SUPPLEMENTARY TABLES

TRABECTEDIN REVEALS A STRATEGY OF IMMUNOMODULATION IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Supplementary Table S1. Clinical and biological features of CLL patients from San Raffaele Hospital, Milan, Italy

Patient no.	Age (yr)	RAI stage*	BINET Stage*	Clinical course	HC	LC	CD38 (%)†	ZAP70 %†	IGHV MS	FISH
1	57	I	B	Progressive	MD	Neg	0.1	32.4	M	Del(13q)
2	62	0	A	Progressive	MD	Neg	24.8	NA	U	Del(13q)
3	63	II	A	Stable	NA	NA	NA	NA	NA	Trisomy12
4	66	0	A	Progressive	Neg	L	0	NA	M	NA
5	88	0	A	Stable	MD	K	32.7	35.01	U	Normal
6	80	0	A	Stable	A	neg	0.2	16.2	M	Del(13q)
7	43	0	A	Stable	Neg	Neg	0.2	NA	NA	Normal
8	79	0	A	Stable	D	neg	0.1	NA	NA	Del(13q)
9	78	0	A	Stable	M	neg	0.2	NA	M	NA
10	78	0	A	Stable	D	neg	0.1	NA	NA	NA
11	78	0	A	Stable	M	κ	0.1	NA	U	NA
12	50	N/A	N/A	Stable	neg	λ	11	NA	NA	Normal

Abbreviations: HC, heavy chain; LC, light chain; IGHV MS, immunoglobulin heavy chain variable mutation status (M, mutated; U: unmutated); FISH, fluorescence *in situ* hybridization; D, IgD surface expression; M, IgM surface expression; MD, IgM/IgD surface expression; NA, not available

* Determined at diagnosis
† Determined by flow cytometry

Supplementary Table S2. Clinical and biological features of CLL patients from MD Anderson Cancer Center

Patient no.	Age (yr)	Sex	WBC	Treatment	ZAP70 %†	IGHV MS	FISH
13	76	M	33.5	PRIOR RX	NEG	M	Trisomy12 & Del(13q)
14	81	F	62.1	PRIOR RX	NA	M	Del(13q)
15	63	M	20.5	UNT	NA	U	Trisomy12
16	61	M	11.9	UNT	NEG	M	Trisomy12
17	68	M	24.2	UNT	NEG	M	Trisomy12
18	67	M	35.9	UNT	NEG	M	Del(13q)
19	39	M	38.9	UNT	NEG	M	Del(13q)
20	67	F	8.4	PRIOR RX	NEG	M	Del(13q)
21	65	M	54.7	UNT	NEG	M	Del(13q)
22	78	F	65.4	PRIOR RX	NA	M	NA
23	87	F	11.2	UNT	NA	NA	NEG
24	72	M	45.9	PRIOR RX	POS	M	NEG
25	51	M	54.6	UNT	NEG	NA	NEG
26	81	M	57.5	UNT	POS	NA	Del(13q)
173	81	M	67.1	PRIOR RX	NEG	M	NEG
915	69	M	24.7	UNT	NEG	U	Trisomy12
665	49	F	19.7	UNT	NEG	M	NEG
538	59	M	32.9	UNT	POS	NA	Del(13q)
089	70	M	57.8	PRIOR RX	NA	NA	NEG
642	73	M	27.7	UNT	NEG	NA	Trisomy12
33	66	M	70.5	PRIOR RX	POS	M	Del(13q)
34	56	M	58.8	UNT	POS	U	Del(13q) & Del (11q)
35	52	M	78.7	UNT	NEG	NA	NEG
36	75	M	87.2	UNT	NA	U	Del(11q)
37	82	M	51.6	PRIOR RX	POS	NA	Del(13q)
38	70	M	10.3	PRIOR RX	NEG	M	Del(13q)
39	66	F	119.6	PRIOR RX	NEG	U	Del(17p)
40*	58	M	3.3	PRIOR RX	NA	NA	Del(17p)
41*	69	M	4.1	PRIOR RX	NA	NA	Del(13q)
42	60	M	21.7	UNT	NEG	M	NEG
43	70	M	7.6	PRIOR RX	NA	NA	NA
44*	82	M	53.3	UNT	POS	NA	Del(13q)
45*	73	M	45,4	PRIOR RX	POS	U	Del(13q)
46	79	F	60.5	PRIOR RX	NEG	NA	NEG
47	65	M	28	UNT	NA	M	Del(13q)
48	75	F	19.3	UNT	NEG	M	Del(17p) & Del(13q)
49	89	F	27.2	UNT	NEG	M	Del(13q)

Abbreviations and notes: WBC, white blood cell count; IGHV MS, immunoglobulin heavy chain variable mutation status (M, mutated; U: unmutated); FISH, fluorescence *in situ* hybridization; PRIOR RX, prior therapies; NA, not available

*Patient 40 had Richter's syndrome, resistant to ibrutinib, venetoclax; patient 41 had CLL and sarcoma; intolerant to ibrutinib, venetoclax; patient 44 had CLL and multiple skin cancers; patient 45 had CLL in relapse

† Determined by flow cytometry

Supplementary Table S3. List of anti-human antibodies used for flow cytometry, myeloid cell panel

Antibody	Fluorochrome	Clone	Company
hCD16	BUV 737	3G8	BD Biosciences
hCD14	Brilliant Violet 786	MφP9	BD Biosciences
hCD45	Brilliant Violet 711	HI30	BioLegend
hCD11b	BUV 395	ICRF44	BD Biosciences
hHLA DR	APC-CY7	L243	BioLegend
hCSF1R	PE	12-3A3-8E6	eBioscience
Lineage Cocktail (hCD3/hCD19/hCD20/hCD56)	APC	UCHTI/HIB19, 2H7/5.1H11	BioLegend
hCD66b	AlexaFluor 700	G10F5	BioLegend

Supplementary Table S4. List of anti-human antibodies used for flow cytometry, lymphoid cell panel

Antibody	Fluorochrome	Clone	Company
hCD8a	eFluor 450	SK1	eBioscience
hCD45RO	BUV 395	UCHL1	BD Biosciences
hCD45RA	PE-Cy7	L48	BD Biosciences
hCD62L	Brilliant Violet 605	DREG-56	BioLegend
hCD4	PE-Dazzle 594	SK3	BioLegend
hCD25	PE	4E3	Miltenyi Biotec
hFoxp3	APC	3G3	Miltenyi Biotec
hCD19	PE-Cy5.5	J3-119	Beckman Coulter
hCD20	Alexa Fluor 700	B9E9	Beckman Coulter
hCD5	APC-Alexa 750	BL1a	Beckman Coulter
hCD23	FITC	TU1	Thermo Fisher

Supplementary Table S5. List of anti-human antibodies used for flow cytometry intracellular cytokine detection on human myeloid cells

Antibody	Fluorochrome	Clone	Company
hIFNα	FITC	LT27:295	Miltenyi Biotec
hTNFα	Brilliant Violet 650	MAb11	BioLegend
hIL-6	PE-CF594	MQ2-13A5	BD Biosciences
hIL-12	PE- Vio770	REA121	Miltenyi Biotec
hCCL2	PerCP	2D8	Novus
hTRAILR2	PE	DJR2-4 (7-8)	BioLegend
hCD14	Brilliant Violet 786	MΦP9	BD Biosciences
hCD16	BUV737	3G8	BD Biosciences
hCD45	Brilliant Violet 711	HI30	BioLegend
hHLA DR	APC- Cy7	L243	BioLegend
Lineage Cocktail (CD3/CD19/CD20/CD56)	APC	UCHT1, HIB19, 2H7, 5.1H11	BioLegend
hCD66b	Alexa Fluor 700	G10F5	BioLegend

Supplementary Table S6. List of anti-human antibodies used for flow cytometry-based phagocytosis assay of human monocytes and macrophages

Antibody/Dye	Fluorochrome	Clone	Company
hCD16	BUV 737	3G8	BD Biosciences
hCD14	Brilliant Violet 786	MφP9	BD Biosciences
Calcein Violet-AM Proliferation Dye (VPD)			BioLegend
hCD68	PE	Y1/82A	BioLegend
hHLA DR	APC-CY7	L243	BioLegend
Lineage Cocktail (hCD3/hCD19/hCD20/hCD56)	APC	UCHTI/HIB19, 2H7/5.1H11	BioLegend
hCD66b	AlexaFluor 700	G10F5	BioLegend

Supplementary Table S7. List of anti-human antibodies used for flow cytometry checkpoint and intracellular cytokine detection on human lymphocytes

Antibody	Fluorochrome	Clone	Company
hCD8a	eFluor 450	SK1	eBioscience
hCD45RO	BUV 395	UCHL1	BD Biosciences
hCD45RA	PE-Cy7	L48	BD Biosciences
hCD62L	Brilliant Violet 605	DREG-56	BioLegend
hCD19	PE-Cy5.5	J3-119	Beckman Coulter
hGRANZYME B	Alexa Fluor 700	GB11	BD Biosciences
hPD-1	Brilliant Violet 650	EH12.2H7	BioLegend
hCD5	APC-Alexa 750	BL1a	Beckman Coulter
hIFNγ	FITC	25723.11	BD Biosciences

Supplementary Table S8. Primers for qRT-PCR analysis

HUMAN

GENE	FORWARD	REVERSE
CCL2	5'-AGTCTCTGCCGCCCTTCT-3'	5'-GTGACTGGGGCATTGATTG-3'
IL10	5'-GATGCCTTCAGCAGAGTGAA-3'	5'-GCAACCCAGGTAACCCTTAAA-3'
IFN-α	5'-GACTCCATCTTGGCTGTGA-3'	5'- TGATTTCTGCTCTGACAACCT-3'
TNF-α	5'-TCCTTCAGACACCCTCAACC-3'	5'- CAGGGATCAAAGCTGTAGGC-3'
IL12A	5'- CTTTTATGATGGCCCTGTGC -3'	5'-TCAAGGGAGGATTTTTGTGG-3'
IL6	5'-TACCCCCAGGAGAAGATTCC-3'	5'-CCATCTTTGGAAGGTTCAGG-3'
TGF-β	5'-CCCAGCATCTGCAAAGCTC-3'	5'-GTCAATGTACAGCTGCCGCA-3'
IFN-γ	5'- TGACCAGAGCATCCAAAAGA -3	5'-CTCTTCGACCTCGAAACAGC -3'
GZMB	5'-CCCTGGGAAAACACTCACACA-3'	5'-CACAACTCAATGGTACTGTCGT-3'
FOXP3	5'-CCCACTTACAGGCACTCCTC-3	5'-CTTCTCCTTCTCCAGCACCA-3'
TRAIL-R2	5'-AGACCCTTGTGCTCGTTGTC-3'	5'-TTGTTGGGTGATCAGAGCAG-3'
TNFR1	5'-TGCTCCAAATGCCGAAAG-3'	5'-AATGCCGGTACTGGTTCTTC-3'
FAS	5'-ATGGCCAATTCTGCCATAAG-3'	5'-TGACTGTGCAGTCCCTAGCTT-3'
FASL	5'-TGGGGATGTTTCAGCTCTTC-3'	5'-TGTGCATCTGGCTGGTAGAC-3'
FADD	5'-CCGAGCTCAAGTTCCTATGC-3'	5'-AGGTCTAGGCCGCTCTGC-3'
CASP 8	5'-CAGCAGCCTTGAAGGAAGTC-3'	5'-CGAGATTGTCATTACCCCACA-3'
CASP 3	5'-TTGTGGAATTGATGCGTGAT-3'	5'-GGCTCAGAAGCACACAAACA-3'
CASP 9	5'-AAGCCCAAGCTCTTTTTCATC-3'	5'-ACTCGTCTTCAGGGGAAGTG-3'
BAX	5'-CAAGACCAGGGTGGTTGG-3'	5'-CACTCCCGCCACAAAGAT-3'
BID	5'-GTGCTGGGGTCATGATGG-3'	5'-CGACTCACTCCTGGTTCACA-3'
MCL1	5'-AAGCCAATGGGCAGGTCT-3'	5'-GAACTCCACAAACCCATCCTT-3'
β-ACTIN	5'-ACCGAGCGCGGCTACAG-3'	5'-CTTAATGTCACGCACGATTTCC-3'
ERCC6	5'-GCAGAAGAAAGTGCCA-3'	5'-CATTACCTAAGATATGTGAAAGAG-3'
ERCC6L	5'- TATTCCATCTCCTTAACCG-3'	5'-TCATCATCTCCCTGTTCT-3'
ERCC8	5'- TTCTACAGGGTCACAGAC-3'	5'-CCCATTATGTTGATCAAGAG-3'
XAB2	5'- ATGCACAAGATGCCTCGT-3'	5'- AATTCGAGAGTGCTGCGT-3'
RAD51	5'- CCTGCTTGGTCTTTCATTGCTA-3'	5'- CACACTGGCCTGAAATGCTTT-3'
RAD51B	5'- GGATATGGGTTCTGGGCTTTAG-3'	5'- GTGAGGCAGGCCATAGTATTT-3'
RAD51D	5'- GGGAGCTTCAGCGTACTAATG-3'	5'- CCAGATTCCAGCAACACAAATG-3'

Supplementary Table S9. List of anti-murine antibodies used for flow cytometry, myeloid cell panel

Antibody	Fluorochrome	Clone	Company
mCD45	BUV 395	30-F11	BD Biosciences
mCD11b	Brilliant Violet 750	M1/70	BioLegend
mCD206	FITC	MR5D3	BioLegend
mF4/80	PE-CY7	BM8	BioLegend
mLy6C	APC-CY7	HK1.4	BioLegend
mLy6G	PerCP	1A8	BioLegend
mCSF1R	PE	604B5 2E11	Bio-Rad
mIA[b]	eFluor 450	AF6-120.1	eBioscience
mCD169	APC	3D6.112	BioLegend
mPD-L1	AlexaFluor 700	MIH5	Novus

Supplementary Table S10. List of anti-murine antibodies used for flow cytometry, lymphoid cell panel

Antibody	Fluorochrome	Clone	Company
mCD62L	BUV 395	MEL-14	BD Biosciences
mCD4	Brilliant Violet 650	GK1.5	BD Biosciences
mKappa	FITC	187.1	BD Biosciences
mCD19	PE-CY7	1D3	BD Biosciences
mCD3ε	APC-CY7	145-2C11	BioLegend
mCD8a	PerCP	53-6.7	BioLegend
mLambda	PE	JC5-1	Thermo Fisher
mPD-1	eFluor 450	RMP1-30	eBioscience
mCD5	APC	53-7.3	BD Biosciences
mPD-L1	AlexaFluor 700	MIH5	Novus
mCD44	BUV737	IM7	BD Biosciences
mCD25	Brilliant Violet 786	PC61	BD Biosciences
mCD45	Brilliant Violet 711	30-F11	BioLegend

Supplementary Table S11. Comparisons between human myeloid and lymphoid cells in depletion studies

Comparison A vs B	Method	p-value	Expr A	Expr B	FDR
$CD19^+$ vs $CD19^+CD5^+$	t-test	0.1934	16.6	26.59	0.3113
$CD19^+$ vs Intermediate	t-test	0.0533	16.6	43.02	0.1067
$CD19^+$ vs $Foxp3^+$Treg	t-test	0.0092	16.6	55	0.0494
$CD19^+CD5^+$ vs Intermediate	t-test	0.2273	26.59	43.02	0.3469
$CD19^+CD5^+$ vs $Foxp3^+$Treg	t-test	0.0469	26.59	55	0.0999
Intermediate vs $Foxp3^+$Treg	t-test	0.4713	43.02	55	0.6100
$CD19^+$ vs $CD19^+CD5^+CD23^+$	MWW-test	0.9694	13.15	13.1	0.9845
$CD19^+$ vs $CD8^+$	MWW-test	0.0433	13.15	0	0.0952
$CD19^+$ vs Classical	MWW-test	0.0116	13.15	0	0.0511
$CD19^+$ vs M-MDSC	MWW-test	0.5693	13.15	13.41	0.7089
$CD19^+$ vs $CD8^+$naive	MWW-test	0.8499	13.15	10.6	0.9195
$CD19^+$ vs Non classical	MWW-test	0.1426	13.15	0	0.2413
$CD19^+$ vs $CD8^+T_{CM}$	MWW-test	0.0239	13.15	0	0.0717
$CD19^+$ vs $CD8^+T_{EM}$	MWW-test	0.0213	13.15	0	0.0717
$CD19^+CD5^+$ vs $CD19^+CD5^+CD23^+$	MWW-test	0.5678	27.05	13.1	0.7089
$CD19^+CD5^+$ vs $CD8^+$	MWW-test	0.0100	27.05	0	0.0494
$CD19^+CD5^+$ vs Classical	MWW-test	0.0036	27.05	0	0.0343
$CD19^+CD5^+$ vs M-MDSC	MWW-test	0.7910	27.05	13.41	0.8701
$CD19^+CD5^+$ vs $CD8^+$naive	MWW-test	0.2567	27.05	10.6	0.3764
$CD19^+CD5^+$ vs Non classical	MWW-test	0.0350	27.05	0	0.0873
$CD19^+CD5^+$ vs $CD8^+T_{CM}$	MWW-test	0.0079	27.05	0	0.0476
$CD19^+CD5^+$ vs $CD8^+T_{EM}$	MWW-test	0.0063	27.05	0	0.0476
$CD19^+CD5^+CD23^+$ vs $CD8^+$	MWW-test	0.1476	13.1	0	0.2435
$CD19^+CD5^+CD23^+$ vs Classical	MWW-test	0.0371	13.1	0	0.0875
$CD19^+CD5^+CD23^+$ vs Intermediate	MWW-test	0.2312	13.1	44	0.3469
$CD19^+CD5^+CD23^+$ vs M-MDSC	MWW-test	0.7886	13.1	13.41	0.8701
$CD19^+CD5^+CD23^+$vs CD8+naive	MWW-test	0.9696	13.1	10.6	0.9845
$CD19^+CD5^+CD23^+$vs Non classical	MWW-test	0.1996	13.1	0	0.3137
$CD19^+CD5^+CD23^+$ vs $Foxp3^+$Treg	MWW-test	0.0521	13.1	65.65	0.1067
$CD19^+CD5^+CD23^+$ vs $CD8^+T_{CM}$	MWW-test	0.1258	13.1	0	0.2185
$CD19^+CD5^+CD23^+$vs $CD8^+T_{EM}$	MWW-test	0.0803	13.1	0	0.1472
$CD8^+$ vs Classical	MWW-test	0.3305	0	0	0.4544
$CD8^+$ vs Intermediate	MWW-test	0.0292	0	44	0.0803
$CD8^+$ vs M-MDSC	MWW-test	0.0433	0	13.41	0.0952
$CD8^+$ vs $CD8^+$naive	MWW-test	0.0235	0	10.6	0.0717
$CD8^+$ vs Non classical	MWW-test	0.9257	0	0	0.9698
$CD8^+$ vs $Foxp3^+$Treg	MWW-test	0.0030	0	65.65	0.0330

Abbreviations and notes: Cell depletion data were tested for normality using a Shapiro-Wilk normality test. ExprA/B: median, respectively mean, according to the normality of the distribution of the data. t-test was applied to normally distributed data, otherwise the Mann-Whitney-Wilcoxon (MWW) test.

Supplementary Table S12. Comparisons between human myeloid and lymphoid cells in depletion studies

Comparison A vs B	Method	p-value	Expr A	Expr B	FDR
$CD8^+$ vs $CD8^+T_{CM}$	MWW-test	1.0000	0	0	1.0000
$CD8^+$ vs $CD8^+T_{EM}$	MWW-test	0.6548	0	0	0.7840
Classical vs Intermediate	MWW-test	0.0105	0	44	0.0494
Classical vs M-MDSC	MWW-test	0.0072	0	13.41	0.0476
Classical vs CD8+naive	MWW-test	0.0028	0	10.6	0.0330
Classical vs Non classical	MWW-test	0.3870	0	0	0.5109
Classical vs $Foxp3^+$Treg	MWW-test	0.0009	0	65.65	0.0330
Classical vs $CD8^+T_{CM}$	MWW-test	0.3305	0	0	0.4544
Classical vs $CD8^+T_{EM}$	MWW-test	0.6704	0	0	0.7840
Intermediate vs M-MDSC	MWW-test	0.3410	44	13.41	0.4594
Intermediate vs $CD8^+$naive	MWW-test	0.2881	44	10.6	0.4134
Intermediate vs Non classical	MWW-test	0.0357	44	0	0.0873
Intermediate vs $CD8^+T_{CM}$	MWW-test	0.0292	44	0	0.0803
Intermediate vs $CD8^+T_{EM}$	MWW-test	0.0184	44	0	0.0701
M-MDSC vs $CD8^+$naive	MWW-test	0.6771	13.41	10.6	0.7840
M-MDSC vs Non classical	MWW-test	0.0746	13.41	0	0.1406
M-MDSC vs Foxp3+Treg	MWW-test	0.0636	13.41	65.65	0.1235
M-MDSC vs CD8+T_{CM}	MWW-test	0.0239	13.41	0	0.0717
M-MDSC vs CD8+T_{EM}	MWW-test	0.0138	13.41	0	0.0568
$CD8^+$naive vs Non classical	MWW-test	0.0858	10.6	0	0.1530
$CD8^+$naive vs $Foxp3^+$Treg	MWW-test	0.0342	10.6	65.65	0.0873
$CD8^+$naive vs $CD8^+T_{CM}$	MWW-test	0.0191	10.6	0	0.0701
$CD8^+$naive vs $CD8^+T_{EM}$	MWW-test	0.0079	10.6	0	0.0476
Non classical vs $Foxp3^+$Treg	MWW-test	0.0030	0	65.65	0.0330
Non classical vs $CD8^+T_{CM}$	MWW-test	0.9257	0	0	0.9698
Non classical vs $CD8^+T_{EM}$	MWW-test	0.6548	0	0	0.7840
$Foxp3^+$Treg vs $CD8^+T_{CM}$	MWW-test	0.0023	65.65	0	0.0330
$Foxp3^+$Treg vs $CD8^+T_{EM}$	MWW-test	0.0017	65.65	0	0.0330
$CD8^+T_{CM}$ vs $CD8^+T_{EM}$	MWW-test	0.7281	0	0	0.8285

Abbreviations and notes: Cell depletion data were tested for normality using a Shapiro-Wilk normality test. ExprA/B: median, respectively mean, according to the normality of the distribution of the data. t-test was applied to normally distributed data, otherwise the Mann-Whitney-Wilcoxon (MWW) test.