US UNIVERSITY OF SUSSEX

Sussex Research

Long-term follow-up of 415 patients with chronic lymphocytic leukemia treated with fludarabine and cyclophosphamide-based chemoimmunotherapy in the frontline ADMIRE and ARCTIC trials: a comprehensive assessment of prognostic factors

David John Allsup, Zoe Craig, David Cairns, Dena Howard, Anna Hockaday, Adrian Bloor, Zarni Soe, Christopher Pepper, Valter Gattei, Antonella Zucchetto, Pauline Robbe, Ruth Clifford, Anna Schuh, Talha Munir, Andrew Rawstron, Peter Hillmen

Publication date
10-06-2023

Licence
This work is made available under the Copyright not evaluated licence and should only be used in accordance with that licence. For more information on the specific terms, consult the repository record for this item.

Document Version
Accepted version

Citation for this work (American Psychological Association 7th edition)
Allsup, D. J., Craig, Z., Cairns, D., Howard, D., Hockaday, A., Bloor, A., Soe, Z., Pepper, C., Gattei, V., Zucchetto, A., Robbe, P., Clifford, R., Schuh, A., Munir, T., Rawstron, A., & Hillmen, P. (2022). *Long-term follow-up of 415 patients with chronic lymphocytic leukemia treated with fludarabine and cyclophosphamide-based chemoimmunotherapy in the frontline ADMIRE and ARCTIC trials: a comprehensive assessment of prognostic factors* (Version 1). University of Sussex. https://hdl.handle.net/10779/uos.23488052.v1

Published in
American Journal of Hematology

Link to external publisher version
https://doi.org/10.1002/ajh.26483

Copyright and reuse:
This work was downloaded from Sussex Research Open (SRO). This document is made available in line with publisher policy and may differ from the published version. Please cite the published version where possible. Copyright and all moral rights to the version of the paper presented here belong to the individual author(s) and/or other copyright owners unless otherwise stated. For more information on this work, SRO or to report an issue, you can contact the repository administrators at sro@sussex.ac.uk. Discover more of the University's research at https://sussex.figshare.com/

Supplementary material

Long-term follow-up of 415 chronic lymphocytic leukaemia patients treated with fludarabine and cyclophosphamide-based chemoimmunotherapy in the frontline ADMIRE and ARCTIC trials, a comprehensive assessment of prognostic factors

David Allsup PhD[1]*, Zoe Craig MSc[2]*, David Cairns PhD[2], Dena Howard PhD[2], Anna Hockaday PhD[2], Adrian Bloor PhD[3], Zarni Soe MD[4], Christopher Pepper PhD[5], Valter Gattei PhD[6], Antonella Zucchetto PhD[6], Pauline Robbe PhD[7], Ruth Clifford PhD[8], Anna Schuh PhD[9], Tahla Munir PhD[4], Andrew Rawstron PhD[10], Peter Hillmen PhD[4,11].

DA and ZC co-first authors.

Affiliations: -

1. Centre for Atherothrombosis and Metabolic Disease, Hull York Medical School, Hull, UK.

2. Clinical Trials Research Unit, University of Leeds, Leeds, UK.

3. The Christie NHS Foundation Trust, Manchester, UK.

4. St James Institute of Oncology, Leeds, UK.

5. Clinical and Experimental Medicine, Brighton and Sussex Medical School, Brighton, UK.

6. Clinical and Experimental Onco-Hematology Unit, Centro di Riferimento Oncologico di Aviano (CRO), IRCCS, Aviano (PN), Italy.

7. Laboratory for Transcriptome Technology, RIKEN Centre for Integrative Medical Sciences, Yokohama, Japan.

8. Department of Haematology, University Hospital Limerick, Limerick, Ireland.

9. Department of Oncology, University of Oxford, Oxford, UK.

10. Haematological Malignancy Diagnostic Service, St James Hospital, Leeds, UK.

11. Leeds Institute of Medical Research at St James's, University of Leeds, Leeds, UK.

Running title.

ARCTIC ADMIRE long-term follow-up.

Corresponding author.

David Allsup.

hycda1@hyms.ac.uk

Methods

Clinical trial design

ADMIRE was a superiority trial comparing FCR with and without mitoxantrone in previously untreated CLL. ARCTIC was a non-inferiority trial comparing FCM-miniR with FCR in previously untreated patients with CLL and results have been published[1,2].

Treatment was with fludarabine cyclophosphamide rituximab (FCR), fludarabine cyclophosphamide mitoxantrone rituximab (FCM-R) or fludarabine cyclophosphamide mitoxantrone with reduced dose rituximab (FCM-miniR) was repeated every 28 days for a total of six cycles. Fludarabine and cyclophosphamide were administered orally at doses of 24 and 150 mg/m^2/day, respectively, for the first five days of each cycle. These doses are pharmacologically equivalent to the doses used when FCR is given intravenously for CLL.[3]

Prognostic factors

Pre-treatment prognostic factors assessed by local investigators included: age (≤65 years, >65 years), Binet stage, beta-2 microglobulin (mg/L), immunoglobulins and direct Coombs' test (DCT). Centrally assessed flow cytometric parameters included: CD20 mean fluorescence intensity (MFI), CD23 MFI, CD24 MFI, CD25 MFI, CD27 MFI, CD38 MFI, CD38 % positive cells, CD38 % positive cells category (<2%,2%-<30%,≥30%), CD49d MFI, CD49d % positive cells, CD62L MFI, CD81 MFI, CD86 MFI, chemokine receptor 6 (CCR6) MFI, CCR6 % positive cells, CCR6 % positive cells category (<30%,≥30%), IgM MFI, IgD MFI, IgM/IgD ratio, leucocyte-associated immunoglobulin receptor 1 (LAIR1) MFI, fluorescent in-situ hybridisation (FISH) assessment of chromosomes 11 and 17, IGHV mutation analysis and sequencing of *TP53, ATM, BIRC3, NOTCH1 and SF3B1*.

Flow cytometric assessment of cell-surface antigens and minimal residual disease (MRD).

Leucocytes were prepared from whole blood or bone marrow by incubation with a ten-fold excess of ammonium chloride (8.6 g/L in distilled water) for 10 minutes at 37°C and washed twice in FACSFlow (BD Biosciences) containing 0.3% bovine serum albumin (FACSFlow/BSA). An aliquot of either 5 x 10^5 (for B-cell clonality analysis) or 2 x 10^6 leucocytes (for MRD assessments) was pipetted into a microtitre plate wells, centrifuged to 2,000rpm and the supernatant discarded. The cell pellet was resuspended in pre-titred antibody mixtures and incubated for 30 minutes at 4°C in the dark, washed twice in FACSFlow/BSA, and resuspended in FACSFlow for acquisition using a Canto II flow

cytometer with FACSDiva software. The B-cell enumeration and clonality assessment applied to all cases comprised acquisition and analysis of at least 1 x 10^5 cells stained with the following antibody and fluorochrome conjugates: Lambda FITC, Kappa PE, CD19 PerCP-Cy5.5, CD5 PE-Cy7, CD20 APC and CD45 APC-H7 (BD Biosciences). Assessment of CD49d was performed as previously described.[4]

MRD was assessed in the bone marrow (BM) three months following completion of therapy with a threshold of greater than 0.01% CLL cells used to define MRD positivity. MRD analysis applied to all cases comprised acquisition and analysis of at least 5 x 10^5cells stained with the following antibody and fluorochrome conjugates: CD20 Pacific Blue (Coulter), CD81 FITC, CD79b PE, CD19 PerCP-Cy5.5, CD5 PE-Cy7, CD43 APC (BD Biosciences). Repeat MRD analysis with the addition of CD3 APC-H7 was performed in cases with 0.008-0.012% with suspected residual disease from the first data acquisition. Patient cases were classified as having measurable residual disease if a discrete population of chronic lymphocytic leukaemia (CLL) phenotype cells comprising ≥ 50 events was identified per event file (0.01% limit of quantitation for a 5 x 10^5 event file) and no detectable disease if <20 CLL-phenotype events were identifiable (0.004% limit of detection for a 5 x 10^5 event file). Cases with 20-50 CLL-phenotype events were classified as having MRD below the quantitative range.

Genes involved in CLL pathogenesis were sequenced by targeted massively parallel sequencing (Illumina): *SF3B1*, *ATM*, *TP53*, *NOTCH1*, *BIRC3*. Mutations predicted as somatic, defined as not being present in databases of common polymorphisms, and functional consequences: non-/frameshift indels, stop codon mutations, missense, and splice site mutations with a variant allele frequency (VAF) ≥ 5% were included for all genes. In addition, mutations in *TP53* with a VAF between 1% and 5% were also included and defined as minor subclones. IGHV gene sequences were analysed using IgBlast (v1.3.0) (https://www.ncbi.nlm.nih.gov/igblast/) and those with at least 98% homology to germline were regarded as unmutated.

Potential prognostic factors and markers analysed

The following potential prognostic factors and markers were analysed in univariable Cox regression models of progression free survival (PFS) and overall survival (OS): -

Potential prognostic factors:

- Age at randomisation (≤65 years, >65 years).
- Binet stage (A Progressive, B, C).
- B2 microglobulin (mg/L) (≤3.5mg/L, >3.5mg/L).

- IgA category (≤detectable (low), ≥normal).
- IgG category (≤detectable (low), ≥normal).
- IgM category (≤detectable (low), ≥normal).
- Direct Coombs Test (positive, negative).
- 3-month post-treatment BM MRD status (positive, negative): Patients with missing MRD status (n=60) were not included in the univariable analysis as their PFS and OS was found to fall between the MRD positive and MRD negative groups so it was deemed not appropriate to include these patients as MRD positive. For these patients, their MRD status was imputed in the imputation model specified below for the multivariable model.
- IGHV mutation status (mutated/unmutated/equivocal).
- IGHV mutation and 3-month post-treatment BM MRD status (IGHV mutated/MRD positive, IGHV mutated/MRD negative, IGHV unmutated/MRD positive, IGHV unmutated/MRD negative).
- 17p deletion status (deleted, not deleted).
- 17p and 11q deletion status (either deleted, neither deleted).
- CLL-IPI score.
- CLL-IPI risk category (low risk, intermediate risk, high risk, very high risk).
- TP53 (mutated, unmutated).
- ATM (mutated, unmutated).
- BIRC3 (mutated, unmutated).
- NOTCH1 (mutated, unmutated).
- SF3B1 (mutated, unmutated).
- Deletion 17p &/or mutated TP53 (yes, no).

Potential prognostic markers:

- CD20 mean fluorescence intensity (MFI).
- CD23 MFI.
- CD24 MFI.
- CD25 MFI.
- CD27 MFI.
- CD38 MFI.
- CD38 % positive cells.
- CD38 % positive cells category (<2%, 2%-<30%, ≥30%).
- CD49d MFI.
- CD49d % positive cells.

- CD62L MFI.
- CD81 MFI.
- CD86 MFI.
- CCR6 MFI.
- CCR6 % positive cells.
- CCR6 % positive cells category (<30%, ≥30%).
- IgM MFI.
- IgD MFI.
- IgM/IgD ratio.
- LAIR1 MFI.

Statistics

PFS and OS were estimated according to allocated treatment, toxicities, and the number of treatment cycles using the Kaplan-Meier method and Cox regression models, adjusting for the minimisation factors Binet staging (A or B, C), age (≤65 years, >65 years) and gender. Where PFS was analysed by the number of treatment cycles, only those who prematurely discontinued treatment due to toxicity, rather than disease progression, were included in the ≤3 cycles group. PFS was defined as time from randomisation to progression or death. OS was defined as time from randomisation to death. Individuals were censored at the last date they were known to be alive and progression-free for PFS, and alive for OS. The cumulative incidence function of death was estimated by nonparametric maximum likelihood estimation.

Grade three and four adverse events (AEs) were summarised by baseline immunoglobulin levels. Selected prognostic factors were compared according to number of treatment cycles. Continuous and categorical variables were evaluated with the two-sample *t*-test and chi-squared test respectively; non-parametric equivalents were used where appropriate.

PFS and OS according to potential prognostic factors were estimated using the Kaplan-Meier method, using the observed data. All variables except MRD status were measured at baseline; MRD status was measured three months post-treatment. Multiple imputation by chained equations accounted for missing data, with 42 imputed datasets generated[5]. Univariable Cox regression models estimated the hazard of PFS and OS for each prognostic factor. Penalised Cox models using the least absolute shrinkage and selection operator method selected the most important predictors of PFS and OS[6]. All reported P values are 2-sided and considered significant at the 5% significance level. Statistical analyses were performed using SAS (version 9.4).

Multiple imputation by chained equations (MICE)

Prior to imputation, the data were explored to assess the relationship between missing prognostic factors and survival time using Kaplan Meier curves stratified by a missing value indicator. The log-rank method was used to test for differences between the distribution of survival times for the missing data and non-missing data for each potential prognostic factor for both PFS and OS. Based on this data exploration, data were assumed to be missing at random.

To account for missing data, multiple imputation by chained equations (MICE) was used, with 42 imputed dataset generated, based on the fraction of missing information. Non-normally distributed variables underwent log or shifted-log transformation prior to imputation.

For each missing variable, all other potential prognostic factors were included in the imputation model and the order of imputation was from least missing to most missing. Predictive mean matching (PMM) was used for continuous variables and logistic regression was used for categorical variables. Log-transformed variables were transformed back following imputation and composite and categorical variables of interest were calculated from their parts. Each imputed dataset was then analysed and results were combined following Rubin's rules. Summary statistics of the imputed and observed data were compared to check the imputation process. Univariable PFS and OS Cox regression models for each prognostic factor were generated using the imputed data, as described below.

Penalised Cox regression multivariable model

The penalised Cox models included in the analysis of PFS and OS were generated using the least absolute shrinkage and selection operator (lasso) method, using the following steps:

1. A correlation matrix was generated to explore the correlation between variables within each imputed dataset. Those with a correlation >0.7 were queried with the investigators. CD38 and CD81 were the only highly correlated variables with a correlation of ~0.85 in all imputed datasets. The investigators confirmed that they are not directly associated with each other or interact therefore, both variables were included in the analysis.
2. All continuous variables were standardised prior to inclusion in the model. These variables were the mean fluorescent intensity (MFI) for CD20, CD22, CD24, CD25, CD27, CD38, CD49d, CD62L, CD81, CD86, IgM, IgD and LAIR1 and CD49d % positive cells.

3. Categorical variables were included in the model as dummy variables. The variables included were: age at randomisation, Binet stage, β_2 microglobulin, IgA, IgG, direct Coombs' test, 3 months post-treatment BM MRD status, IGHV mutation status, *TP53* mutation status &/or 17p deletion status, 11q deletion status, *ATM, BIRC3, NOTCH1* and *SF3B1* mutation status, and categorised CCR6 % of positive cells.
4. The optimum value of λ was calculated within each imputed dataset using likelihood cross-validation. The obtained λ was then averaged (mean) across the 42 imputed datasets and this applied as the penalty term within each imputed dataset.
5. The coefficients were extracted from the model for each dataset and the variance calculated via the bootstrap method for each dataset.
6. The coefficients and standard errors were averaged across all 42 imputed datasets and hazard ratios and 95% confidence intervals were estimated from these.

It is important to note that standard errors calculated from penalised regression models may not accurately reflect variance of each estimator but have been included to roughly provide some confidence intervals, which again should be interpreted with caution.

References

1. Munir T, Howard DR, McParland L, et al. Results of the randomized phase IIB ADMIRE trial of FCR with or without mitoxantrone in previously untreated CLL. Leukemia 2017;31(10):2085–2093.
2. Howard DR, Munir T, McParland L, et al. Results of the randomized phase IIB ARCTIC trial of low-dose rituximab in previously untreated CLL. Leukemia 2017;31(11):2416–2425.
3. Dearden CE, Richards S, Else M, Catovsky D, Hillmen P. A comparison of the efficacy and safety of oral and intravenous fludarabine in chronic lymphocytic leukemia in the LRF CLL4 trial. Cancer 2011;117(11):2452–2460.
4. Bulian P, Shanafelt TD, Fegan C, et al. CD49d is the strongest flow cytometry-based predictor of overall survival in chronic lymphocytic leukemia. Journal of Clinical Oncology 2014;32(9):897–904.
5. Kunkel D, Kaizar EE. A comparison of existing methods for multiple imputation in individual participant data meta-analysis. Statistics in medicine 2017;36(22):3507–3532.
6. Vasquez MM, Hu C, Roe DJ, Chen Z, Halonen M, Guerra S. Least absolute shrinkage and selection operator type methods for the identification of serum biomarkers of overweight and obesity: simulation and application. BMC Medical Research Methodology 2016 16:1 2016;16(1):1–19.

Supplementary Tables

Table 1. Baseline characteristics.

	Total (n=415)
Age at randomisation	
Mean (s.d.)	61.9 (8.17)
Median (range)	63.0 (33, 80)
Patient gender	
Male	298 (71.8%)
Female	117 (28.2%)
Ethnicity	
White	400 (96.4%)
Black (black Caribbean, black African, other)	5 (1.2%)
Asian (Indian, Pakistani, Bangladeshi, other)	4 (1.0%)
Other	6 (1.4%)
Unknown	0 (0.0%)
Binet Stage	
A Progressive	60 (14.5%)
B	206 (49.6%)
C	149 (35.9%)
Time from diagnosis to randomisation (months)	
Mean (s.d.)	36.2 (41.86)
Median (range)	24.9 (0, 273)

Table 2. Allocated treatment by trial.

	ADMIRE (n=215)	**ARCTIC (n=200)**	**Total (n=415)**
Randomised treatment			
FCR	107 (49.8%)	100 (50.0%)	207 (49.9%)
FCM-R	108 (50.2%)	N/A	108 (26.0%)
FCM-miniR	N/A	100 (50.0%)	100 (24.1%)

Table 3A. Univariable Cox regression analysis of PFS by prognostic factors**.**

	Estimate	Standard Error	Hazard ratio and 95% CI	p-value
Age at randomisation				
>65 years vs. ≤65 years	0.23036	0.13312	1.26 (0.97 to 1.63)	0.0835
Binet stage				
B vs. A Progressive	0.02794	0.19574	1.03 (0.70 to 1.51)	0.8865
C vs. A Progressive	-0.10322	0.20688	0.90 (0.60 to 1.35)	0.6178
B2 microglobulin (mg/L)				
>3.5mg/L vs. ≤3.5mg/L	0.21605	0.14980	1.24 (0.93 to 1.66)	0.1493
IgA category				
≤detectable (low) vs. ≥normal	-0.13961	0.13278	0.87 (0.67 to 1.13)	0.2931
IgG category				
≤detectable (low) vs. ≥normal	-0.07595	0.13600	0.93 (0.71 to 1.21)	0.5766
IgM category				
≤detectable (low) vs. ≥normal	-0.20514	0.13962	0.81 (0.62 to 1.07)	0.1418
Direct Coombs Test				
Positive vs. Negative	0.33799	0.18618	1.40 (0.97 to 2.02)	0.0697
3 month post-treatment BM MRD status				
Positive vs. Negative	1.50120	0.15149	4.49 (3.33 to 6.04)	<.0001
IGHV mutation status				
Unmutated vs. mutated	0.962209	0.158659	2.62 (1.92 to 3.57)	<.0001
Equivocal vs. mutated	0.830538	0.325650	2.29 (1.21 to 4.35)	0.0109
IGHV mutation and 3 month post-treatment BM MRD status				
IGHV mutated/MRD positive vs. IGHV mutated/MRD negative	1.53913	0.28294	4.66 (2.68 to 8.12)	<.0001
IGHV unmutated/MRD negative vs. IGHV mutated/MRD negative	0.92233	0.26912	2.52 (1.48 to 4.26)	0.0006
IGHV unmutated/MRD positive vs. IGHV mutated/MRD negative	2.40658	0.25304	11.10 (6.76 to 18.22)	<.0001
17p deletion status				
Deleted vs. Not deleted	0.71003	0.29731	2.03 (1.13 to 3.65)	0.0175
17p and 11q deletion status				
Either deleted vs. Neither deleted	0.68680	0.14972	1.99 (1.48 to 2.67)	<.0001
CLL-IPI score				

	Estimate	Standard Error	Hazard ratio and 95% CI	p-value
HR per 1 point increase	0.18454	0.04429	1.20 (1.10 to 1.31)	<.0001
CLL-IPI risk category				
Intermediate risk vs. Low risk	-0.05563	0.26361	0.95 (0.56 to 1.59)	0.8329
High risk vs. Low risk	0.59770	0.24477	1.82 (1.12 to 2.94)	0.0147
Very high risk vs. Low risk	1.01571	0.33072	2.76 (1.44 to 5.29)	0.0023
TP53				
Mutated vs. Unmutated	0.68283	0.25493	1.98 (1.19 to 3.28)	0.0084
ATM				
Mutated vs. Unmutated	0.19368	0.20870	1.21 (0.80 to 1.83)	0.3543
BIRC3				
Mutated vs. Unmutated	0.00417	0.28137	1.00 (0.58 to 1.75)	0.9882
NOTCH1				
Mutated vs. Unmutated	-0.11224	0.22324	0.89 (0.58 to 1.39)	0.6156
SF3B1				
Mutated vs. Unmutated	0.31434	0.19137	1.37 (0.94 to 2.00)	0.1020
Deletion 17p &/or mutated TP53				
Yes vs. No	0.65524	0.24034	1.93 (1.20 to 3.10)	0.0073

Table 3B. Univariable Cox regression analysis of PFS by flow cytometrically assessed prognostic markers.

	Estimate	Standard Error	Hazard ratio and 95% CI	p-value
CD20 MFI	-0.000098058	0.000033049	0.99990 (0.99984 to 0.99997)	0.0030
CD23 MFI	-0.000023869	0.000022516	0.99998 (0.99993 to 1.00002)	0.2891
CD24 MFI	-0.000001061	0.000007426	1.00000 (0.99998 to 1.00001)	0.8864
CD25 MFI	-0.000012619	0.000076114	0.99999 (0.99984 to 1.00014)	0.8683
CD27 MFI	-0.000026689	0.000024199	0.99997 (0.99993 to 1.00002)	0.2701
CD38	0.000008874	0.000011038	1.00001 (0.99999 to 1.00003)	0.4214

	Estimate	Standard Error	Hazard ratio and 95% CI	p-value
CD38 (% positive cells)	0.006403	0.002306	1.00642 (1.00188 to 1.01098)	0.0055
CD38 % positive cells (category)				
≥30% vs. 2%-<30%	0.181157	0.153326	1.20 (0.89 to 1.62)	0.2375
<2% vs. 2%-<30%	-0.350016	0.184474	0.70 (0.49 to 1.01)	0.0578
CD49d (% positive cells)	0.005031	0.002261	1.00504 (1.00059 to 1.00952)	0.0266
CD62L	-0.000019553	0.000012060	0.99998 (0.99996 to 1.00000)	0.1050
CD81	-0.000013493	0.000024028	0.99999 (0.99994 to 1.00003)	0.5744
CD86	-0.000472	0.000341	0.99953 (0.99886 to 1.00020)	0.1665
CCR6	-0.000597	0.000144	0.99940 (0.99912 to 0.99969)	<.0001
CCR6 (% positive cells)	-0.011056	0.002730	0.98900 (0.98373 to 0.99431)	<.0001
CCR6 % positive cells (category)				
≥30% vs. <30%	-0.543797	0.178840	0.58 (0.41 to 0.82)	0.0024
IgM	-0.000019894	0.000073010	0.99998 (0.99984 to 1.00012)	0.7853
IgD	-0.000001037	0.000020263	1.00000 (0.99996 to 1.00004)	0.9592
IgM/IgD ratio	-0.052252	0.057495	0.94909 (0.84781 to 1.06247)	0.3637
LAIR1	-0.000089916	0.000037793	0.99991 (0.99984 to 0.99998)	0.0174

Table 3C. Multivariable penalised Cox regression analysis of PFS.

	Parameter Estimate	Standard Error	Hazard ratio (HR) and 95% CI	Number of times variable selected out of 42 imputed datasets^
IGHV mutation status Unmutated vs. Mutated	0.328	0.13	1.39 (1.08 to 1.79)	42
3 month post-treatment BM MRD status Positive vs. Negative	1.03	0.12	2.81 (2.22 to 3.56)	42
Standardised CD49d (% of positive cells)	0.0302	0.0416	1.03 (0.95 to 1.12)	31
Deletion 17p &/or mutated TP53 Yes vs. No	0.0862	0.1	1.09 (0.896 to 1.33)	20
Mutated SF3B1 Yes vs. No	0.00477	0.028	1 (0.951 to 1.06)	2
Mutated BIRC3 Yes vs. No	0.000146	0.00737	1 (0.986 to 1.01)	1
Standardised LAIR1	0.0000961	0.0188	1 (0.964 to 1.04)	1

Table 4A. Univariable Cox regression analysis of OS by prognostic factors.

	Estimate	Standard Error	Hazard ratio and 95% CI	p-value
Age at randomisation				
>65 years vs. ≤65 years	0.60005	0.18257	1.82 (1.27 to 2.61)	0.0010
Binet stage				
B vs. A Progressive	-0.46935	0.24694	0.63 (0.39 to 1.01)	0.0573
C vs. A Progressive	-0.47436	0.26245	0.62 (0.37 to 1.04)	0.0707
B2 microglobulin (mg/L)				
>3.5mg/L vs. ≤3.5mg/L	0.32944	0.21554	1.39 (0.91 to 2.12)	0.1264
IgA category				
≤detectable (low) vs. ≥normal	-0.35035	0.18461	0.70 (0.49 to 1.01)	0.0577
IgG category				
≤detectable (low) vs. ≥normal	-0.24542	0.19420	0.78 (0.53 to 1.14)	0.2063
IgM category				
≤detectable (low) vs. ≥normal	-0.21973	0.19092	0.80 (0.55 to 1.17)	0.2498
Direct Coombs Test				
Positive vs. Negative	0.61119	0.23348	1.84 (1.17 to 2.91)	0.0089
3 month post-treatment BM MRD status				
Positive vs. Negative	0.85283	0.19977	2.35 (1.59 to 3.47)	<.0001
IGHV mutation status				
Unmutated vs. mutated	0.498099	0.210053	1.65 (1.09 to 2.48)	0.0178
Equivocal vs. mutated	0.687293	0.411765	1.99 (0.89 to 4.46)	0.0952
IGHV mutation and 3 month post-treatment BM MRD status				
IGHV mutated/MRD positive vs. IGHV mutated/MRD negative	0.99342	0.34709	2.70 (1.37 to 5.33)	0.0042
IGHV unmutated/MRD negative vs. IGHV mutated/MRD negative	0.45922	0.33624	1.58 (0.82 to 3.06)	0.1721
IGHV unmutated/MRD positive vs. IGHV mutated/MRD negative	1.23804	0.29330	3.45 (1.94 to 6.13)	<.0001
17p deletion status				
Deleted vs. Not deleted	0.96280	0.33073	2.62 (1.37 to 5.01)	0.0037
17p and 11q deletion status				
Either deleted vs. Neither deleted	0.18971	0.21477	1.21 (0.79 to 1.84)	0.3771
CLL-IPI score				
HR per 1 point increase	0.17644	0.05433	1.19 (1.07 to 1.33)	0.0013

	Estimate	Standard Error	Hazard ratio and 95% CI	p-value
CLL-IPI risk category				
Intermediate risk vs. Low risk	-0.09091	0.37250	0.91 (0.44 to 1.90)	0.8072
High risk vs. Low risk	0.37625	0.33548	1.46 (0.75 to 2.81)	0.2621
Very high risk vs. Low risk	1.00441	0.41730	2.73 (1.20 to 6.20)	0.0164
TP53				
Mutated vs. Unmutated	0.60761	0.30271	1.84 (1.01 to 3.34)	0.0461
ATM				
Mutated vs. Unmutated	0.15409	0.31056	1.17 (0.63 to 2.15)	0.6204
BIRC3				
Mutated vs. Unmutated	0.23376	0.37486	1.26 (0.60 to 2.65)	0.5338
NOTCH1				
Mutated vs. Unmutated	0.20749	0.27046	1.23 (0.72 to 2.09)	0.4434
SF3B1				
Mutated vs. Unmutated	-0.00818	0.26727	0.99 (0.59 to 1.68)	0.9756
Deletion 17p &/or mutated TP53				
Yes vs. No	0.63079	0.28305	1.88 (1.08 to 3.28)	0.0268

Table 4B. Univariable Cox regression analysis of OS by flow cytometrically assessed prognostic markers.

	Estimate	Standard Error	Hazard ratio and 95% CI	p-value
CD20 MFI	-0.000058031	0.000038084	0.99994 (0.99987 to 1.00002)	0.1276
CD23 MFI	-0.000035049	0.000035136	0.99996 (0.99990 to 1.00003)	0.3186
CD24 MFI	0.000000709	0.000010873	1.00000 (0.99998 to 1.00002)	0.9480
CD25 MFI	-0.000057383	0.000113	0.99994 (0.99972 to 1.00016)	0.6113
CD27 MFI	0.000000212	0.000029336	1.00000 (0.99994 to 1.00006)	0.9942
CD38	0.000007855	0.000018073	1.00001 (0.99997 to 1.00004)	0.6638

	Estimate	Standard Error	Hazard ratio and 95% CI	p-value
CD38 (% positive cells)	-0.000132	0.003416	0.99987 (0.99319 to 1.00659)	0.9693
CD38 positive cells (category)				
≥30% vs. 2%-<30%	-0.168244	0.224697	0.85 (0.54 to 1.31)	0.4541
<2% vs. 2%-<30%	-0.249071	0.244707	0.78 (0.48 to 1.26)	0.3088
CD49d (% positive cells)	0.007801	0.003260	1.00783 (1.00139 to 1.01432)	0.0173
CD62L	-0.000023537	0.000017937	0.99998 (0.99994 to 1.00001)	0.1895
CD81	0.000014155	0.000016678	1.00001 (0.99998 to 1.00005)	0.3960
CD86	-0.000193	0.000451	0.99981 (0.99892 to 1.00069)	0.6691
CCR6	-0.000439	0.000186	0.99956 (0.99920 to 0.99993)	0.0184
CCR6 (% positive cells	-0.005789	0.003600	0.99423 (0.98724 to 1.00127)	0.1079
CCR6 % positive cells (category)				
≥30% vs. <30%	-0.219794	0.234972	0.80 (0.51 to 1.27)	0.3496
IgM	0.000018675	0.000097771	1.00002 (0.99983 to 1.00021)	0.8485
IgD	0.000012436	0.000026735	1.00001 (0.99996 to 1.00006)	0.6418
IgM/IgD ratio	-0.000089260	0.043345	0.99991 (0.91846 to 1.08858)	0.9984
LAIR1	-0.000118	0.000058830	0.99988 (0.99977 to 1.00000)	0.0452

Table 5. Number and proportion of patients with PFS and OS events by occurrence of grade 3 or 4 adverse events (AE).

Any type of AE	**Grade 3 or 4 AE (n=299)**	**No Grade 3 or 4 AE (n=116)**	**Total (n=415)**
PFS event?			
No	118 (39.5%)	57 (49.1%)	175 (42.2%)
Yes	181 (60.5%)	59 (50.9%)	240 (57.8%)
OS event?			
No	203 (67.9%)	91 (78.4%)	294 (70.8%)
Yes	96 (32.1%)	25 (21.6%)	121 (29.2%)
Haematological AEs	**Grade 3 or 4 AE (n=264)**	**No Grade 3 or 4 AE (n=151)**	**Total (n=415)**
PFS event?			
No	107 (40.5%)	68 (45.0%)	175 (42.2%)
Yes	157 (59.5%)	83 (55.0%)	240 (57.8%)
OS event?			
No	179 (67.8%)	115 (76.2%)	294 (70.8%)
Yes	85 (32.2%)	36 (23.8%)	121 (29.2%)
Infection AEs	**Grade 3 or 4 AE (n=49)**	**No Grade 3 or 4 AE (n=366)**	**Total (n=415)**
PFS event?			
No	13 (26.5%)	162 (44.3%)	175 (42.2%)
Yes	36 (73.5%)	204 (55.7%)	240 (57.8%)
OS event?			
No	28 (57.1%)	266 (72.7%)	294 (70.8%)
Yes	21 (42.9%)	100 (27.3%)	121 (29.2%)

Table 6A. Cox regression analysis of PFS by occurrence of any grade 3 or 4 AE, haematological AEs and infections AEs accounting for the stratification factors (age, sex and Binet stage).

	Degrees of freedom	Estimate	Adjusted Hazard Ratio and 95% CI	Test Statistic	p-value
Any grade 3/4 toxicity experienced?*					
Yes vs. No	1	0.17	1.19 (0.89 to 1.60)	1.35	0.2454
Grade 3/4 haematological toxicity experienced?*					
Yes vs. No	1	0.04	1.04 (0.80 to 1.36)	0.09	0.7585
Grade 3/4 infection toxicity experienced?*					
Yes vs. No	1	0.42	1.52 (1.07 to 2.17)	5.38	0.0204

* Each toxicity type was included in its own model, adjusted for the stratification factors only.

Table 6B. Cox regression analysis of OS by occurrence of any grade 3 or 4 AE, haematological AEs and infections AEs accounting for the stratification factors (age, sex and Binet stage).

	Degrees of freedom	Estimate	Adjusted Hazard Ratio and 95% CI	Test Statistic	p-value
Any grade 3/4 toxicity experienced?*					
Yes vs. No	1	0.39	1.47 (0.94 to 2.29)	2.91	0.0881
Grade 3/4 haematological toxicity experienced?*					
Yes vs. No	1	0.28	1.32 (0.89 to 1.95)	1.89	0.1687
Grade 3/4 infection toxicity experienced?*					
Yes vs. No	1	0.49	1.64 (1.02 to 2.63)	4.22	0.0398

* Each toxicity type was included in its own model, adjusted for the stratification factors only.

Table 7A. Number and proportion of patients with any grade 3/4 toxicity by immunoglobulin levels.

	Grade 3 or 4 (n=299)	No Grade 3 or 4 (n=116)	Total (n=415)
IgA level			
Undetectable	9 (3.0%)	5 (4.3%)	14 (3.4%)
Detectable (low)	156 (52.2%)	52 (44.8%)	208 (50.1%)
Normal	117 (39.1%)	51 (44.0%)	168 (40.5%)
High	7 (2.3%)	4 (3.4%)	11 (2.7%)
Missing	10 (3.3%)	4 (3.4%)	14 (3.4%)
IgG level			
Undetectable	4 (1.3%)	2 (1.7%)	6 (1.4%)
Detectable (low)	112 (37.5%)	40 (34.5%)	152 (36.6%)
Normal	156 (52.2%)	64 (55.2%)	220 (53.0%)
High	13 (4.3%)	6 (5.2%)	19 (4.6%)
Paraprotein	2 (0.7%)	0 (0.0%)	2 (0.5%)
N/A (or IVIg)	2 (0.7%)	0 (0.0%)	2 (0.5%)
Missing	10 (3.3%)	4 (3.4%)	14 (3.4%)
IgM level			
Undetectable	13 (4.3%)	5 (4.3%)	18 (4.3%)
Detectable (low)	178 (59.5%)	81 (69.8%)	259 (62.4%)
Normal	93 (31.1%)	24 (20.7%)	117 (28.2%)
High	3 (1.0%)	2 (1.7%)	5 (1.2%)
Paraprotein	2 (0.7%)	0 (0.0%)	2 (0.5%)
Missing	10 (3.3%)	4 (3.4%)	14 (3.4%)

Table 7B. Number and proportion of patients with a haematological grade 3/4 toxicity by immunoglobulin levels.

	Grade 3 or 4 (n=264)	No Grade 3 or 4 (n=151)	Total (n=415)
IgA level			
Undetectable	8 (3.0%)	6 (4.0%)	14 (3.4%)
Detectable (low)	137 (51.9%)	71 (47.0%)	208 (50.1%)
Normal	105 (39.8%)	63 (41.7%)	168 (40.5%)
High	6 (2.3%)	5 (3.3%)	11 (2.7%)
Missing	8 (3.0%)	6 (4.0%)	14 (3.4%)

	Grade 3 or 4 (n=264)	No Grade 3 or 4 (n=151)	Total (n=415)
IgG level			
Undetectable	4 (1.5%)	2 (1.3%)	6 (1.4%)
Detectable (low)	101 (38.3%)	51 (33.8%)	152 (36.6%)
Normal	138 (52.3%)	82 (54.3%)	220 (53.0%)
High	10 (3.8%)	9 (6.0%)	19 (4.6%)
Paraprotein	2 (0.8%)	0 (0.0%)	2 (0.5%)
N/A (or IVIg)	1 (0.4%)	1 (0.7%)	2 (0.5%)
Missing	8 (3.0%)	6 (4.0%)	14 (3.4%)
IgM level			
Undetectable	12 (4.5%)	6 (4.0%)	18 (4.3%)
Detectable (low)	155 (58.7%)	104 (68.9%)	259 (62.4%)
Normal	85 (32.2%)	32 (21.2%)	117 (28.2%)
High	2 (0.8%)	3 (2.0%)	5 (1.2%)
Paraprotein	2 (0.8%)	0 (0.0%)	2 (0.5%)
Missing	8 (3.0%)	6 (4.0%)	14 (3.4%)

Table 7C. Number and proportion of patients with an infection grade 3/4 toxicity by immunoglobulin levels.

	Grade 3 or 4 (n=49)	No Grade 3 or 4 (n=366)	Total (n=415)
IgA level			
Undetectable	2 (4.1%)	12 (3.3%)	14 (3.4%)
Detectable (low)	23 (46.9%)	185 (50.5%)	208 (50.1%)
Normal	22 (44.9%)	146 (39.9%)	168 (40.5%)
High	1 (2.0%)	10 (2.7%)	11 (2.7%)
Missing	1 (2.0%)	13 (3.6%)	14 (3.4%)
IgG level			
Undetectable	1 (2.0%)	5 (1.4%)	6 (1.4%)
Detectable (low)	15 (30.6%)	137 (37.4%)	152 (36.6%)
Normal	30 (61.2%)	190 (51.9%)	220 (53.0%)
High	2 (4.1%)	17 (4.6%)	19 (4.6%)
Paraprotein	0 (0.0%)	2 (0.5%)	2 (0.5%)
N/A (or IVIg)	0 (0.0%)	2 (0.5%)	2 (0.5%)
Missing	1 (2.0%)	13 (3.6%)	14 (3.4%)

	Grade 3 or 4 (n=49)	No Grade 3 or 4 (n=366)	Total (n=415)
IgM level			
Undetectable	3 (6.1%)	15 (4.1%)	18 (4.3%)
Detectable (low)	24 (49.0%)	235 (64.2%)	259 (62.4%)
Normal	20 (40.8%)	97 (26.5%)	117 (28.2%)
High	0 (0.0%)	5 (1.4%)	5 (1.2%)
Paraprotein	1 (2.0%)	1 (0.3%)	2 (0.5%)
Missing	1 (2.0%)	13 (3.6%)	14 (3.4%)

Table 8. Characteristics of patients with Richter's transformation.

	Total (n=12)
VH mutation status	
Mutated	5 (41.7%)
Unmutated	7 (58.3%)
17p deletion status	
No	11 (91.7%)
Missing	1 (8.3%)
TP53 mutated	
Yes	1 (8.3%)
No	7 (58.3%)
Missing	4 (33.3%)
NOTCH1 mutated	
Yes	1 (8.3%)
No	7 (58.3%)
Missing	4 (33.3%)

Table 9. Patients with AML/MDS.

	FCR	FCMR	FCMminiR	Total
Randomised treatment	9 (52.9%)	5 (29.4%)	3 (17.6%)	17 (100%)
Time from end of treatment to AML/MDS diagnosis (months)				
Mean (s.d.)	36.4 (18.43)	50.5 (32.15)	16.3 (12.93)	37.0 (24.22)
Median (range)	35.0 (5, 64)	45.7 (14, 84)	22.6 (1, 25)	34.9 (1, 84)
IQR	34, 45	26, 83	1, 25	23, 46
Patient received next line of CLL therapy? (of those who progressed)				
Yes	1 (14.3%)	1 (25.0%)	1 (33.3%)	3 (21.4%)
No	6 (85.7%)	3 (75.0%)	2 (66.7%)	11 (78.6%)

Table 10. Prognostic factors by number of treatment cycles received.

	≤ 3 cycles (n=55)	> 3 cycles (n=360)	Total (n=415)	p-value
Age at randomisation				0.0380
Mean (s.d.)	64.0 (7.85)	61.6 (8.17)	61.9 (8.16)	
Median (range)	65.0 (40, 74)	62.0 (33, 80)	63.0 (33, 80)	
Binet stage				0.5833
A Progressive	8 (14.5%)	52 (14.4%)	60 (14.5%)	
B	24 (43.6%)	182 (50.6%)	206 (49.6%)	
C	23 (41.8%)	126 (35.0%)	149 (35.9%)	
IGHV mutation status				0.2100
Mutated	25 (45.5%)	126 (35.0%)	151 (36.4%)	
Unmutated	23 (41.8%)	182 (50.6%)	205 (49.4%)	
Equivocal	1 (1.8%)	17 (4.7%)	18 (4.3%)	
Missing	6 (10.9%)	35 (9.7%)	41 (9.9%)	
Beta2 microglobulin concentration*				0.6464
Mean (s.d.)	4.6 (1.41)	4.6 (1.87)	4.6 (1.81)	
Median (range)	4.1 (3, 8)	4.3 (2, 14)	4.2 (2, 14)	
BM MRD 3 months post-treatment				0.0000
MRD positive	28 (50.9%)	141 (39.2%)	169 (40.7%)	
MRD negative	3 (5.5%)	183 (50.8%)	186 (44.8%)	
Missing	24 (43.6%)	36 (10.0%)	60 (14.5%)	
PB MRD 3 months post-treatment				0.0000
MRD positive	19 (34.5%)	63 (17.5%)	82 (19.8%)	
MRD negative	6 (10.9%)	254 (70.6%)	260 (62.7%)	
Missing	30 (54.5%)	43 (11.9%)	73 (17.6%)	
13q14 deleted				0.3896
Yes	14 (25.5%)	87 (24.2%)	101 (24.3%)	
No	6 (10.9%)	58 (16.1%)	64 (15.4%)	
Missing	35 (63.6%)	215 (59.7%)	250 (60.2%)	
Trisomy12*				1.0000
Yes	2 (3.6%)	18 (5.0%)	20 (4.8%)	
No	18 (32.7%)	127 (35.3%)	145 (34.9%)	
Missing	35 (63.6%)	215 (59.7%)	250 (60.2%)	
11q23 deleted				0.3987

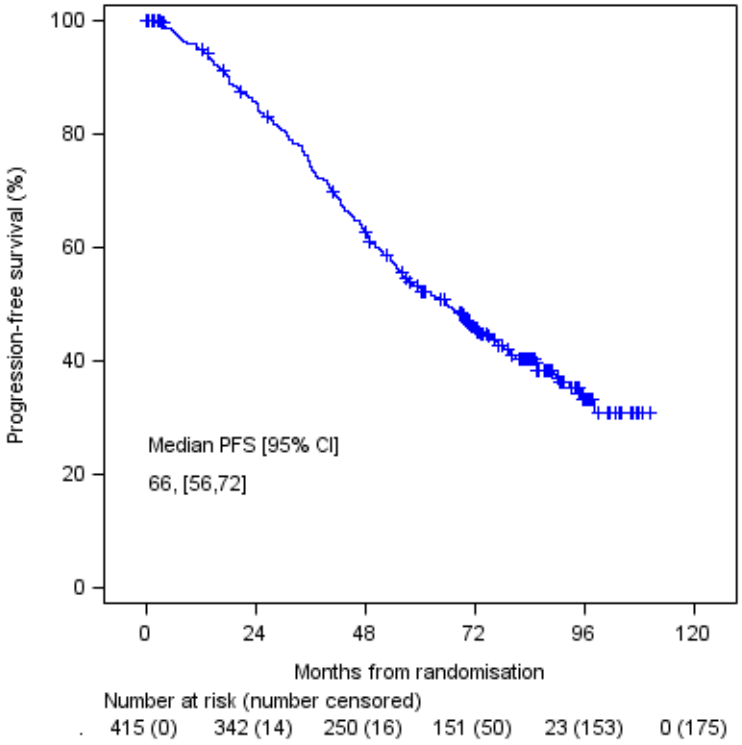
	≤ 3 cycles (n=55)	> 3 cycles (n=360)	Total (n=415)	p-value
Yes	11 (20.0%)	57 (15.8%)	68 (16.4%)	
No	40 (72.7%)	283 (78.6%)	323 (77.8%)	
Missing	4 (7.3%)	20 (5.6%)	24 (5.8%)	
17p deleted*				0.0021
Yes	8 (14.5%)	13 (3.6%)	21 (5.1%)	
No	41 (74.5%)	324 (90.0%)	365 (88.0%)	
Missing	6 (10.9%)	23 (6.4%)	29 (7.0%)	

* Non-parametric test used

Supplementary Figures

Figure 1. Survival curves for the whole cohort displaying progression-free (1A) and overall survival (1B).

A



B

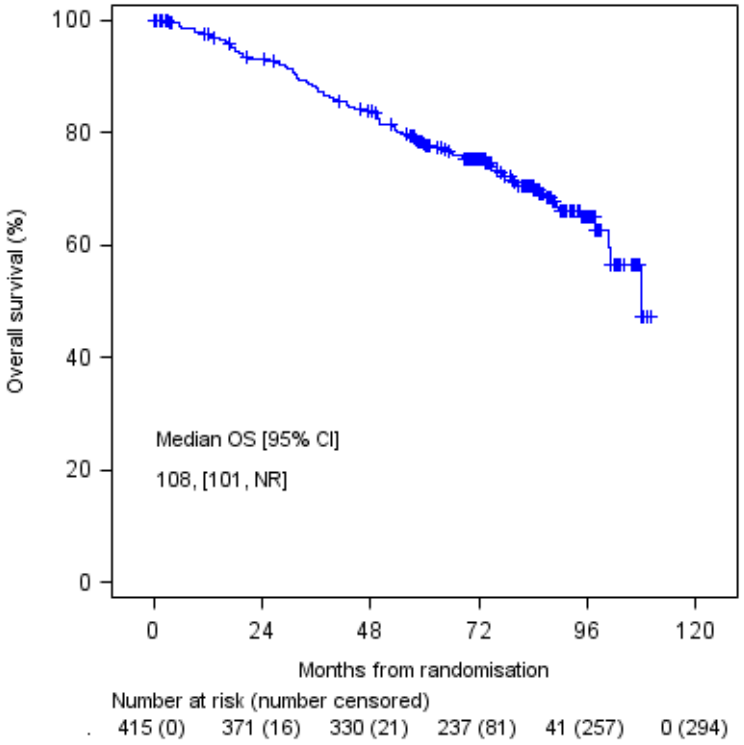


Figure 2. Survival curves for FCR, FCMR and FCM-miniR displaying progression free (2A) and overall survival (2B).

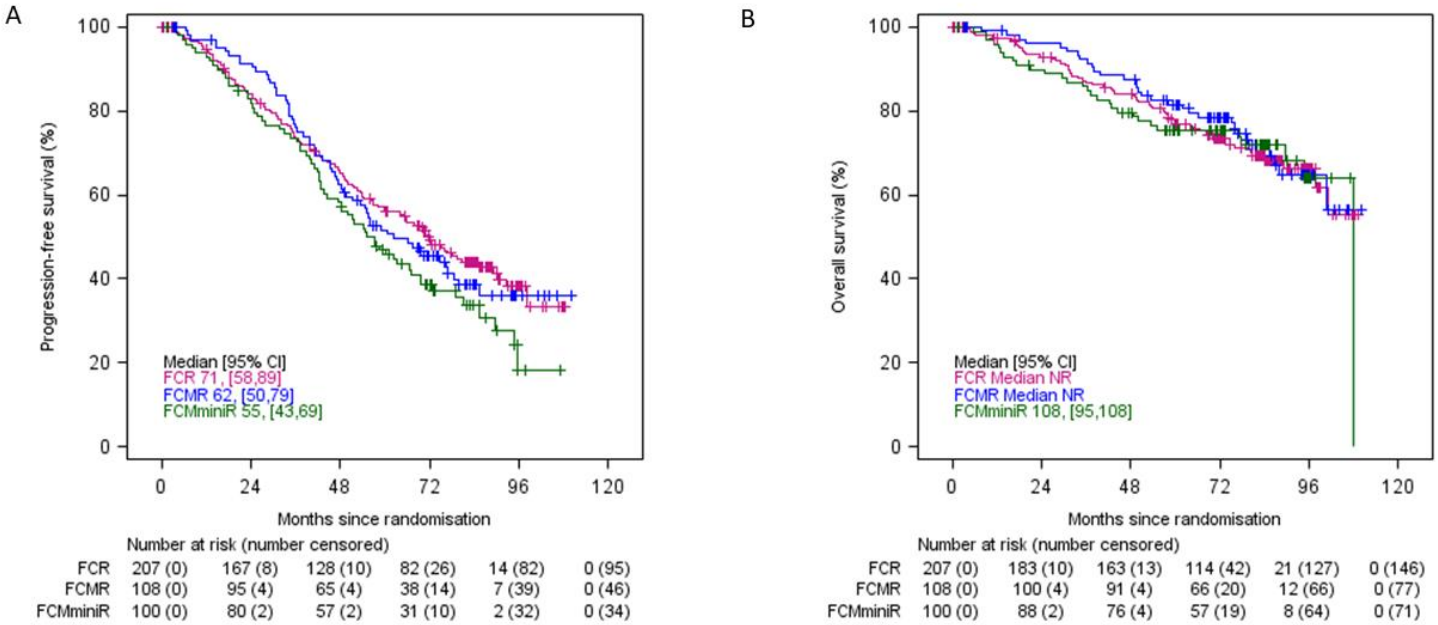


Figure 3. Survival curves for all patients according to IGHV mutational status displaying progression free (3A) and overall survival (3B).

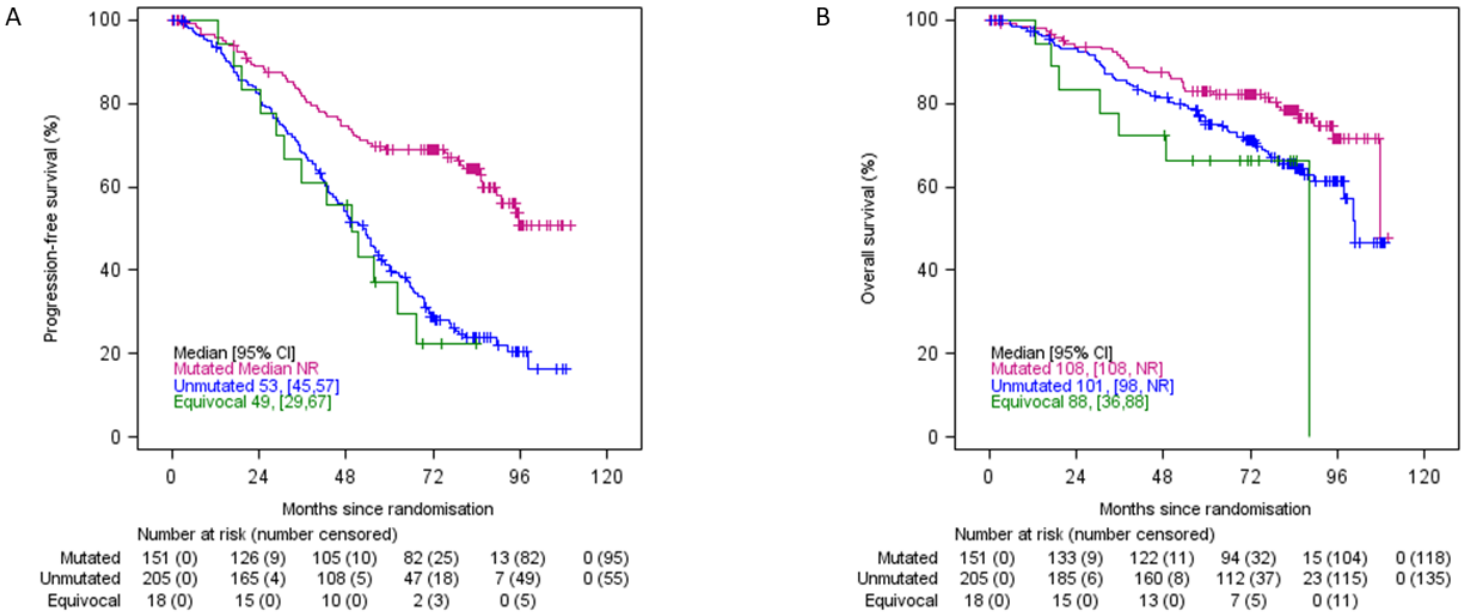
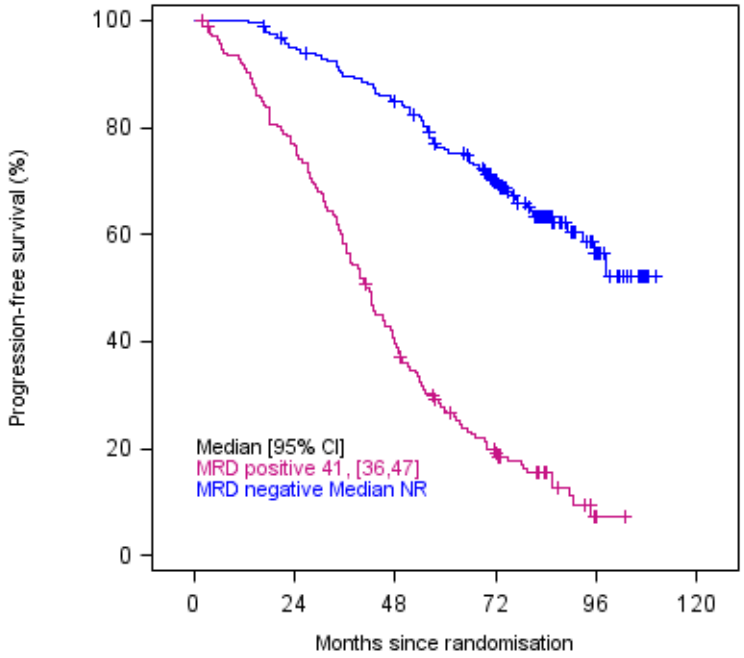


Figure 4. Survival curves, from randomisation, for all patients according to 3-month post-treatment bone marrow minimal residual disease status displaying progression-free (2A) and overall survival (2B).

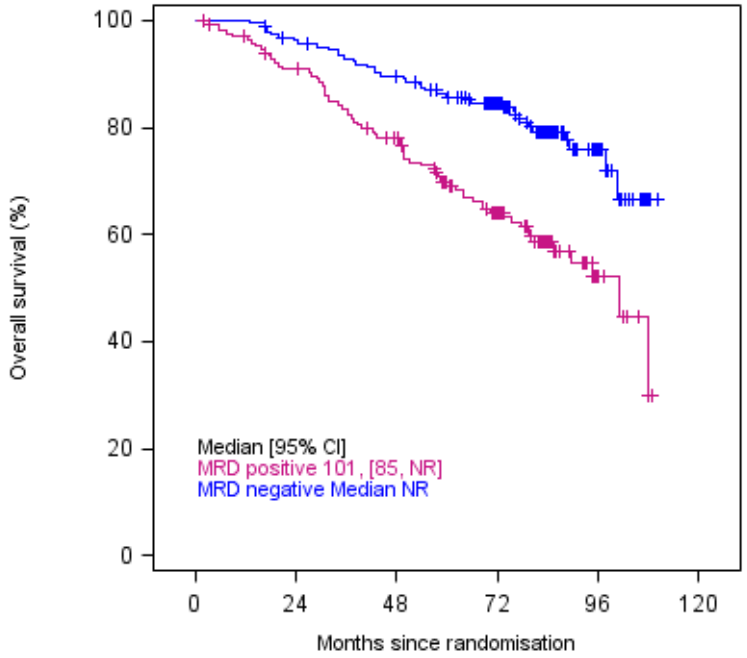
A



Number at risk (number censored)

MRD positive	169 (0)	127 (3)	66 (4)	28 (9)	2 (25)	0 (27)
MRD negative	186 (0)	175 (2)	155 (3)	110 (22)	19 (100)	0 (118)

B



Number at risk (number censored)

MRD positive	169 (0)	149 (5)	124 (9)	83 (29)	12 (91)	0 (101)
MRD negative	186 (0)	177 (2)	164 (3)	130 (28)	25 (124)	0 (147)

Figure 5. Survival curves for all patients according to IGHV mutational and MRD status displaying progression free (5A) and overall survival (5B).

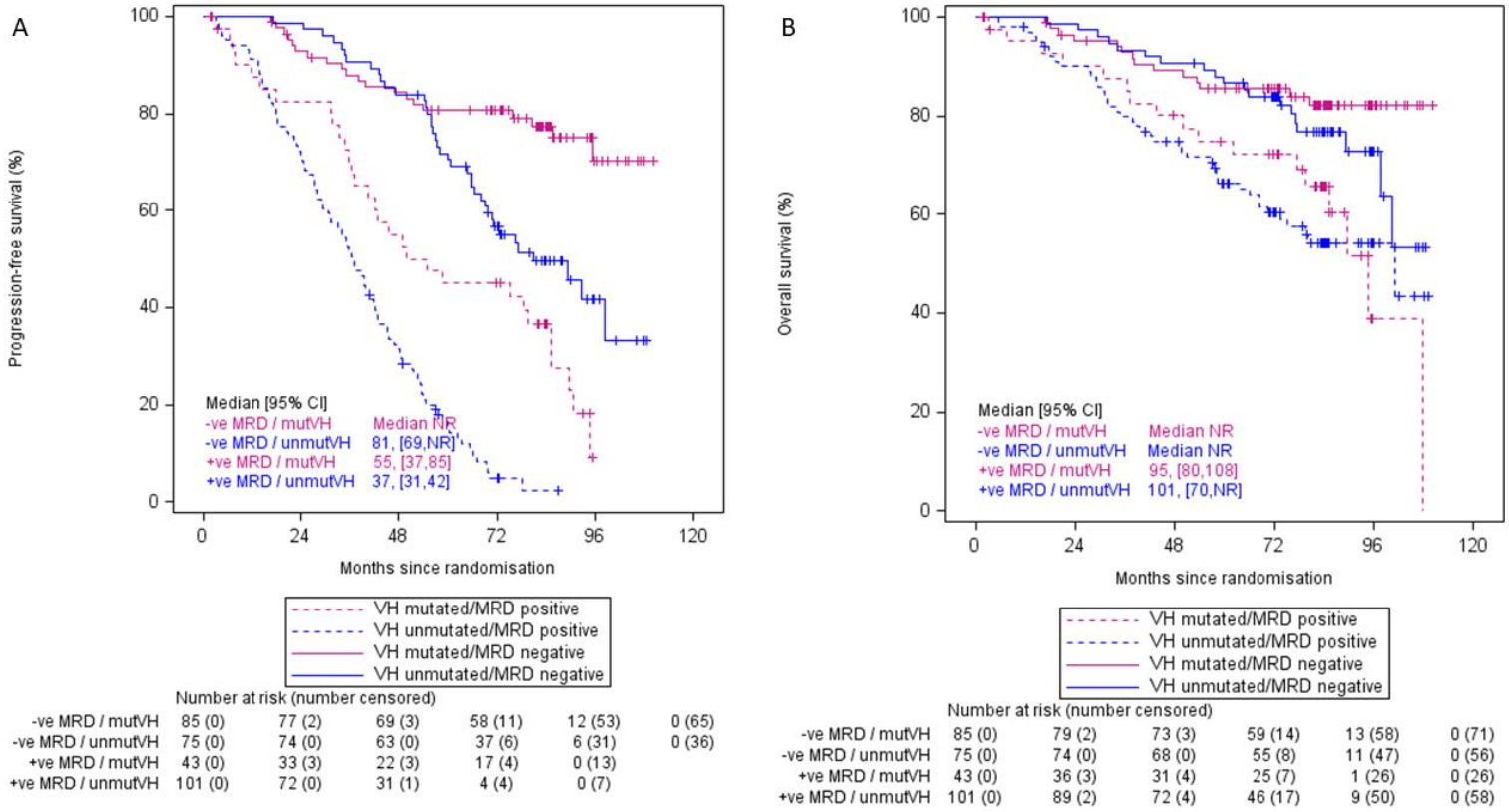


Figure 6. Survival curves for all patients according to the presence or absence of a grade 3/4 infectious episode displaying progression free (6A) and overall survival (6B).

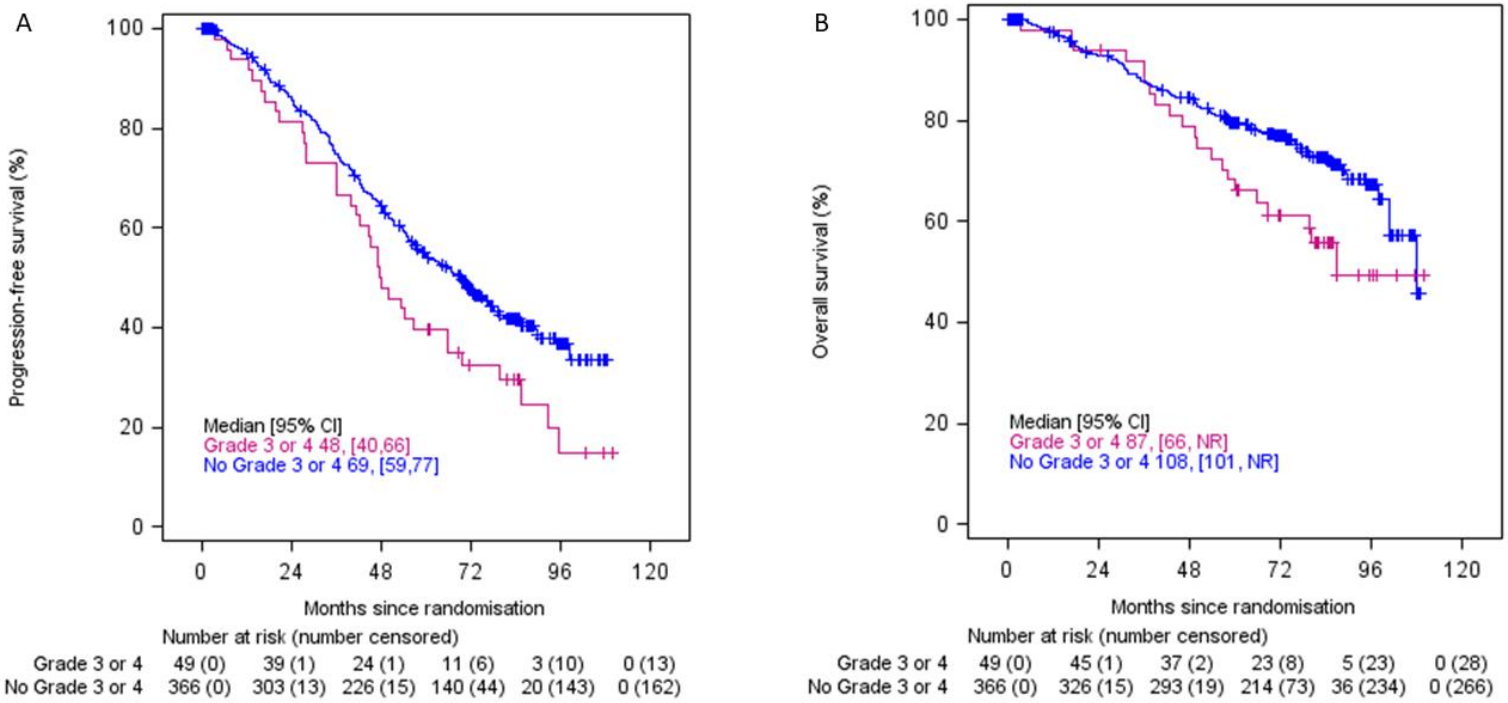


Figure 7. Survival curves for all patients according to whether <=3 or >3 cycles of trial therapy were delivered displaying progression free (5A) and overall survival (5B). For PFS, only data for patients whose therapy was discontinued for reasons other than disease progression are displayed in the <=3 cycles group.

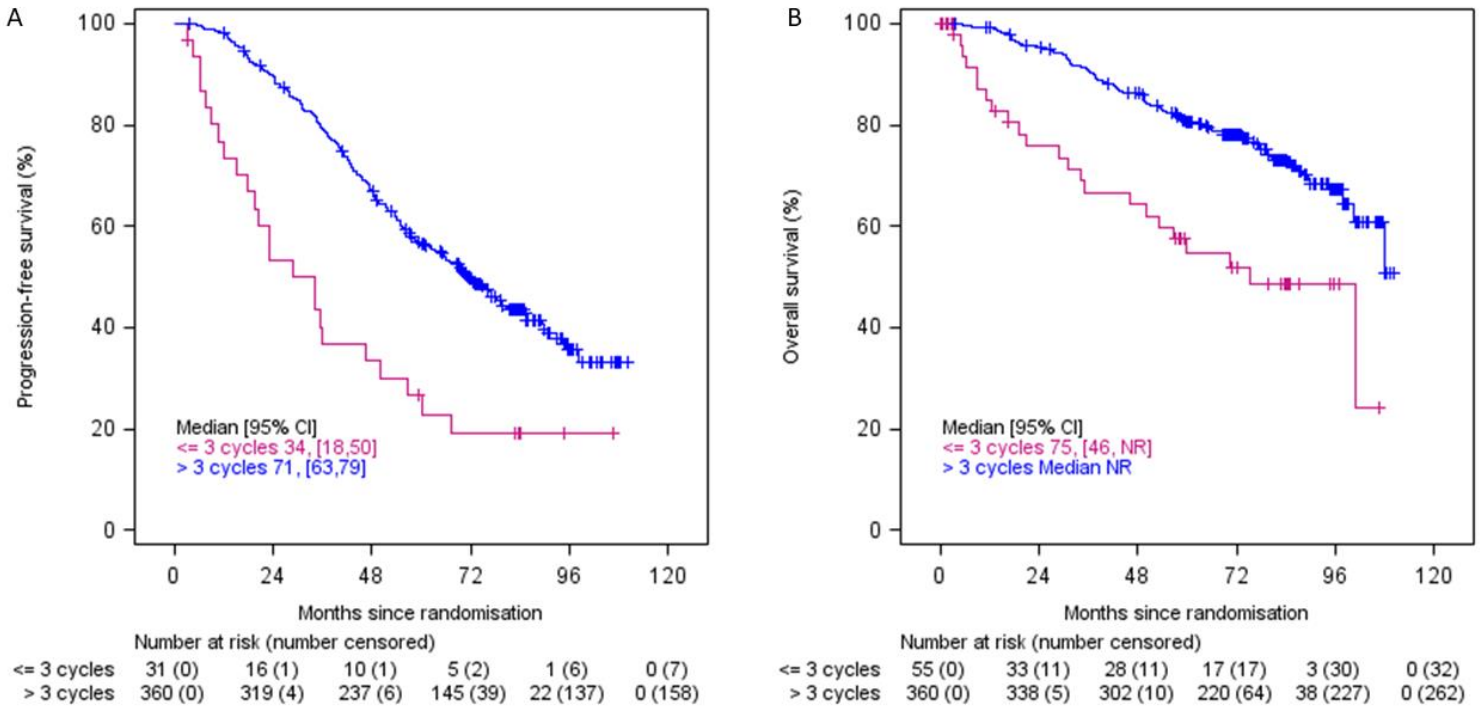


Figure 8. Post-progression treatment by year (NB: Includes all lines of treatment post-progression).