The novel CD19-targeting antibody-drug conjugate huB4-DGN462 shows improved anti-tumor activity compared to SAR3419 in CD19-positive lymphoma and leukemia models

Stuart W. Hicks,[1*] Chiara Tarantelli,[2*] Alan Wilhem,[1] Eugenio Gaudio,[2] Min Li,[1] Alberto J. Arribas,[2] Filippo Spriano,[2] Roberta Bordone,[3] Luciano Cascione,[2,4] Katharine C. Lai,[1] Qifeng Qiu,[1] Monica Taborelli,[5] Davide Rossi,[2,3] Georg Stussi,[3] Emanuele Zucca,[3] Anastasios Stathis,[3] Callum M. Sloss[1] and Francesco Bertoni[2]

These authors contributed equally to this work.

[1]ImmunoGen Inc., Waltham, MA, USA; [2]Università della Svizzera italiana, Institute of Oncology Research, Bellinzona, Switzerland; [3]Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; [4]Swiss Institute of Bioinformatics, Lausanne, Switzerland and [5]Cytogenetics Laboratory, Ente Ospedaliero Cantonale, Bellinzona, Switzerland

 doi:10.3324/haematol.2018.211011

Received: November 3, 2018.

Accepted: February 7, 2019.

Pre-published: February 7, 2019.

Correspondence: *FRANCESCO BERTONI* - frbertoni@mac.com

CALLUM SLOSS - callum.sloss@immunogen.com

The novel CD19-targeting antibody-drug conjugate huB4-DGN462 shows improved anti-tumor activity than SAR3419 in CD19-positive lymphoma and leukemia models

Stuart W. Hicks[1], Chiara Tarantelli[2], Alan Wilhem[1], Eugenio Gaudio[2], Min Li[1], Alberto J. Arribas[2], Filippo Spriano[2], Roberta Bordone[3], Luciano Cascione[2,4], Katharine C. Lai[1], Qifeng Qiu[1], Monica Taborelli[5], Davide Rossi[2,3], Georg Stussi[3], Emanuele Zucca[3], Anastasios Stathis[3], Callum M. Sloss[1], Francesco Bertoni[2]

[1] ImmunoGen, Inc., Waltham, MA, USA; [2] Università della Svizzera italiana, Institute of Oncology Research, , Bellinzona, Switzerland; [3] Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; [4] Swiss Institute of Bioinformatics, Lausanne, Switzerland; [5], Cytogenetics Laboratory, Ente Ospedaliero Cantonale, Bellinzona, Switzerland.

Supplementary materials

Supplementary Table S1. Anti-tumor activity of huB4-DGN462 and SAR3419 in B-cell lymphomas.

Cell Line	Histology	huB4-DGN462 IC50 (pM)	DGN462 IC50 (pM)	Apoptosis, huB4-DGN462	SAR3419 IC50 (pM)	Apoptosis, SAR3419	CD19 (MFI)	*BCL2* translocation	*MYC* translocation	inactive *TP53*
DB	GCB-DLBCL	800	800	0	2000	0	1174	yes*	no #	yes [1]
DoHH2	GCB-DLBCL	0.2	0.1	1	3000	1	1483	yes [2]	yes ^	no [3]
ESKOL	MZL	12	0.1	1	8000	1	366		no #	
Farage	GCB-DLBCL	0.8	0.8	1	20	1	3858	no [4]	no ^	yes [5]
Granta 519	MCL	40	0.1	1	3000	1	392	no *,[6]	no #	yes [7]
HAIR-M	MZL	100	0.1	0	5000	1	92		no #	
HBL-1	GCB-DLBCL	750	750	1	8000	0	346		no ^	yes [8]
HC-1	MZL	30	0.1	1	5000	1	210		no #	
JeKo-1	MCL	100	0.1	0	12000	0	198	no *	no #	yes [9]
JVM-2	MCL	50	50	0	3000	0	65	no *	no #	no [10]
Karpas-1106	PMBCL	200	10	1	10000	1	2966		no #	
Karpas-1718	MZL	5	0.1	1	1500	1	586		no #	yes [11]
Karpas-422	GCB-DLBCL	8000	3000	1	2000	1	1913	yes [12, 13]	no ^	yes [10, 12]
MAVER-1	MCL	50	0.1	1	2000	1	1799		yes #	yes [14]
MEC-1	CLL	1500	2	1	40000	0	712		no #	yes [15]
Mino	MCL	50	0.1	1	5000	1	1365		yes #	yes [16]
OCI-LY-1	GCB-DLBCL	12	200	1	600	1	1544	yes [12, 17, 18]	no #	yes [18]
OCI-LY-10	ABC-DLBCL	0.75	0.1	1	3500	1	627	no [18, 19]	no ^	yes [15]
OCI-LY-18	GCB-DLBCL	3	0.75	1	1500	1	1286	yes [18]	yes ^	yes [18]
OCI-LY-19	GCB-DLBCL	0.75	0.1	1	1200	1	4342	yes *,[20]		no [18, 21]
OCI-LY-3	ABC-DLBCL	30	5	1	7000	0	156	no [1]	no ^	no [18, 22]
OCI-LY-7	GCB-DLBCL	50	50	1	5000	0	984	no *	yes ^	yes [12, 18]
OCI-LY-8	GCB-DLBCL	3	3	1	50	0	961	yes [17, 18, 20]	yes ^	yes [18]
PCL12	CLL	3000	1000	1	10000	1	634		no #	
Pfeiffer	GCB-DLBCL	2500	2500	0	8000	0	997	yes **	no ^	yes [3]
RCK8	ABC-DLBCL	500	500	1	6000	0	1791		no ^	no [23]
Rec-1	MCL	40000	1500	0	15000	0	312		no #	yes [15]
RI-1	ABC-DLBCL	3000	2000	0	4500	1	358			yes [15]
SP-49	MCL	5	1.5	0	2	0	1479		no #	

SP-53	MCL	30	0.1	1	2000	1	2397		no #	
SSK41	MZL	1000	800	1	1500	1	507		no #	
SU-DHL-10	GCB-DLBCL	2000	2000	1	100	1	1109	yes [1]	yes ^	yes [15]
SU-DHL-16	GCB-DLBCL	200	10	1	7000	1	15		no ^	
SU-DHL-2	ABC-DLBCL	200	200	1	7000	1	68	no [20]	no ^	no [1]
SU-DHL-4	GCB-DLBCL	2000	2000	1	7000	1	9568	yes [20]	no #	yes [15]
SU-DHL-5	GCB-DLBCL	3	0.1	1	800	1	2086	no *	no ^	
SU-DHL-6	GCB-DLBCL	100	1000	1	200	1	746	yes *	no #	yes [15]
SU-DHL-8	GCB-DLBCL	40	100	1	3000	1	40	no *	yes #	
TMD8	ABC-DLBCL	3000	800	1	7000	1	402		no ^	no [24, 25]
Toledo	GCB-DLBCL	300	200	1	12500	1	2306	yes [17, 26]	yes ^	yes [15]
U2932	ABC-DLBCL	700	700	1	4000	1	465	no *	no ^	yes [27]
UPN1	MCL	2	1	1	1000	1	537		no #	yes [28]
VAL	GCB-DLBCL	200	200	1	2500	1	2158	yes *	yes ^	no [21]
VL51	MZL	100	0.1	1	10000	1	20		no #	
WSU-DLCL2	GCB-DLBCL	3500	2500	1	2500	1	1134	yes *	no #	yes [21]
Z-138	MCL	5	0.1	0	5000	1	157		yes #	

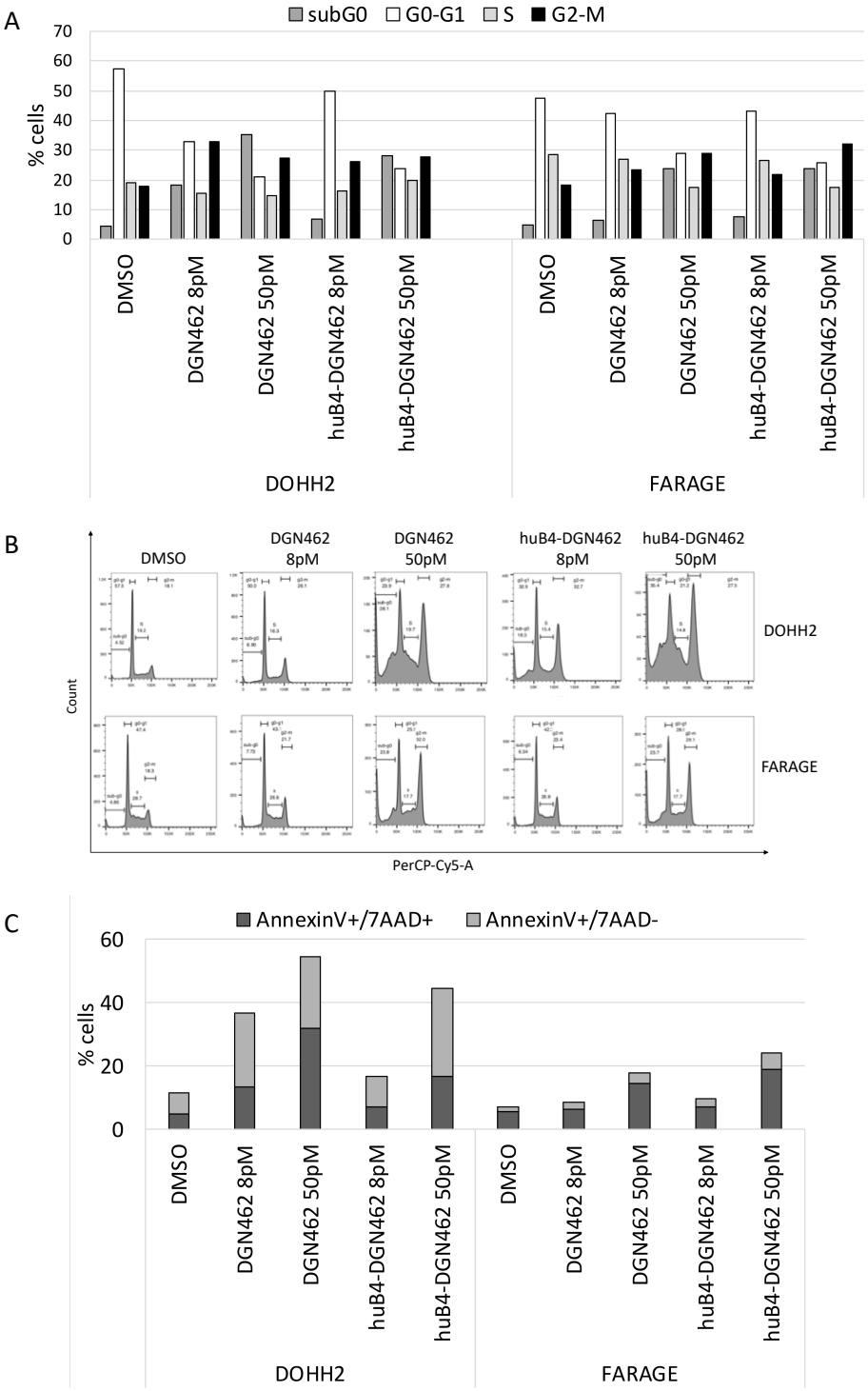
GCB-DLBCL, germinal center B-cell type diffuse large B-cell lymphoma; ABC-DLBCL, activated B-cell like diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; CLL, chronic lymphocytic leukemia; PMBCL, primary mediastinal large B-cell lymphoma; n.d., not determined. ; *, as described in the catalogue of the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) (www.dsmz.de); **, as described in the catalogue of the American Type Culture Collection (ATCC)(http://www.lgcstandards-atcc.org); # FISH, XL MYC BA Triple-color Probe, D-6030-100-TC (MetaSystems Probes GmbH, Germany); ^ FISH using the XL t(8;14) Dual Fusion Probe, D-5008-100-OG (MetaSystems Probes GmbH). FISH analyses were performed as previously described [29].

Supplementary Table S2. Anti-tumor activity of huB4-DGN462 and SAR3419 in six acute lymphoblastic leukemia cell lines. ABC, antibody binding capacity

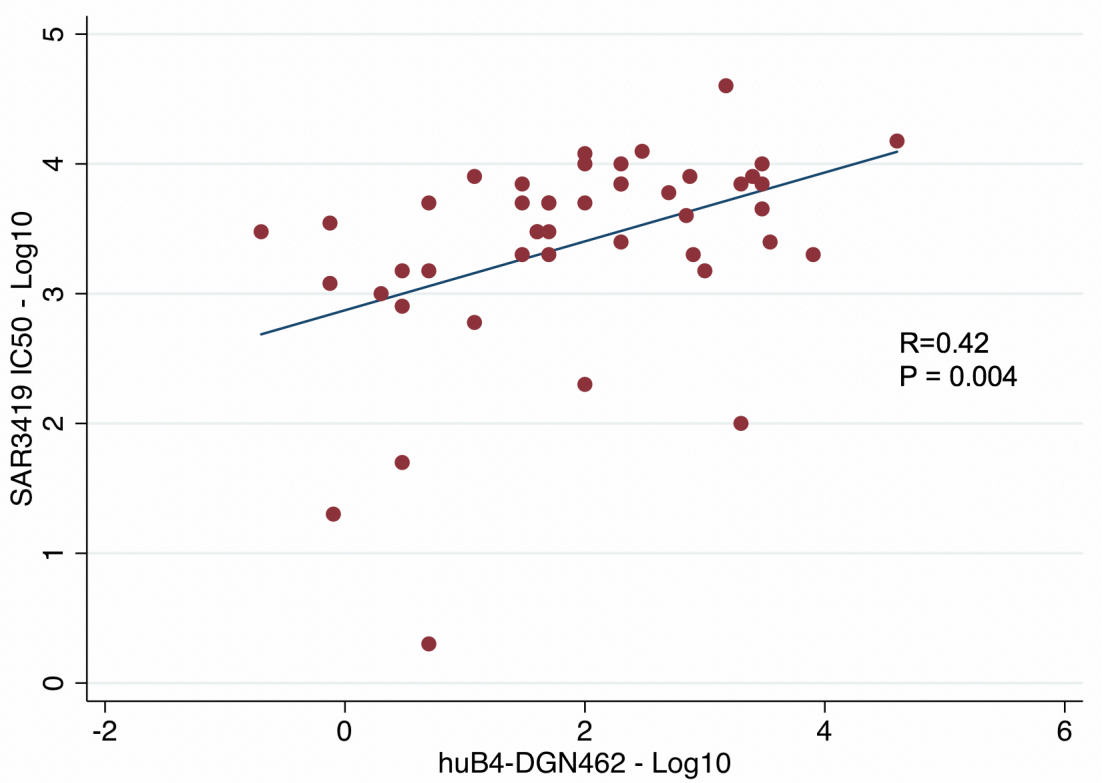
		EC50 (pM)	
Cell line	**CD19 ABC (x1,000)**	**huB4-DGN462**	**SAR3419**
RS4;11	32	10.2	2,117
NALM-6	25	8.4	1,260
TOM-1	16	15.6	3517
Mutz-5	13	7.2	6,259
BALL-1	12	4.0	9,285
GRANTA-452	6	9.1	5,685

ABC, antibody binding capacity

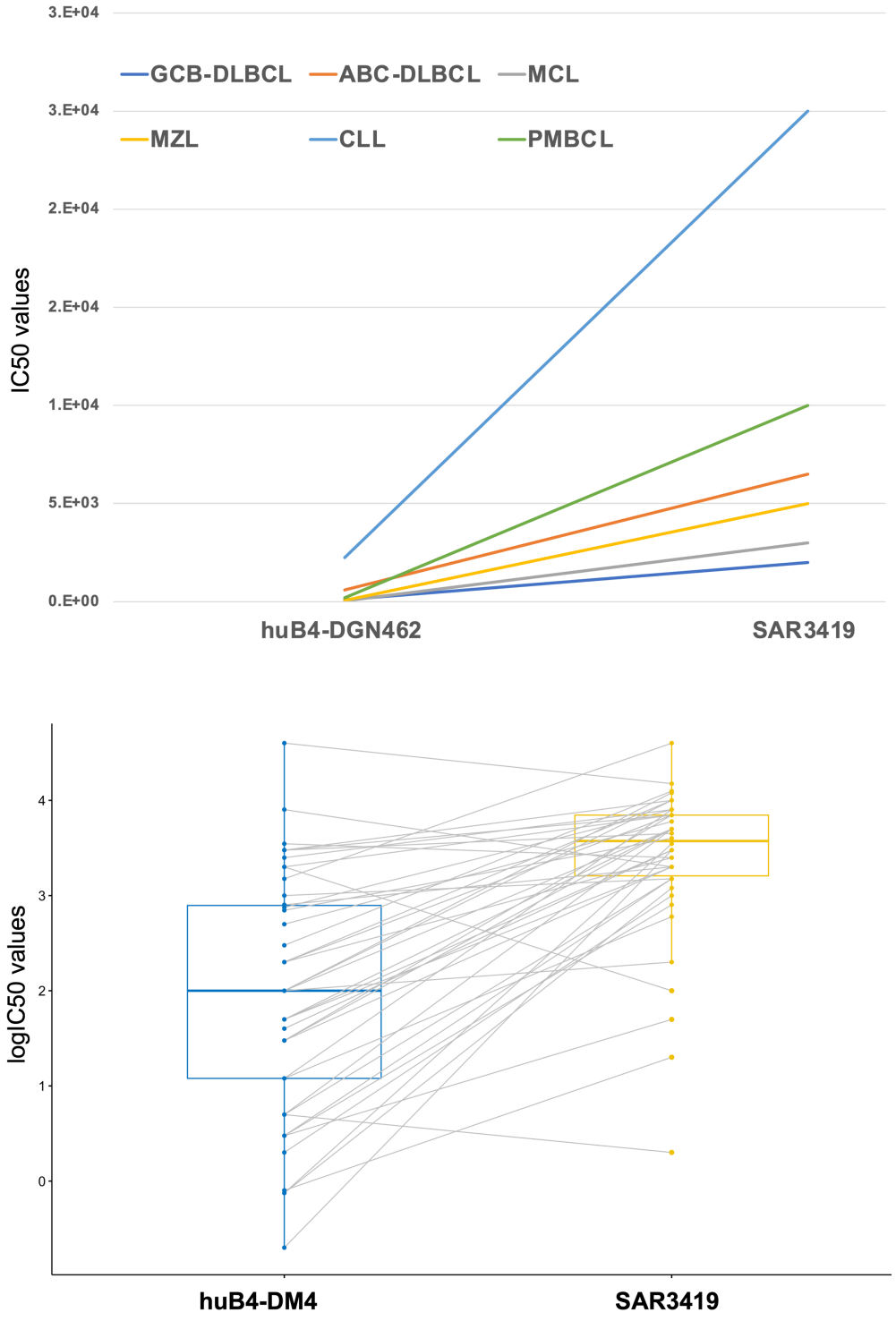
Supplementary Figure S1. huB4-DGN462 and DGN462 induce apoptosis and G2-M arrests in lymphoma cell lines. A. Cell cycle distribution after huB4-DGN462, DGN462 treatment (8pM or 50pM) or DMSO (72 hours). B. Representative histograms of cell cycle distribution and (C) induction of apoptosis in DOHH2 and FARAGE cells treated with huB4-DGN462, DGN462 treatment (8pM or 50pM) or DMSO (72 hours).



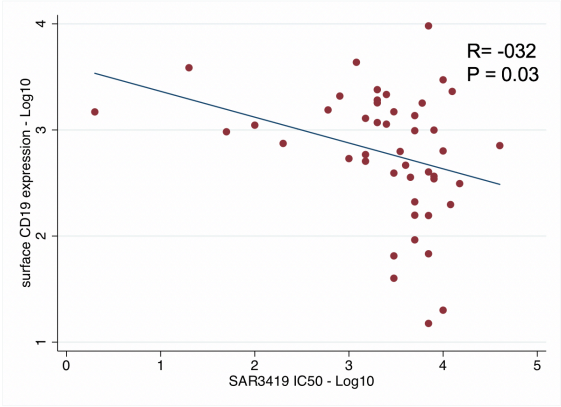
Supplementary Figure S2. The *in vitro* antitumor activity of huB4-DGN462 and SAR3419 are correlated across B-cell lymphoma lines. Y-axis, Log10 IC50 values of SAR3419 (pM). X-axis, Log10 IC50 values of huB4-DGN462 (pM). R, correlation coefficient. P, p-value.

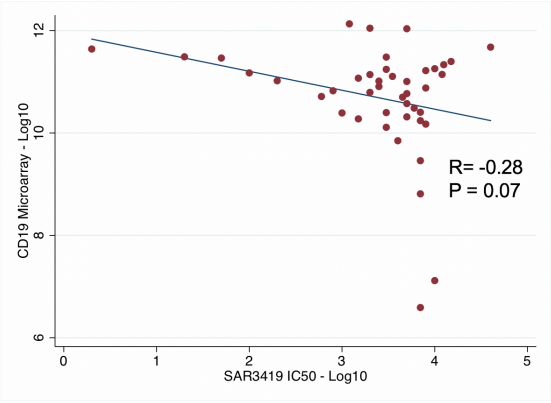


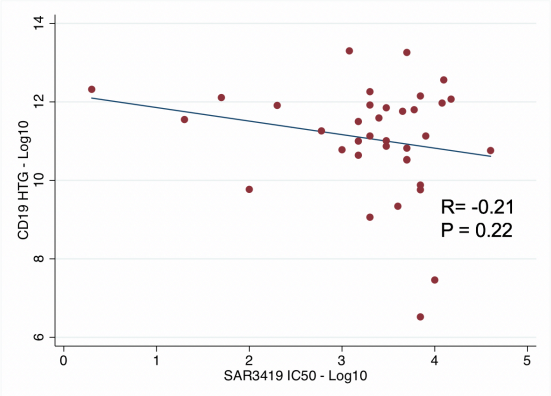
Supplementary Figure S3. Paired line plots showing the higher *in vitro* antitumor activity of huB4-DGN462 than SAR3419. Upper plot shows the differences in median IC50 values by histotype as detailed in Table 1. Lower plot shows the differences for each individual cell line as detailed in Table 1. median IC50 values by histotype as detailed in Supplementary Table S1. GCB-DLBCL, germinal center B-cell type diffuse large B-cell lymphoma; ABC-DLBCL, activated B-cell like diffuse large B-cell lymphoma; MCL, mantle cell lymphoma; MZL, marginal zone lymphoma; CLL, chronic lymphocytic leukemia; PMBCL, primary mediastinal large B-cell lymphoma



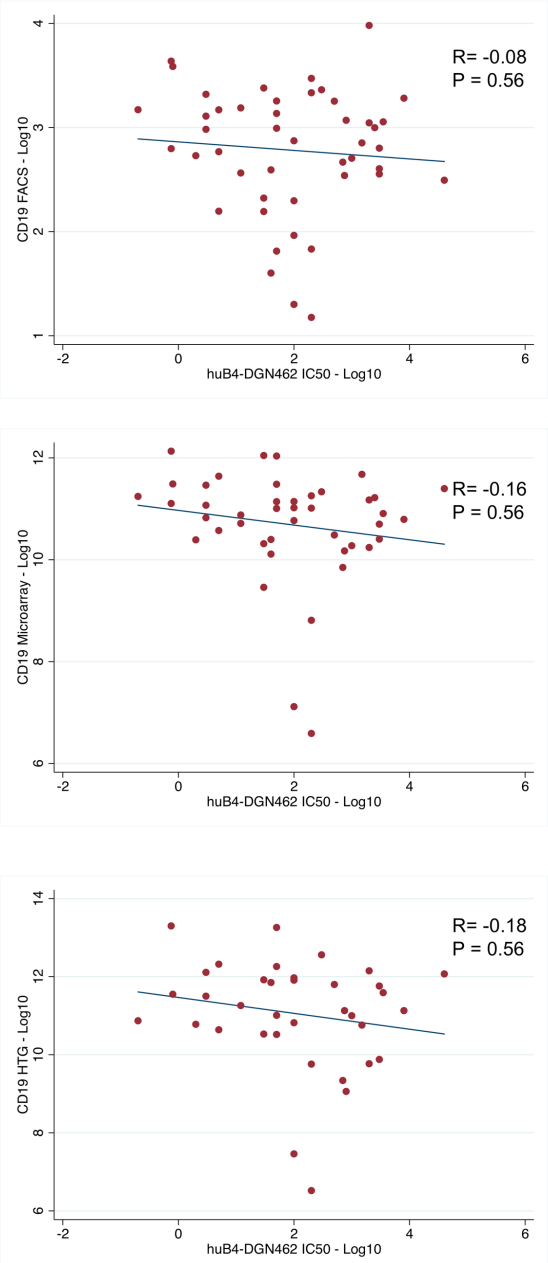
Supplementary Figure S4. The *in vitro* antitumor activity of SAR3419 is correlated with surface CD19 expression, but not CD19 RNA levels in B-cell lymphoma lines. Y-axis, CD19 expression levels (Log10) as measured by flow cytometry analysis (upper panel), microarray analysis (middle panel) or next generation sequencing (lower panel). X-axis, Log10 IC50 values of SAR3419 (pM). R, correlation coefficient. P, p-value.



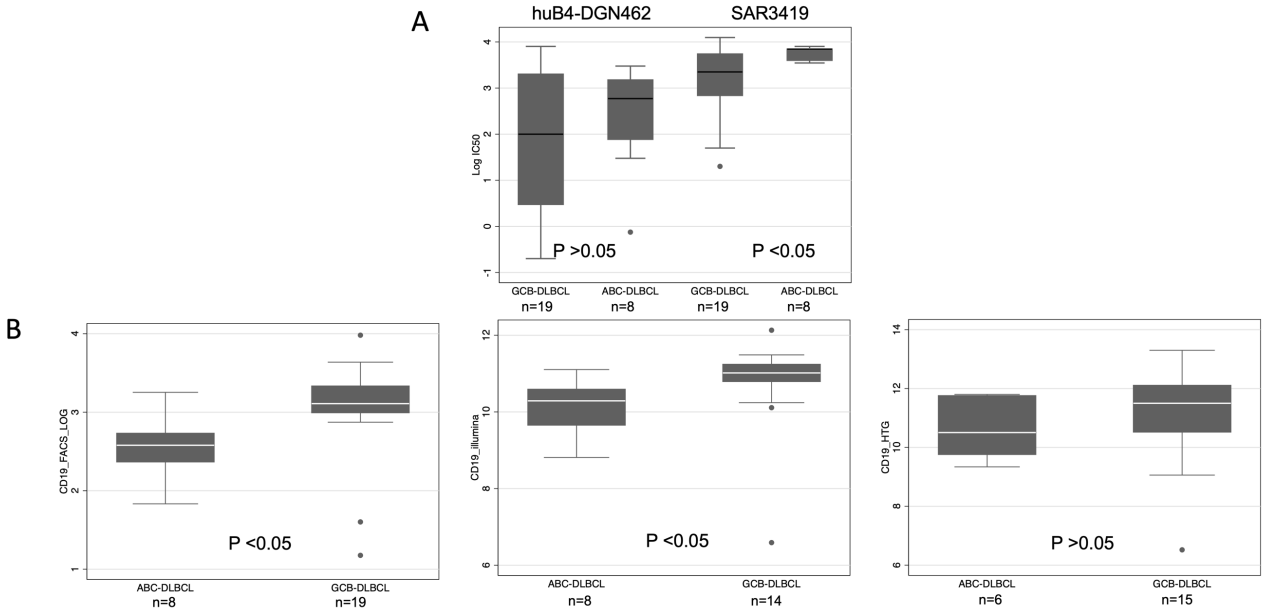




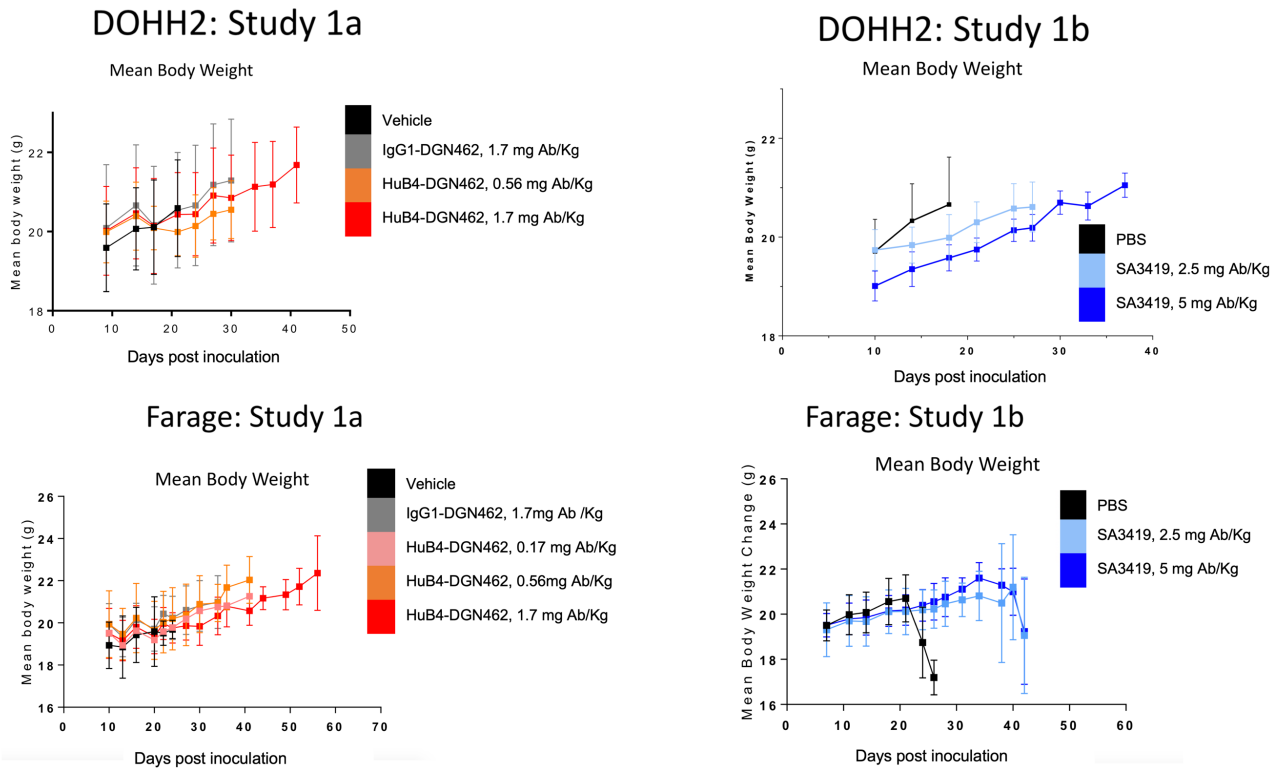
Supplementary Figure S5. The *in vitro* antitumor activity of huB4-DGN462 is not correlated to CD19 expression in B-cell lymphoma lines. Y-axis, CD19 expression levels (Log10) as measured by flow cytometry analysis (upper panel), microarray analysis with Illumina arrays (middle panel) or NGS with HTG platform (lower panel). X-axis, Log10 IC50 values of huB4-DGN462 (pM). R, correlation coefficient. P, p-value.



Supplementary Figure S6. DLBCL cell of origin, CD19 expression and sensitivity to huB4-DGN462 or SAR3419. A, Sensitivity to SAR3419 but not to huB4-DGN462 is affected by the DLBCL cell of origin. B, GCB-DLBCL cell lines express higher CD19 levels than ABC-DLBCL cell lines. CD19 expression levels were measured (Log10) by flow cytometry analysis (left panel), microarray analysis with Illumina arrays (central panel) or NGS with HTG platform (right panel).



Supplementary Figure S7. Body weight changes after exposure to vehicles, huB4-DGN462 (left panels) or to SAR3419 (right panels) in subcutaneous (upper panel) or disseminated (lower panel) DLBCL xenograft models as shown in Figure 2. Body weights were measured twice weekly.



References

1. Deng W, Clipson A, Liu H, et al. Variable Responses of MYC Translocation Positive Lymphoma Cell Lines To Different Combinations of Novel Agents: Impact of BCL2 Family Protein Expression. Transl Oncol. 2018;11(5):1147-1154.
2. Kluin-Nelemans HC, Limpens J, Meerabux J, et al. A new non-Hodgkin's B-cell line (DoHH2) with a chromosomal translocation t(14;18)(q32;q21). Leukemia. 1991;5(3):221-224.
3. Drakos E, Singh RR, Rassidakis GZ, et al. Activation of the p53 pathway by the MDM2 inhibitor nutlin-3a overcomes BCL2 overexpression in a preclinical model of diffuse large B-cell lymphoma associated with t(14;18)(q32;q21). Leukemia. 2011;25(5):856-867.
4. Baruch M, Hochberg M, Gabay C, Ben-Bassat H, Shlomai Z, Laskov R. Molecular characterization of an unusual non-Hodgkin's B-lymphoma cell line ("Farage") lacking the ability to produce immunoglobulin polypeptide chains. Leuk Lymphoma. 1996;21(5-6):485-495.
5. Snuderl M, Kolman OK, Chen YB, et al. B-cell lymphomas with concurrent IGH-BCL2 and MYC rearrangements are aggressive neoplasms with clinical and pathologic features distinct from Burkitt lymphoma and diffuse large B-cell lymphoma. Am J Surg Pathol. 2010;34(3):327-340.
6. Rudolph C, Steinemann D, Von Neuhoff N, et al. Molecular cytogenetic characterization of the mantle cell lymphoma cell line GRANTA-519. Cancer Genet Cytogenet. 2004;153(2):144-150.
7. Drexler HG, MacLeod RA. Malignant hematopoietic cell lines: in vitro models for the study of mantle cell lymphoma. Leuk Res. 2002;26(9):781-787.
8. Derenzini E, Agostinelli C, Imbrogno E, et al. Constitutive activation of the DNA damage response pathway as a novel therapeutic target in diffuse large B-cell lymphoma. Oncotarget. 2015;6(9):6553-6569.
9. Camps J, Salaverria I, Garcia MJ, et al. Genomic imbalances and patterns of karyotypic variability in mantle-cell lymphoma cell lines. Leuk Res. 2006;30(8):923-934.
10. Forbes SA, Beare D, Boutselakis H, et al. COSMIC: somatic cancer genetics at high-resolution. Nucleic Acids Research. 2017;45(D1):D777-D783.
11. Fresquet V, Robles EF, Parker A, et al. High-throughput sequencing analysis of the chromosome 7q32 deletion reveals IRF5 as a potential tumour suppressor in splenic marginal-zone lymphoma. Br J Haematol. 2012;158(6):712-726.
12. Farrugia MM, Duan LJ, Reis MD, Ngan BY, Berinstein NL. Alterations of the p53 tumor suppressor gene in diffuse large cell lymphomas with translocations of the c-MYC and BCL-2 proto-oncogenes. Blood. 1994;83(1):191-198.
13. Dyer MJ, Fischer P, Nacheva E, Labastide W, Karpas A. A new human B-cell non-Hodgkin's lymphoma cell line (Karpas 422) exhibiting both t (14;18) and t(4;11) chromosomal translocations. Blood. 1990;75(3):709-714.
14. Zamo A, Ott G, Katzenberger T, et al. Establishment of the MAVER-1 cell line, a model for leukemic and aggressive mantle cell lymphoma. Haematologica. 2006;91(1):40-47.
15. Barretina J, Caponigro G, Stransky N, et al. The Cancer Cell Line Encyclopedia enables predictive modelling of anticancer drug sensitivity. Nature. 2012;483(7391):603-607.
16. Lai R, McDonnell TJ, O'Connor SL, et al. Establishment and characterization of a new mantle cell lymphoma cell line, Mino. Leuk Res. 2002;26(9):849-855.
17. Dutta C, Day T, Kopp N, et al. BCL2 suppresses PARP1 function and nonapoptotic cell death. Cancer Res. 2012;72(16):4193-4203.
18. Chang H, Blondal JA, Benchimol S, Minden MD, Messner HA. p53 mutations, c-myc and bcl-2 rearrangements in human non-Hodgkin's lymphoma cell lines. Leuk Lymphoma. 1995;19(1-2):165-171.
19. Kunkalla K, Liu Y, Qu C, et al. Functional inhibition of BCL2 is needed to increase the susceptibility to apoptosis to SMO inhibitors in diffuse large B-cell lymphoma of germinal center subtype. Ann Hematol. 2013;92(6):777-787.
20. Souers AJ, Leverson JD, Boghaert ER, et al. ABT-199, a potent and selective BCL-2 inhibitor, achieves antitumor activity while sparing platelets. Nat Med. 2013;19(2):202-208.
21. Oricchio E, Katanayeva N, Donaldson MC, et al. Genetic and epigenetic inactivation of SESTRIN1 controls mTORC1 and response to EZH2 inhibition in follicular lymphoma. Sci Transl Med. 2017;9(396):
22. Houldsworth J, Petlakh M, Olshen AB, Chaganti RS. Pathway activation in large B-cell non-Hodgkin lymphoma cell lines by doxorubicin reveals prognostic markers of in vivo response. Leuk Lymphoma. 2008;49(11):2170-2180.
23. Haery L, Mussakhan S, Waxman DJ, Gilmore TD. Evidence for an oncogenic modifier role for mutant histone acetyltransferases in diffuse large B-cell lymphoma. Leuk Lymphoma. 2016;57(11):2661-2671.
24. Pulvino M, Liang Y, Oleksyn D, et al. Inhibition of proliferation and survival of diffuse large B-cell lymphoma cells by a small-molecule inhibitor of the ubiquitin-conjugating enzyme Ubc13-Uev1A. Blood. 2012;120(8):1668-1677.
25. Tohda S, Sato T, Kogoshi H, Fu L, Sakano S, Nara N. Establishment of a novel B-cell lymphoma cell line with suppressed growth by gamma-secretase inhibitors. Leuk Res. 2006;30(11):1385-1390.
26. Bellas C, Garcia D, Vicente Y, et al. Immunohistochemical and molecular characteristics with prognostic significance in diffuse large B-cell lymphoma. PLoS One. 2014;9(6):e98169.

27. Amini RM, Berglund M, Rosenquist R, et al. A novel B-cell line (U-2932) established from a patient with diffuse large B-cell lymphoma following Hodgkin lymphoma. Leuk Lymphoma. 2002;43(11):2179-2189.
28. Pinyol M, Bea S, Pla L, et al. Inactivation of RB1 in mantle-cell lymphoma detected by nonsense-mediated mRNA decay pathway inhibition and microarray analysis. Blood. 2007;109(12):5422-5429.
29. Tarantelli C, Gaudio E, Arribas AJ, et al. PQR309 Is a Novel Dual PI3K/mTOR Inhibitor with Preclinical Antitumor Activity in Lymphomas as a Single Agent and in Combination Therapy. Clin Cancer Res. 2018;24(1):120-129.