Resistance to ABT-199 induced by microenvironmental signals in chronic lymphocytic leukemia can be counteracted by CD20 antibodies or kinase inhibitors

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Supplemental Table 1: Patients' characteristics

Patient #	Age	Gender	IgVH mutational status	WBC 10^9/L	% lymphocytes	%CD19+/CD5+ cells	FISH	Treatment before sampling
1	79	M	U	122,0	n.d.	82,0	tris 12	chlorambucil
2	62	M	U	155,0	96,0	79,1	tris 12, 17p-	chlorambucil, prednisone, dexamethasone
3A	72	F	U	115,3	n.d.	90,3	tris 12	none
3B	73	F	U	89,9	n.d.	91,4	tris 12	none
4	68	F	U	100,5	89,0	84,8	tris 12	none
5	60	F	U	265,0	n.d.	99,8	13q-	FCR
6	74	M	n.d.	58,4	86	92,7	n.d.	none
7	66	M	M	93,4	n.d.	90,8	none	none
8	68	F	M	65,1	91,4	78,4	13q-	chlorambucil+prednisone, steroids
9	69	F	n.d.	27,8	n.d.	81,3	n.d.	chlorambucil
10A	73	M	U	116,8	n.d.	98,4	n.d.	none
10B	73	M	U	166,6	n.d.	84,2	n.d.	chlorambucil
11	67	F	M	88,7	n.d.	86,9	13q- IgH/CCND1	chlorambucil, fludarabine, prednisone
12	72	M	U	108,0	94,7	96,4	17p-	chlorambucil, fludarabine, Alemtuzumab, FCR
13A	62	F	M	173,0	n.d.	99,9	3q-, 17p-, IgH/CCNI	none
13B	63	F	M	153,0	96,0	95,7	3q-, 17p-, IgH/CCNI	chlorambucil, FCR
14	80	M	M	149,4	92,0	98,2	11q-, 13q-	chlorambucil
15	59	M	M	85,5	94,0	95,8	n.d.	none
16	61	F	M	46,1	n.d.	91,1	13q-	none
17	59	M	U	74,0	n.d.	92,3	11q-	Ofatumumab
18	66	M	U	232,0	96,1	98,5	n.d.	unknown
19	63	F	M	79,2	91,0	92,3	n.d.	none
20	55	F	U	272,0	n.d.	95,4	none	none
21	76	F	M	112,0	n.d.	91,8	n.d.	none
22	57	F	M	170,9	n.d.	97,3	tris 12,13q-	chorambucil, fludarabine
23	64	F	U	64,8	82	88	n.d.	none
24	74	F	n.d.	55,0	n.d.	86,4	n.d.	none
25	60	M	n.d.	n.d.	n.d.	97,2	n.d.	none

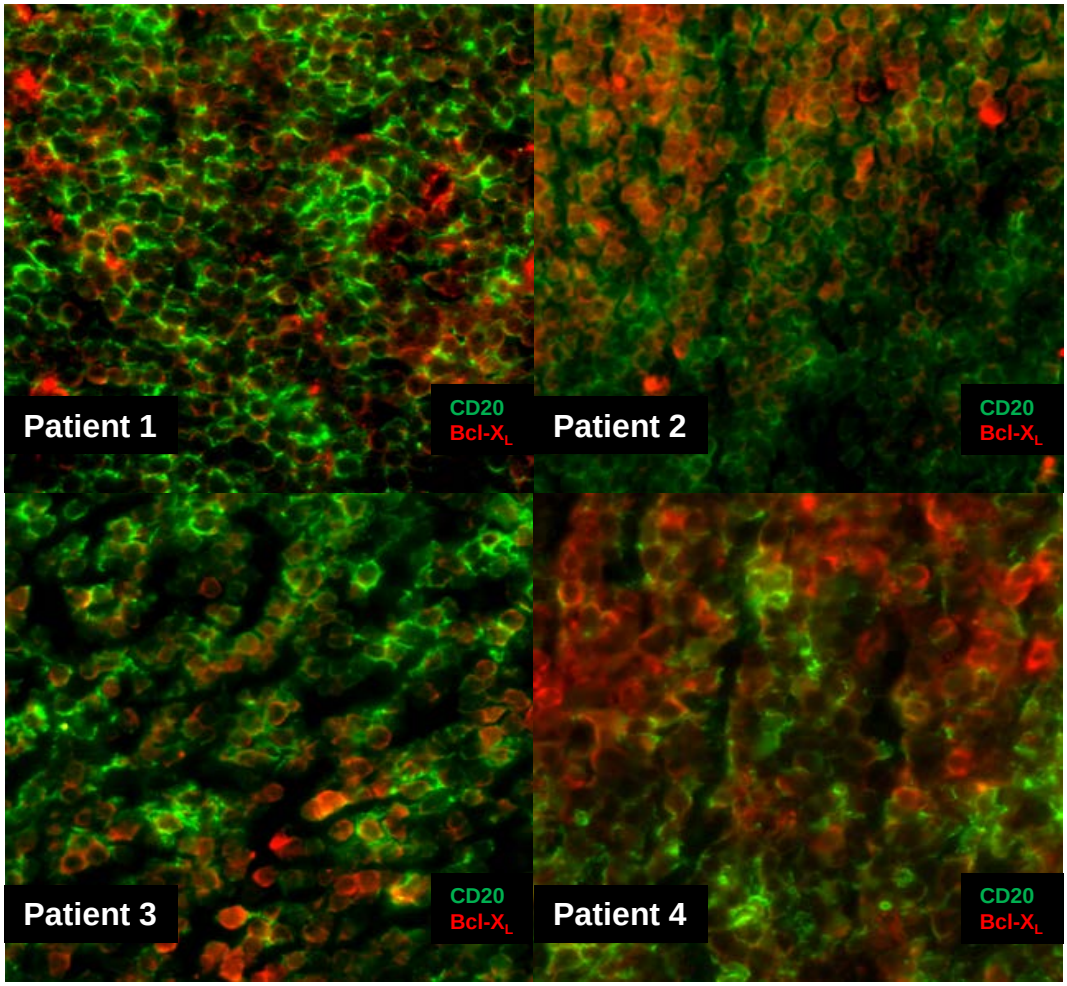
n.s. not specified; n.d. not determined; A, B, C refer to different sampling times of the same patient; FCR: fludarabine + cyclophosphamide + rituximab

Supplemental Table 2. LC50 values for ABT-199 and ABT-737 for CLL cells under various conditions.

LC50 values were calculated from averaged data from CLL samples tested for sensitivity for ABT-199 or ABT-737 under the indicated conditions, n=8, n=3 for GA-101. (RXL: Rituximab plus crosslinker, n.d. not determined)

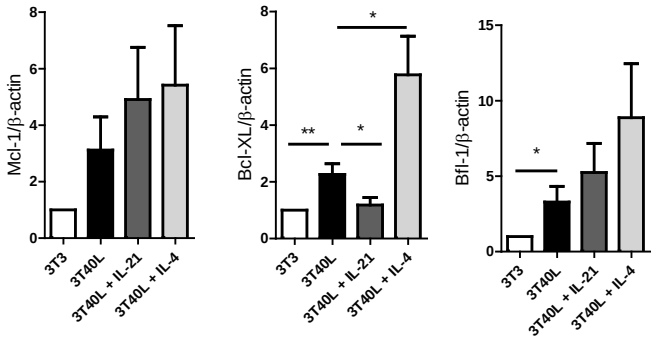
Stimulation	LC50 (µM)	
	ABT-199	ABT-737
3T3 (Control)	0.001	0.005
3T40L	>10	0.781
3T40L + 0.1 µM dasatinib	0.066	0.081
3T40L + 1 µM dasatinib	0.020	0.037
3T40L + 10 ug/ml GA-101	0.044	n.d.
3T40L + 10 ug/ml RXL	0.065	n.d.

Supplemental Figure 1/Thijssen et al



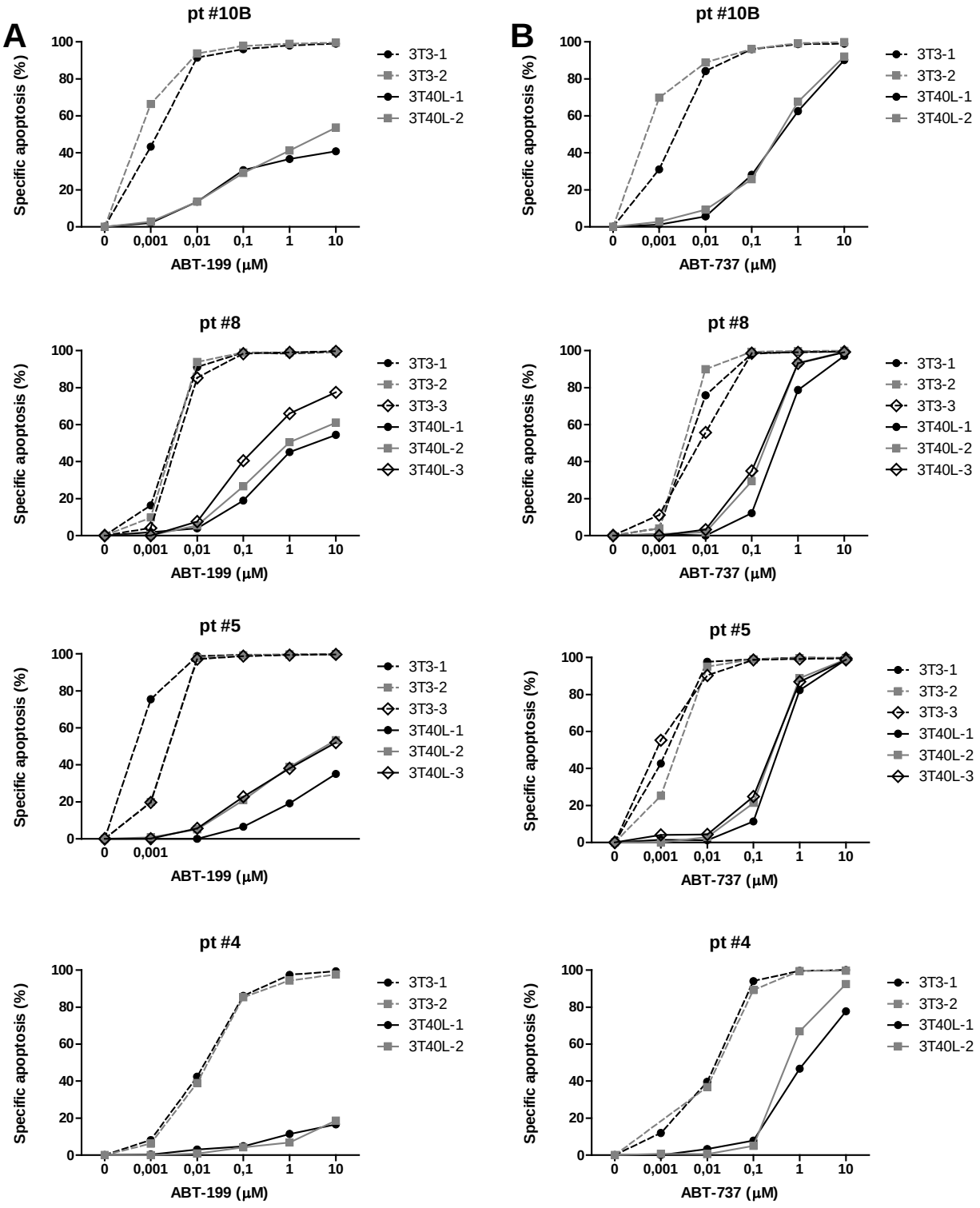
Supplemental Figure 1. Bcl-XL staining in lymph nodes from different CLL patients. Immunofluorescent staining for CD20 (green) and Bcl-XL (red) showing presence of Bcl-XL in CD20-positive cells in the lymph node of 4 CLL patients. Additional staining for Patient 1 is also shown in Figure 1 of the main article.

Supplemental Figure 2/Thijssen et al



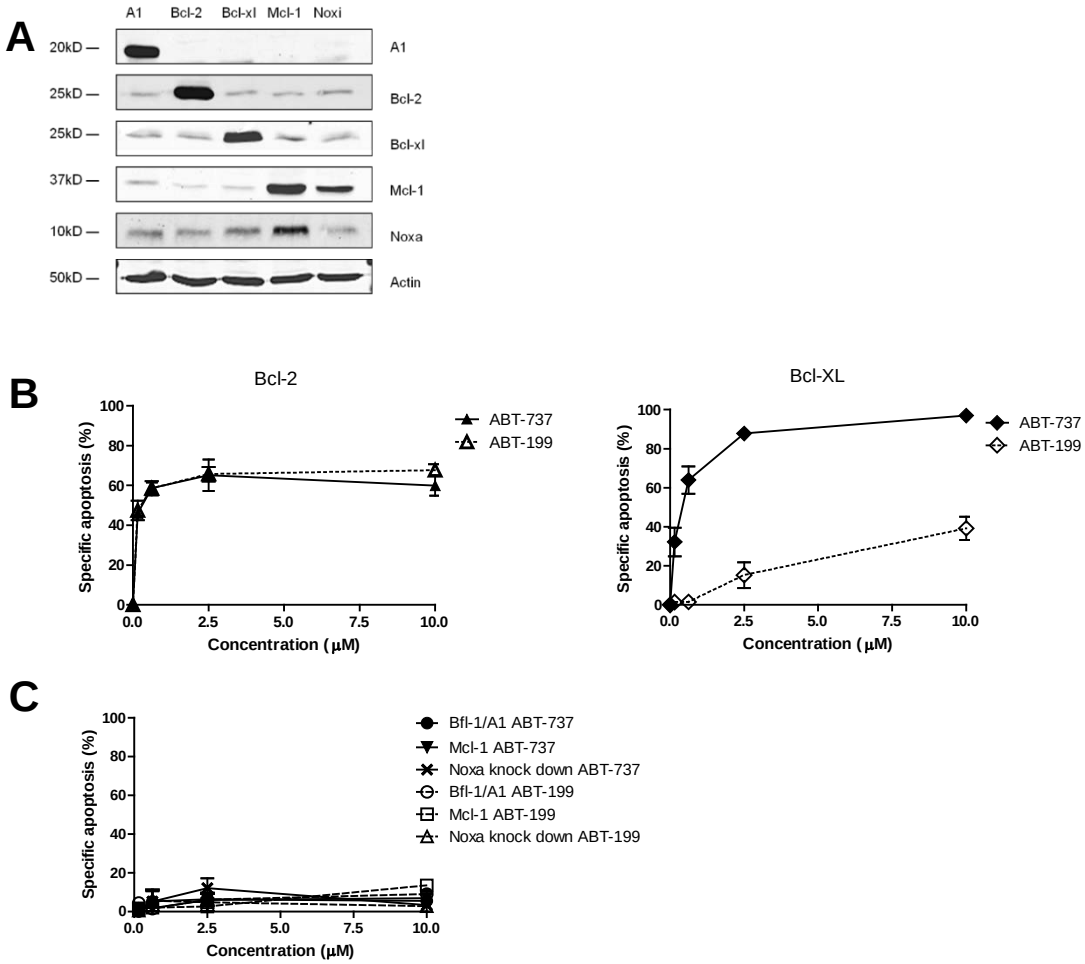
Supplemental Figure 2. Stimulation via CD40 plus IL-4 or IL-21 differentially induces expression of Mcl-1, Bcl-XL and Bfl-1/A1. Densitometric analysis of Mcl-1/actin levels, Bcl-XL/actin levels and Bfl-1/actin levels of seven CLL samples is shown. Bars represent the mean SEM, * $p < 0.05$;** $p < 0.01$. Western blots from 2 patients are shown in Figure 2A of the main article.

Supplemental Figure 3/Thijssen et al



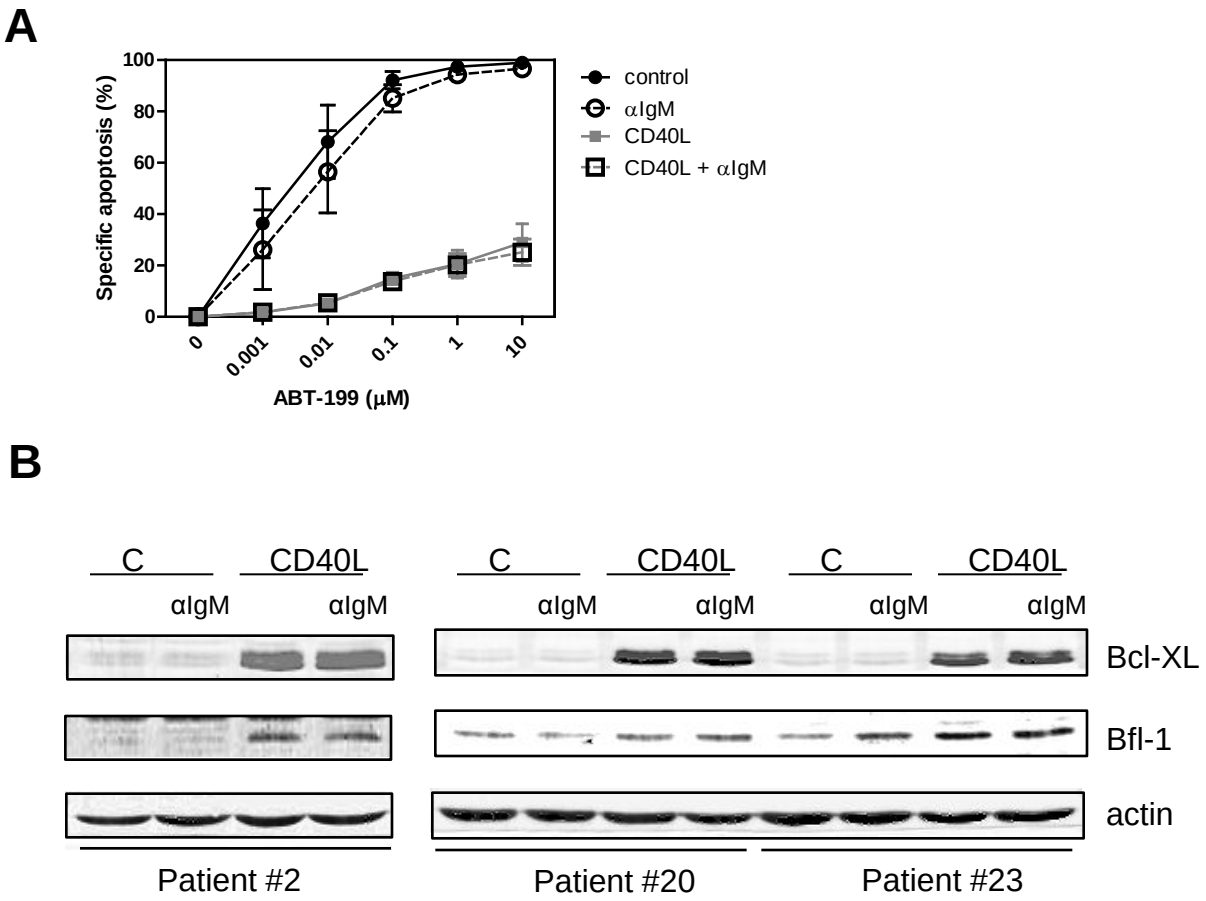
Supplemental Figure 3. Heterogeneity among patients in the response to BH3-mimetics after CD40 stimulation is reproducible. A-B. CLL cells were stimulated with 3T3 or 3T40L cells for 3 days. After detachment, cells were incubated with ABT-199 (**A**) or ABT-737 (**B**) for 24 hours. Specific apoptosis of two or three independent experiments are shown for 4 patient samples.

Supplemental Figure 4/Thijssen et al



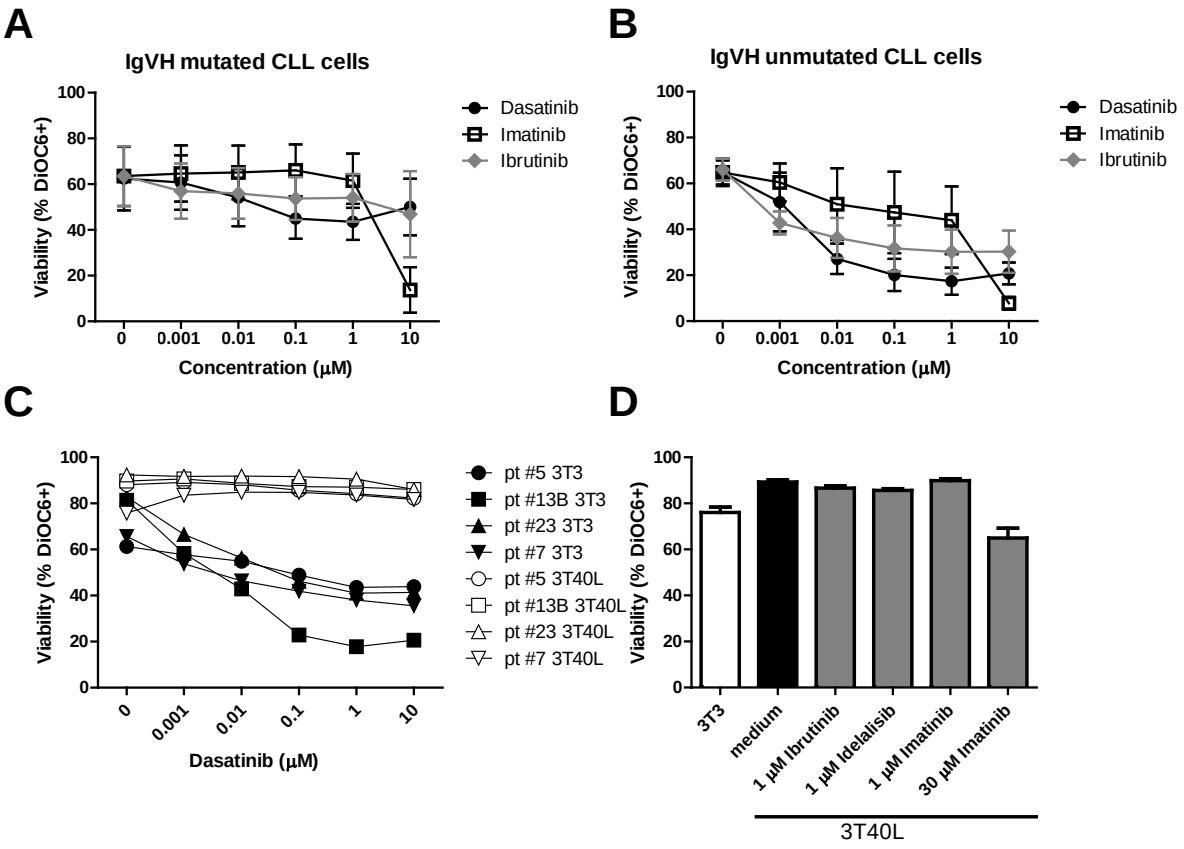
Supplemental Figure 4. Immortalized primary B cells with overexpression of Mcl-1, Bfl-1, Bcl-XL or knockdown of Noxa are resistant to ABT-199. A. Primary human memory B cells were immortalized by overexpressing Bfl-1/A1, Bcl-2, Bcl-XL, Mcl-1 or knockdown of Noxa (described in Tromp et al, Clin Cancer res 2012, 18: 487). Overexpression or knockdown was confirmed by Western blot analysis. **B.** Immortalized primary B cells with overexpression of Bcl-2 or Bcl-XL were incubated with different concentration of ABT-737 or ABT-199. **C.** Immortalized primary B cells with overexpression of Bfl-1/A1, Mcl-1 or knockdown of Noxa were incubated with different concentration of ABT-737 or ABT-199. Graphs represent the mean specific apoptosis SEM, n=3.

Supplemental Figure 5/Thijssen et al



Supplemental Figure 5. Effect of combined CD40+ BCR stimulation on sensitivity for ABT-199, and expression of Bcl-2 family members. CLL cells were cultured with medium or 3T40L in the presence or absence of 500ng/ml goat (Fab')2 anti-human IgM (Sanbio, Uden, The Netherlands) for 3 days. **A.** After detachment, cells were incubated with 0.001-10 μM ABT-199. Results are shown as mean SEM, n=3 (IgV_H unmutated) (Supplemental table 1; patient #2, 20, 23). **B.** protein lysates were probed for Bcl-XL, Bfl-1 and actin as loading control.

Supplemental Figure 6/Thijssen et al



Supplemental Figure 6. Direct killing effects of various kinase inhibitors before and after CD40 stimulation. A-B) CLL cells were thawed and incubated with dasatinib, imatinib and ibrutinib for 48 hours. Results are shown as mean SEM, n=3 for IgVH mutated CLL cells **(A)** and n=3 for IgVH unmutated CLL cells **(B)**. **C)** CLL cells were stimulated with 3T3 or 3T40L cells for 3 days. After detachment, cells were incubated with dasatinib for 48 hours. Viability of 4 CLL samples are shown. **D)** CLL cells were cocultured with 3T3 or 3T40L for 72 hours, in the presence of imatinib, ibrutinib or idelalsib as indicated. After detachment, viability was assessed and averaged data of 8 CLL samples are shown, error bars represent SEM.