

# 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](mailto:sro@sussex.ac.uk). Discover more of the University's research at <https://sussex.figshare.com/>

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

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

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](mailto:hycda1@hyms.ac.uk)

Word count.

Main text 1484 words.

Trial registration.

ADMIRE was registered as an International Standard Randomized Controlled Trial (ISRCTN42165735) and on the European Clinical Trials Database (EudraCT: 2008-006342-122 25). ARCTIC was registered as an International Standard Randomized Controlled Trial (ISRCTN16544962) and on the European Clinical Trials Database (EudraCT: 2009-010998-20).

#### Funding Statement

ADMIRE was a National Institute for Health Research (NIHR) Portfolio Study developed in association with the NCRI CLL Subgroup and funded by Roche Products Ltd. ARCTIC was funded by the NIHR Health Technology Assessment (HTA) programme. This work was also supported by Core Clinical Trials Unit Infrastructure from Cancer Research UK (C7852/A25447). We also acknowledge funding from Progetto Ricerca Finalizzata PE-2016-02362756 (to VG), and RF-2018-12365790 (to AZ), Italian Ministry of Health, Rome, Italy; Associazione Italiana Ricerca Cancro (AIRC), Investigator Grant IG-21687 (to VG).

#### Conflict of Interest

Dr Allsup reports personal fees and research funding from Roche Pharmaceuticals. Prof. Hillmen received research funding and speakers' fees from Roche Pharmaceuticals. Dr. Rawstron reports personal fees from Roche Pharmaceuticals. Dr. Munir reports personal fees from Roche Pharmaceuticals. Dr. Bloor reports personal fees, consultancy/advisory fees and speakers' fees from Roche Pharmaceuticals.

Ethics approval statement.

Both trials were approved by relevant institutional ethical committees and regulatory review bodies and were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice.

Chemoimmunotherapy (CIT) with fludarabine, cyclophosphamide and rituximab (FCR) has been the mainstay treatment of previously untreated chronic lymphocytic leukaemia (CLL) for many years<sup>1</sup>. A minority of FCR-treated patients are identified as a favourable risk group by the presence of mutated immunoglobulin heavy chain variable genes (M-IGHV), weak CD49d expression, long telomeres and post-therapy assessment of minimal residual disease (MRD)<sup>2-4</sup>. Most patients treated with FCR relapse, and this has driven the development of efficacious therapies targeting the B-cell receptor (BCRi) but exposes patients to risks such as hypertension and other cardiovascular toxicities<sup>5</sup>. Therefore, there may remain a case for CIT in untreated patients with CLL with favourable features.

We report the outcomes of previously untreated patients with CLL after prolonged follow-up following fludarabine and cyclophosphamide-based CIT in two phase II randomised controlled trials, ADMIRE and ARCTIC. ADMIRE compared FCR with fludarabine, cyclophosphamide, mitoxantrone and rituximab (FCMR) whilst ARCTIC compared FCR with fludarabine, cyclophosphamide mitoxantrone and reduced dose rituximab (FCM-minR). In addition to reporting long-term-year outcomes we assessed a range of factors reported to be prognostic to identify robust markers associated with favourable outcomes. Treatment-related toxicities and premature discontinuation of therapy may be associated with inferior outcomes, so we assessed how these events impact patient outcomes. Methods including trial design and assessment of prognostic factors are presented as supplementary information.

ADMIRE/ARCTIC provided outcome data on 415 previously untreated patients with CLL recruited between July 2009-September 2012 (supplementary Tables 1 and 2). Median follow-up is 84 months (interquartile range (IQR): 72-94 months), median PFS is 66 months (95% confidence interval (CI) 56-72 months), and median OS is 108 months (95% CI 101 months-not reached) (Supplementary Figure 1A and B). There is no difference in PFS or OS between FCR and FCM-R (PFS adjusted hazard ratio (aHR) 1.09, 95% CI 0.80-1.49; OS aHR 0.95, 95% CI 0.62-1.47). For FCR and FCM-miniR, a small difference was observed for PFS but not OS (PFS aHR 1.35, 95% CI 1.00-1.84; OS aHR 1.01, 95% CI 0.65-1.57) (Supplementary Figures 2A and 2B).

Five years post-randomisation, 68.9% (95% CI 60.6-75.9%) of patients with M-IGHV CLL were progression-free and 83.0% were alive (95% CI 75.8-88.3%). Unadjusted Cox regression analysis found unmutated IGHV (UM-IGHV) is associated with shortened PFS and OS (PFS HR 2.62, 95% CI 1.92-3.57; OS HR 1.65, 95% CI 1.09-2.48) (Supplementary Figure 3A and 3B). Three months post-treatment 169 patients (40.7%) were bone marrow (BM) MRD positive, 84.0% had a PFS event and 40.2% had an OS event during follow-up, compared to 36.6% and 21.0% who were MRD negative (n=186). Unadjusted Cox regression analysis of

MRD found that MRD positivity is associated with shortened PFS and OS (PFS HR 4.49, 95% CI 3.33-6.04; OS HR 2.35, 95% CI 1.59-3.47) (Supplementary Figure 4A and 4B). The combination of MRD positivity and UM-IGHV resulted in highly shortened PFS and OS compared to M-IGHV and MRD negativity (PFS HR 11.1, 95% CI 6.76-18.22; OS HR 3.45, 95% CI 1.94-6.13) (Supplementary Figure 5A and 5B).

Univariable Cox regression analysis of PFS revealed UM-IGHV, 17p/11q deletion, TP53 mutations (mutTP53) and increasing international prognostic index for CLL (CLL-IPI) are associated with shortened PFS (Supplementary Table 3A). Increasing CD38 (% positive cells), CD49d (% positive cells), and decreasing CD20 (MFI), CCR6 (MFI and % positive cells) and LAIR1 (MFI) are also associated with shortened PFS (Supplementary Table 3B). Multivariable penalised Cox regression found IGHV, MRD, standardised CD49d (% positive cells) and deletion 17p (d17p) &/or mutTP53 are, in combination, most prognostic of PFS (Supplementary Table 3C).

Univariable Cox regression analysis of OS found age greater than 65 years, positive DCT, UM-IGHV, d17p, mutTP53 and increasing CLL-IPI are associated with shortened OS (Supplementary Table 4A). Increasing CD49d (% positive cells), decreasing CCR6 (MFI) and LAIR1 (MFI) are associated with shortened OS (Supplementary Table 4B). Mutations in ATM, BIRC3, NOTCH1 and SF3B1 are not independently associated with PFS or OS. Multivariable penalised Cox regression found MRD, age, CD49d (% positive cells), DCT and d17p and/or mutTP53 are, in combination, most prognostic of OS (Table 1).

The proportion of PFS and OS events is higher in those who experienced any grade 3 or 4 adverse event (AE), a haematological-related grade 3 or 4 AE, or an infection-related grade 3 or 4 AE (Supplementary Table 5), but the presence of any grade 3 or 4 AE or haematological-related grade 3 or 4 AEs are not associated with shortened PFS, or OS (Supplementary Table 6A and B). However, the presence of infection-related grade 3 or 4 AEs are associated with both shortened PFS and OS (PFS aHR 1.52, 95% CI 1.07-2.27; OS HR 1.64, 95% CI 1.02-2.63) (Supplementary Figure 6A and B). Baseline IgA and IgG levels are not associated with grade 3 or 4 AEs, a haematological or an infection grade 3 or 4 AE (Supplementary Table 7A-C).

122 second cancers were diagnosed in 102 of the 415 patients and comprised Richter's transformations 12 (2.9%), acute myeloid leukaemia/myelodysplasia (AML/MDS) 19 (4.6%), skin (non-melanoma) 37 (8.9%), skin (melanoma) 9 (2.2%) and solid tumours 35 (8.4%). In those diagnosed with a second cancer, the median time to diagnosis from randomisation was 34.5 months (IQR: 22, 60 months). For patients developing Richter's there was no preponderance of poor prognostic features (Supplementary Table 8). In patients who

developed AML/MDS, this was not associated with trial therapy and occurred at a median of 34.9 months (IQR 23, 46 months) post-therapy. Of patients who developed AML/MDS three had received more than one line of therapy (Supplementary Table 9).

Patients who receive three or less treatment cycles tend to be older, more likely to have d17p and more likely to be MRD positive (Supplementary Table 10). Receiving three or less treatment cycles is associated with shorter PFS, and OS (PFS aHR 2.66, 95% CI 1.73-4.07; OS aHR 2.62, 95% CI 1.65-4.17) (Supplementary Figure 7A and B). For PFS, only patients who prematurely discontinued therapy due to toxicity, without progression, were included in the analysis.

Of 192 patients who experienced disease progression, 79 (41.1%) received further treatment with 70 (88.6%) receiving 1 line, 8 (10.1%) receiving 2 lines and 1 (1.3%) receiving 3 lines of subsequent treatment. Of these, 46.3% received CIT, 37.5% received BCRi, 5% received BCRi with a BCL2 inhibitor, 13.8% received a monoclonal antibody and 3.8% received steroids. The proportion of patients treated with novel agents at relapse increased over the duration of follow-up with a corresponding decrease in CIT (supplementary figure 8).

We report excellent long-term outcomes in patients with CLL treated with FC-based CIT in two UK clinical trials. With a median follow-up of 84 months the PFS and OS are 65 and 108 months. Patients with favourable factors such as M-IGHV and non-disrupted TP53 have prolonged survival outcomes following FC-based CIT and the incorporation of BM-MRD following therapy further refines the identification of a cohort with prolonged survival.

We assessed all available parameters previously described as prognostic in similar patient populations and identified multiple factors predictive of PFS and OS in univariable analysis. However, the parameters most predictive of PFS on multivariable analysis were CD49d, d17p and mutTP53 combined with post-treatment BM-MRD. Likewise multivariable analysis revealed age, DCT and d17P/mutTP53 combined with post-treatment BM-MRD were most predictive for OS. Thus, a relatively simple combination of prognostic factors and MRD can identify a cohort with prolonged survival. Our results complement those of others who demonstrate that IGHV, d17p, mutTP53 and post-treatment MRD are prognostic following CIT and facilitate the identification of patients with prolonged survival.<sup>2,4</sup>

In addition to assessing the prognostic power of established biomarkers we sought to assess the role of patient specific factors in responses to FC-based CIT. Our finding that infection-related AEs were associated with shortened survival could reflect the impact of infectious events in an immunosuppressed patient population or could be a surrogate for other comorbidities not captured within our data collection. It is likely that a combination of reduced dose intensity due to premature discontinuation of therapy with associated suboptimal disease

control and the consequences of grade 3/4 infections could contribute to inferior outcomes for a subset of patients.

CLL is associated with an increased risk of second malignancies, a risk exacerbated by treatment with regimens such as FC. After prolonged follow-up of around 10 years 25% of patients were diagnosed with at least one new malignancy. Our results confirm that second malignancies remain a significant concern in FC-treated patients and support the role of health promotion interventions known to reduce cancer.

In conclusion we find that treatment of CLL with FC-based CIT is associated with excellent long-term outcomes. We show that the main markers for long-term progression-free and overall survival are MRD, age, IGHV, CD49d, DCT, d17p and mutTP53. The results presented in this paper further aid in the consideration of which patients with CLL may benefit from initial treatment with FCR as opposed to targeted therapy and who may need less intensive follow-up following completion of therapy.

## References

1. Thompson PA, Tam CS, O'Brien SM, et al. Fludarabine, cyclophosphamide, and rituximab treatment achieves long-term disease-free survival in IGHV-mutated chronic lymphocytic leukemia. *Blood* 2016;127(3):303–309.
2. Böttcher S, Ritgen M, Fischer K, et al. Minimal residual disease quantification is an independent predictor of progression-free and overall survival in chronic lymphocytic leukemia: a multivariate analysis from the randomized GCLLSG CLL8 trial. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2012;30(9):980–988.
3. Pepper AGS, Zucchetto A, Norris K, et al. Combined analysis of IGHV mutations, telomere length and CD49d identifies long-term progression-free survivors in TP53 wild-type CLL treated with FCR-based therapies. *Leukemia* 2022;36(1):271–274.
4. Fischer K, Bahlo J, Fink AM, et al. Long-term remissions after FCR chemoimmunotherapy in previously untreated patients with CLL: Updated results of the CLL8 trial. *Blood* 2016;127(2):208–215.
5. Caldeira D, Alves D, Costa J, Ferreira JJ, Pinto FJ. Ibrutinib increases the risk of hypertension and atrial fibrillation: Systematic review and meta-analysis. *PloS one* 2019;14(2):e0211228.



## Tables

### Table Legends

**Table 1. Multivariable penalised Cox regression analysis of OS.** Variables frequently selected from the imputed datasets by the penalised Cox model are more predictive of the outcome than those not selected or selected infrequently. Each variable selected contributes to predicting the outcome in combination with the other selected variables, even if it is not significantly associated with the outcome itself. BM, bone marrow; MRD, minimal residual disease.

**Table 1**

	Parameter Estimate	Standard Error	Hazard ratio (HR) and 95% CI	Number of times variable selected out of the 42 imputed datasets^
<b>3-month post-treatment BM MRD status</b>				
Positive vs. Negative	0.39	0.18	1.48 (1.04 to 2.1)	42
<b>Age at randomisation</b>				
>65 years vs. ≤65 years	0.129	0.15	1.14 (0.847 to 1.53)	42
<b>Standardised CD49d (% of positive cells)</b>	0.0737	0.0686	1.08 (0.941 to 1.23)	36
<b>Direct Coombs Test</b>				
Positive vs. Negative	0.0496	0.147	1.05 (0.788 to 1.4)	24
<b>Deletion 17p &amp;/or mutated TP53</b>				
Yes vs. No	0.098	0.146	1.1 (0.828 to 1.47)	24
<b>Standardised LAIR1</b>	-0.000638	0.0407	0.999 (0.923 to 1.08)	5
<b>Mutated BIRC3</b>				
Yes vs. No	0.00785	0.0725	1.01 (0.874 to 1.16)	3
<b>Mutated ATM</b>				
Yes vs. No	0.00426	0.0316	1 (0.944 to 1.07)	1
<b>Mutated NOTCH1</b>				
Yes vs. No	0.000673	0.0477	1 (0.911 to 1.1)	1