Targeting CD205 with the antibody drug conjugate MEN1309/OBT076 is an active new therapeutic strategy in lymphoma models

Eugenio Gaudio,[1*] Chiara Tarantelli,[1*] Filippo Spriano,[1] Francesca Guidetti,[1] Giulio Sartori,[1] Roberta Bordone,[2] Alberto J. Arribas,[1] Luciano Cascione,[1,3] Mario Bigioni,[4] Giuseppe Merlino,[4] Alessio Fiascarelli,[4] Alessandro Bressan,[4] Afua Adjeiwaa Mensah,[1] Gaetanina Golino,[1] Renzo Lucchini,[5] Elena Bernasconi,[1] Davide Rossi,[1,2] Emanuele Zucca,[2] Georg Stussi,[2] Anastasios Stathis,[2] Robert S. Boyd,[6] Rachel L. Dusek,[6] Arnima Bisht,[7] Nickolas Attanasio,[7] Christian Rohlff,[7] Andrea Pellacani,[8] Monica Binaschi[4] and Francesco Bertoni[1,2]

[1]Università della Svizzera italiana, Istituto Oncologico di Ricerca, Bellinzona, Switzerland; [2]Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; [3]Swiss Institute of Bioinformatics (SIB), Lausanne, Switzerland; [4]Menarini Ricerche S.p.A., Pomezia, Italy; [5]Laboratorio di Diagnostica Molecolare, Dipartimento di Medicina di Laboratorio EOLAB, Bellinzona, Switzerland; [6]Oxford BioTherapeutics Ltd., Abingdon, UK; [7]Oxford BioTherapeutics Inc., San Jose, CA, USA and [8]Menarini Ricerche S.p.A - Menarini Group, Florence, Italy

**EG and CT contributed equally as co-first authors.*

 doi:10.3324/haematol.2019.227215

Received: May 16, 2019.

Accepted: January 2, 2020.

Pre-published: January 9, 2020.

Correspondence: *FRANCESCO BERTONI* - francesco.bertoni@ior.usi.ch

Targeting CD205 with the antibody drug conjugate MEN1309/OBT076 is an active new therapeutic strategy in lymphoma models

Eugenio Gaudio [1*], Chiara Tarantelli [1*], Filippo Spriano [1], Francesca Guidetti [1], Giulio Sartori [1], Roberta Bordone [2], Alberto J. Arribas [1], Luciano Cascione [1,3], Mario Bigioni [4], Giuseppe Merlino [4], Alessio Fiascarelli [4], Alessandro Bressan [4], Afua Adjeiwaa Mensah [1], Gaetanina Golino [1], Renzo Lucchini [5], Elena Bernasconi [1], Davide Rossi [1,2], Emanuele Zucca [2], Georg Stussi [2], Anastasios Stathis [2], Robert S. Boyd [6], Rachel L. Dusek [6], Arnima Bisht[7], Nickolas Attanasio [7], Christian Rohlff [7], Andrea Pellacani[8], Monica Binaschi [4], Francesco Bertoni [1]

[1] Università della Svizzera italiana, Istituto Oncologico di Ricerca, Bellinzona, Switzerland; [2] Oncology Institute of Southern Switzerland, Bellinzona, Switzerland; [3] Swiss Institute of Bioinformatics (SIB), Lausanne, Switzerland; [4] Menarini Ricerche S.p.A., Pomezia, Italy; [5] Laboratorio di Diagnostica Molecolare, Dipartimento di Medicina di Laboratorio EOLAB, Bellinzona, Switzerland*; [6] Oxford BioTherapeutics, Ltd., Abingdon, UK; [7] Oxford BioTherapeutics, Inc., San Jose, CA, U;* [8] Menarini Ricerche S.p.A - Menarini Group, Florence, Italy.

SUPPLEMENTARY METHODS

Proliferation and apoptosis assays

Anti-proliferative activity after treatment with and assessed as previously described [12]. Briefly, cells were exposed to MEN1309/OBT076 or to IgG-DM4 for 72h and assayed by MTT. Cells (n.=10^4) were seeded in each well of a 96-well plate and compounds were added in a range of concentrations between 1000nM and 0.5pM, following a 1:5 dilution factor. For apoptosis detection, cells (3 x 10^3) were seeded in 384-well plates and exposed to MEN1309/OBT076 (50pM and 1000pM) or to IgG-DM4 (1nM and 20nM for IgG-DM4) for 72h and assayed with Caspase-Glo 3/7 Assay (Promega, Madison, WI, USA). Apoptosis was defined by at least a 1.5-fold increase in signal activation with respect to controls. Peripheral blood mononuclear cells from healthy donors were isolated by using the Ficoll-Paque PLUS (Ge Heathcare Lifesciences) reagent in accord with its guidelines. $CD19^+$ B-cell lymphocytes were isolated from PBMCs by using the CD19 MicroBeads (MACS Miltenyi Biotec).

In vitro combinations were assessed as previously described [10, 12]. Based on the Chou-Talalay Combination Index (CI) [13], the effect of the combinations was defined as beneficial if synergistic (CI < 0.9) or additive (CI, 0-9-1.1).

Immunohistochemistry

Immunohistochemical staining was performed on 4-5 µm-thick sections of formalin-fixed paraffin embedded cores on tumor microarray slides (LY1501, LY2086, LYM401, LY2088, NHL482 and LM482; US Biomax, Rockville, MD) or gross biopsy sections (Origene, Rockville, MD). The slides were deparaffinized and heat induced epitope retrieval was performed for 20 minutes in Target Retrieval Solution citrate pH6 (Dako, Carpenteria, CA). Endogenous peroxidase activity was blocked with Dako Peroxidase Blocking Solution (Dako, Ely, Cambrideshire, UK). The primary antibody (NCL-L-DEC205, Leica Biosystems, Peterborough, Cambridgeshire, UK) was diluted to 1/160 in Dako Antibody Diluent (Dako, Ely, Cambrideshire, UK) and applied to the slides for 45 minutes. The secondary antibody HRP-conjugated polymer (Envision +, Dako, Carpenteria, CA) was applied to the tissues for 30 minutes. DAB chromogen was prepared in substrate dilution buffer (Dako, Ely, Cambrideshire, UK) and applied to the slides for 10 minutes. The slides were washed and counterstained with haematoxylin before coverslips were mounted with aqueous mounting media (Aquatex, VWR, Lutterworth, Leicestershire, UK). Slides were analyzed under brightfield microscopy according to the following scoring scheme: -, no staining; +/-, ambiguous staining; 1+, weak staining; 2+, moderate staining; and 3+, intense staining; 0, not evaluable. Only membranous and cytoplasmic staining specific to the cancer cells was considered.

CD205 surface expression by cytofluorimetry

CD205 expression was determined by flow cytofluorimetry (FACS) on fresh cells. Cells were washed with ice-cold FACS buffer (PBS + 0.5% BSA) and divided $1x10^6$ cells per tube. A pretreatment with human FcR blocking (Miltenyi Biotec Inc., Auburn, CA, USA) was performed according to manufacturer's instructions. MEN1309-PE (5µl, 1µg) or control isotype were incubated with cells at 4°C for 30 minutes; cells were washed twice in FACS buffer and the final cell pellets were re-suspended in FACS buffer. Flow-cytometry analysis was carried out with a FACSCanto II instrument (BD Biosciences). Median Fluorescence intensity (MFI) of each sample was defined using FacsDiva v8.0.1 software (BD Biosciences, Allschwil, Switzerland). Unstained cells and cells stained with isotype control antibody were used as controls.

RT-PCR

Total RNA was extracted from cells by using TRIZOL. cDNA was prepared by using the Super script double strand cDNA synthesis kit (Thermo Fisher Scientific, Waltham, MA, USA). In order to determine the expression levels of both CD205 and the reported intergenically spliced forms, duplicates of cDNA, corresponding to 50 ng of total RNA, were amplified in 25 µl of TaqMan Universal PCR Master Mix, using both inventoried assays and custom assays designed with the Primer Express software (Applied Biosystem, CA, USA). The inventoried assays Hs00158966_m1, Hs00994886_m1 and Hs99999905_m1 were used for the amplification of the CD205, CD302 and GAPDH transcripts, respectively. Custom assays were used for the amplification of the two splicing variants [Chimera1, 5' - GTGAACATGGTTTTGGAAGAGTTG - 3' (forward primer), 5' - ACTGAATCCAAGTAGATGAAGGACAGT - 3' (reverse primer), 5' – CTGCAAAGTGCCTCTG - 3' (probe); Chimera2, 5' - CCATGAGAGTTTGGCTTGGATTA - 3' (forward primer), 5' - AAATGTAACAACTGTCTTGGAACTGAA - 3' (reverse primer), and 5' - ATTCTGTTGACTGTCCTTC - 3' (probe)]. The amplification and quantitative real time detection were performed in a 7300 Real Time PCR system (Applied Biosystems). The cycling conditions for all the amplifications were as follows: 95°C for 10 min, 45 cycles at 95°C for 15 sec and 60°C for 1 min. Threshold cycle (Ct) values were selected from the linear phase of the amplification curves and values greater than 34 were considered as not detectable. The mRNA expression levels of the target genes were calculated by relative quantification using the $2^{-\Delta\Delta CT}$ method. The data were normalized with respect to the endogenous gene GAPDH and the results were expressed as fold change with respect to the expression levels obtained with the calibrator cell line THP-1. RNA expression levels obtained with the HumanHT-12 v4 Expression BeadChip (Illumina, San Diego, CA, USA) were retrieved from our previous publication (GSE94669) [10].

Animal studies

Mice maintenance and animal experiments were performed under the institutional guidelines established for the Animal Facility and with study protocols approved by the local Cantonal Veterinary Authority (license TI-22-2015). NOD-SCID mice were obtained from The Harlan Laboratory (S. Pietro al Natisone, Udine, IT). Xenografts were established by injecting lymphoma cells (15 x 10^6 cells/mouse, 200 µL of PBS) into the left flanks of female NOD-SCID mice (6 weeks of age, approximately 20 gr of body weight). Tumor size was measured on regular basis and until tumors reached around 5 mm in diameter (100 mm^3). Tumor volumes were calculated as previously described [14]. Differences in tumor volumes were calculated using the Wilcoxon rank-sum test (Stata/SE 12.1 for Mac, Stata Corporation, College Station, TX). The P-value (P) for significance was < 0.05.

SUPPLEMENTARY TABLES AND FIGURE

Supplementary Table S1. Cell lines fingerprinting using STR. For each cell line, the upper row shows our own results, the lower row shows the reference profile.

			D5S818	D13S317	D7S820	D16S539	VWA	TH01	AM	TPOX	CSF1PO
1	sample:	DB	11,11	11,12	11,11	11,11	15,16	6,8	X,Y	8,8	10,11
	reference:	DB (DSMZ#539)	11,11	11,12	11,11	11,11	15,16	6,8	X,Y	8,8	10,11
2	sample:	DOHH2	11,12	12,13	11,11	11,14	19,19	9,9	X,Y	8,11	11,12
	reference:	DOHH-2 (DSMZ#ACC-47)	11,12	12,13	11,11	11,14	14,19	9,9	X,Y	8,11	11,12
3	sample:	ESKOL	12,12	9,13	8,12	9,9	14,19	9.3,9.3	X,Y	8,8	11,12
	reference:	No available reference	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
4	sample:	FARAGE	11,12	11,13	12,12	11,12	14,15	8,9	X,X	9,9	11,12
	reference:	Farage (DSMZ#CRL-2630)	12,12	11,13	12,12	11,12	14,15	8,9	X,X	9,9	11,12
5	sample:	GRANTA519	9,13	9,11	10,14	12,12	17,17	7,8	X,X	8,8	10,10
	reference:	GRANTA-519 (DSMZ#342)	9,13	9,11	10,14	12,12	17,17	7,8	X,X	8,8	10,10
6	sample:	HAIR-M	11,13	9,10	12,12	9,11	16,17	6,6	X,Y	9,11	10,13
	reference:	HAIR-M (DSMZ#762)	11,13	9,10	12,12	9,11	16,17	6,6	X,Y	9,11	10,13
7	sample:	HBL-1	10,11	8,9	11,12	9,11	16,17	6,9	X,X	8,9	10,11
	reference:	No available reference	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
8	sample:	HC-1	11,12	11,12	10,12	10,11	15,18	9,9.3	X,Y	8,8	11,12
	reference:	HC-1 (DSMZ#301)	11,12	11,12	10,12	10,11	15,18	9,9.3	X,Y	8,8	11,12
9	sample:	JEKO1	10,13	8,9	10,11	12,12	14,14	7,7	X,X	8,8	9,12
	reference:	JEKO-1 (DSMZ#553)	10,13	8,9	10,11	12,12	14,14	7,7	X,X	8,8	9,12
10	sample:	JVM2	11,12	11,13	10,11	12,13	17,17	6,9	X,X	8,11	11,11
	reference:	JVM-2 (DSMZ#12)	11,12	11,13	10,11	12,13	17,17	6,9	X,X	8,11	11,11

11	sample:	KARPAS 1718	11,12	8,13	8,8	11,13	14,14	6,6	X,Y	8,11	11,13
	reference:	Karpas-1718 (CVCL_2539)	11,12	8,13	8,8	11,13	14,14	6,6	X,Y	8,11	11,13
12	sample:	KARPAS 422	11,12	12,12	12,13	13,13	19,19	6,9	X,X	8,8	10,11
	reference:	KARPAS-422 (DSMZ#32)	11,12	12,12	12,13	13,13	19,19	6,9	X,X	8,8	10,11
13	sample:	MAVER 1	12,12	8,13	8,8	9,12	14,18	8,9	X,Y	8,12	10,11
	reference:	MAVER-1 (DSMZ#CRL-3008)	12,12	8,13	8,8	9,12	14,18	8,9	X,Y	8,12	10,11
14	sample:	MEC 1	10,12	11,12	7,11	9,11	16,17	6,8	X,Y	8,11	11,12
	reference:	MEC-1 (DSMZ#497)	10,12	11,12	7,11	9,11	16,17	6,8	X,Y	8,11	11,12
15	sample:	MINO	11,12	12,12	10,11	11,12	14,17	9.3,9.3	X,Y	8,11	9,11
	reference:	MINO (DSMZ#687)	11,12	12,12	10,11	11,12	14,17	9.3,9.3	X,Y	8,11	9,11
16	sample:	OCILY1	11,12	12,12	11,11	9,9	15,16	9.3,9.3	XY	8,9	10,11
	reference:	OCI-LY1 (DSMZ#722)	11,12	12,12	11,11	9,9	15,16	9.3,9.3	X,Y	8,9	10,11
17	sample:	OCILY-3	12,12	11,11	11,12	11,12	17,17	7,9.3	X,Y	8,12	10,12
	reference:	OCI-LY3 (DSMZ#761)	12,12	11,11	11,12	11,12	17,17	7,9.3	X,Y	8,12	10,12
18	sample:	OCILY 8	10,13	11,11	11,12	9,11	17,19	6,9.3	X,X	9,11	11,12
	reference:	BMC Genomics 2012, 13:596	10,13	11,11	11,12	9,11	17,19	6,9.3	X,X	9,11	11,12
19	sample:	OCILY10	10,12	11,12	10,11	13,13	16,17	6,7	X,X	8,8	11,12
	reference:	OCI-LY10 (CVCL_8795)	10,12	11,12	10,11	13,13	16,17	6,7	X,X	8,8	11,12
20	sample:	OCILY 18	11,12	10,12	10,12	11,13	15,19,20	6,9	X,X	8,11	10,12
	reference:	OCI-LY18 (DSMZ#699)	11,12	10,12	10,12	11,13	15,19	6,9	X,X	8,11	10,12
21	sample:	OCILY19	9,11	11,12	12,12	12,12	16,16	6,7	X,X	8,9	11,12
	reference:	OCI-LY19 (DSMZ#528)	9,11	11,12	12,12	12,12	16,16	6,7	X,X	8,9	11,12
22	sample:	PCL12	11,13	8,14	10,10	11,12	14,18	9.3,9.3	X,Y	8,11	11,12
	reference:	PCL-12 (DSMZ#794)	11,13	8,13	10,10	11,12	14,18	9.3,9.3	X,Y	8,11	11,12

23	sample:	PFEIFFER	10,13	11,12	8,12	12,12	17,18	9,9.3	X,Y	9,9	10,11
	reference:	Pfeiffer (DSMZ#CRL-2632)	10,13	11,12	8,12	12,12	17,18	9,9.3	X,Y	9,9	10,11
24	sample:	RCK8	10,11	9,9	11,12	10,10	18,19	7,7	X,Y	8,8	11,13
	reference:	RC-K8 (DSMZ#561)	11,11	9,9	11,12	10,10	18,19	7,7	X,Y	8,8	11,13
25	sample:	REC-1	12,13	10,10	10,11	11,11	17,17	9,9.3	X,Y	8,9	10,12
	reference:	REC-1 (DSMZ#584)	12,13	10,10	10,11	11,11	17,17	9,9.3	X,Y	8,9	10,12
26	sample:	RI-1	11,11	9,14	9,13	11,12	17,17	6,6	X,X	8,8	11,11
	reference:	RI-1 (DSMZ#585)	11,11	9,14	9,13	11,12	17,17	6,6	X,X	8,8	11,11
27	sample:	SP49	11,12	9,10	7,12	9,11	14,16	7,9	X,X	8,9	12,13
	reference:	No available reference	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
28	sample:	SP53	11,12	9,10	7,12	9,11	14,16	7,9	X,X	8,9	12,13
	reference:	[1]	11,12	9,10	7,12	9,11	14,16	7,9	X,X	8,9	12,13
29	sample:	SSK41	10,12	10,10	10,12	9,12	19,19	9,9	X,Y	11,11	12,12
	reference:	No available reference	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
30	sample:	SUDHL 2	13,13	11,12	11,13	14,14	17,17	6,9	X,X	8,8	12,12
	reference:	SU-DHL-2 (ATCC#CRL-2956)	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -	- - - -
31	sample:	SUDHL-4	11,12	11,12	8,11	11,13	18,19	6,9.3	X,Y	9,11	12,12
	reference:	SU-DHL-4 (DSMZ#495)	11,12	11,12	8,11	11,13	18,19	6,9.3	X,Y	9,11	12,12
32	sample:	SUDHL-8	11,13	11,13	8,8	12,12	15,19	6,9	X,X	8,8	11,12
	reference:	SU-DHL-8 (DSMZ#573)	11,13	11,13	8,8	12,12	15,19	6,9	X,X	8,8	11,12
33	sample:	SUDHL 10	11,11	10,11	8,11	10,11	17,17	8,9.3	X,Y	8,8	13,13
	reference:	SU-DHL-10 (DSMZ#576)	11,**12**	10,11	8,11	10,11	17,17	8,9.3	X,Y	8,8	10,13
34	sample:	SUDHL-16	10,10	12,12	9,10	11,13	15,18	7,9.3	X,Y	8,11	10,11

	reference:	SU-DHL-16 (DSMZ#577)	10,10	9,12	9,10	11,13	15,18	7,9.3	X,Y	8,11	10,11
35	sample:	TOLEDO	10,12	11,11	9,10	9,9	16,17	8,9.3	X,X	8,11	12,12
	reference:	Toledo (DSMZ#CRL-2631)	10,12	11,11	9,10	9,9	16,17	8,9.3	X,X	8,11	12,12
36	sample:	TMD8	12,13	9,11	8,10	9,11	14,18	6,7	X,Y	11,11	11,12
	reference:	Leukemia 2015, 29(8);1702-	12,13	9,11	8,10	9,11	14,18	6,7	X,Y	11,11	11,12
37	sample:	U2932	12,12	11,11	9,13	12,12	14,17	9.3,9.3	X,X	11,12	10,12
	reference:	U-2932 (DSMZ#633)	12,12	11,11	9,13	12,12	14,17	9.3,9.3	X,X	11,12	10,12
38	sample:	UPN1	12, 13	13, 13	11, 13	13, 13	16, 18	8, 9.3	X,X	8, 9	12, 12
	reference:	Tarantelli et al, [1]	12, 13	13, 13	11, 13	13, 13	16, 18	8, 9.3	X,X	8, 9	12, 12
39	sample:	VAL	11,11	10,14	10,10	9,13	14,15	6,9	X,X	8,8	10,12
	reference:	VAL (DSMZ#586)	11,11	10,14	10,10	9,13	14,15	6,9	X,X	8,8	10,12
40	sample:	VL51	11,12	8,10	10,11	12,12	17,18	8,9	X,X	8,8	11,12
	reference:	SLVL (DSMZ#RCB1702)	11,12	8,10	10,11	12,12	17,18	8,9	X,X	8,8	11,12
41	sample:	WSU-DLCL2	11,12	11,14	10,12	10,13	16,17	8,9	X,Y	8,8	11,11
	reference:	WSU-DLCL-2 (DSMZ#575)	11,12	11,14	10,12	10,13	16,17	8,9	X,Y	8,8	11,11
42	sample:	Z138	11,13	9,12	8,8	11,11	15,18	6,6	X,Y	8,8	10,11
	reference:	Z-138 (DSMZ#CRL-3001)	11,13	9,12	8,8	11,11	15,18	6,6	X,Y	8,8	10,11

Supplementary Table S2. IC50s, expressed in pM, obtained in human lymphoma cell lines after 72 hours of exposure to MEN1309 or IgG-DM4. GCB-DLBCL, germinal center B-cell type diffuse large B-cell lymphoma; ABC-DLBCL, activated B-cell like diffuse large B-cell lymphoma; MZL, marginal zone lymphoma; CLL, chronic lymphocytic leukemia; PMBCL, primary mediastinal large B-cell lymphoma. *BCL2*, *MYC* and *TP53* status obtained as previously described [2].

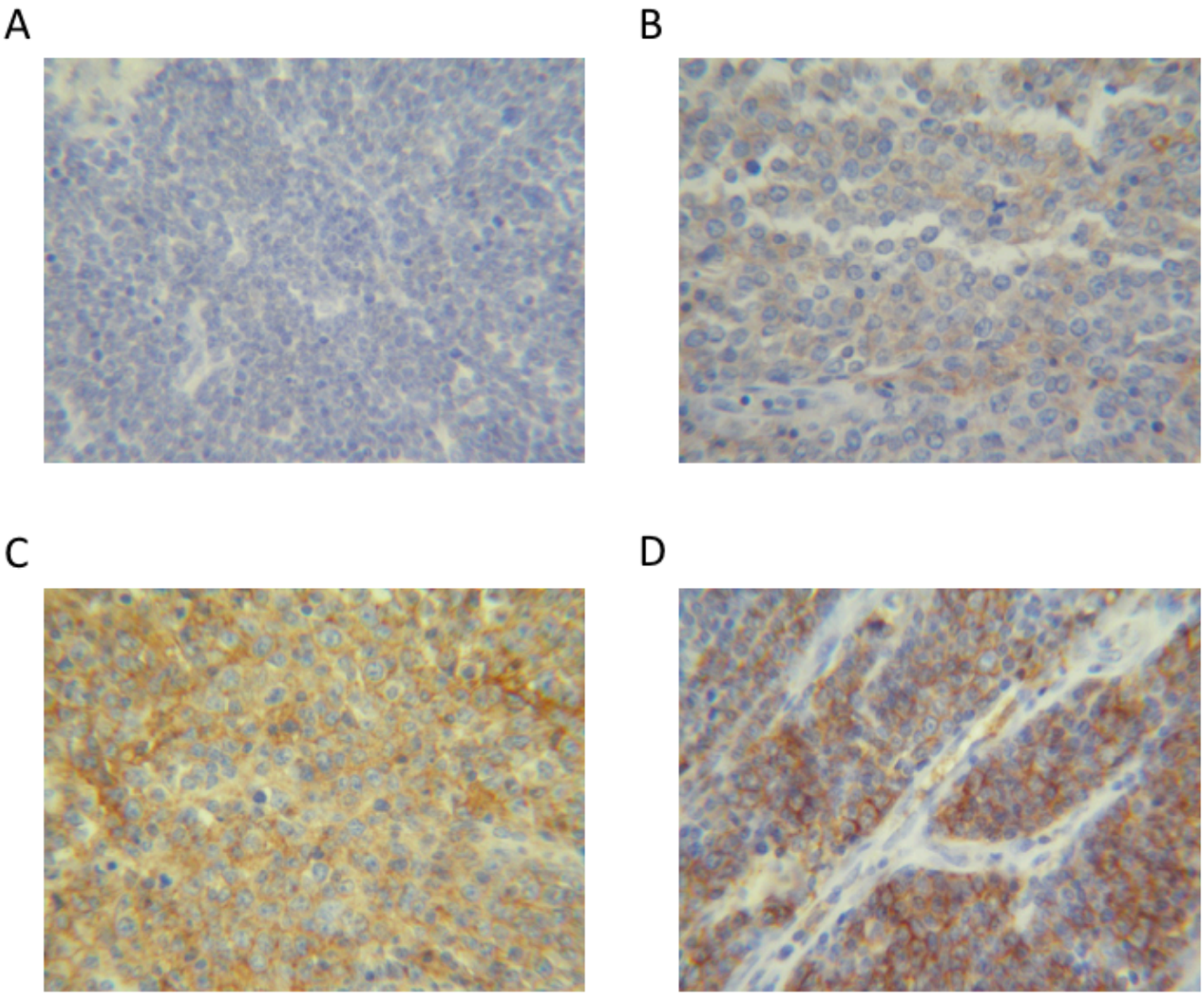
Cell Line	Histology	MEN1309	IgG-DM4	*BCL2* translocation	*MYC* translocation	inactive *TP53*
DB	GCB-DLBCL	10000	15000	yes	no	yes
DoHH2	GCB-DLBCL	300	30000	yes	yes	no
ESKOL	MZL	120	25000		no	
Farage	GCB-DLBCL	15	30000	no	no	yes
Granta 519	MCL	100	13000	no	no	yes
HAIR-M	MZL	50	40000		no	
HBL-1	GCB-DLBCL	300	30000		no	yes
HC-1	MZL	100	15000		no	
JeKo-1	MCL	80	15000	no	no	yes
JVM-2	MCL	100	20000	no	no	no
Karpas-1106	PMBCL				no	
Karpas-1718	MZL	500	15000		no	yes
Karpas-422	GCB-DLBCL	5000	30000	yes	no	yes
MAVER-1	MCL	600	70000		yes	yes
MEC-1	CLL	500	50000		no	yes
Mino	MCL	100	15000		yes	yes
OCI-LY-1	GCB-DLBCL	50	20000	yes	no	yes
OCI-LY-10	ABC-DLBCL	200	20000	no	no	yes
OCI-LY-18	GCB-DLBCL	200	20000	yes	yes	yes
OCI-LY-19	GCB-DLBCL	200	20000	yes		no
OCI-LY-3	GCB-DLBCL	200	20000	no	no	no
OCI-LY-7	GCB-DLBCL			no	yes	yes
OCI-LY-8	GCB-DLBCL	100	20000	yes	yes	yes
PCL12	CLL	120	18000		no	
Pfeiffer	GCB-DLBCL	200	40000	yes	no	yes
RCK8	ABC-DLBCL	300	40000		no	no
Rec-1	MCL	200000	70000		no	yes
RI-1	ABC-DLBCL	8000	40000			yes
SP-49	MCL	60	15000		no	
SP-53	MCL	2500	16000		no	
SSK41	MZL	100	15000		no	
SU-DHL-10	GCB-DLBCL	10000	15000	yes	yes	yes
SU-DHL-16	GCB-DLBCL				no	
SU-DHL-2	ABC-DLBCL	1500	30000	no	no	no
SU-DHL-4	GCB-DLBCL	8000	40000	yes	no	yes
SU-DHL-5	GCB-DLBCL			no	no	
SU-DHL-6	GCB-DLBCL			yes	no	yes
SU-DHL-8	GCB-DLBCL	200	40000	no	yes	
TMD8	ABC-DLBCL	300	40000		no	no
Toledo	GCB-DLBCL	300	40000	yes	yes	yes
U2932	GCB-DLBCL	400	30000	no	no	yes
UPN1	MCL	500	15000		no	yes
VAL	GCB-DLBCL	60	30000	yes	yes	no
VL51	MZL	150	20000		no	

WSU-DLCL2	GCB-DLBCL	300	30000	yes	no	yes
Z-138	MCL	100	15000		yes	

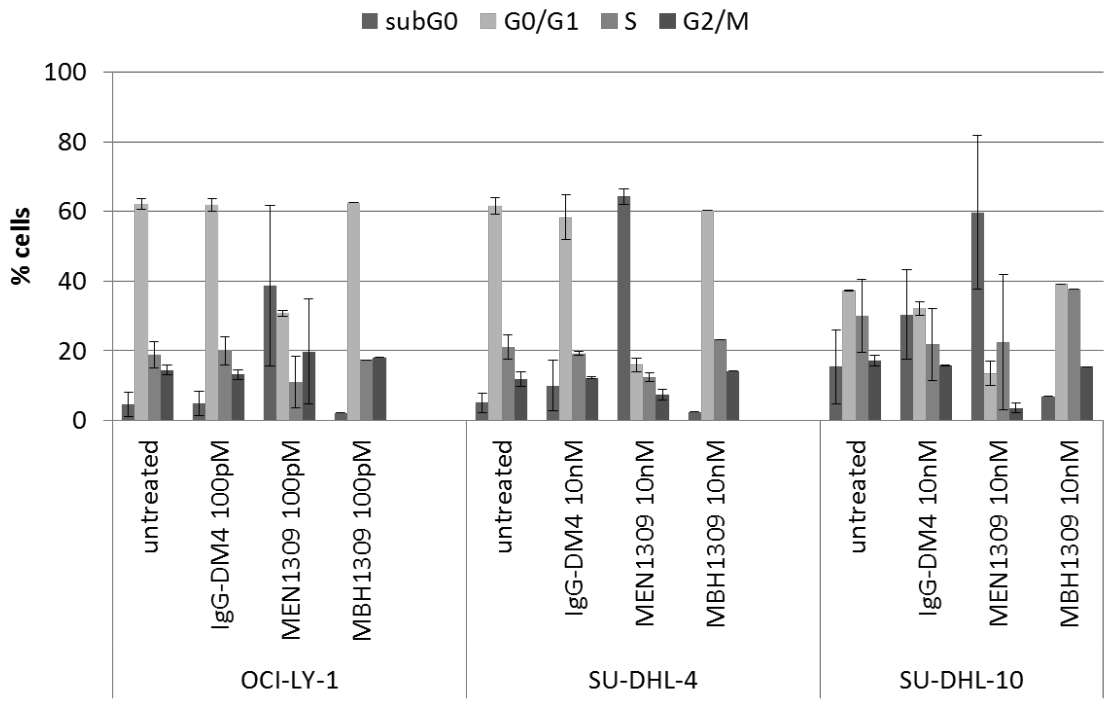
Supplementary Table S3. CD205 expression in lymphoma cell lines. *, data normalized with respect to the endogenous gene GAPDH and the results expressed as fold change with respect to the expression levels obtained with the calibrator cell line THP-1.

Histology	Cell Line	CD205 - MFI	LY75, RT-PCR *	CD302, RT-PCR *	LY75-CD302 chimera 1, RT-PCR *	LY75-CD302 chimera 2, RT-PCR *
DLBCL	DB	33	0	0	0	0
DLBCL	DOHH2	31	0.24	0.01	0.06	0.18
DLBCL	FARAGE	432	1.55	0.05	0.81	1.22
DLBCL	HBL1	63	0.44	0.06	0.5	1.64
DLBCL	KARPAS422	11	0	0	0	0
DLBCL	OCI-LY-1	159	0.2	0.24	0.14	0.29
DLBCL	OCI-LY-10	106	1.21	0.06	1.66	2.68
DLBCL	OCI-LY-18	30	0.39	0.1	0.19	0.31
DLBCL	OCI-LY-19	61	0.38	0.07	0.38	0.62
DLBCL	OCI-LY-3	88	0.41	0.01	0.33	0.21
DLBCL	OCI-LY-8	80	0.23	0.05	0.1	0.07
DLBCL	PFEIFFER	99	0.56	0.08	1.03	0.43
DLBCL	RCK8	45	0.9	0	1.65	1.35
DLBCL	RI-1	33	0.18	0.06	0.08	0.26
DLBCL	SU-DHL-10	14	0	0	0	0
DLBCL	SU-DHL-2	20	0.12	0.09	0.12	0.56
DLBCL	SU-DHL-4	15	0.02	0	0	0.02
DLBCL	SU-DHL-8	33	0.3	0.05	0.09	0.14
DLBCL	TMD8	87	0.64	0.18	0.79	0.81
DLBCL	TOLEDO	14	0.08	0.08	0.07	0.05
DLBCL	U2932	37	0.25	0.02	0.15	0.41
DLBCL	VAL	89	0.22	0.01	0.09	0.07
DLBCL	WSU-DLCL2	16	0.08	0	0.03	0.06
CLL	MEC1	63	n.a.	n.a.	n.a.	n.a.
CLL	PCL-12	207	n.a.	n.a.	n.a.	n.a.
MCL	GRANTA519	73	n.a.	n.a.	n.a.	n.a.
MCL	JEKO1	21	n.a.	n.a.	n.a.	n.a.
MCL	JVM2	146	n.a.	n.a.	n.a.	n.a.
MCL	MAVER1	41	n.a.	n.a.	n.a.	n.a.
MCL	MINO	50	n.a.	n.a.	n.a.	n.a.
MCL	REC-1	23	n.a.	n.a.	n.a.	n.a.
MCL	SP-49	54	n.a.	n.a.	n.a.	n.a.
MCL	SP53	46	n.a.	n.a.	n.a.	n.a.
MCL	UPN-1	24	n.a.	n.a.	n.a.	n.a.
MCL	Z138	24	n.a.	n.a.	n.a.	n.a.
MZL	ESKO-L	47	n.a.	n.a.	n.a.	n.a.
MZL	HAIR-M	116	n.a.	n.a.	n.a.	n.a.
MZL	HC1	118	n.a.	n.a.	n.a.	n.a.
MZL	KARPAS1718	25	n.a.	n.a.	n.a.	n.a.
MZL	SSK41	48	n.a.	n.a.	n.a.	n.a.
MZL	VL51	107	n.a.	n.a.	n.a.	n.a.

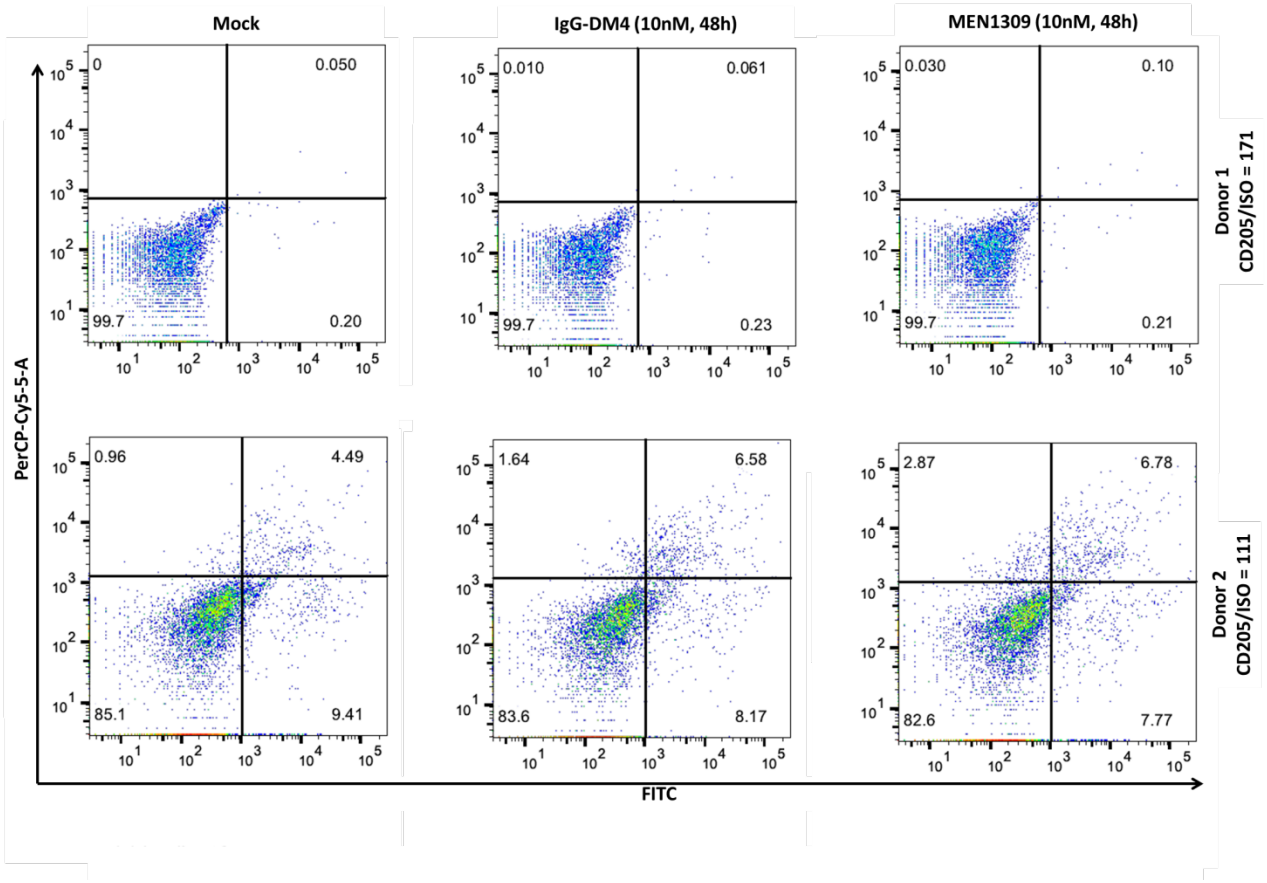
Supplementary Figure S1. The expression of CD205 in DLBCL tissue sections from tissue microarrays (TMA) using immunohistochemistry. The FFPE tissue sections were stained with 11A10 primary antibody. The tissue sections show one negative staining DLBCL tissue section (A, -), one weakly staining DLBCL tissue section (B, 1+), one moderate staining DLBCL tissue section (C, 2+) and one intense staining DLBCL tissue section (D, 3+). The staining was specific to the cancer cells, the tumor cells displayed membranous and cytoplasmic staining.



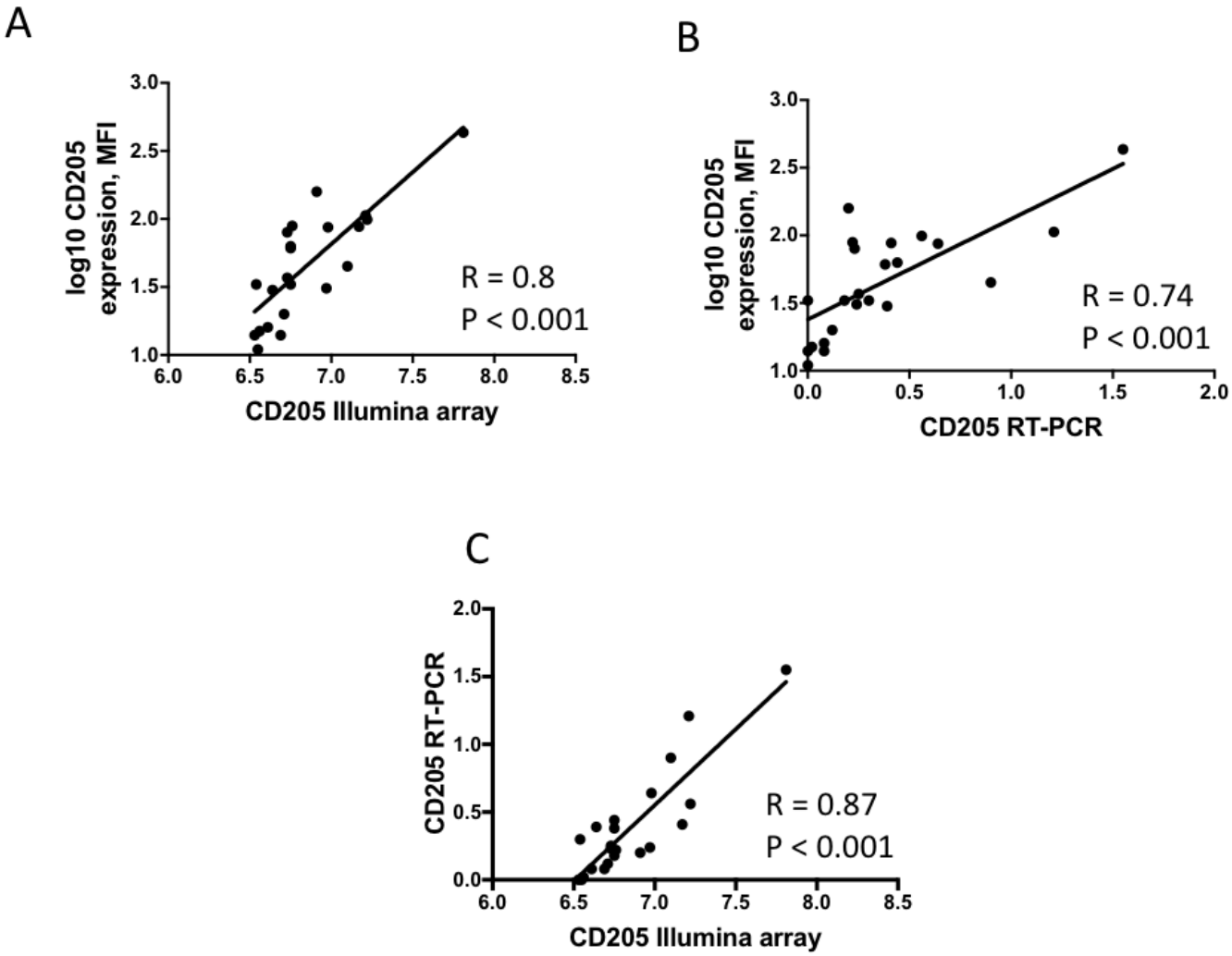
Supplementary Figure S2. Cell cycle distribution after 100pM of IgG-DM4 or MEN1309 (72 hours) in three lymphoma cell lines. Graph plots show mean ± STDEV for values from two biological replicates.



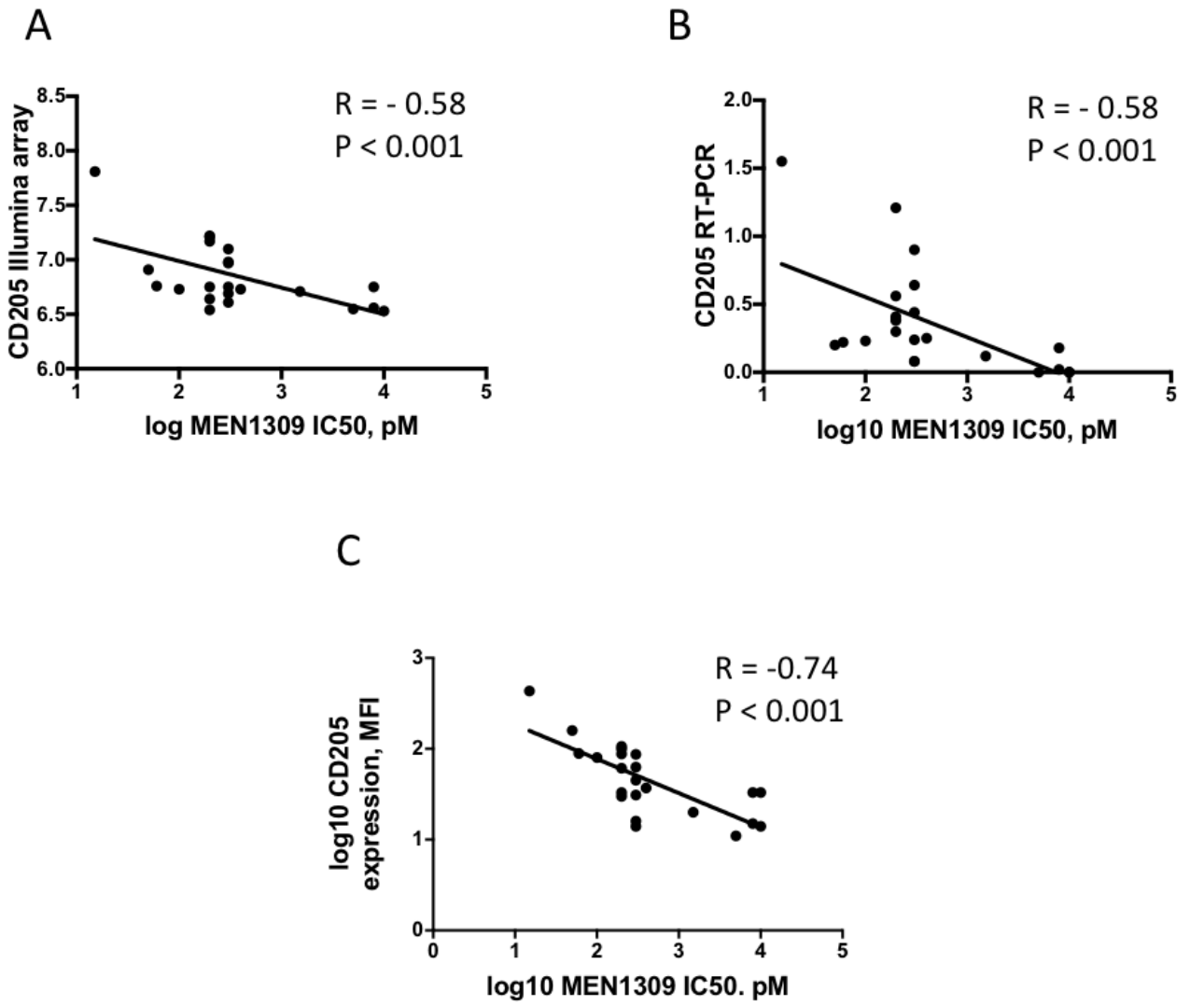
Supplementary Figure S3. MEN1309 activity against peripheral mononuclear B cells (PMBCs) from healthy donors. Healthy PMBCs were incubated with 10 nM of MEN1309 or IgG-DM4 for 48 h and Annexin V measured by FACS. For both samples were also reported the levels of CD205 measured on $CD19^{+}$ B-cell lymphocytes.



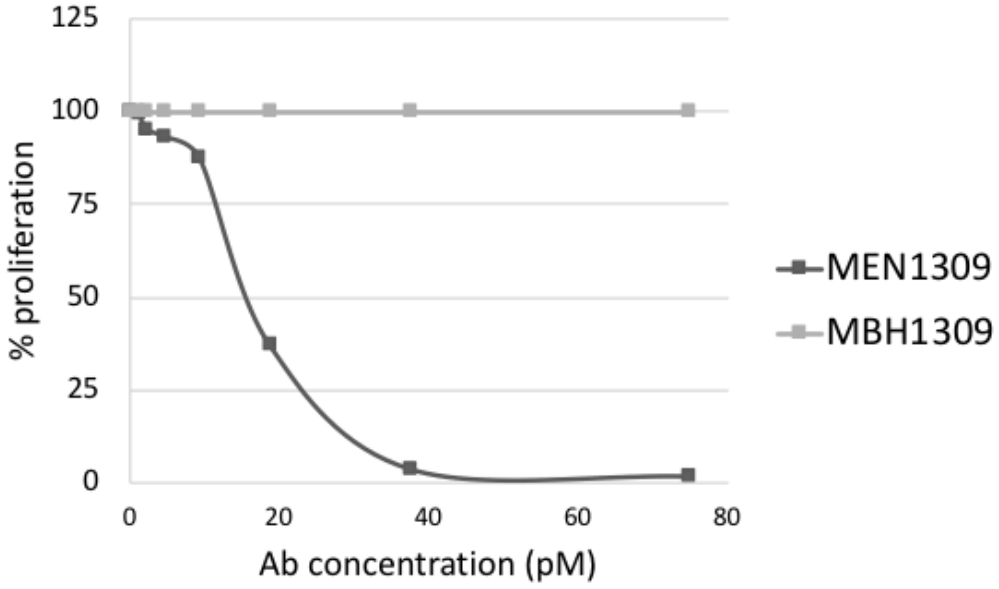
Supplementary Figure S4. CD205 measurements by FACS, microarray and RT-PCR in 22 DLBCL cell lines are highly correlated. (A) FACS vs microarray. Y-axis, Log10 CD205 MFI values as measured with flow cytometry. X-axis, CD205 mRNA expression values as measured with Illumina arrays. (B) FACS vs RT-PCR. Y-axis, Log10 CD205 MFI values as measured with flow cytometry. X-axis, CD205 mRNA expression values as measured by RT-PCR. (C) RT-PCR vs microarray. Y-axis, CD205 mRNA expression values as measured with RT-PCR. X-axis, CD205 mRNA expression values as measured with Illumina arrays.



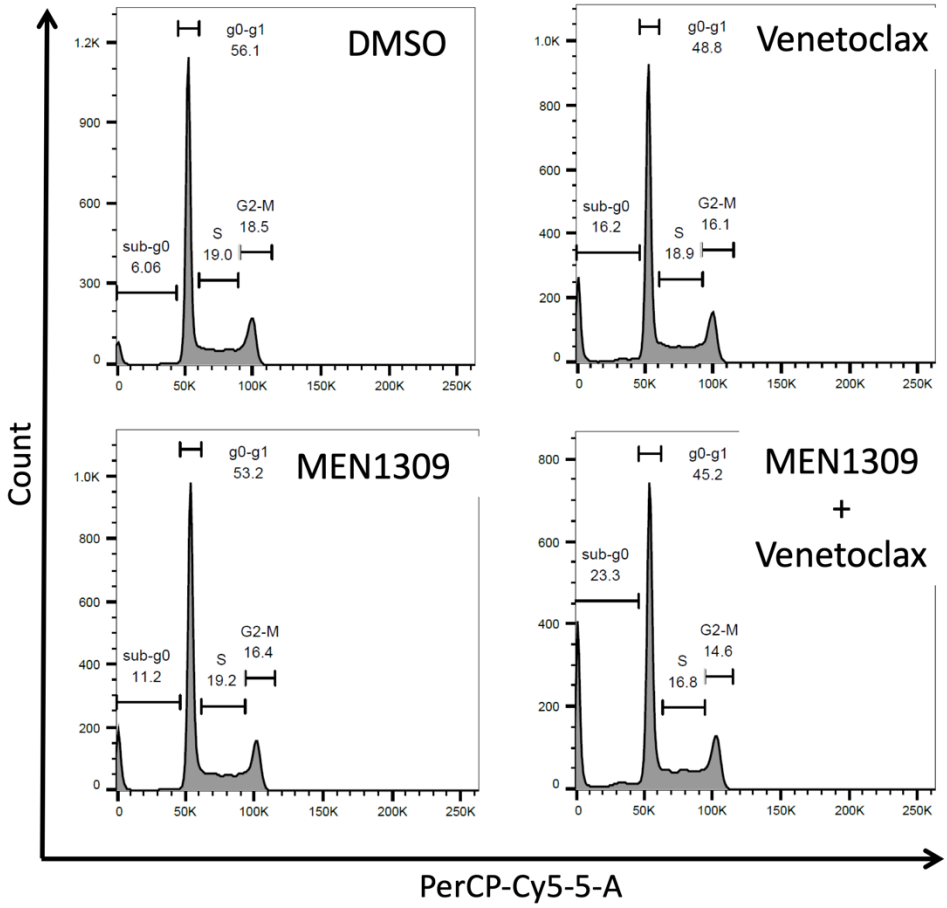
Supplementary Figure S5. The antitumor activity of MEN1309 in 21 DLBCL cells is correlated to CD205 RNA or protein surface expression. In each plot, X-axis, Log10 MEN1309 IC50 values (pM). (A) Y-axis, CD205 mRNA expression values as measured with Illumina arrays. (B) Y-axis, CD205 mRNA expression values as measured by RT-PCR. (C) Y-axis, Log10 MFI values of CD205 as measure by flow cytometry.



Supplementary Figure S6. MEN1309 but not its naked Ab MBH1309 has anti-tumor activity in a DLBCL cell line. Proliferation curve of FARAGE cell line treated with MEN1309 or MBH1309. Representative plot of two biologic replicates.



Supplementary Figure S7. Active combinations with MEN1309 in lymphoma. Representative histograms of cell cycle distribution in OCI-LY-10 cells treated with MEN1309, venetoclax, combination, or DMSO (72 hours).



References

1. Tarantelli C, Bernasconi E, Gaudio E, et al. BET bromodomain inhibitor birabresib in mantle cell lymphoma: in vivo activity and identification of novel combinations to overcome adaptive resistance. ESMO Open. 2018;3(6):e000387.
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.