High rate of minimal residual disease responses in young and fit patients with IGHV mutated chronic lymphocytic leukemia treated with front-line fludarabine, cyclophosphamide, and intensified dose of ofatumumab (FCO2)

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SUPPLEMENTARY MATERIAL

SUPPLEMENTARY PATIENTS AND METHODS

Statistics

The primary endpoint of this study, the expected CR rate, was considered to calculate the sample size of patients to include in this study. Based on the CR rate recorded with the FCR regimen in the CLL8 trial, 44%, it was assumed that treatment with the FCO2 regimen would lead to a 60% or higher CR rate. With this assumption, to reject the null hypothesis that $p \leq 0.45$ vs the alternative hypothesis that $p \geq 0.6$ with type I error probability (α) equal to 5% and 80% power ($1-\beta$), 70 patients needed to be enrolled in the study. If the number of responses was 39 or higher, the treatment would be deemed worthy of further studies. Conversely, if the total number of responses was 38 or lower, the combination therapy would not be recommended for further studies. Due to an expected drop-out rate of about 10%, the estimated final number of required patients was 80. According to the intention-to-treat (ITT) basis, patients who received at least one dose of the study drugs were included in the efficacy and safety analyses. In univariate analysis (UVA) non-parametric tests were performed for comparisons between groups (Chi-Squared and Fisher Exact test in case of categorical variables or response rate, Mann-Whitney and Kruskal-Wallis test in case of continuous variables). OS was defined as the time from the start of treatment to death or to the last follow-up. PFS was defined as the time from the start of treatment to disease progression, death or last follow-up. Survival curves were calculated according to the Kaplan and Meier method. Differences in survival were analyzed by means of the Log-Rank test in UVA and by means of the Cox logistic regression model in multivariate analysis (MVA), after the assessment of the proportionality of hazards. Factors included in the MVA were obtained from UVA. Confidence intervals (CIs) were calculated at the 95% level. All statistical tests were two-sided. A p value of less than 0.05 was considered significant. All analyses were performed by using the SAS (version 9.4) and the R (R Foundation for Statistical Computing, Vienna, Austria) system software.

Ethics

This phase 2, single-arm, open-label study was approved by the Ethical Committees of all participating institutions. Patients provided written informed consent before the central screening. The study is registered at ClinicalTrials gov, Identifier: NCT01762202.

Supplementary Table 1. Baseline clinical and biologic characteristics of patients

	N (%)
No patients	78 (00)
Median follow-up, months (range)	31.1 (13.7-36.2)
Median age, years (range)	55.6 (36.2-65.1)
Gender, M/F	51(65.4)/27(34.6)
Hb, g/dl	12.95 (7.9-15.7)
Lymphocyte count x 10^9/L	54.8 (5-480.0)
Platelet count x 10^9/L	145.6 (27.0-371.0)
B symptoms	15 (19.2)
Binet stage B/C	69 (88.5)
Bulky nodes (lymph nodes size ≥5 cm)	7 (9)
Beta-2 microglobulin ≥3.5 mg/L	52/76 (68.4)
ECOG performance status 0-1	68 (87.2)/10/78(12.8)
Median CIRS	1 (0-5)
CD38 positive	46(68.7)
FISH cytogenetic aberrations (77 evaluated patients)	
del(13q)	29 (37.7)
12q+	9 (11.7)
del(11q)	9 (11.7)
del(17p)	5 (6.5)
No aberrations	25 (32.5)
*TP*53 mutations	6 (7.7)
Del(17p) and/or *TP*53 mutations	8/72 (11.1)
Mutated IGHV	26 (35.6)
Unmutated IGHV	47 (64.4)
IPI score	
Low risk/Intermediate risk	35 (50.7)
High risk/Very high risk	34 (49.3)

Abbreviations. ECOG, Eastern Cooperative Oncology Group; CIRS, Cumulative Illness Rating Scale; FISH, fluorescence-in-situ hybridization; IPI, International Prognostic Index.

Supplementary Table 2. Factors predicting CR, CR with uMRD by flow-cytometry and by PCR.

	All patients	Patients with CR	p value	Patients with CR and uMRD by flow-cytometry	p value	Patients with CR and uMRD by PCR	p value
	N (%)	N (%)		N (%)		N (%)	
All patients	78	60 (77)	-	36 (46.15)	-	17 (21.8)	--
Gender							
male	51	37 (72.5)	0.328	24 (47)	1	11 (21.6)	1
female	27	23 (85.2)		12 (44.4)		6 (22.3)	
Binet stage							
A	9	8 (88.9)	0.627	7 (77.8)	**0.095**	3 (33.3)	0.644
B/C	69	52 (36.2)		29 (42)		14 (20.3)	
Increased B2M							
yes	15	9 (60)	0.165	6 (40)	0.807	4 (26.7)	0.872
no	63	51 (80.9)		30 (47.6)		13 (20.6)	
Lymph nodes >5 cm							
yes	7	3 (42.8)	**0.076**	2 (28.6)	0.561	0 (0)	0.325
no	71	57 (80.3)		34 (47.9)		17 (24)	
IGHV							
mutated	26	22 (84.6)	0.61	16 (61.5)	**0.097**	11 (42.3)	**0.01**
unmutated	47	36 (76.6)		18 (38.3)		6 (12.8)	
***TP*53 disruption**							
yes	8	3 (37.5)	0.009	1 (12.5)	0.103	1 (12.5)	0.802
no	64	54 (84.4)		32 (50)		15 (23.4)	
Del11q							
yes	9	6 (7.69)	0.740	1 (1.28)	**0.065**	1 (1.28)	0.677
no	68	53 (67.95)		34 (43.59)		16 (20.51)	
CD38							
negative	46	34 (74)	0.75	20 (95.2)	0.959	11 (52.4)	0.294
positive	21	17 (81)		10 (21.7)		2(9.5)	
IPI score							
Low-intermediate	35	31 (88.6)	0.197	18 (51.4)	0.540	11 (31.4)	0.174
High-very high	34	25 (73.5)		14 (41.2)		5 (14.7)	

Abbreviations.CR, complete response; uMRD, undetectable minimal residual disease; beta-2 microglobulin, B2M; IGHV, immunoglobulin heavy-chain variable region gene; PCR, polymerase chain reaction.

Supplementary Table 3. Multivariate analysis: factors predicting CR, uMRD-CR, PCR uMRD-CR, PFS and OS

All patients										
	CR		**Flow-uMRD-CR**		**PCR-uMRD-CR**		**PFS**		**OS**	
	OR (95%CI)	**P value**	**OR (95%CI)**	**P value**	**OR (95%CI)**	**P value**	**OR (95%CI)**	**P value**	**OR (95%CI)**	**P value**
*TP*53 disruption	0.126 (0.024-0.657)	0.014	-	-	-	-	6.96 (2.02-23.97)	0.002	31.19 (3.21-303.15)	0.003
Lymph-node size	0.182 (0.031-1.055)	0.057	-	-	-		-	-	-	-
Binet stage	-		0.084 (0.007-0.920)	0.042	-	-	-	-	-	
IGHV	-		2.634 (0.871-7.963)	0.086	5.011 (1.575-15.942)	0.006	-	-	-	-
Patients without *TP*53 disruptions										
IGHV	-		3.35 (1.12-10.01)	0.030	6.00 (1.71-21.08)	0.005	-	-	-	-

Abbreviations.CR, complete response; MRD, minimal residual disease; uMRD, ndetectable minimal residual disease; IGHV, immunoglobulin heavy-chain variable region gene; Flow, flow-cytometry; PCR, *polymerase chain reaction;* PFS, progression-free survival; OS, overall survival.

Supplementary Table 4. Factors predicting CR, CR with uMRD by flow-cytometry and by PCR in patients without *TP*53 disruption

	All patients	Patients with CR	p value	Patients with CR and uMRD by flow-cytometry	p value	Patients with CR and uMRD by PCR	p value
	N (%)	N (%)		N (%)		N (%)	
All patients	64	54 (84.4)	-	32 (50)	-	15 (23.4)	-
Gender							
Male	41	33 (80.5)	0.433	20 (48.8)	1.000	9 (21.9)	0.946
Female	23	21 (91.3)		12 (52.2)		6 (26.1)	
Stage							
A	6	6 (100)	0.605	5 (83.3)	0.198	2 (33.3)	0.924
B/C	58	48 (82.7)		27 (46.5)		13 (22.4)	
Increased B2M							
Yes	12	8 (66.7)	0.152	5(41.6)	0.749	4 (33.3)	0.603
No	52	46 (88.4)		27 (51.9)		11 (21.1)	
Lymph nodes >5 cm							
Yes	5	3 (60)	0.357	2 (40)	1.000	0 (0)	0.460
No	59	51 (86.4)		30 (50.8)		15 (25.4)	
IGHV							
Mutated	22	20 (90.9)	0.473	15 (68.2)	0.036	10(45.4)	0.005
Unmutated	41	33 (80.5)		16 (39)		5 (12.2)	
Del11q							
Yes	9	6 (66.7)	0.279	1 (11.1)	0.031	1 (11.1)	0.605
No	55	48 (87.3)		31 (56.4)		14 (25.4)	
CD38							
negative	37	31 (83.8)	1.000	18 (48.6)	1.000	11 (29.7)	0.091
positive	18	15 (83.3)		9 (50)		1(5.5)	
IPI score							
Low-intermediate	35	31 (88.6)	0.673	18 (51.4)	1.000	11 (31.4)	0.224
High-very high	27	22 (81.5)		13 (48.1)		4 (14.8)	

Abbreviations.CR, complete response; uMRD, undetectable minimal residual disease; beta-2 microglobulin, B2M; IGHV, immunoglobulin heavy-chain variable region gene; PCR, polymerase chain reaction.

Supplementary Table 5. Prognostic factors for progression-free survival.

Variables	**HR**	**Lower 95%CI**	**Higher 95%CI**	**p**
Age, as continuous variable	1	0.93	1.08	0.9616
IGHV, mutated vs unmutated	0.322	0.0704	1.4756	0.1446
Binet stage, A vs B/C	1.59	0.21	12.14	0.657
*TP*53, disruption present vs absent	6.96	2.02	23.97	0.0021
Del11q	1.95	0.54	7.12	0.3112
CD38, positive vs negative	2.15	0.47	9.9	0.3259
B2M, normal vs increased	2.137	0.657	6.949	0.207
Lymph node size, >5 cm vs ≤5 cm	2.532	0.556	11.532	0.2297
Gender, male vs female	0.333	0.074	1.501	0.1522
IPI score,l ow/intermediate vs high/very high	1.821	0.507	6.531	0.358

Abbreviations. IGHV, immunoglobulin heavy-chain variable region gene; B2M, beta2-microglobulin; IPI, International Prognostic Index.

Supplementary Table 6. Prognostic factors for Progression-Free Survival in patients without *TP*53 disruption.

	HR	Lower 95%CI	Higher 95%CI	p
Age as continuous variable	0.95	0.86	1.05	0.2986
IGHV, mutated vs unmutated	0.231	0.0282	1.8862	0.1713
Binet stage, B-C vs A	0.77	0.1	6.2	0.8101
CD38, positive vs negative	0.86	0.16	4.57	0.8578
B2M, normal vs increased	2.947	0.703	12.366	0.1396
Lymph node size, >5 cm vs ≤5 cm	1.81	0.224	14.628	0.5777
Gender, male vs female	0.188	0.023	1.526	0.1177
IPI Score, low /intermediate vs high/ very high	1.105	0.244	4.994	0.8972
Del11q, present vs absent	3.32	0.79	13.94	0.1016

Abbreviations. IGHV, immunoglobulin heavy-chain variable region gene; B2M, beta2-microglobulin; IPI, International Prognostic Index.

Supplementary Table 7. Prognostic factors for Overall Survival.

	HR	Lower 95%CI	Higher 95%CI	p
Age as continuous variable	1.01	0.88	1.17	0.8496
Gender, male vs female	0.616	0.064	5.92	0.6744
IGHV, mutated vs unmutated	0.853	0.0773	9.4126	0.8968
Binet stage, B-C vs A	0.46	0.05	4.18	0.4942
Del17p and/or*TP*53 aberrations, present vs absent	31.19	3.21	303.15	0.003
Del 11q	1.28	0.13	12.26	0.8285
CD19/CD38, positive vs negative	1.62	0.18	14.78	0.6669
B2M normal vs increased	1.531	0.159	14.736	0.7124
Lymph node size, >5 cm vs ≤5 cm	12.095	1.693	86.418	0.013
IPI Score low/intermediate vs high/very high	0.47	0.043	5.184	0.5376

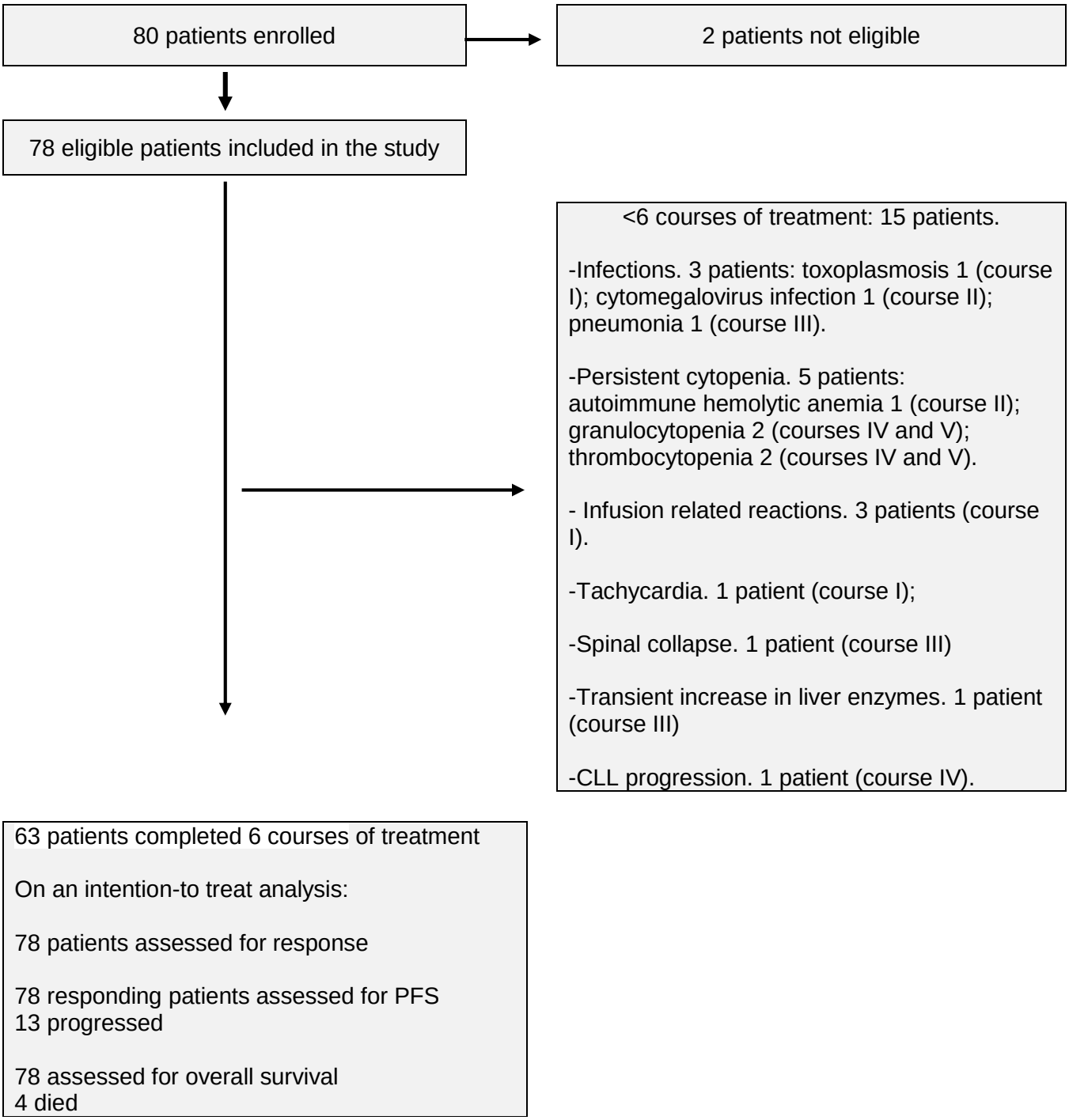
Abbreviations. IGHV, immunoglobulin heavy-chain variable region gene; B2M, beta2-microglobulin; IPI, International Prognostic Index.

Supplementary Table 8. Adverse events (AEs) per distinct patient

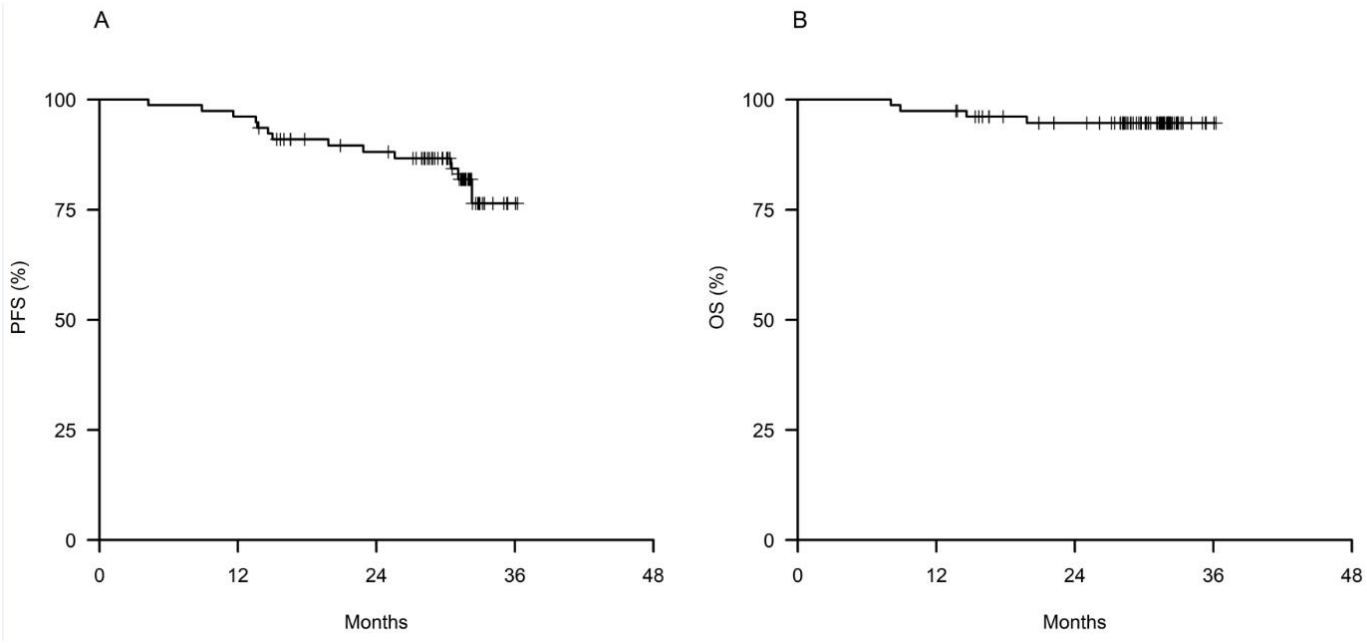
	All grades N (%)	Grade 1-2 N (%)	Grade ≥3[(1)] N (%)
Patients with one or more adverse events	68 (87.18)	57 (73.08)	53 (67.95)
Hematologic toxicity	44 (56.4)	30 (38.5)	39 (50)
• Neutropenia	38 (48.72)	5 (6.41)	33 (42.31)
• Thrombocytopenia	23 (29.49)	15 (19.23)	8 (10.26)
• Anemia	18 (23.07)	14 (17.95)	4 (5.13)
Febrile neutropenia	2 (2.56)	1 (1.28)	1 (1.28)
Fever of unknown origin	20 (25.64)	17 (21.79)	3 (3.85)
Infections, total	37 (47.43)	27 (34.61)	10 (12.82)
Upper respiratory tract infections	9 (11.54)	7 (8.97)	2 (2.56)
• Pneumonia	5 (6.41)	4 (5.13)	1 (1.28)
• Bronchitis	2 (2.56)	2 (2.56)	0 (0)
• Gastroenteric	2 (2.56)	2 (2.56)	0 (0)
• Urogenital tract infections	4 (5.13)	4 (5.13)	0 (0)
• Sepsis	2 (2.56)	0 (-)	2 (2.56)
• Soft tissue infections	6 (7.69)	5 (6.41)	1 (1.28)
• Opportunistic infections[(1)]	7 (8.97)	3 (3.85)	4 (5.13)
Gastroenteric	21 (26.92)	21 (26.92)	0 (0)
Infusion reactions	23 (29.49)	14 (17.94)	9 (11.54)
Fatigue	4 (6.41)	4 (6.41)	0 (0)
Neurological and psychiatric disorders	4 (5.13)	4 (5.13)	0 (0)
Arthritis and arthralgia; trauma and orthopedic problems	9 (11.54)	7 (8.97)	2 (2.56)
Cardiovascular disorders	4 (5.13)	3 (3.85)	1 (1.28)
Laboratory abnormalities	7 (8.97)	4 (5.13)	3 (3.85)

[(1)]Opportunistic infections: toxoplasmosis 1; cytomegalovirus infection 2; herpes simplex 2; enterovirus 1; influenza-like illness 1.

Supplementary Figure 1. Consort diagram: trial profile.



Supplementary Figure 2. A. Progression survival probability (36 months PFS: 76.4%; 95% CI 63.9-91.5) **B. Overall survival probability** (36 months OS: 94.7%;(95% CI 89.7-99.9)**.**



Supplementary Figure 3. Prognostic impact of biologic factors on progression-free survival (PFS). **A. PFS by *TP*53 disruption** (24 months PFS, *TP*53 disruption absent vs present: 93.6% vs 46.9% [HR, 6.96; 95%CI: 2.02-23.97] p=0.002). **B. PFS by IGHV mutational status** (36 months PFS, M-IGHV vs UM-IGHV, 92% vs 65.5% [HR, 0.322; 95%CI: 0.07-1.47] p=0.14). Abbreviations:*TP*53 disruption present, *TP*53+; *TP*53 disruption absent, *TP*53-; unmutated IGHV, UM-IGHV; mutated IGHV, M-IGHV.