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Supplemental Data

AP24534, a Pan-BCR-ABL Inhibitor for Chronic Myeloid Leukemia, Potently Inhibits the T315I Mutant and Overcomes Mutation-Based Resistance

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Figure S1: Inhibition of BCR-ABL phosphorylation in Ba/F3 cells expressing native BCR-ABL or BCR-ABL^{T315I}. BCR-ABL phosphorylation was evaluated in Ba/F3 cells expressing either (A) native BCR-ABL or (B) BCR-ABL^{T315I} treated for 4 hr with imatinib, nilotinib, dasatinib, or AP24534. Samples were analyzed by immunoblot analysis with antibodies against pBCR-ABL and eIF4E (loading control). The phosphorylation status of CrkL in these same lysates was determined by immunoblot analysis as described in Figure 3.

Figure S2: Colony formation assays for CML T315I patient and normal primary cells against AP24534. Mononuclear cells from a CML accelerated phase (AP) patient harboring BCR-ABL^{T315I} and from a healthy individual were plated in methylcellulose containing nilotinib, dasatinib, or AP24534 and cultured for 14-18 days. Colonies were counted under an inverted microscope, and results were expressed as the mean of three replicates (error bars represent S.E.M.).

(A) Colony formation assays in the presence of AP24534 using mononuclear cells from a CML AP patient harboring BCR-ABL^{T315I}.

(B) Colony formation assays in the presence of AP24534 using mononuclear cells from a healthy individual.

Figure S3: Effect of dasatinib in mouse models using Ba/F3 cells expressing BCR-ABL^{T315I}. Survival curves are shown for mice injected intravenously with Ba/F3 cells expressing BCR-ABL^{T315I} treated during the indicated dosing period with vehicle or dasatinib by oral gavage. Median survival was calculated using the Kaplan-Meier method, and statistical significance was evaluated with a Log-rank test (GraphPad PRISM) by comparing the survival time of each treatment group with the vehicle group.

Table S1: AP24534 kinase panel screening data

Table S2: Tabulated AP24534 single-agent mutagenesis data (starting from Ba/F3 native BCR-ABL cells)

Table S3: Tabulated AP24534 single-agent mutagenesis data (starting from Ba/F3 BCR-ABL^{T315I} cells)

Table S4: Tabulated AP24534 single-agent mutagenesis data (starting from Ba/F3 BCR-ABL^{E255V} cells)

Table S5: BCR-ABL compound mutations involving T315I or E255V conferring moderate to high level resistance to AP24534

Figure S1.

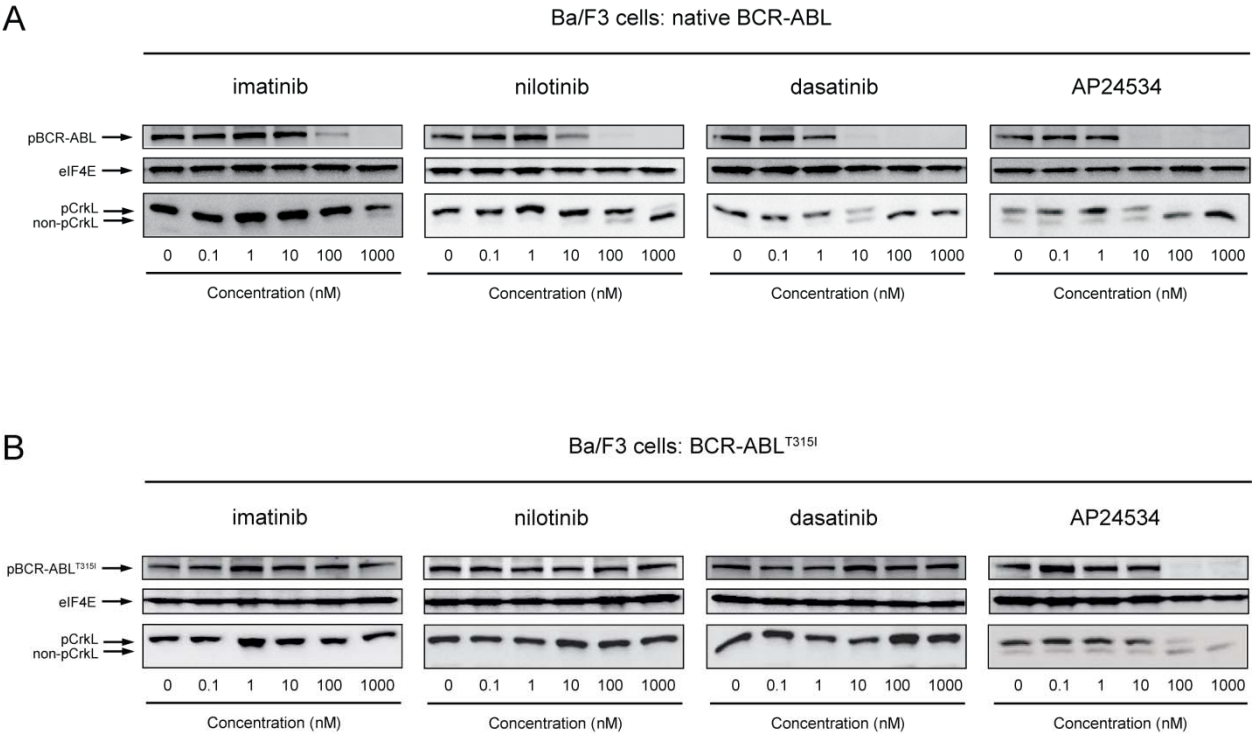
A
Ba/F3 cells: native BCR-ABL
imatinib
nilotinib
dasatinib
AP24534
pBCR-ABL
eIF4E
pCrkL
non-pCrkL
0 0.1 1 10 100 1000
Concentration (nM)
B
Ba/F3 cells: BCR-ABL T315I
imatinib
nilotinib
dasatinib
AP24534
pBCR-ABL T315I
eIF4E
pCrkL
non-pCrkL
0 0.1 1 10 100 1000
Concentration (nM)

Figure S2.

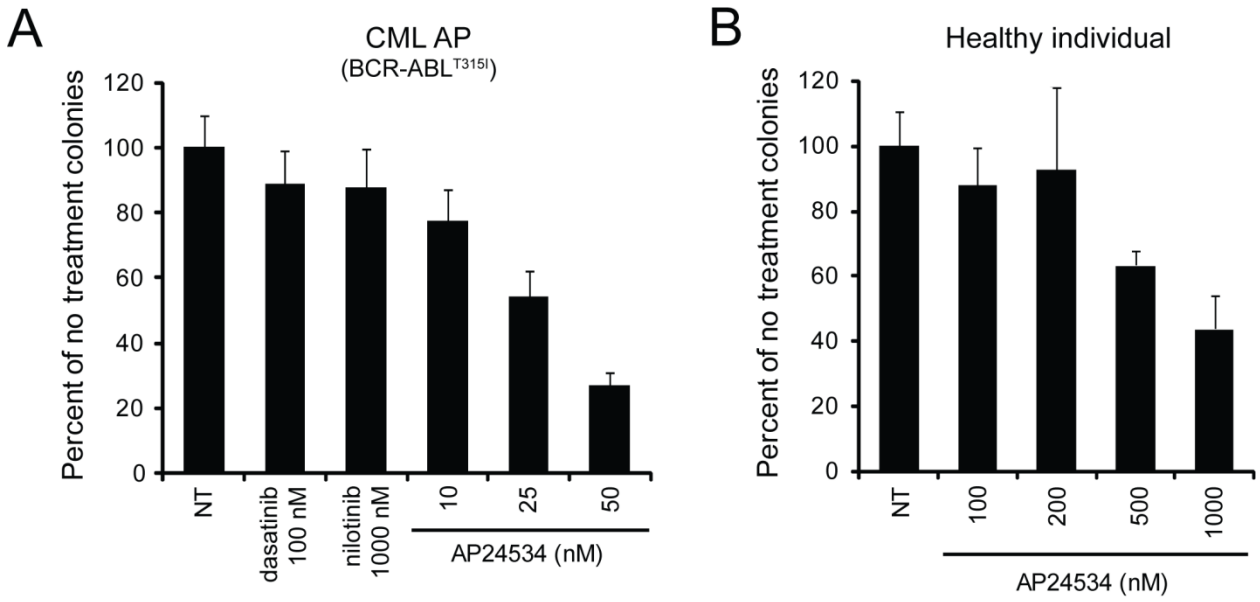
A
CML AP
(BCR-ABL^{T315I})
Percent of no treatment colonies
120
100
80
60
40
20
0
NT
dasatinib 100 nM
nilotinib 1000 nM
10
25
50
AP24534 (nM)
B
Healthy individual
Percent of no treatment colonies
120
100
80
60
40
20
0
NT
100
200
500
1000
AP24534 (nM)

Figure S3.

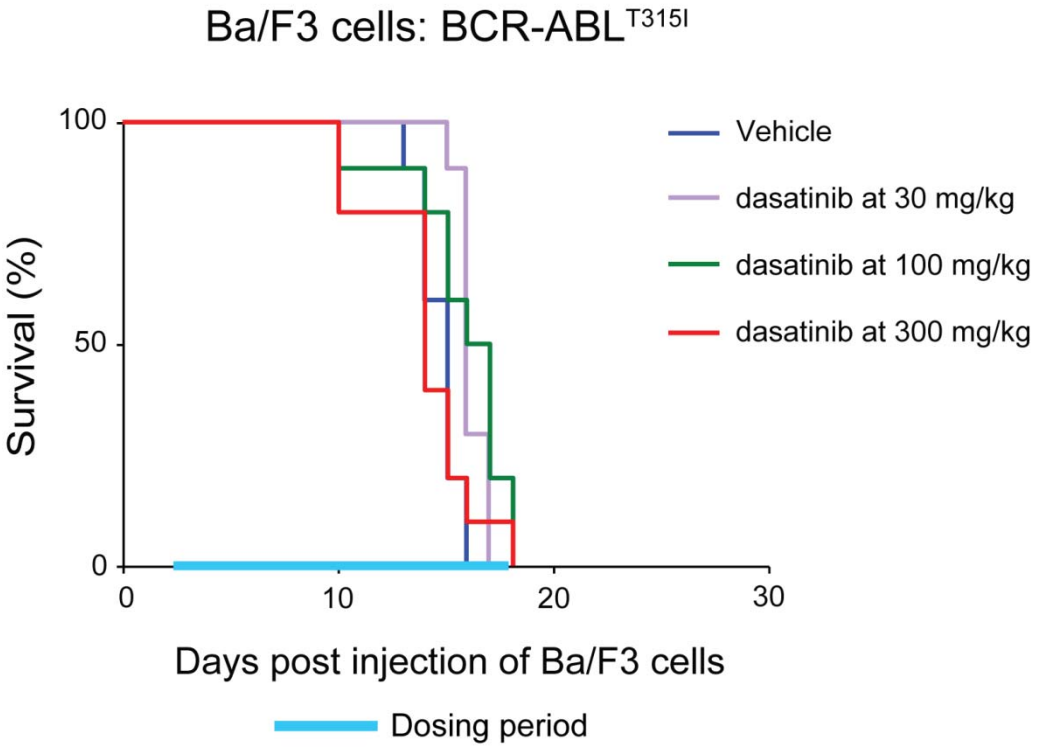
Ba/F3 cells: BCR-ABL T315I
Survival (%)
100
50
0
0
10
20
30
Days post injection of Ba/F3 cells
Vehicle
dasatinib at 30 mg/kg
dasatinib at 100 mg/kg
dasatinib at 300 mg/kg
Dosing period

Table S1. AP24534 Kinase Panel Screening Data

IC_{50} < 10 nM		IC_{50} < 50 nM		IC_{50} ≤ 250 nM		IC_{50} > 250 nM	
Kinase	IC_{50} (nM)	Kinase	IC_{50} (nM)	Kinase	IC_{50} (nM)	Kinase	IC_{50} (nM)
ABL	0.37	BMX	47.2	BRK	50.6	AKT2	>1000
ABL^{Q252H}	0.44	CSK	12.7	$EGFR^{L858R}$	211	ALK	>1000
ABL^{Y253F}	0.3	DDR2	16.1	EPHA1	143	Aurora A	>1000
ABL^{T315I}	2	EPHB4	10.2	ERBB4	176	Aurora B	543
ABL^{M351T}	0.3	FGFR3	18.2	JAK2	169	Aurora C	>1000
ABL^{H396P}	0.34	FLT3	12.6	JAK3	91.1	AXL	>1000
ARG	0.76	JAK1	32.2	KIT^{V654A}	77.8	BTK	849
BLK	6.1	c-KIT	12.5	KIT^{D816V}	152	BTK^{E41K}	>1000
EPHA2	2.1	KIT^{D816H}	16	TYK2	177	CDK2/CyclinE	>1000
EPHA3	6.7	$PDGFR\alpha^{D842V}$	15.6			CTK	>1000
EPHA4	1.1	PYK2	35.1			EGFR	>1000
EPHA5	0.69	TIE2	14.3			$EGFR^{L861Q}$	536
EPHA7	8.5	TRKA	11.4			$EGFR^{T790M}$	>1000
EPHA8	2.5	TRKB	15.1			ERBB2	>1000
EPHB1	1.2	TRKC	13.2			FAK	>1000
EPHB2	0.63					FER	560
EPHB3	1.1					FES	768
FGFR1	2.23					$FLT3^{D835Y}$	948
$FGFR1^{V561M}$	7.3					IGF-1R	>1000
FGFR2	1.6					IR	>1000
$FGFR2^{N549H}$	0.45					IRR	>1000
FGFR4	7.7					ITK	>1000
FGR	0.45					c-MER	406
FMS	8.6					c-MET	>1000
FRK	1.3					mTOR	>1000
FYN	0.36					MUSK	694
HCK	0.11					PI3Kα	>1000
KIT^{V560G}	0.41					PKA	613
LCK	0.28					PKCθ	>1000
LYN	0.24					RON	>1000
LYNB	0.21					ROS	>1000
PDGFRα	1.1					SRC^{T341M}	>1000
$PDGFR\alpha^{V561D}$	0.84					SYK	>1000
$PDGFR\alpha^{T674I}$	3					TEC	>1000
PDGFRβ	7.7					TYK1	>1000
RET	0.16					TYRO3	>1000
RET^{V804L}	3.7					ZAP70	>1000
RET^{V804M}	1.4						
c-SRC	5.4						
VEGFR1	3.7						
VEGFR2	1.5						
VEGFR3	2.3						
YES	0.89						

Table S2. AP24534 cell-based mutagenesis assay (starting from native BCR-ABL)

Ba/F3 cells expressing native BCR-ABL

				By specific mutation				By residue		
Concentration	Wells surveyed	Wells with outgrowth	Clones sequenced (N)	Mutant(s)	Occurrences (n)	Frequency among clones (%)	Frequency among mutants (%)	Residue	Occurrences (n)	Frequency by residue (%)
5 nM	576	576	51	Native BCR-ABL	46	90.2	---	---	---	---
				G250E	1	2.0	20.0	G250	1	20.0
				Y253H	1	2.0	20.0	Y253	1	20.0
				E255K	1	2.0	20.0	E255	1	20.0
				T315I	1	2.0	20.0	T315	1	20.0
				F317I	1	2.0	20.0	F317	1	20.0
10 nM	1440	168	157	Native BCR-ABL	105	66.9	---	---	---	---
				G250E	1	0.6	1.9	G250	1	1.9
				Q252H	4	2.5	7.7	Q252	4	7.7
				Y253F	1	0.6	1.9	Y253	7	13.5
				Y253H	6	3.8	11.5			
				E255K	12	7.6	23.1	E255	19	36.5
				E255V	7	4.5	13.5			
				K285N	1	0.6	1.9	K285	1	1.9
				E292V	1	0.6	1.9	E292	1	1.9
				L298V	2	1.3	3.8	L298	2	3.8
				T315I	7	4.5	13.5	T315	7	13.5
				F317I	1	0.6	1.9	F317	1	1.9
				V339G	1	0.6	1.9	V339	1	1.9
				F359C	2	1.3	3.8	F359	5	9.6
				F359I	3	1.9	5.8			
				L387F	2	1.3	3.8	L387	2	3.8
				S438C	1	0.6	1.9	S438	1	1.9
20 nM	1440	3	3	E255V	1	33.3	33.3	E255	1	33.3
				T315I	2	66.7	100.0	T315	2	66.7
40 nM	1440	0	0	---	---	---	---	---	---	---

Table S3. AP24534 cell-based mutagenesis assay (starting from BCR-ABL^{T315I})

Ba/F3 cells expressing BCR-ABL^{T315I}

				By specific compound mutation (with T315I)				By residue		
Concentration	Wells surveyed	Wells with outgrowth	Clones sequenced (N)	Mutant(s)	Occurrences (n)	Frequency among clones (%)	Frequency among mutants (%)	Residue	Occurrences (n)	Frequency by residue (%)
10 nM	480	480	10	T315I only	9	90.0	---	---	---	---
				A365V	1	10.0	100.0	A365	1	100.0
20 nM	480	480	20	T315I only	20	100.0	---	---	---	---
40 nM	480	192	140	T315I only	6	4.3	---	---	---	---
				G250E	3	2.1	2.2	G250	3	2.2
				Q252H	5	3.6	3.7	Q252	5	3.7
				Y253F	3	2.1	2.2	Y253	46	34.3
				Y253H	41	29.3	30.6			
				Y253N	2	1.4	1.5			
				E255K	7	5.0	5.2	E255	12	9.0
				E255V	5	3.6	3.7			
				E281K	1	0.7	0.7	E281	1	0.7
				K285N	2	1.4	1.5	K285	2	1.5
				I293N	4	2.9	3.0	N293	4	3.0
				F311I	24	17.1	17.9	F311	39	29.1
				F311V	15	10.7	11.2			
				I315L	3	2.1	2.2	I315	4	3.0
				I315M	1	0.7	0.7			
				L327M	1	0.7	0.7	L327	1	0.7
				F359C	7	5.0	5.2	F359	9	6.7
				F359I	1	0.7	0.7			
				F359V	1	0.7	0.7			
				A380S	3	2.1	2.2	A380	3	2.2
				H396P	4	2.9	3.0	H396	5	3.7
				H396R	1	0.7	0.7			
80 nM	480	75	71	Q252H	3	4.2	4.2	Q252	3	4.2
				Y253H	51	71.8	71.8	Y253	51	71.8
				E255K	8	11.3	11.3	E255	8	11.3
				F311I	2	2.8	2.8	F311	3	4.2
				F311V	1	1.4	1.4			
				I315L	3	4.2	4.2	I315	3	4.2
				A380S	3	4.2	4.2	A380	3	4.2
160 nM	480	42	32	Y253H	29	90.6	90.6	Y253	29	90.6
				E255V	3	9.4	9.4	E255	3	9.4
320 nM	480	1	1	E255V	1	100.0	100.0	E255	1	100.0
640 nM	480	0	0	---	---	---	---	---	---	---

Table S4. AP24534 cell-based mutagenesis assay (starting from BCR-ABL^{E255V})

Ba/F3 cells expressing BCR-ABL^{E255V}

				By specific compound mutation (with E255V)				By residue		
Concentration	Wells surveyed	Wells with outgrowth	Clones sequenced (N)	Mutant(s)	Occurrences (n)	Frequency among clones (%)	Frequency among mutants (%)	Residue	Occurrences (n)	Frequency by residue (%)
80 nM	480	152	123	E255V only	104	84.6	---	---	---	---
				G250E	2	1.6	10.5	G250	2	10.5
				Q252H	1	0.8	5.3	Q252	1	5.3
				Y253H	5	4.1	26.3	Y253	5	26.3
				E292V	1	0.8	5.3	E292	1	5.3
				F311I	2	1.6	10.5	F311	2	10.5
				T315I	1	0.8	5.3	T315	1	5.3
				E355G	1	0.8	5.3	E355	1	5.3
				F359C	3	2.4	15.8	F359	5	26.3
				F359I	2	1.6	10.5			
				H396R	1	0.8	5.3	H396	1	5.3
160 nM	480	9	6	Y253F	1	16.7	16.7	Y253	3	50.0
				Y253H	2	33.3	33.3			
				T315I	3	50.0	50.0	T315	3	50.0
320 nM	480	1	1	T315I	1	100.0	100.0	T315	1	100.0
640 nM	480	0	0	---	---	---	---	---	---	---

Table S5. BCR-ABL compound mutations involving T315I or E255V conferring moderate to high level resistance to AP24534

Compound mutant	AP24534 concentration at which recovered in screen			Reported clinically (refs.)
	80 nM	160 nM	320 nM	
T315I / Q252H	✓			NR
T315I / Y253H	✓	✓		(1), (2)
T315I / E255K	✓			(3)
T315I / E255V	✓	✓	✓	NR
T315I / F311I	✓			(2)
T315I / F311V	✓			NR
T315I / A380S	✓			NR
E255V / G250E	✓			NR
E255V / Q252H	✓			NR
E255V / Y253F		✓		NR
E255V / Y253H	✓	✓		NR
E255V / E292V	✓			NR
E255V / F311I	✓			NR
E255V / E355G	✓			NR
E255V / F359C	✓			NR
E255V / F359I	✓			NR
E255V / H396R	✓			NR

(1) Shah et al. (2007). JCI *117*, 2562-2569.
(2) Khorashad et al. (2008). Blood *111*, 2378-2381.
(3) Stagno et al. (2008). Leuk. Res. *32*, 673-674.
NOTE: The following clinically reported compound mutants were not detected in this screen: V299L / E255V.
Abbreviations: NR, not reported.