Lenalidomide plus bendamustine-rituximab does not overcome the adverse impact of *TP53* mutations in mantle cell lymphoma

Christian Winther Eskelund,[1,2] Alexandra Albertsson-Lindblad,[3] Arne Kolstad,[4] Anna Laurell,[5] Riikka Räty,[6] Lone Bredo Pedersen,[1] Christian Hartmann Geisler,[1] Mats Jerkeman[3] and Kirsten Grønbæk[1,2]

[1]Department of Hematology, Rigshospitalet, Copenhagen, Denmark; [2]Biotech Research and Innovation Centre, Copenhagen, Denmark; [3]Lund University, Skåne University Hospital, Department of Clinical Sciences Lund, Oncology and Pathology, Sweden; [4]Department of Oncology, Oslo University Hospital, Norway; [5]Department of Oncology, Uppsala University Hospital, Sweden; [6]Department of Hematology, Helsinki University Hospital, Finland

CWE and AA-L Contributed equally to this work.

Correspondence: kirsten.groenbaek@regionh.dk
doi:10.3324/haematol.2018.194399

SUPPLEMENTARY MATERIAL

Supplementary Methods

Patients

Patients older than 65 years, or ≤65 years and considered unfit for high-dose chemotherapy, with previously untreated, stage II-IV, histologically confirmed, diagnosis of MCL were included in the Nordic Lymphoma group phase I/II trial MCL4 (#NCT00963534).[1] Treatment consisted of an induction phase with six cycles of LBR (lenalidomide [days 1-14, cycles 1-6], bendamustine [90 mg/m2 IV, days 1-2], rituximab [375 mg/m2 IV, day 1]), cycle duration 28 days, followed by a maintenance phase with single-agent lenalidomide ([days 1-21], cycle 7-13, cycle duration 28 days). In the early phase I portion (after 12 patients included), the protocol was amended due to unexpected high portion of treatment-related toxicity. Lenalidomide was omitted from cycle 1 and included in cycles 2-6. Details on the regimen are found in supplement figure 1.

The diagnosis of MCL was confirmed by central pathology/histology review board according to WHO criteria by detection of t(11;14) or overexpression of cyclin D1.

The study was performed in agreement with the Declaration of Helsinki and was conducted according to the guidelines for Good Clinical Practice, issued by The International Conference on Harmonization (ICH). The protocol was approved by all national Ethical Review Boards. All patients signed a written informed consent to participate and to donate/provide samples from peripheral blood, bone marrow and tissue for biologic studies. The study was registered at www.ClinicalTrials.gov as #NCT00963534.

Patient samples

Bone marrow (BM) and peripheral blood (PB) samples were collected centrally for MRD measurements, and DNA was purified from unsorted specimens by QIAamp DNA Blood Midi Kit (Qiagen, Valencia, CA). Inclusion criteria in this study were available pre-treatment BM or PB sample with measurable MCL by flow cytometry or positive minimal residual disease (MRD) marker. BM samples were available from 39 patients, and PB samples from another 7 patients. Two of the PB samples did not reach sufficient quality for next generation sequencing (NGS) analyses, and were thus only included in deletion analyses, both described below.

Mutational analysis with Next Generation Sequencing

Targeted NGS was performed of selected coding regions, splice sites and untranslated regions (UTRs) of eight recurrently mutated genes in MCL: *ATM, KMT2D, CCND1, TP53, WHSC1, BIRC3, NOTCH1* and *NOTCH2,* as previously described.[2] Libraries were constructed based on the Ion Ampliseq technology (Thermo Fischer Scientific, Waltham, MA), and quantitative polymerase chain reaction (qPCR) measurements performed using the TaqMan Ion library quantification kit. Template preparation was carried out on the Ion Chef instrument and sequencing was performed on the Ion PGM System, using Hi-Q view technology and reagents. All steps were carried out according to manufacturer's instructions, and reagents and equipment were manufactured by ThermoFisher Scientific. Median coverage of all runs was >3000X.

Cut-off for calling a variant was variant allele frequency (VAF) of ≥5% and coverage of ≥400X. For *TP53*, the lower limit for calling a variant was 3%, as described previously.[2] Variants were carefully reviewed in the IGV software (Broad Institute). All known common single nucleotide polymorphisms (SNPs) (>1% in the SNP database, dbSNP) were excluded prior to analyses, and only variants giving rise to amino acid changes were reported, unless in splice sites or UTR regions. Variants with a VAF 40-60% and a SNP database (dbSNP) reference were considered rare SNPs and excluded. If both dbSNP and COSMIC references were present, the variant was reported here, including both references (supplemental table 2).

Deletion analysis by Droplet Digital PCR

Deletion analyses for the *TP53* gene and *CDKN2A* locus were performed by Droplet Digital PCR (ddPCR) using the QX200 system (Bio-Rad Laboratories, Hercules, CA). RPP30 was used as a reference gene. All samples were run at least twice. QuantaSoft software was used for data analyses, and copy number (CN) below 1.95 was interpreted as a deletion, as previously described.[2]

Statistics

Overall survival (OS), progression-free survival (PFS) and cumulative incidence of relapses or progression (CIR) were used as patient and disease-specific endpoints with starting point at date of inclusion in the trial. OS was measured until date of death of any cause, PFS until date of documented progression, lack of response, first relapse, or death of any cause and CIR until date of documented relapsing or progressive disease. The Kaplan-Meier method was used to estimate survival curves for PFS, OS and CIR and subgroup analyses by specific gene alterations or mutations were compared by log-rank test. Analyses on adverse events (grade 3-5 infections, cutaneous reactions and incidence of SPM) in relation to presence of specific gene alterations or mutations were made by using Fisher's exact t-test. All analyses were made by using SPSS v.22.

REFERENCES

1. Albertsson-Lindblad A, Kolstad A, Laurell A, et al. Lenalidomide-bendamustine-rituximab in patients older than 65 years with untreated mantle cell lymphoma. *Blood*. 2016;128(14):1814–1820.

2. Eskelund CW, Dahl C, Hansen JW, et al. TP53 mutations identify younger mantle cell lymphoma patients who do not bene fi t from intensive chemoimmunotherapy. *Blood*. 2017;130(17):1903–1911.

Supplementary table I: Patient characteristics

	n patients (%)
Male/Female	37/13
Median age (range)	71 (62-84)
Ann Arbor stage	
II	2 (4%)
III	4 (8%)
IV	44 (88%)
Performance score	
0	25 (50%)
1	22 (44%)
2	3 (6%)
Elevated LDH	31 (62%)
LPK, median ($x10^9/mm^3$)	8.4 (1.7-136.9)
Bone marrow involvement	44 (88%)
MIPI	
median (range)	6,3 (5,2-7,5)
Low	5 (10)
Intermediate	19 (38)
High	26 (52)
Morphological subtype	
Classic*	39 (76%)
Blastoid	2 (4%)
Ki-67%**	
<30%	29 (58%)
≥ 30%	9 (18%)
Prior treatment (1 cycle)	4 (8%)
R-CHOP	2 (4%)
R-Bendamustine	1 (2%)
R-Ara-C	1 (2%)
Clinical outcome	median (95% CI)
Overall survival	69 (58-50)
Progression-free survival	42(29-55)
Cumulative incidence of rel	53 (34-72)

* Diffuse, nodal or mantle zone growth pattern

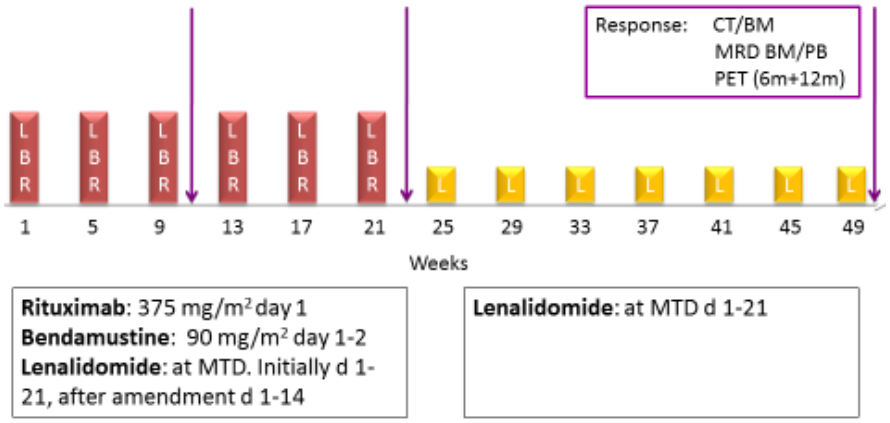
** MIB-1 in a few cases, values converted 1:1

Supplementary table 2: Mutations overview

UPN	NM	Gene	Exon	Genotype Variant(ref/mut)	Chromosome	c.	p.	consequence	VAF, %	dbSNP/COSMIC ref *
2	NM_000051.3	ATM	37	A/T	chr11:108175463	c.5558A>T	p.Asp1853Val	missense	47,01	rs1801673 / COSP17291
3	NM_000051.3	ATM	17	CG/AT	chr11:108137925	c.2494_2495delCGinsAT	p.Arg832Ile	missense	48,27	rs199875915 / COSP25858
4	NM_000051.3	ATM	34	A/C	chr11:108170506	c.5071A>C	p.Ser1691Arg	missense	45,6	rs1800059 / COSP42470
6	NM_000051.3	ATM	50	G/C	chr11:108200993	c.7360G>C	p.Ala2454Pro	missense	5,31	
8	NM_000051.3	ATM	49	T/C	chr11:108199935	c.7277T>C	p.Leu2426Pro	missense	25,8	
13	NM_000051.3	ATM	25	AATTTTTGGACTTTTTTTCCAAGGCT/A	chr11:108114806	c.627_651delTTTGGACTTTTTTTCCAAGGCTATT	p.Leu210fs	deletion-frameshift	18,1	
19	NM_000051.3	ATM	56	A/T	chr11:108206594	c.8174A>T	p.Asp2725Val	missense	48,9	
19	NM_000051.3	ATM	18	G/A	chr11:108139336	c.2838G>A	p.Met946Ile	missense	46,2	
21	NM_000051.3	ATM	59	A/T	chr11:108218036	c.8615A>T	p.His2872Leu	missense	6,85	
22	NM_000051.3	ATM	42	G/A	chr11:108186757	c.6115G>A	p.Glu2039Lys	missense	30,59	
24	NM_000051.3	ATM	6	GAATAAT/G	chr11:108114749	c.567_572delAATAAT	p.Arg189_Ile191delinsSer	deletion-frameshift	5,93	
36	NM_000051.3	ATM	49	T/C	chr11:108199938	c.7280T>C	p.Leu2427Pro	missense	13,16	
42	NM_000051.3	ATM	17	C/T	chr11:108137985	c.2554C>T	p.Gln852Ter	nonsense	48,3	
43	NM_000051.3	ATM	50	G/A	chr11:108200975	c.7342G>A	p.Asp2448Asn	missense	6,38	
47	NM_000051.3	ATM	52	G/A	chr11:108202765			3' splice site	25,65	
47	NM_000051.3	ATM	63	C/T	chr11:108236086	c.9022C>T	p.Arg3008Cys	missense	23,02	
48	NM_000051.3	ATM	17	T/C	chr11:108138003	c.2572T>C	p.Phe858Leu	missense	51,4	rs1800056 / COSP37973
24	NM_182962.2	BIRC3	8	T/G	chr11:102206706	c.1334T>G	p.Leu445Ter	nonsense	6,51	
24	NM_053056.2	CCND1		A/ACC	chr11:69456042			5' UTR	11,32	
28	NM_053056.2	CCND1	1	CC/TG	chr11:69456209	c.128_129delCCinsTG	p.Ser43Leu	missense	29,16	
37	NM_053056.2	CCND1		C/T	chr11:69455955			5' UTR	5,24	
37	NM_053056.2	CCND1		C/T	chr11:69456037			5' UTR	5,24	
38	NM_053056.2	CCND1		G/A	chr11:69456049			5' UTR	33,82	
8	NM_003482.3	KMT2D	15	T/TCACACA	chr12:49440518	c.4291_4292insTGTGTG	p.Cys1430_Glu1431insValCys	insertion-frameshift	15,99	
13	NM_003482.3	KMT2D	39	TC/T	chr12:49425194	c.13293delG	p.Lys4432fs	deletion-frameshift	15,23	
14	NM_003482.3	KMT2D	48	T/C	chr12:49420120	c.15629A>G	p.Tyr5210Cys	missense	21,3	
22	NM_003482.3	KMT2D	42	TC/T	chr12:49424113	c.13948delG	p.Glu4650fs	deletion-frameshift	23,33	
29	NM_003482.3	KMT2D	10	C/CG	chr12:49445222	c.2243_2244insC	p.Glu748fs	insertion-frameshift	5,72	
40	NM_003482.3	KMT2D	31	C/T	chr12:49434759	c.6794G>A	p.Gly2265Glu	missense	55,56	
46	NM_003482.3	KMT2D	31	G/A	chr12:49434801	c.6752C>T	p.Ser2251Leu	missense	47,63	rs189199944 / COSU540
48	NM_003482.3	KMT2D	34	G/A	chr12:49432396	c.8743C>T	p.Arg2915Ter	nonsense	36,45	
29	NM_017617.4	NOTCH1	34	CAG/C	chr9:139390648	c.7541_7542delCT	p.Pro2514fs	deletion-frameshift	5,39	
49	NM_017617.4	NOTCH1	34	G/A	chr9:139390690	c.7501C>T	p.Gln2501Ter	nonsense	24,15	
4	NM_000546.5	TP53	7	AG/A	chr17:7577557	c.723delC	p.Cys242fs	deletion-frameshift	10,87	
4	NM_000546.5	TP53	5	T/TGAGG	chr17:7578539	c.390_391insCCTC	p.Asn131fs	insertion-frameshift	8,32	
8	NM_000546.5	TP53	8	GC/AA	chr17:7577121	c.816_817delGCinsTT	p.Arg273Cys	missense	21,52	
28	NM_000546.5	TP53	7	A/T	chr17:7577517	c.764T>A	p.Ile255Asn	missense	42,33	
29	NM_000546.5	TP53	9	C/T	chr17:7576852			3' splice site	24,14	
37	NM_000546.5	TP53	8	G/A	chr17:7577094	c.844C>T	p.Arg282Trp	missense	3,85	
48	NM_000546.5	TP53	5	C/A	chr17:7578370			3' splice site	60,89	
29	NM_001042424.2	WHSC1	18	G/A	chr4:1962801	c.3295G>A	p.Glu1099Lys	missense	20	
38	NM_001042424.2	WHSC1	18	G/A	chr4:1962801	c.3295G>A	p.Glu1099Lys	missense	15,6	

** Listed are mutations with VAF 40-60% and both a dbSNP and COSMIC reference.*

Supplementary figure 1: MCL4 regimen. Top: Overview of the regimen and doses of rituximab and bendamustin. Bottom: Lenalidomide dosing, before and after amendment.



Lenalidomide dosing schedule:

trial phase I	cohort	**c 1-6 (d1-21)**	**c7-13 (d1-21)**	
	1	5	25	
	2	10	25	
	3	15	25	
I (after amendment)		**c1**	**c 2-6 (d1-14)**	**c 7-8 (d1-21)**	**c 9-13 (d1-21)**
	A	0	10 mg	10 mg	15 mg
	B		10 mg	10 mg	15 mg
	C		5	10 mg	15 mg
Trial phase II		**c1**	**c 2-6 (d1-14)**	**c 7-8 (d1-21)**	**c 9-13 (d1-21)**
		0	10 mg	10 mg	15 mg

Supplementary figure 2: Prognostic impact of deletions of *TP53* and *CDKN2A*. Kaplan-Meier estimates of OS, PFS and CIR by subgroups according to presence of deletion of *TP53* or not (A-C); deletion of *CDKN2A* or not (D-F) and both deletions (G-I) and compared by log-rank test.