Anti-tumor activity of the dual BET and CBP/EP300 inhibitor NEO2734

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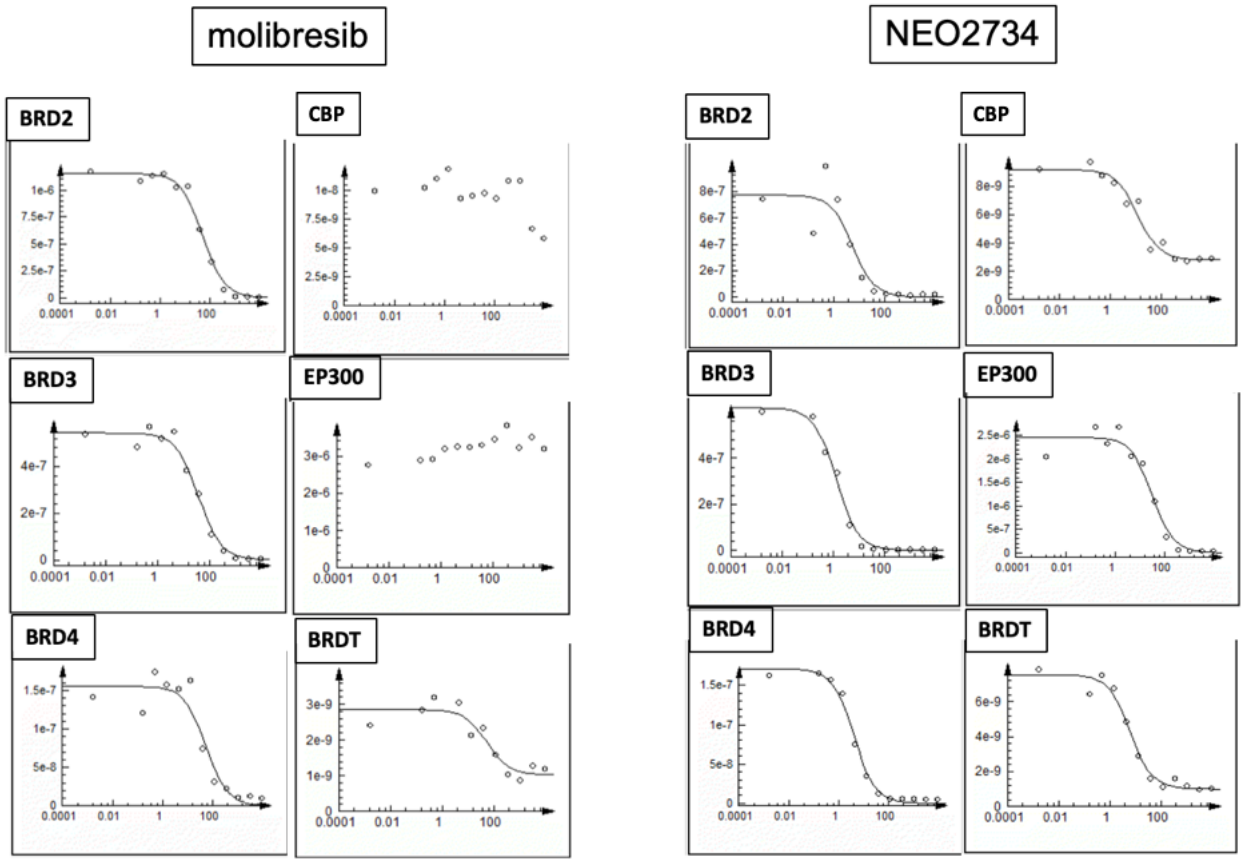
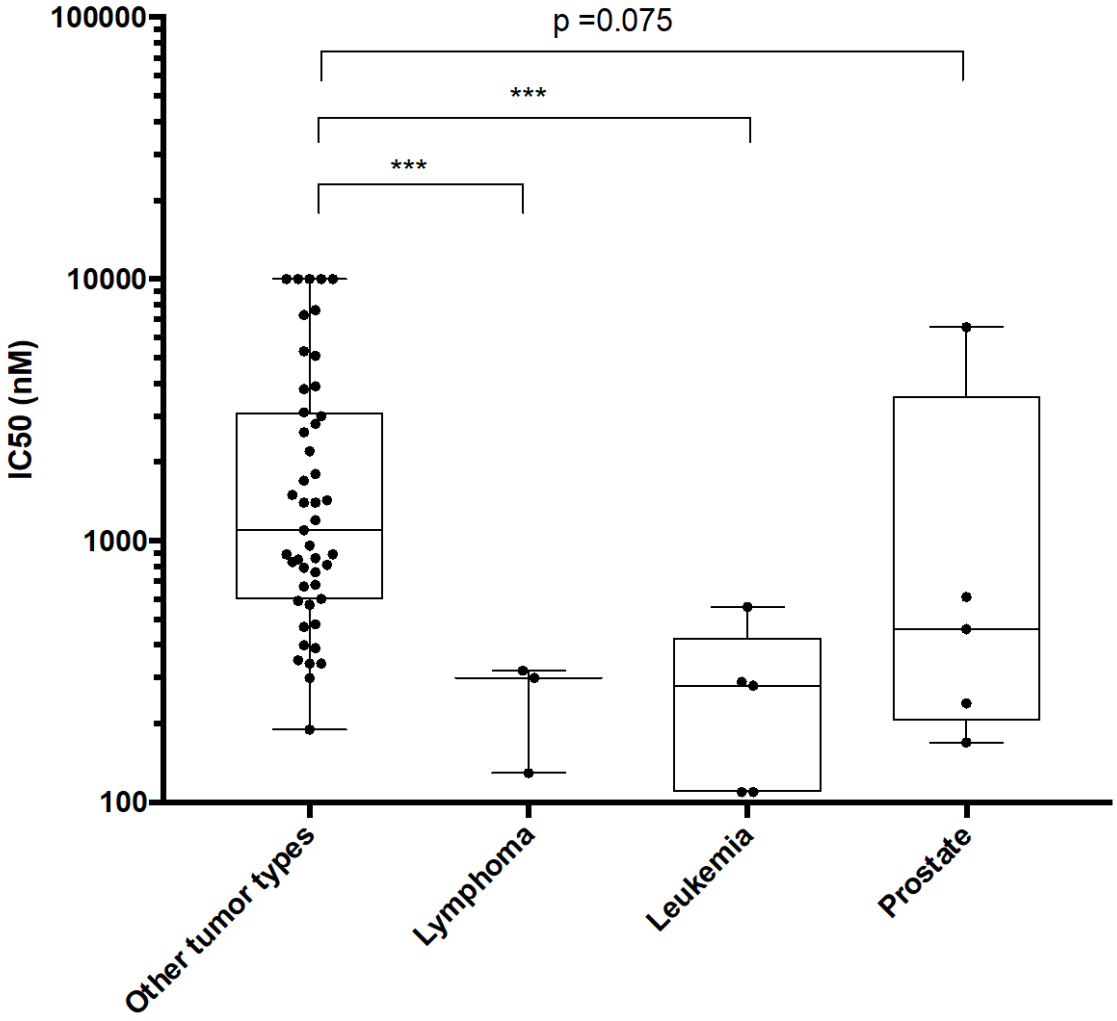
Supplementary Material

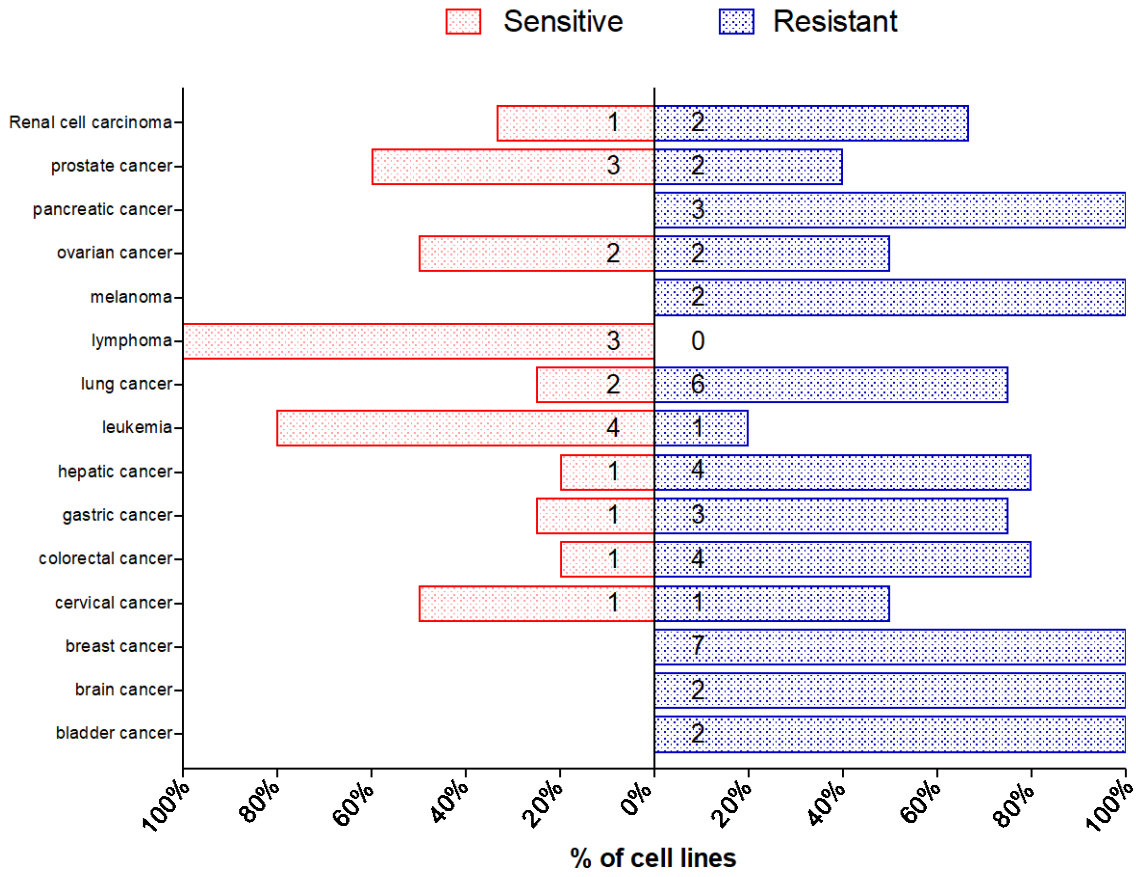
Supplementary Figure 1. Curves showing binding of molibresib and NEO2734 to bromodomains. Bromodomain levels measured by qPCR (Signal; y-axis) are plotted against the corresponding compound concentration in nM in log10 scale (x-axis). Experiments performed in duplicate. BRD2, BRD3, BRD4, BRDT, CBP AND EP300 shown as examples.



Supplementary Figure 2. Activity of NEO2734 in Lymphoma or Leukemia or Prostate cancer cells compared to other tumor types. IC50s calculated **for 60 cell lines** after 72 hours treatment with NEO2734. Each dot represents a cell line. *** $p < 0.001$ (Mann-Whitney test)

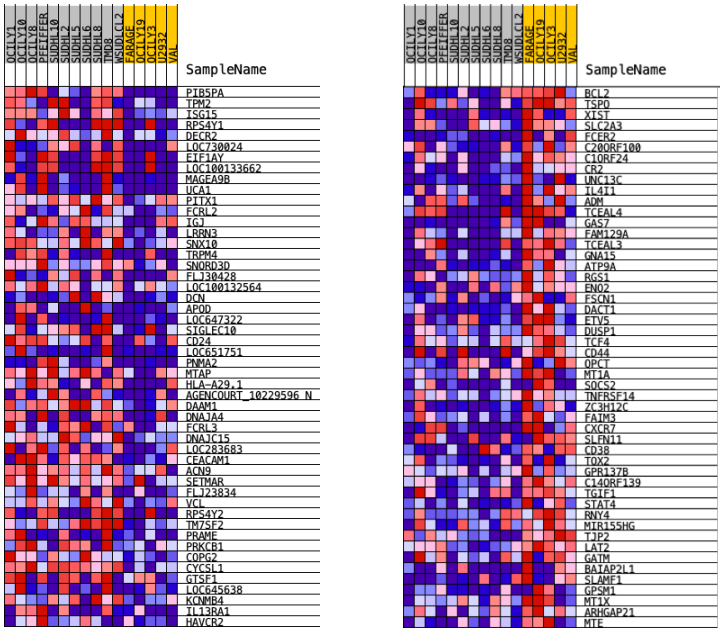


Supplementary Figure 3. Percentage of cell lines for each tumor type that are sensitive or resistant to NEO2734. Sensitive = IC50 < 500 nM; Resistant = IC50 > 500 nM. The numbers inside the bars represent the number of cell lines in each group. X axis: percentage of cell lines that are sensitive or resistant for each tumor type.



Supplementary Figure 4. *BCL2* is upregulated in DLBCL cell lines resistant to NEO2734. A) Heatmap generated with the GSEA software (Broad Institute) showing the top 50 upregulated genes in sensitive cell lines (left panel) and the top 50 upregulated genes in resistant cell lines (right panel). B) Box-plot showing CI (Chou-Talalay combination index) for NEO2734 plus venetoclax combination treatment. Y-axis: CI values. In each box-plot, the line in the middle of the box represents the median and the box extends from the 25th to the 75th percentile (interquartile range, IQ); the whiskers extend to the upper and lower adjacent values (i.e., ±1.5 IQ). CI < 0.9 = synergy; 0.9 < CI < 1.1 = additivity; CI > 1.1 = no benefit. C) Dose response curve after 72h treatment with venetoclax alone. D) Dose response curves for NEO2734 plus a fixed concentration of venetoclax and vice versa. In SU-DHL10 and TMD8 sensitive cell lines the combination curves are below the predicted additivity curves, indicating synergism. In Farage and U2932 resistant cell lines the combination and predicted additivity curves overlap, indicating no synergism.

A)



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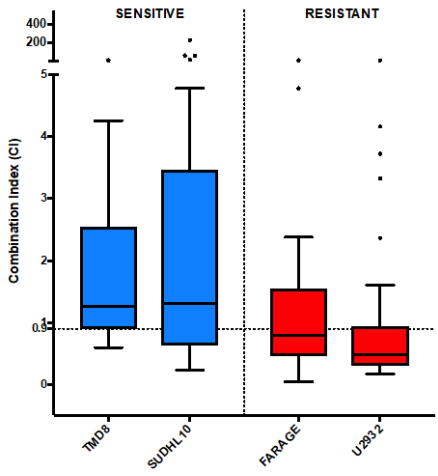

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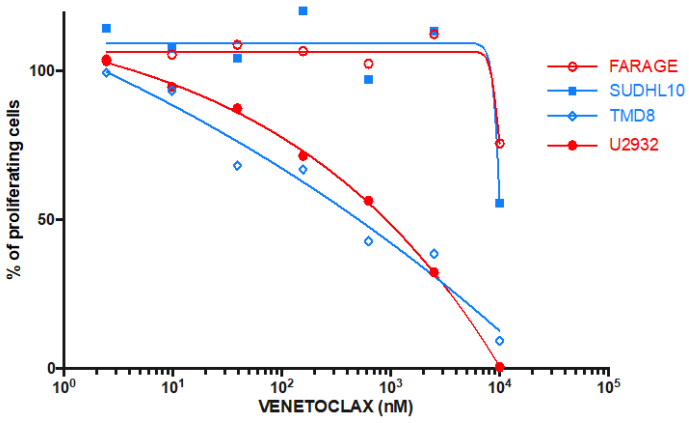
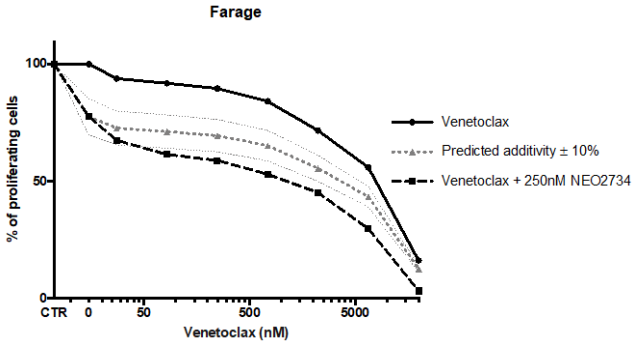
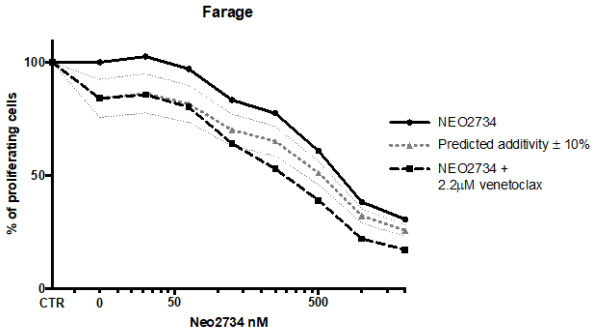
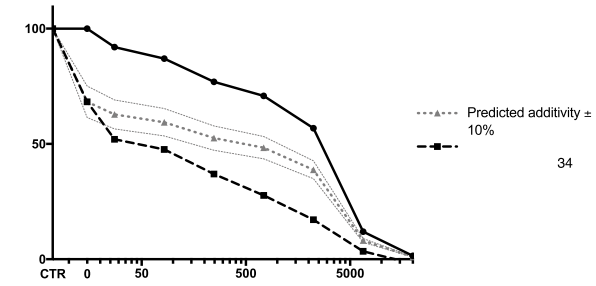
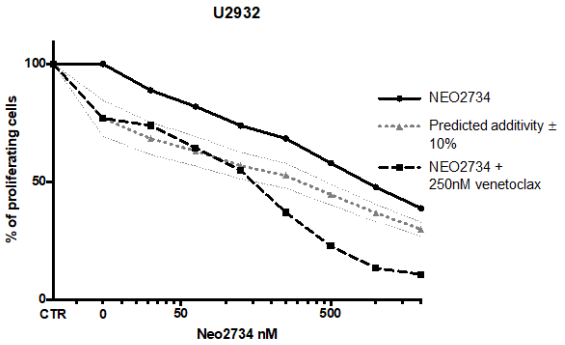


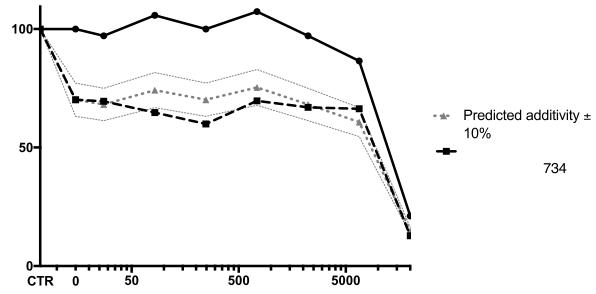
D)

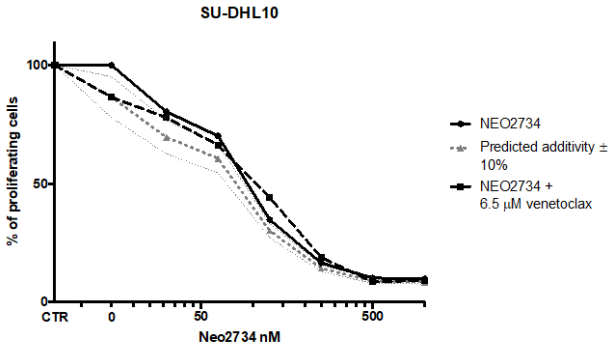

Farage
% of proliferating cells
100
50
0
CTR 0 50 500 5000
Venetoclax (nM)
Venetoclax
Predicted additivity ± 10%
Venetoclax + 250nM NEO2734

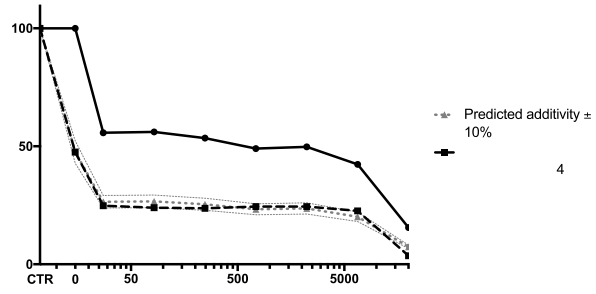

Farage
% of proliferating cells
100
50
0
CTR 0 50 500
Neo2734 nM
NEO2734
Predicted additivity ± 10%
NEO2734 +
2.2μM venetoclax

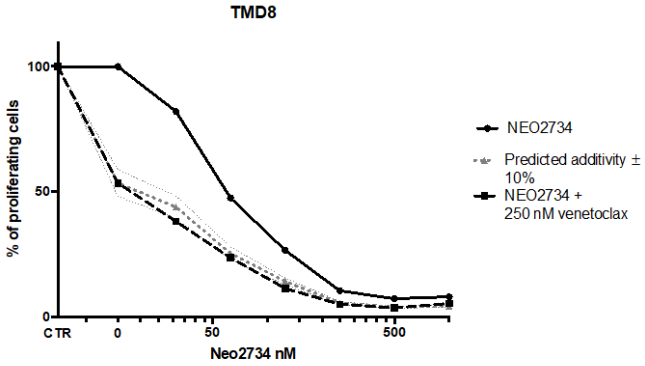

100
50
0
CTR 0 50 500 5000
Predicted additivity ±
10%
34


U2932
% of proliferating cells
100
50
0
CTR 0 50 500
Neo2734 nM
NEO2734
Predicted additivity ±
10%
NEO2734 +
250nM venetoclax

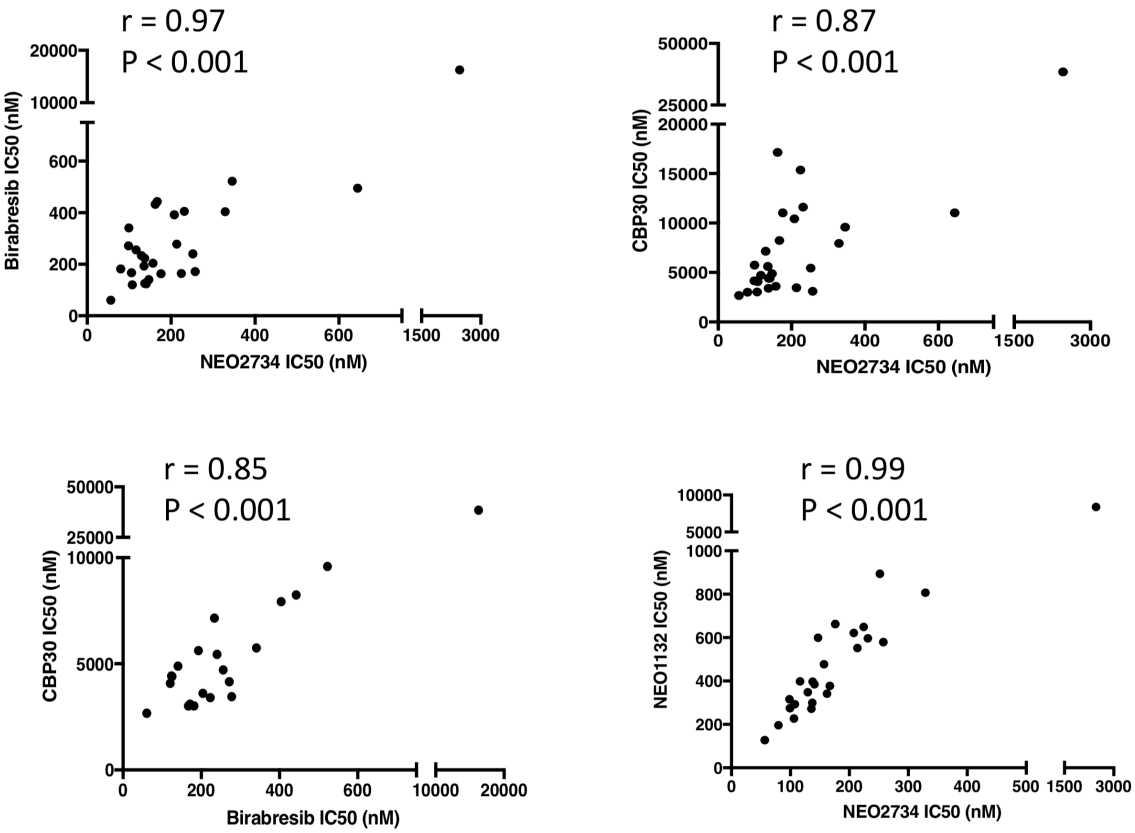

100
50
0
CTR 0 50 500 5000
Predicted additivity ±
10%
734


SU-DHL10
% of proliferating cells
100
50
0
CTR 0 50 500
Neo2734 nM
NEO2734
Predicted additivity ±
10%
NEO2734 +
6.5 μM venetoclax


100
50
0
CTR 0 50 500 5000
Predicted additivity ±
10%
4

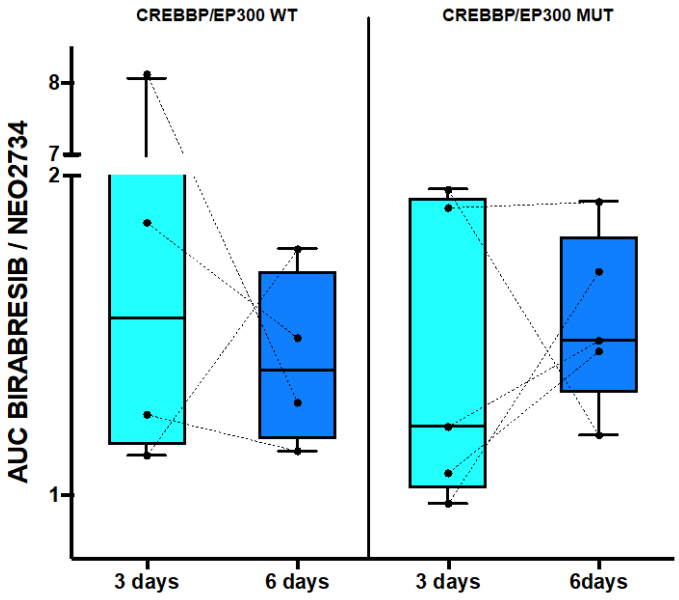

TMD8
% of proliferating cells
100
50
0
CTR 0 50 500
Neo2734 nM
NEO2734
Predicted additivity ±
10%
NEO2734 +
250 nM venetoclax

Supplementary Figure 5. NEO2734, NEO1132, birabresib (OTX015) and SGC-CBP30 show correlated patterns of activity in 27 DLBCL cell lines. Each dot represents a cell line. Pearson's correlation was used for correlation analysis.



Supplementary Figure 6. Effect of three days versus six days exposure to NEO2734 and birabresib. A) Box plot comparing AUC Birabresib / AUC NEO2734 after 3 days or 6 days treatment in CREBBP/EP300 WT or mutated DLBCL cell lines. B) Box plot comparing AUC birabresib/AUC NEO2734 after 3 days or 6 days treatment in *BCL2* low expressor or *BCL2* high expressor DLBCL cell lines. AUC (Area Under the Curve) was calculated using Prism7.

A)



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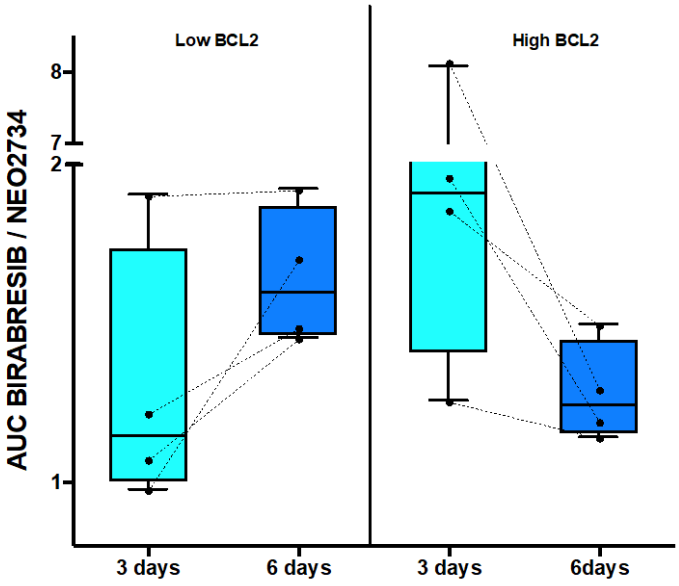
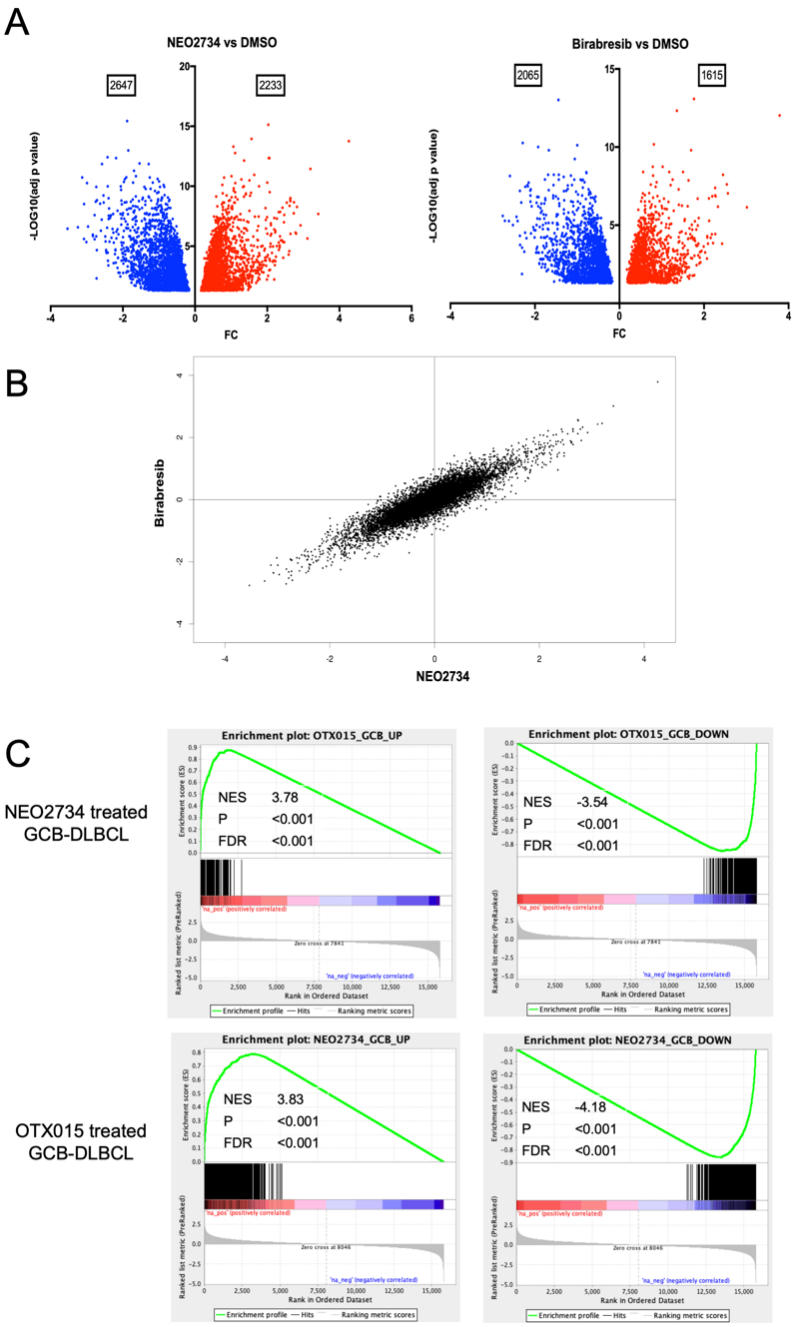
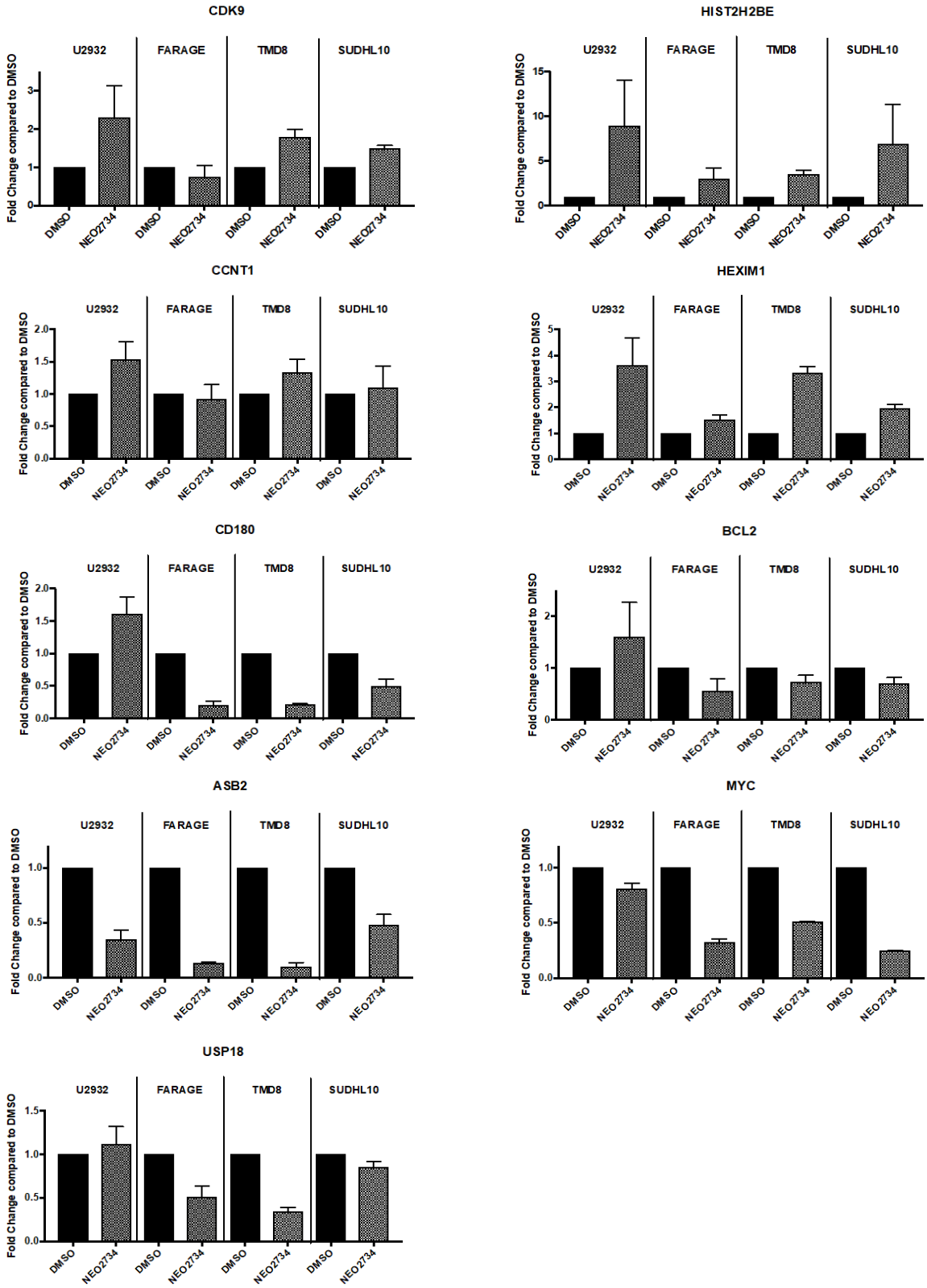


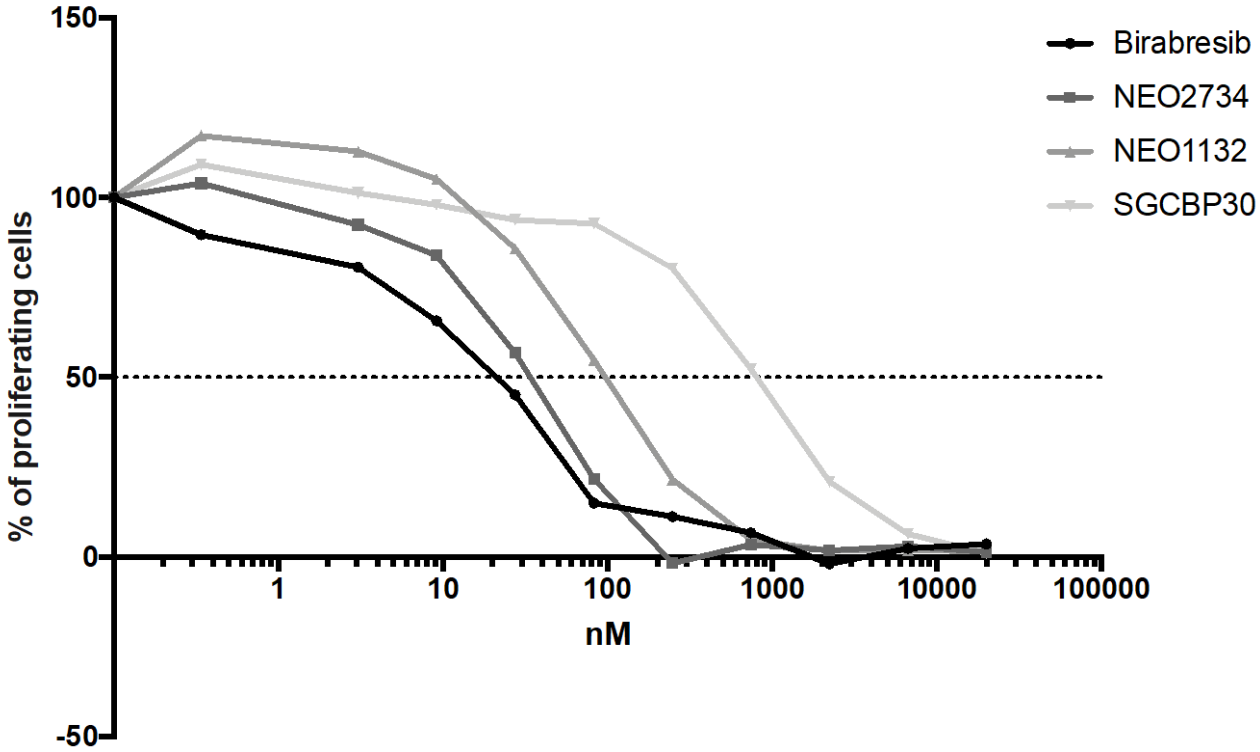
Supplementary Figure 7. RNA-Seq of DLBCL cell lines treated with NEO2734 or birabresib in four DLBCL cell lines. (A) volcano plots showing significantly upregulated (red) or down-regulated (blue) genes after exposure to NEO2734 (left panel) or birabresib (right panel). (B) Scatter plots of log2-fold gene-expression changes after NEO2734 and birabresib 6h treatment in four DLBCL cell lines. Y axis, log2-fold changes after birabresib treatment; X axis, log2-fold changes after NEO2734 treatment. Pearson rho = 0.89, P < 0.001. (C) GSEA plots for NEO2734-treated GCB-DLBCL analyzed for enrichment of genes affected by birabresib treatment (logfc > |0.5|; adj. P < 0.05) in GCB-DLBCL (above) and GSEA plots in birabresib-treated GCB-DLBCL analyzed for enrichment of genes affected by NEO2734 in GCB-DLBCL (below). Green line, enrichment score; bars in the middle portion of the plots show where the members of the gene set appear in the ranked list of genes; Positive or negative ranking metric indicates, respectively, correlation or inverse correlation with the profile; NES, normalized enrichment score.



Supplementary Figure 8. Real-time PCR performed after 6h NEO2734 treatment of DLBCL cell lines. Real-time PCR was performed in 4 cell lines SU-DHL10 and TMD8 (sensitive) and U2932 and Farage (resistant) treated for 6h with 100 nM NEO2734 or DMSO. At least two biological replicates were performed. Primers: ASB2 Fw: 5'-CATTGGGCAGGAGGAGTACA-3', Rev: 5'- TCTCAGGAGGTGCAGTGGA-3';
CD180 Fw: 5'- AAACATCGCTTAATGGGCCC -3' Rev: 5'- TGGGAAGTCTTTGGGGAACT -3';
CDK9 Fw: 5'- GTTCCCCATTACAGCCTTGC -3' Rev: 5'- CAGACAGCGTGAACTTGACC -3';
USP18 Fw: 5'- CTGACAATCCACCTCATGCG -3' Rev: 5'-AAAGCTCATACTGCCCTCCA -3';
CNT1 Fw5'- TTGTTCGAGCAAGCAAGGAC -3'Rev: 5'- ATACTCCCACCAGTGCTTCC -3';
Primer sequences for BCL2, MYC, HIST2H2BE and HEXIM1 were obtained from Lucile et al.[1]



Supplementary Figure 9. Antiproliferative activity of NEO2734, NEO1132, birabresib (OTX015) and CBP30 in a canine DLBCL cell line. CLBL-1 cells were treated with increasing doses of NEO2734, NEO1132, Birabresib or CBP30 for 72h and the percentage of proliferating cells was determined with an MTT assay.



SUPPLEMENTARY TABLES AND FIGURE

Supplementary Table 1. Binding activity of NEO1132 against bromodomains, as detected using a ligand binding site-directed competition assay.

Target	NEO2734 (Kd, nM)	Molibresib (Kd, nM)	NEO1132 (Kd, nM)
ATAD2A	>10000	>10000	>10000
ATAD2B	>10000	>10000	5600
BAZ2A	150	>10000	120
BAZ2B	130	>10000	150
BRD1	1100	>10000	1200
BRD2(1)	4.6	55	6.2
BRD2(1,2)	9	64	42
BRD2(2)	4.1	20	57
BRD3(1)	1.4	29	4
BRD3(1,2)	3.5	40	26
BRD3(2)	2.6	26	48
BRD4(1)	5.1	60	7.2
BRD4(1,2)	7.1	31	63
BRD4(2)	6.2	19	190
BRD4(full-length,short-iso.)	2.5	40	7.4
BRD7	200	>10000	110
BRD8(1)	>10000	>10000	4700
BRD8(2)	>10000	>10000	>10000
BRD9	59	>10000	43
BRDT(1)	4.6	150	24
BRDT(1,2)	3.5	50	87
BRDT(2)	17	37	190
BRPF1	75	>10000	6.5
BRPF3	2600	>10000	1900
CECR2	25	>10000	290
CBP	19	>10000	61
EP300	31	>10000	80
FALZ	2300	>10000	140
GCN5L2	>10000	>10000	>10000
PBRM1(2)	3800	>10000	2400
PBRM1(5)	1100	>10000	1500
PCAF	3300	>10000	5300
SMARCA2	4700	>10000	3700
SMARCA4	9100	>10000	8300
TAF1(2)	100	>10000	1300
TAF1L(2)	490	>10000	7600
TRIM24(Bromo.)	680	>10000	410
TRIM24(PHD,Bromo.)	2000	>10000	890
TRIM33(PHD,Bromo.)	3300	>10000	1900
WDR9(2)	4100	>10000	2400

Supplementary Table 2. Antiproliferative activity, expressed in nM, obtained in 60 cell lines after 72 hours exposure to NEO2734 or molibresib (iBET-762, GSK525762).

			Molibresib IC50 nM	NEO2734 IC50 nM
1	Bladder	SW780	10000	1090
2		T24	7230	590
3	Brain & Nerves	M059K	10000	1430
4		SK-N-SH	10000	1530
5	Breast	BT474	10000	1710
6		CAL-120	10000	2160
7		HCC1937	10000	10000
8		HCC1954	10000	10000
9		MX-1	10000	7340
10		SK-BR-3	10000	760
11		ZR-75-1	10000	3050
12	Cervix	HeLa	1640	350
13		HeLa 229	10000	2630
14	Colorectum	COLO 205	2340	400
15		DLD-1	10000	670
16		HCT-8	10000	1770
17		SW1116	10000	860
18		SW1417	9370	680
19	Kidney	786-O	10000	3010
20		OS-RC-2	10000	10000
21		UO.31	5770	480
22	Liver	Hep3B	10000	890
23		SNU-182	10000	5330
24		SNU-354	10000	470
25		SNU-368	10000	3840
26		SNU-387	10000	1430
27	Lung	Hs 618.T	10000	7620
28		NCI-H1417	3750	390
29		NCI-H1688	10000	5080
30		NCI-H1836	10000	10000
31		NCI-H1963	1070	190
32		NCI-H209	10000	10000
33		NCI-H69	10000	1400

34		SW1271	10000	600
35	Ovary	A2780cis	1700	340
36		OVCAR-8	2300	340
37		SW626	10000	790
38		SW756	10000	830
39	Pancreas	CFPAC-1	6360	570
40		PL45	10000	1210
41		SW1990	10000	2790
42	Prostate	22Rv1	10000	610
43		C4-2	2290	460
44		DU 145	10000	6550
45		LNCaP clone FGC	990	240
46		VCAP	790	170
47	Skin	SK-MEL-28	10000	3880
48		SK-MEL-5	10000	960
49	Stomach	SNU-1	1120	300
50		SNU-216	10000	810
51		SNU-484	10000	850
52		SNU-601	10000	890
53	Blood	Molt-4	2380	560
54		KG-1	580	110
55		THP-1	1010	290
56		KHYG-1	1070	180
57		Daudi	1170	300
58		Raji	1780	320
59		RL	840	130
60		U-937	1110	280

Supplementary Table 3. Antiproliferative activity, expressed in nM, obtained in DLBCL cell lines after 72 hours exposure to NEO2734, birabresib (OTX015), SGC-CBP30 and NEO1132. DLBCL, diffuse large B-cell lymphoma; GCB, germinal center B-cell type; ABC, activated B-cell like. *MYD88*, *BCL2*, *MYC* and *TP53* status were obtained as previously described [2,3]. *CREBBP* and *EP300* status was obtained as described in the methods.

DLBCL type	Cell.line	Neo2734	Birabresib	Neo1132	SGC-CBP30	MYC translocation	MYD88 mutation	BCL2 translocation	p53 inactive	CREBBP	EP300
ABC	SUDHL2	56.6	60.6	127.9	2670.9	no	N.A.	no	no	0	1
ABC	TMD8	79.9	181.5	196.5	3013.6	no	N.A.	N.A.	no	0	0
ABC	RCK8	98.3	271.5	316.0	4157.4	no	N.A.	N.A.	no	0	0
ABC	OCILY10	99.3	341.2	274.4	5749.9	no	yes	no	yes	0	0
GCB	TOLEDO	106.0	167.0	227.2	3018.0	yes	N.A.	yes	yes	0	1
GCB	DOHH2	107.5	120.5	292.5	4079.9	yes	N.A.	yes	no	0	1
GCB	DB	116.4	255.8	398.5	4711.5	N.A.	N.A.	N.A.	N.A.	0	1
GCB	SUDHL10	129.4	233.7	348.3	7148.2	yes	no	yes	yes	0	1
ABC	HBL1	135.6	192.9	272.0	5619.8	no	yes	N.A.	yes	0	0
GCB	PFEIFFER	137.1	222.9	299.7	3405.1	no	N.A.	yes	yes	1	1
GCB	SUDHL6	137.6	125.3	397.4	4408.7	no	yes	yes	yes	0	1
GCB	SUDHL16	140.8	123.8	384.8	4422.1	no	N.A.	N.A.	N.A.	1	1
ABC	RI1	146.9	140.4	599.2	4891.8	N.A.	no	N.A.	yes	1	0
GCB	OCILY1	157.0	204.0	477.3	3607.7	no	N.A.	yes	yes	0	0
ABC	OCILY3	162.2	432.5	341.6	17135.5	no	yes	no	no	0	0
GCB	WSUDLCL2	167.0	442.9	377.2	8244.1	no	no	yes	yes	0	1
ABC	U2932	176.1	163.3	662.8	11027.2	no	no	no	yes	0	0
GCB	OCILY7	207.6	392.0	621.6	10430.7	yes	no	no	yes	0	1
GCB	SUDHL5	213.7	278.0	551.5	3454.9	no	no	no	N.A.	0	1
GCB	OCILY8	224.5	164.3	648.7	15358.0	yes	no	yes	yes	1	0
GCB	SUDHL4	231.5	405.2	596.6	11618.9	no	no	yes	yes	0	1
GCB	SUDHL8	251.9	240.5	893.7	5447.2	yes	no	no	N.A.	1	1
GCB	OCILY18	257.7	171.4	579.1	3096.4	yes	no	yes	yes	0	1
GCB	KARPAS422	328.9	404.1	806.7	7929.2	no	N.A.	yes	yes	1	1
GCB	OCILY19	345.9	522.7	1194.1	9586.9	N.A.	N.A.	yes	no	1	0
GCB	VAL	644.6	495.0	1064.8	11035.9	yes	no	yes	no	1	0
GCB	FARAGE	2462.7	16274.5	8381.0	38441.4	no	N.A.	no	yes	0	1

Supplementary Table 4. Gene set enrichment analysis data. Cell lines were divided into sensitive and resistant cell lines to NEO2734. Selected gene sets, P value < 0.05 and FDR <0.05.

Supplementary Table 5. Supervised analysis of RNA-Seq data of DLBCL cell lines exposed to NEO2734. Data are presented for all the cell lines together, for ABC- or GCB- DLBCL subtypes. In each worksheet: Red, transcripts with log fold change >1 and adjusted P < 0.05; Green, transcripts with log fold change <-1 and adjusted P < 0.05; B) Gene sets significantly enriched after treatment.

Supplementary Table 6. Supervised analysis of RNA-Seq data of DLBCL cell lines exposed to birabresib (OTX015). Data are presented for all the cell lines together, for ABC- or GCB- DLBCL subtypes. In each worksheet: Red, transcripts with log fold change >1 and adjusted P < 0.05; Green, transcripts with log fold change <-1 and adjusted P < 0.05; B) Gene sets significantly enriched after treatment.

References

1. Astorgues-Xerri L, Vazquez R, Odore E, et al. Insights into the cellular pharmacological properties of the BET-inhibitor OTX015/MK-8628 (birabresib), alone and in combination, in leukemia models. *Leuk Lymphoma*. 2019;60(12):3067-3070.
2. Hicks SW, Tarantelli C, Wilhem A, et al. The novel CD19-targeting antibody-drug conjugate huB4-DGN462 shows improved anti-tumor activity compared to SAR3419 in CD19-positive lymphoma and leukemia models. *Haematologica*. 2019;104(8):1633-1639.
3. Spriano F, Chung EYL, Gaudio E, et al. The ETS Inhibitors YK-4-279 and TK-216 Are Novel Antilymphoma Agents. *Clin Cancer Res*. 2019;25(16):5167-5176.