from at least three independent experiments performed in quadruplicate.

Table S7. Summary of cell-based resistance screen for combinations of asciminib and ponatinib starting from Ba/F3 pSRα BCR-ABL1^{T315I} cells, related to Figure 6.

Treatment condition	Total wells surveyed, n	Wells with outgrowth, n (%)	Clones sequenced, n	Mutant(s) recovered	n	Frequency among all clones (%)	Frequency among compound mutant clones (%)
Ponatinib 20 nM + Asciminib 200 nM	96	0	0	---	---	---	---
Ponatinib 20 nM + Asciminib 400 nM	96	3	1	Y253H/T315I	1	100	100
Ponatinib 20 nM + Asciminib 800 nM	96	1	0	---	---	---	---
Ponatinib 20 nM + Asciminib 1600 nM	96	4	4	T315I only Q252H/T315I	2 2	50 50	--- 100
Ponatinib 40 nM + Asciminib 200 nM	96	0	0	---	---	---	---
Ponatinib 40 nM + Asciminib 400 nM	96	1	1	T315I only	1	100	---
Ponatinib 40 nM + Asciminib 800 nM	96	0	0	---	---	---	---
Ponatinib 40 nM + Asciminib 1600 nM	96	1	1	E255V/T315I	1	100	100
Ponatinib 80 nM + Asciminib 200 nM	96	0	0	---	---	---	---
Ponatinib 80 nM + Asciminib 400 nM	96	0	0	---	---	---	---
Ponatinib 80 nM + Asciminib 800 nM	96	0	0	---	---	---	---
Ponatinib 80 nM + Asciminib 1600 nM	96	0	0	---	---	---	---
Ponatinib 160 nM + Asciminib 200 nM	96	0	0	---	---	---	---
Ponatinib 160 nM + Asciminib 400 nM	96	0	0	---	---	---	---
Ponatinib 160 nM + Asciminib 800 nM	96	0	0	---	---	---	---
Ponatinib 160 nM + Asciminib 1600 nM	96	0	0	---	---	---	---

Table S8. Summary of cell-based resistance screen for combinations of asciminib and ponatinib starting from Ba/F3 MIG BCR-ABL1^{T315I} cells, related to Figure 6.

Treatment condition	Total wells surveyed, n	Wells with outgrowth, n (%)	Clones sequenced, n	Mutant(s) recovered	n	Frequency among all clones (%)	Frequency among compound mutant clones (%)
Ponatinib 40 nM + Asciminib 40 nM	192	127	127	T315I only	13	10.2	---
				Q252H/Y253F/T315I	1	0.8	0.9
				Q252H / T315I	17	13.4	14.9
				Y253H / T315I	27	21.3	23.7
				E255K / T315I	3	2.4	2.6
				E255V / T315I	5	3.9	4.4
				E279K/T315I/E462K	1	0.8	0.9
				K285N / T315I	3	2.4	2.6
				E292V / T315I	1	0.8	0.9
				F311I / T315I	20	15.7	17.5
				F311V / T315I	6	4.7	5.3
				T315I / V339A	1	0.8	0.9
				T315I / E355G	4	3.1	3.5
				T315I / F359C	4	3.1	3.5
				T315I / F359I	5	3.9	4.4
				T315I/L387F M388L	1	0.8	0.9
				T315I / H396P	4	3.1	3.5
				T315I/E459K/E462K	1	0.8	0.9
				T315I / P465S	9	7.1	7.9
				T315I / F486S	1	0.8	0.9
Ponatinib 80 nM + Asciminib 80 nM	192	54	54	T315I only	50	92.6	---
				Q252H/E255K/T315I	1	1.9	25.0
				Y253H / T315I	1	1.9	25.0
				E255V / T315I	1	1.9	25.0
				T315I / E462K	1	1.9	25.0
Ponatinib 160 nM + Asciminib 160 nM	192	33	33	T315I only	33	100	---

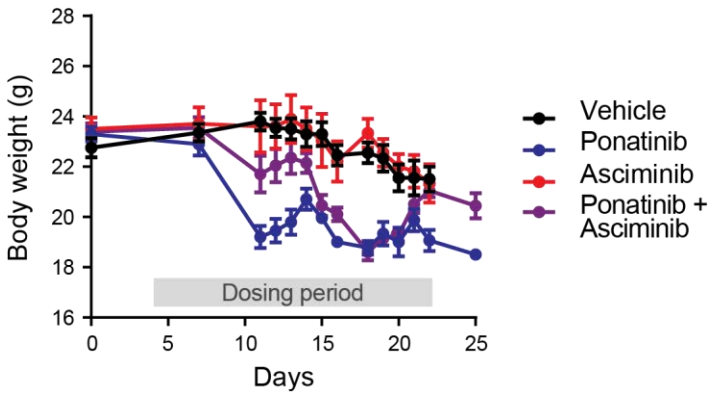

Figure S7. Body weight summary following in vivo combination treatment with asciminib and ponatinib in a T315I-inclusive BCR-ABL1 compound mutant-driven mouse model, related to Figure 7. Female Nod-SCID mice were injected by tail vein with Ba/F3 pMIG-BCR-ABL1$^{T315I/H396R}$ cells and after 3 days commenced oral drug treatment with vehicle, asciminib (30 mg/kg), ponatinib (25 mg/kg), or the combination once daily (n=10 mice/group). Body weight for all animals was measured over the course of 25 days, and the graph shows the mean weight ± SEM. Dosing was held if mice lost >10% of body weight and maintained this loss on two consecutive days or if a mouse lost 4 grams in 24 hours. Mice were re-enrolled if they gained two grams or more.