Supplemental Material

High throughput flow cytometry identifies small molecule inhibitors for drug repurposing in T-ALL

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Suppl. Table 1

Patient ID	PDX ID	Origin Patient	Karyotype	Lesion	ETP
15-093#	15-093	UNM	46,XY[20]	ND	Not ETP
12-089#	12-089	UNM	46,XY,t(?;11)(?;q23). Ish (MLL x 2),(5'MLL sep 3'MLL x 1)	KMT2A-R	ND
ALL82*	PATZZM	COG	46,XY,t(5;14)(q35;q32)[3]/47,idem,+mar[3]/46,XY[19]	ND	not ETP
ALL68*	PATRAP	COG	46,X,add(X)(q26),del(5)(q31),t(10;11)(p12;q21)[13]/46,XX[7]	MLLT10-R	ETP
ALL27*	PASNXS	COG	46,XX,del(2)(q33),del(12)(p12)[20]	CCDC91-R	near ETP
ALL16*	PASGJG	COG	46,Y,t(X;10)(p10;p10)[18]/46,XY[2]	MLLT10-R	ND
ALL31*	PASSPP	COG	46,XY,t(11;19)(q23;p13.3)[20]/46,XY[3]	KMT2A-R	not ETP

Suppl. Table 1. Kariotypic and molecular classification of primary T-ALL samples used for the development of PDX.
primary T-ALL samples used in this study; ND, not determined; R, rearrangements; * reported by Matlawska-Wasowska *et al.* (2016) Leukemia.

Suppl. Table 2. List of the tested inhibitors and their clinical relevance.

Compound	Inhibitor type	Tested Concentration range	FDA approved	Clinical dosage			Steady state plasma concentration (Cmax)					References
				Dose	unit	route	PlasmaConc (µM)	PlasmaConc (M)	MW	Cmax	Unit	
Afatinib	TKI	0.005-100 µM	yes	40	mg	O	0.16	1.58E-07	485			https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5315738/
Alvocidib	**CDK**	**0.05-1000 nM**	no	60	mg	P	2.24	2.24E-06	401			https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3618599/#SD2
Axitinib	TKI	0.005-100 µM	yes	10	mg	O	0.10	9.59E-08	386	37	nanog/milliL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3425526/
Bortezomib	**proteasome**	**0.05-1000 nM**	yes	2.6	mg	P	0.23	2.25E-07	384.24	86.5	nanog/milliL	http://drugcentral.org/label/1521d321-e724-4ffc-adad-34bf4f44fac7/view
Bosutinib	TKI	0.005-100 µM	yes	500	mg	O	0.38	3.77E-07	530	200	nanog/milliL	https://www.drugbank.ca/drugs/DB06616
Cabozantinib	TKI	0.005-100 µM	yes	140	mg	O	0.61	6.11E-07	501	306	nanog/milliL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3646303/
Carfilzomib	**proteasome**	**0.05-1000 nM**	yes	40	mg	P	1.93	1.93E-06	719	1389	nanog/milliL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5438822/
Celecoxib	TKI	0.005-100 µM	yes	200	mg	O	1.80	1.80E-06	381	686	nanog/milliL	https://www.ncbi.nlm.nih.gov/pubmed/16149679
Ceritinib	TKI	0.005-100 µM	yes	750	mg	O	1.43	1.43E-06	558	800	nanog/milliL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4079055/#SD1
Crizotinib	TKI	0.005-100 µM	yes	500	mg	O	0.82	8.18E-07	450	368	nanog/milliL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4982581/
Dabrafenib	TKI	0.005-100 µM	yes	300	mg	O	1.55	1.55E-06	519	806	nanog/milliL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3859275/
Dasatinib	TKI	0.005-100 µM	yes	100	mg	O	0.23	2.29E-07	488	111.6	nanog/milliL	https://www.ncbi.nlm.nih.gov/pubmed/22587422
Erlotinib	TKI	0.005-100 µM	yes	150	mg	O	4.40	4.40E-06	393	1.73	microg/milliL	https://bmccancer.biomedcentral.com/articles/10.1186/1471-2407-11-284
Gefitinib	TKI	0.005-100 µM	yes	250	mg	O	0.19	1.91E-07	446	85	nanog/milliL	https://www.ncbi.nlm.nih.gov/pubmed/16231967
Ibrutinib	TKI	0.005-100 µM	yes	560	mg	O	0.12	1.18E-07	440	51.7	nanog/milliL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4419161/
Imatinib	TKI	0.005-100 µM	yes	400	mg	O	3.24	3.24E-06	493.6	1.6	millig/L	https://pdfs.semanticscholar.org/b9b0/5b7fc6cc9065ef18009e9cbde1752a6c4870.pdf
Lapatinib	TKI	0.005-100 µM	yes	1250	mg	O	6.06	6.06E-06	581	3.52	microg/milliL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4331616/
Nilotinib	TKI	0.005-100 µM	yes	400	mg	O	4.08	4.08E-06	529.5	2160.7	nanog/milliL	https://www.ncbi.nlm.nih.gov/pubmed/19695406
Palbociclib	**CDK**	0.005-100 µM	yes	125	mg	O	0.41	4.15E-07	447.5	185.5	nanog/milliL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4968608/
Ponatinib	TKI	0.005-100 µM	yes	45	mg	O	0.15	1.45E-07	532.5			https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5344956/
Regorafenib	TKI	0.005-100 µM	yes	160	mg	O	7.15	7.15E-06	482.8	3.45	millig/L	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3364125/
Ruxolitinib	TKI	0.005-100 µM	yes	20	mg	O	1.32	1.32E-06	306	1320	nanomol/L	http://www.bloodjournal.org/content/118/21/5162?sso-checked=true
Saracatinib	TKI	0.005-100 µM	yes	175	mg		0.82	8.19E-07	542	444	nanog/milliL	http://clincancerres.aacrjournals.org/content/clincanres/16/19/4876.full.pdf
Selumetinib	TKI	0.005-100 µM	yes	75	mg		1.22	1.22E-06	457.68	557	nanog/milliL	https://www.ncbi.nlm.nih.gov/pubmed/21953275
Sorafenib	TKI	0.005-100 µM	yes	400	mg	O	7.90	7.90E-06	464.8	3.67	millig/L	https://link.springer.com/article/10.1007%2Fs00280-010-1423-9
Sunitinib	TKI	0.005-100 µM	yes	50	mg	O	0.06	6.27E-08	398.5	25	nanog/milliL	https://onlinelibrary.wiley.com/doi/pdf/10.1002/cncr.28554
Tofacitinib	TKI	0.005-100 µM	yes	10	mg	O	1.26	1.26E-06	312.3	392	nanog/milliL	http://dmd.aspetjournals.org/content/dmd/42/4/759.full.pdf
Trametinib	TKI	0.005-100 µM	yes	2	mg	O	0.04	3.61E-08	615.4	22.2	nanog/milliL	https://www.drugbank.ca/drugs/DB08911
Vandetanib	TKI	0.005-100 µM	yes	300	mg	O	0.36	3.58E-07	475.35	170	nanog/milliL	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3586143/
Vemurafenib	TKI	0.005-100 µM	yes	960	mg	O	9.80	9.80E-06	489.92	4.8	microg/milliL	https://link.springer.com/article/10.1007%2Fs00280-013-2324-5
Vismodegib	**Hedghog**	0.005-100 µM	yes	150	mg	O	3.58	3.58E-06	421	3.58	micromol/L	https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3703823/

Suppl. Table 3

Compound	CUTLL1	ALL-SIL	CCRF-CEM	Jurkat	Loucy
Afatinib	3.30	4.44	2.57	2.13	1.91
Alvocidib	0.02	0.03	0.08	0.01	0.01
Axitinib	0.63	1.37	0.07	0.42	0.45
Bortezomib	0.17	0.18	0.01	0.14	0.15
Bosutinib	14.91	7.58	0.03	1.17	1.42
Cabozantinib	10.61	11.27	5.73	6.22	4.10
Carfilzomib	0.03	0.03	0.00	0.02	0.02
Celecoxib	>100	>100	36.29	>100	>100
Ceritinib	45.46	7.62	6.32	3.24	3.03
Crizotinib	2.26	6.79	1.91	2.23	1.90
Dabrafenib	>100	>100	45.75	>100	>100
Dasatinib	>100	7.99	14.96	5.84	6.87
Erlotinib	>100	8.93	>100	6.25	9.18
Gefitinib	21.57	16.42	30.91	19.35	8.87
Ibrutinib	>100	>100	21.59	18.65	14.75
Imatinib	25.85	29.11	39.10	11.55	9.80
Lapatinib	15.51	7.57	7.25	4.15	3.46
Nilotinib	>100	>100	>100	52.12	>100
Palbociclib	>100	>100	>100	>100	>100
Ponatinib	5.66	3.34	4.68	1.12	1.46
Regorafenib	36.34	14.96	10.36	11.95	11.66
Ruxolitinib	12.71	42.56	39.39	55.91	33.10
Saracatinib	>100	60.16	25.49	9.81	8.18
Selumetinib	>100	>100	>100	>100	35.49
Sorafenib	26.66	12.15	11.54	10.72	10.93
Sunitinib	1.71	1.33	4.02	1.62	1.58
Tofacitinib	>100	>100	>100	>100	>100
Trametinib	>100	>100	22.71	29.56	38.18
Vandetanib	>100	>100	13.51	9.21	3.37
Vemurafenib	18.55	9.63	10.31	8.66	9.32
Vismodegib	>100	>100	>100	>100	>100

Suppl. Table 3. *Ex vivo* drug response profiling of T-ALL cell lines. The cells were incubated for 72 hr with the tested agents and the EC_{50} values were calculated from three independent experiments. EC_{50} values were reported for inhibitors that yielded maximum response values of 20% or greater. For EC_{50} values calculated to be beyond the concentration range tested, they were reported as >100 μM. This indicate the cells that were insensitive to the inhibitors at tested concentration range.

Suppl. Table 4

	15-093	12-089	PATZZM	PATRAP	PASNXS	PASGJG	PASSPP	CUTTL1	ALL-SIL	CCRF-CEM	Jurkat	Loucy	PBMC
48 h	82.2	74.8	87.6	76.6	66.4	90.9	89.1	ND	ND	ND	ND	ND	96.2
72 h	77.2	71.5	85.9	72.4	75.3	94.7	92.4	78.3*	85.7*	86.1*	87.9*	83.1*	94.2

Suppl. Table 4. Cell viability (%) of T-ALL cell lines, primary samples, PDX models and PBMC at indicated time points after incubation with DMSO (<1%). * Mean values obtained from three independent experiments. ND, not determined.

Suppl. Table 5

Compound	15-093	12-089	PATZZM	PATRAP	PASNXS	PASGJG	PASSPP
Afatinib	10.12	11.09	9.00	6.76	4.47	>100	>100
Alvocidib	0.25	1.19	>100	>100	48.71	>100	>100
Axitinib	>100	>100	>100	>100	>100	>100	>100
Bortezomib	>100	>100	>100	>100	>100	>100	>100
Bosutinib	48.20	18.73	15.14	1.95	11.85	>100	>100
Cabozantinib	>100	>100	>100	>100	>100	>100	>100
Carfilzomib	0.01	0.005	0.006	0.004	0.012	>100	>100
Celecoxib	>100	>100	>100	>100	>100	>100	>100
Ceritinib	13.38	>100	30.21	>100	>100	>100	>100
Crizotinib	12.21	12.43	6.75	9.62	0.07	>100	>100
Dabrafenib	>100	>100	>100	>100	>100	>100	>100
Dasatinib	>100	>100	>100	2.43	>100	>100	>100
Erlotinib	>100	>100	>100	>100	>100	>100	>100
Gefitinib	>100	>100	>100	20.0	>100	>100	>100
Ibrutinib	>100	54.46	>100	>100	>100	>100	>100
Imatinib	>100	>100	>100	>100	>100	>100	>100
Lapatinib	>100	57.11	>100	13.87	>100	>100	>100
Nilotinib	>100	>100	>100	>100	>100	>100	>100
Palbociclib	>100	>100	>100	>100	>100	>100	>100
Ponatinib	11.21	22.35	5.63	6.98	1.92	>100	>100
Regorafenib	>100	40.4	>100	>100	>100	>100	>100
Ruxolitinib	>100	>100	>100	>100	>100	>100	>100
Saracatinib	>100	>100	>100	>100	>100	>100	>100
Selumetinib	>100	>100	>100	>100	2.71	>100	>100
Sorafenib	>100	>100	96.24	>100	>100	>100	>100
Sunitinib	1.38	5.57	1.35	3.23	1.40	>100	>100
Tofacitinib	>100	>100	>100	>100	>100	>100	>100
Trametinib	>100	>100	>100	>100	>100	>100	>100
Vandetanib	45.71	29.11	26.43	11.57	>100	>100	>100
Vemurafenib	>100	>100	>100	>100	>100	>100	>100
Vismodegib	>100	>100	>100	>100	>100	>100	>100

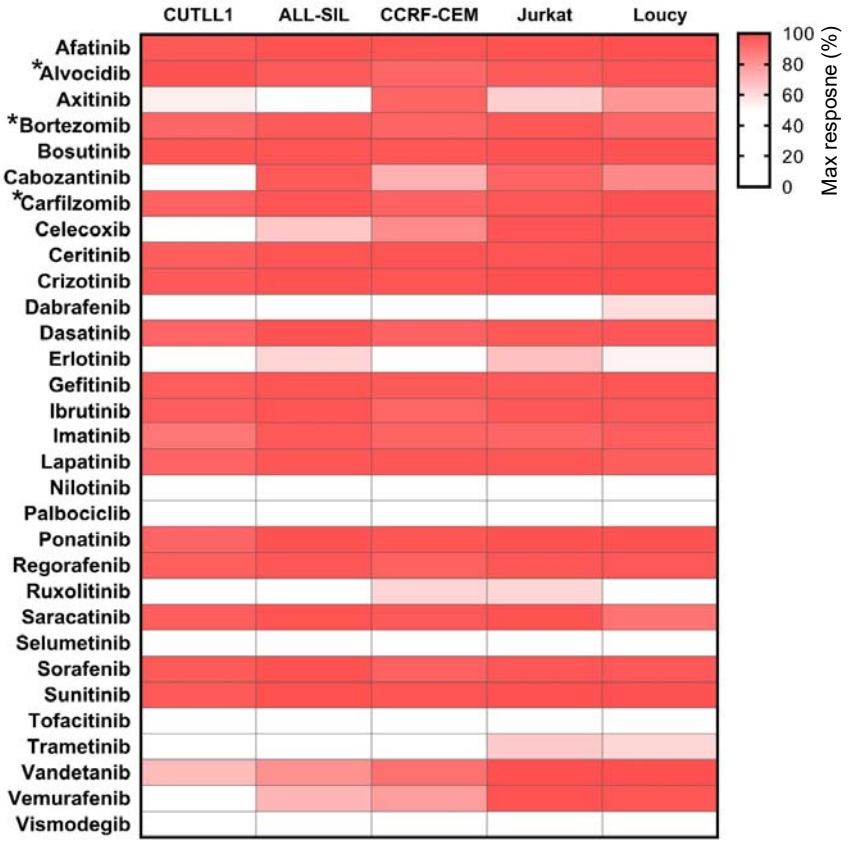
Suppl. Table 5. Response of T-ALL patient samples and patient derived xenografts to 31 small molecule inhibitors. The cells were incubated for 48 hrs with the tested agents. EC_{50} values were reported for inhibitors that yielded maximum response values of 20% or greater. The EC_{50} values that were beyond the concentration range tested are reported as >100 μM. This indicate the cells that were insensitive to the inhibitors at tested concentrations.

Suppl. Figure 1

	CUTLL1	ALL-SIL	CCRF-CEM	Jurkat	Loucy
Afatinib	0.514	0.895*	0.940	0.563	0.729
Alvocidib	0.478	1.011	1.394	0.890*	1.032
Axitinib	0.464	0.782	2.118	1.306	0.627
Bortezomib	0.630	0.974	1.261	0.945	
Bosutinib	0.989	1.484	0.780	0.604	0.544
Cabozantinib	1.619	0.975	0.856	2.019	1.063
Carfilzomib	0.798	1.367*	1.040	1.231	1.356#
Celecoxib			0.902		
Ceritinib		1.032	1.092	0.849	0.860
Crizotinib	0.293	0.562	0.799	0.536	0.959
Dabrafenib					
Dasatinib		0.859	0.977	0.807	1.219
Erlotinib		0.848		0.837	1.103
Gefitinib	1.092	0.844	1.170	1.916	0.863*
Ibrutinib			0.840	0.856	0.563
Imatinib	1.528	1.591	1.473	1.638	1.338
Lapatinib	0.854#	0.812	0.851	1.026	0.686
Nilotinib					
Palbociclib					
Ponatinib	0.801	0.834	0.972	0.936	1.205
Regorafenib	0.392	0.770	0.809	0.897	0.926
Ruxolitinib	1.081	1.122	1.401	1.373	1.003
Saracatinib		1.376	1.071	1.080	0.369
Selumetinib					
Sorafenib	0.537*	0.837	0.903	0.854	0.890
Sunitinib	1.406	1.080	1.329#	1.306	1.216
Tofacitinib					
Trametinib			0.849	0.380	
Vandetanib			1.117	1.022	0.695
Vemurafenib		0.829		0.534	0.658
Vismodegib					

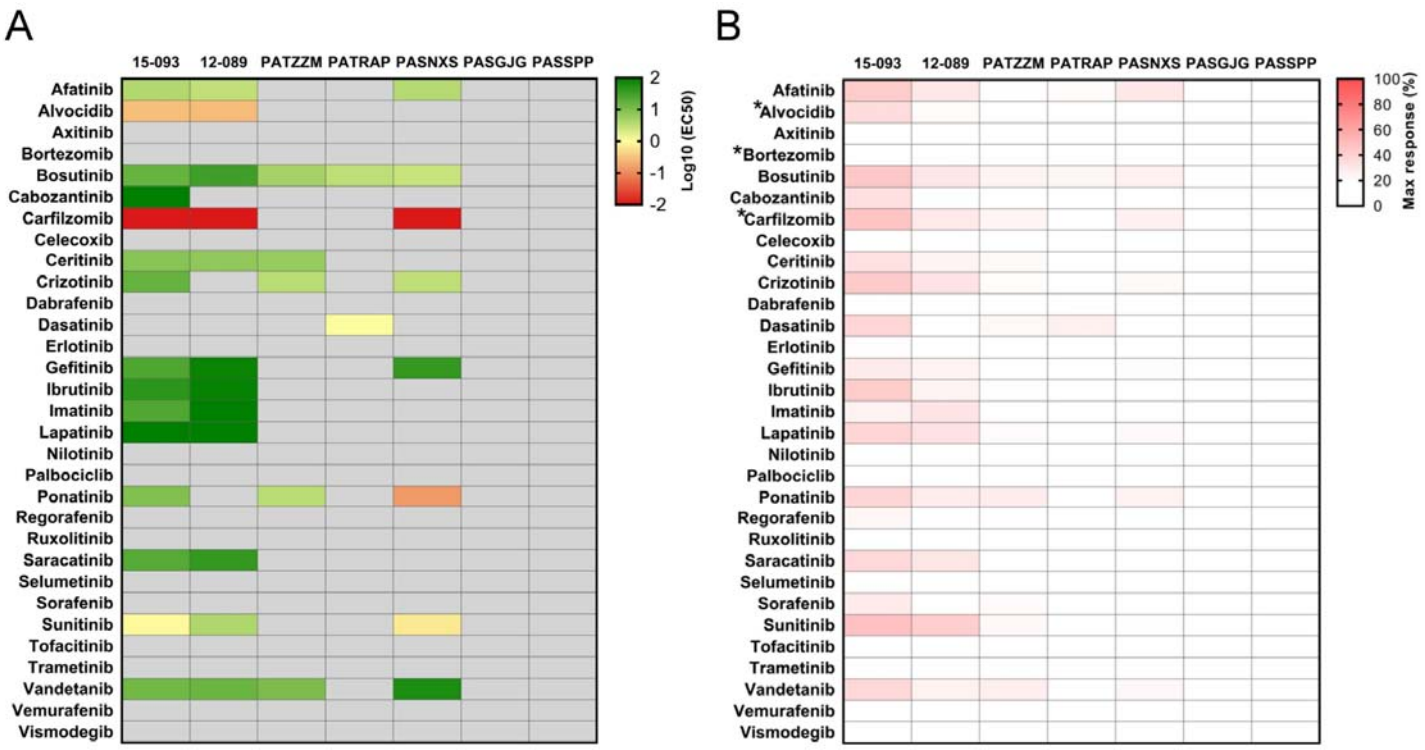
Suppl. Figure 1. Hypoxia Cytotoxity Ratio (HCR) for a panel of T-ALL cell lines treated with 31 inhibitors under normoxia and hypoxia conditions, respectively (72 hr). HCR was determined for each drug and cell line as $EC_{50}^{normoxia}/EC_{50}^{hypoxia}$. Each HCR was calculated from mean EC_{50} values for three independent experiments (paired Student's t test, * $p < 0.05$; # $p < 0.07$).

Suppl. Figure 2



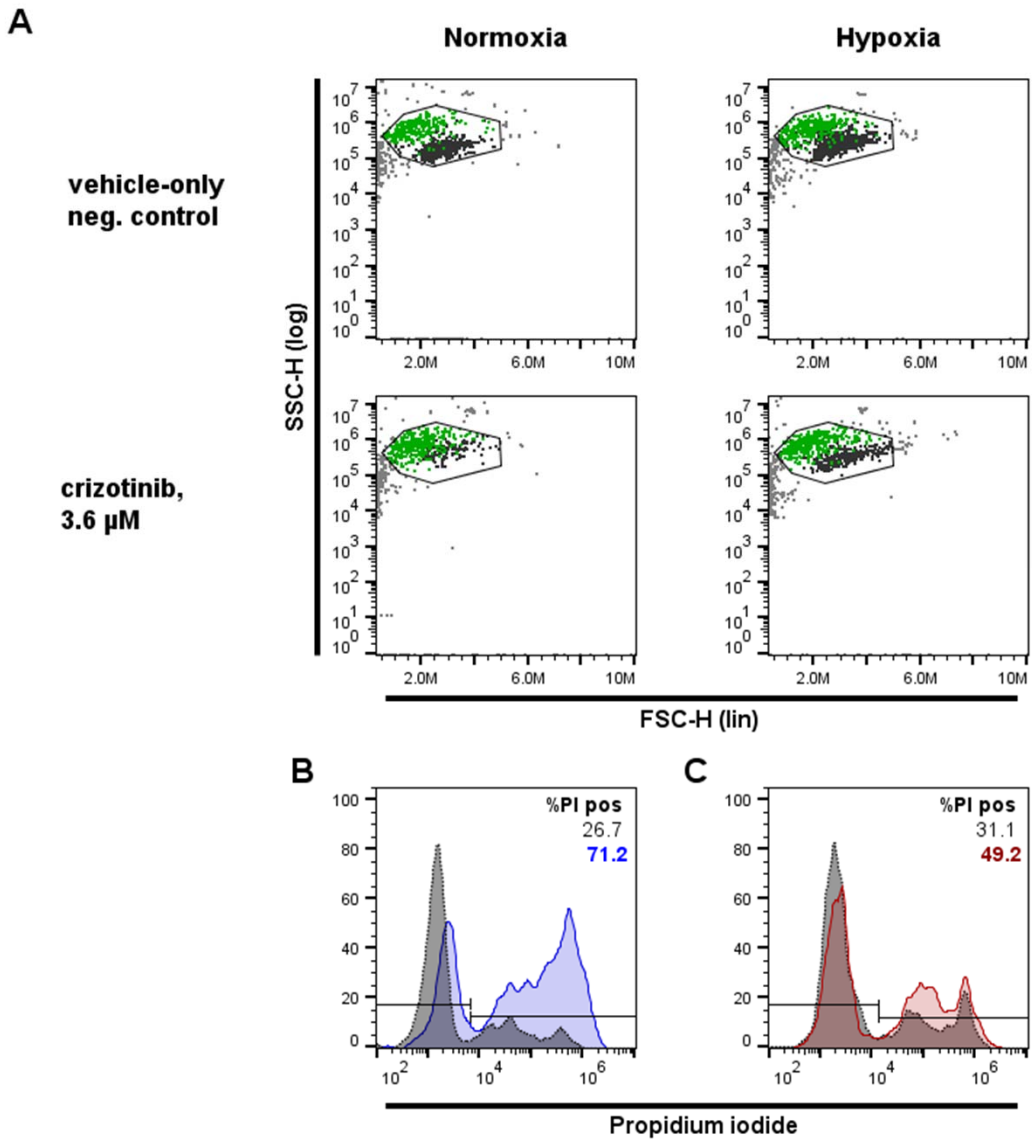
Suppl. Figure 2. Response of T-ALL cell line to the highest tested dose of each inhibitor under hypoxia (100 μM for each drug with exception of *alvocidib, *bortezomib and *carfilzomib for which the highest tested dose was 1 μM). Heatmap indicates the percentage of dead cells (0 -100%).

Suppl. Figure 3



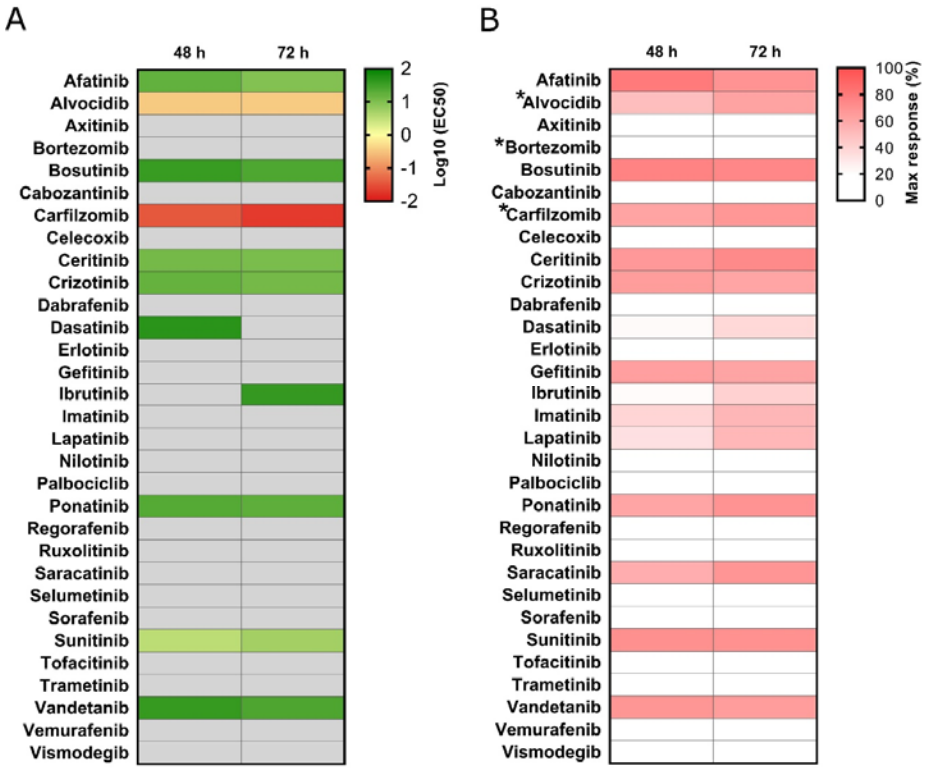
Suppl. Figure 3. (A) The heatmap represents the mean EC_{50} values obtained for the samples treated with the drugs for 72 hr under normoxic conditions. The columns indicate T-ALL samples and the rows indicate the tested drugs. The darkest red indicates the most sensitivity (lowest EC_{50} values) of the cells to the tested inhibitors EC_{50} values are only reported for compounds that yielded a maximum response values of 20% or greater. For those samples which yielded maximal responses < 20% and/or had EC_{50} values greater than 100 μM, we report those EC_{50} values as "100 μM" (light grey). **(B)** Response of T-ALL patient samples and PDX to the highest tested dose of each inhibitor (100 μM for each drug with exception of *alvocidib, *bortezomib and *carfilzomib for which the highest tested dose was 1 μM). Heatmap indicates the percentage of dead cells after 72 hr incubation.

Suppl. Figure 4



Suppl. Figure 4. The HTFC PI assay provides information on the morphological shift that occurs in cells treated with the tested inhibitors. Shown are data from one replicate experiment of CUTLL1 cells incubated with vehicle or TKIs for 72 h in normoxia or hypoxia conditions, then interrogated with the HTFC PI assay. **(A)** Forward- and side-scatter plots of CUTLL1 cells from individual wells in the assay at different conditions. Gated cells that were PI-positive cells are shown in green. Top panels, vehicle-only negative control. Bottom panels, wells treated with 3.6 µM crizotinib. Left panels, wells from the assay plate incubated at normoxia. Right panels, wells from the assay plate incubated in hypoxia. **(B-C)** PI stain histograms from the wells shown in (A). The gated % PI-positive values are shown on the upper right. Grey, negative control well for that oxygen incubation condition. (B) Wells incubated in normoxia. Blue, well treated with 3.6 µM crizotinib. (C) Wells incubated in hypoxia. Red, well treated with 3.6 µM crizotinib.

Suppl. Figure 5



Suppl. Figure 5. Sensitivity of peripheral blood mononuclear cells (PBMC) to 31 small molecule inhibitors. **(A)** The heatmap represents the mean EC_{50} values obtained for normal PBMC treated with the drugs for 42 and 72 hr under normoxic conditions. The columns indicate time points and the rows indicate the tested drugs. The darkest red indicates the most sensitivity (lowest EC_{50} values) of the cells to the tested inhibitors. Light grey, the dose responses indicated EC_{50} > 100 µM indicating limited/lack of response to the tested compounds. **(B)** Response to the highest tested dose of each inhibitor (100 µM for each drug with exception of *alvocidib, *bortezomib and *carfilzomib for which the highest tested dose was 1 µM). Heatmap indicates the percentage of dead cells after 48 and 72 hr incubation.