



This is a repository copy of *Eradication of minimal residual disease improves overall and progression free survival in patients with chronic lymphocytic leukaemia, evidence from NCRN CLL207: A Phase II trial assessing alemtuzumab consolidation.*

White Rose Research Online URL for this paper:
<http://eprints.whiterose.ac.uk/103031/>

Version: Accepted Version

Article:

Varghese, A, Howard, DR orcid.org/0000-0003-3333-9783, Pocock, C et al. (9 more authors) (2017) Eradication of minimal residual disease improves overall and progression free survival in patients with chronic lymphocytic leukaemia, evidence from NCRN CLL207: A Phase II trial assessing alemtuzumab consolidation. *British Journal of Haematology*, 176 (4). pp. 573-582. ISSN 0007-1048

<https://doi.org/10.1111/bjh.14342>

© 2016 John Wiley & Sons Ltd. This is the peer reviewed version of the following article: Varghese, A. M., Howard, D. R., Pocock, C., Rawstron, A. C., Follows, G., McCarthy, H., Dearden, C., Fegan, C., Milligan, D., Smith, A. F., Gregory, W., Hillmen, P. and NCRI CLL Sub-Group (2016), Eradication of minimal residual disease improves overall and progression-free survival in patients with chronic lymphocytic leukaemia, evidence from NCRN CLL207: a phase II trial assessing alemtuzumab consolidation. *British Journal of Haematology*. doi: 10.1111/bjh.14342, which has been published in final form at <https://doi.org/10.1111/bjh.14342>. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Self-Archiving.

Reuse

Unless indicated otherwise, fulltext items are protected by copyright with all rights reserved. The copyright exception in section 29 of the Copyright, Designs and Patents Act 1988 allows the making of a single copy solely for the purpose of non-commercial research or private study within the limits of fair dealing. The publisher or other rights-holder may allow further reproduction and re-use of this version - refer to the White Rose Research Online record for this item. Where records identify the publisher as the copyright holder, users can verify any specific terms of use on the publisher's website.

Takedown

If you consider content in White Rose Research Online to be in breach of UK law, please notify us by emailing eprints@whiterose.ac.uk including the URL of the record and the reason for the withdrawal request.



Eradication of minimal residual disease improves overall and progression free survival in patients with chronic lymphocytic leukaemia, evidence from NCRN CLL207: A Phase II trial assessing alemtuzumab consolidation.



Journal:	<i>British Journal of Haematology</i>
Manuscript ID	BJH-2016-00777.R1
Manuscript Type:	Ordinary Papers
Date Submitted by the Author:	11-Jul-2016
Complete List of Authors:	Varghese, Abraham; Leeds Teaching Hospitals NHS Trust, Haematology Howard, Dena; University of Leeds, Clinical Trials Research Unit Pocock, Christopher; East kent hospitals, haematology Rawstron, Andy; Leeds Teaching Hospitals NHS Trust, HMDS Laboratory Follows, George; Cambridge University Hospitals NHS Foundation Trust, Haematology, Box 234 McCarthy, Helen; The Royal Bournemouth and Christchurch Hospitals, Haematology Dearden, Claire; Royal Marsden Hospital, Haematology Fegan, Christopher; University Hospital of Wales, Department of Haematology Milligan, Don; Heart of England NHS Foundation Trust, Haematology Smith, Alexandra; University of Leeds, Clinical Trials Research Unit Gregory, Walter; University of Leeds, Clinical Trials Research Unit Hillmen, Peter; Leeds Teaching Hospitals NHS Trust, Haematology;
Key Words:	CHRONIC LYMPHOCYTIC LEUKAEMIA, MINIMAL RESIDUAL DISEASE, ALEMTUZUMAB

Eradication of minimal residual disease improves overall and progression free survival in patients with chronic lymphocytic leukaemia, evidence from NCRN CLL207: A Phase II trial assessing alemtuzumab consolidation.

Abraham M.Varghese^{1*}, Dena R Howard^{2*}, Christopher Pocock³, Andy C Rawstron^{1,4}, George Follows⁵, Helen McCarthy⁶, Claire Dearden⁷, Christopher Fegan⁸, Donald Milligan⁹, Alexandra F Smith², Walter Gregory² and Peter Hillmen¹
on behalf of NCRI CLL Sub-Group

* These authors contributed equally to this work.

¹Department of Haematology, Leeds Teaching Hospitals NHS Trust, Leeds, UK

²Leeds Institute of Clinical Trials Research, University of Leeds, Leeds, UK

³East Kent Hospitals NHS Trust, Canterbury, UK

⁴Hull York Medical School, University of York, York, UK

⁵Addenbrooke's Hospital, Cambridge, UK

⁶Royal Bournemouth Hospital, Bournemouth, UK

⁷Royal Marsden Hospital, Sutton, UK

⁸University Hospital of Wales, Cardiff, UK

⁹Centre for Haematology and Stem Cell Transplantation, Heartlands Hospital, Birmingham, UK

1
2
3 **Author for correspondence:** Professor Peter Hillmen, St James's University
4 Hospital, Haematology, 3rd Floor, Bexley Wing, St James's University Hospital,
5
6 Beckett Street, Leeds, LS97TF
7
8

9
10 Phone: +441132068513
11

12
13 Fax: +441132068177
14

15
16 email: Peter.Hillmen@nhs.net
17

18
19 **Running Title:** Alemtuzumab consolidation for eradication of MRD in CLL
20

21
22 **Authors disclosures and potential conflict of interest:**
23

24 There are no disclosures or conflicts of interest to report.
25
26
27

28
29
30 **Author contributions**

31
32 **Conception and design:** Peter Hillmen, Andy C Rawstron, Dena R Howard,
33
34 Abraham M.Varghese
35

36
37 **Provision of study materials or patients:** Peter Hillmen, Christopher Pocock,
38
39 George Follows, Helen McCarthy, Claire Dearden, Christopher Fegan, Donald
40
41 Milligan
42

43
44 **Collection and assembly of data:** Dena R Howard, Alexandra F Smith and Walter
45
46 Gregory
47

48
49 **Data analysis and interpretation:** Dena R Howard, Peter Hillmen, Andy C
50
51 Rawstron, Abraham M.Varghese, Alexandra F Smith and Walter Gregory
52

53 **Manuscript writing:** All authors
54

55 **Final approval of manuscript:** All authors
56
57
58
59
60

Summary

With immunochemotherapy, remission duration and survival in patients with CLL is dependent on the level of minimal residual disease after treatment. This phase II trial assessed alemtuzumab consolidation post-chemotherapy in patients who responded with persistent low levels of detectable disease. Blood was screened for MRD using multi-parameter flow cytometry, 6 to 24 months post-chemotherapy. MRD-positive participants received alemtuzumab 30mg subcutaneously 3 times weekly for 6 weeks. Following a marrow assessment, MRD-negative participants or non-responders stopped therapy and MRD-positive participants with 1+ log reduction had 6 more weeks of alemtuzumab. Alemtuzumab consolidation was received by 47 participants. One death and 19 of 22 serious adverse events reported from 17 (36%) participants were alemtuzumab related. MRD eradication from blood and bone marrow was achieved in 39 (83%) participants at the end of consolidation, with 18 (38%) remaining MRD-negative in the blood 6 months later. Of the 18 MRD-negative participants at 6 months, the median time to MRD relapse was 46 months which was similar to patients who were MRD-negative at baseline and were followed up. The 5-year PFS and OS of MRD-negative participants at 6 months was significantly better than MRD-positive participants (PFS: 78%vs39% (p=0.010), OS: 89%vs64% (p=0.029)).

Keywords: Chronic lymphocytic leukaemia, minimal residual disease, alemtuzumab

Introduction

The treatment of Chronic Lymphocytic Leukaemia (CLL) has seen several advances over the past decade including advent of combination immunochemotherapy with Fludarabine, Cyclophosphamide and Rituximab (FCR) as the standard first line treatment and development of new agents such as several monoclonal antibodies, B-cell receptor signalling pathway inhibitors and BCL-2 inhibitors. **It is well established that with immunochemotherapy patients attaining complete remission (CR) have a better survival rate than those attaining partial response or less.** This led to the concept of improving the depth of response up to the point of eradication of minimal residual disease (MRD), which is a standard practice in many other haematological malignancies (Hallek *et al*, 2008). **In CLL patients treated with different immunochemotherapy, Kwok et al has shown that attainment of MRD-negativity is an independent predictor of Overall Survival (OS) and Progression-Free Survival (PFS) in a retrospective analysis** (Kwok *et al*, 2009). In addition MRD-negativity following front-line FCR is a strong predictor of PFS and OS (Böttcher *et al*, 2012) (Strati *et al*, 2014).

At the time of trial recruitment, alemtuzumab, a humanised monoclonal antibody specific for CD52, was licensed for the treatment of refractory CLL. But from 2012 onwards alemtuzumab is available for this purpose only on patient access programs as it was withdrawn by the manufacturer due to non-medical reasons. However the trial still serves as a proof of principle for consolidation treatment, and there are several other effective monoclonal antibodies which can be used for further trials. Previous trials consistently showed that alemtuzumab is more efficacious when administered to patients with lower levels of disease, providing a rationale to use the

1
2
3 drug as consolidation when patients have only MRD detectable after
4
5 chemoimmunotherapy and no bulk disease. The German CLL4B trial, a phase III trial
6
7 to compare alemtuzumab consolidation against observation after purine analogue
8
9 therapy, was prematurely stopped due to infection related toxicity(Wendtner *et al*,
10
11 2004)(Schweighofer *et al*, 2009). A long term follow up of the 21 participants has
12
13 shown that PFS was significantly prolonged for the 11 treated participants vs. those
14
15 in observation arm. Possible reasons for the toxicity were the proximity to initial
16
17 therapy, the dose and the route of administration. In treatment for clinically
18
19 progressive disease, the median duration of alemtuzumab therapy required to attain
20
21 an MRD-negative CR was 9 weeks in a study of 91 participants(Moreton *et al*, 2005).
22
23 The pharmacokinetics of alemtuzumab in MRD is more likely to be related to that
24
25 observed in the bone marrow transplant setting than with conventional CLL
26
27 treatment due to the low level of disease(Hale *et al*, 2004)(Rebello *et al*, 2001). Thus,
28
29 for MRD level treatment, a reasonable dosing strategy was selected to be 30mg
30
31 three times weekly subcutaneously for 6 weeks, at least 6 months after the last
32
33 chemotherapy, with the treatment period extended to 12 weeks if necessary.
34
35 Moreover careful surveillance for infection and pre-emptive treatment, if needed,
36
37 would help to reduce the infection related toxicity in these patients.
38
39
40
41
42

43 **Methods**

44 **Trial design**

45
46
47 CLL207 was a phase II, multi-centre, single-arm study, to determine the efficacy and
48
49 safety of alemtuzumab consolidation in patients with low levels of MRD, defined as
50
51 >1 CLL in 10,000 leucocytes, following conventional therapy. The joint primary
52
53 endpoints were the MRD-negativity rate at the end of therapy and the proportion of
54
55
56
57
58
59
60

1
2
3 participants experiencing a predefined unacceptable level of toxicity. The secondary
4
5 objectives were to assess clinical response to alemtuzumab therapy as defined by
6
7 NCI Criteria, time to MRD relapse, progression free survival, overall survival and the
8
9 pharmacokinetic profile of alemtuzumab in the MRD setting. An unacceptable toxicity
10
11 was defined as any death or grade 3 or above toxicity attributed to the study
12
13 treatment or its complications, excluding lymphopenia, grade 3 neutropenia or grade
14
15 4 neutropenia responding to GCSF. A two stage design was planned to incorporate
16
17 a stopping rule if the treatment was not felt to be acceptable in terms of either
18
19 efficacy or toxicity. The sample size was determined using the Bryant and Day
20
21 design which incorporates toxicity considerations as well as clinical responses(Bryant
22
23 & Day, 1995). The calculation was based on the assumptions that an MRD rate over
24
25 40% and toxicity rate under 20% is desired, and an MRD rate below 20% and toxicity
26
27 rate above 40% is unacceptable. With 10% significance and 90% power, a total of 54
28
29 participants were planned, with the stage I assessment after 24 participants. An
30
31 independent Data Monitoring and Ethics Committee (DMEC) reviewed the safety
32
33 and ethics of the study. The trial was approved by all relevant institutional ethical
34
35 committees and regulatory review bodies, and was conducted in accordance with the
36
37 Declaration of Helsinki and Good Clinical Practice.
38
39
40
41
42

43 **Patients**

44
45 Eligible patients had completed chemotherapy for CLL between 6 to 24 months prior
46
47 to registering, having attained a complete or partial remission. They did not have
48
49 lymph nodes greater than 2cm in diameter by CT-scan nor a peripheral B-cell count
50
51 more than $5 \times 10^9/l$, and had not received more than 3 prior therapies for CLL or an
52
53 allogeneic transplant. Patients who previously failed alemtuzumab therapy and those
54
55 with persisting severe pancytopenia due to previous therapy rather than disease
56
57
58
59
60

1
2
3 were also excluded. MRD status was assessed at entry; MRD-positive participants
4
5 received consolidation therapy in the Main Study; MRD-negative participants were
6
7 followed up within the study and only treated if they become MRD-positive during the
8
9 eligibility period, otherwise they were followed under a Monitoring Investigation.
10
11

12 13 **Treatment**

14
15 Participants were treated with 30mg subcutaneous alemtuzumab three-times weekly
16
17 for 6 weeks after which bone marrow and peripheral blood were assessed for MRD.
18
19 Alemtuzumab was stopped if bone marrow was MRD-negative or if there was no
20
21 significant improvement in the level of CLL cells. Participants who were responding
22
23 but remained MRD-positive were treated with a further 6 weeks of alemtuzumab and
24
25 then reassessed for MRD. Peripheral blood MRD status was reassessed every 3
26
27 months after treatment. Participants were eligible to be retreated with alemtuzumab
28
29 when they become MRD-positive, if they had remained MRD-negative for at least 6
30
31 months (Figure 1). Standard antimicrobial prophylaxis for alemtuzumab were given
32
33 including monitoring of CMV PCR. Participants with neutrophils below $1.0 \times 10^9/l$
34
35 were treated with G-CSF (filgrastim 300 μ g three-times weekly on the days of
36
37 alemtuzumab).
38
39
40
41

42 Treatment was interrupted in the presence of the following events: platelets $<25 \times$
43
44 $10^9/l$ or neutrophils $<0.25 \times 10^9/l$, grade 4 non hematologic toxicity, and grades 3 to 4
45
46 infection. Following the recovery of platelets to $>50 \times 10^9/l$ and neutrophils $>0.5 \times$
47
48 $10^9/l$, alemtuzumab was re-introduced.
49
50

51 52 **Response assessments**

53
54 MRD response assessments were based on the bone marrow MRD findings, and
55
56 were categorised as follows:
57
58
59
60

1
2
3 *MRD-negative response*: No detectable CLL in the bone marrow and peripheral
4
5 blood by 4 colour flow cytometry and absence of lymphadenopathy and
6
7 organomegaly attributed to CLL(Rawstron *et al*, 2007).
8

9
10 *Partial response*: At least 1 logarithmic reduction in bone marrow CLL cells but still
11
12 MRD-positive.
13

14 *Progressive disease*: At least 1 logarithmic increase in bone marrow CLL cells or
15
16 new clinically evident lymphadenopathy or splenomegaly.
17

18 *Stable disease*: Not falling into any of the above categories.
19

20
21 Clinical response was also assessed according to the NCI response criteria
22
23 1996(Cheson *et al*, 1996)
24

25 26 **Pharmacokinetics study** 27

28 A pharmacokinetics study was planned on consenting participants from selected
29
30 centres. Serum alemtuzumab concentration was analysed using indirect
31
32 immunofluorescence described by Rebello and Hale(Hale *et al*, 2004) at baseline,3, 6,
33
34 9 and 12 weeks of treatment, where appropriate, and at 1, 2, 4, 6 and 12 weeks after
35
36 treatment.
37
38

39 40 **Statistical methods** 41

42 Response rates derived based on bone marrow findings as well as NCI criteria are
43
44 summarised for the Main Study population and 95% confidence intervals (CI)
45
46 reported for the proportion of participants who became MRD-negative. Time to MRD
47
48 relapse, PFS and OS are described using Kaplan-Meier curves. Safety is
49
50 summarised by the number and proportion of participants who suffer an
51
52 unacceptable toxicity, and 95% CIs reported. Details of the toxicities are also
53
54 summarised using adverse event and treatment-related mortality rates. Planned
55
56
57
58
59
60

1
2
3 subgroup analyses summarise the primary endpoints, MRD relapse and survival
4 data (where appropriate).
5
6
7

8 **Results**

9 **Participant Characteristics**

10
11
12 Sixty-one participants with written informed consent were registered between
13
14 December 2006 and January 2010 from 11 UK institutions with local ethical and
15
16 management approval. 47 participants were treated within the Main Study, of which
17
18 43 had detectable disease at registration and 4 became MRD-positive within 24
19
20 months of completing their prior therapy. 11 participants remained in the Monitoring
21
22 Investigation and 3 withdrew or were found to be ineligible before receiving
23
24 treatment. The CONSORT diagram (Schulz *et al*, 2010) (Figure 2) shows the flow of
25
26 participants throughout the trial. The recruitment target of 54 participants to the Main
27
28 Study was not met due to slower than anticipated recruitment.
29
30
31

32
33 Baseline characteristics of the participants in the Main Study are displayed in Table
34
35 1. The median age was 58 years (range 40-77) with 75% men. The relatively young
36
37 population is expected given the concerns of additional toxicity with alemtuzumab.
38
39 49% had received just one prior therapy, and 30% had received two (range 1-4).
40
41 98% received prior fludarabine and 19% prior rituximab. 45% had responded to prior
42
43 therapy with a CR and 55% with a PR. The mean neutrophil count of the participants
44
45 was $2.91 \times 10^9/l$ (range 0.7-5.1), lymphocyte count was $1.45 \times 10^9/l$ (range 0.3-6.2),
46
47 49% had a normal or high IgG level and 28% had a low but detectable IgG.
48
49
50
51

52 **Treatment**

53
54 The duration of treatment received is summarised in Table 2. **Of the 47**
55
56 **participants, 6(12.8%) did not receive the full 6 cycles as protocolled.**
57
58
59
60

1
2
3 **12(25.5%) participants were eligible to be treated for a further 6 weeks, and of**
4 **these 4(33.3%) did not receive all 12 cycles.** Reasons for early stopping included
5 Adverse (AEs), CMV reactivation, cytopenia and participant choice. 20(42.6%)
6 participants had a dose delay or modification for at least one week of treatment.
7
8
9

10 11 12 13 **Primary Endpoint Analysis**

14
15 At the analysis of stage I, reported to the DMEC in December 2008, 18/24(75.0%)
16 participants responded to treatment with MRD-negativity, and 5(20.8%) suffered an
17 unacceptable adverse reaction (AR), which did not pass the planned stopping
18 boundaries. The DMEC felt that the toxicity was significant but manageable, and
19 since the efficacy outcome was encouraging, the trial should proceed.
20
21
22
23

24
25
26
27 At the stage II analysis, summarised in Table 2, 39/47 (83%; 95%CI: 69%-92%)
28 responded to treatment with MRD-negativity, which greatly exceeded the stopping
29 boundary for efficacy of 13 responses. The denominator includes the participant with
30 a missing response, to ensure the most conservative response rate by intention to
31 treat. An unacceptable adverse reaction occurred in 10/47(21.3%), which was less
32 than the stopping boundary of 15 participants, so the pre-defined stopping criteria
33 were not met.
34
35
36
37
38
39
40
41
42

43 **Efficacy Endpoints**

44
45 The overall response rate (ORR), defined as complete or partial remission (CR/PR)
46 by NCI Criteria, was 91.5% as summarised in Table 2. **As of August 2015, the**
47 **median follow-up for survivors was 6.3 years. The five-year PFS was 53.2%,**
48 **and OS was 72.2%.** Kaplan-Meier OS and PFS curves are presented in figures 3A
49 and 3B respectively. The median time to progression was 70 months, and the
50 median OS was not yet reached.
51
52
53
54
55
56
57
58
59
60

1
2
3 **Of the 39 participants who became MRD-negative following treatment, the five-**
4 **year survival for MRD relapse, progression or death was 11.2%.** The median
5 duration of MRD-negativity was 6.7 months (95%CI: 5.6,25.0). 21/39 MRD-negative
6 participants (53.8%) became MRD-positive within 6 months of completing therapy,
7 and participants who remained MRD-negative beyond 6 months appeared to have a
8 greater chance of sustained MRD-negativity, as illustrated by the Kaplan-Meier MRD
9 curve presented in figure 3C. If MRD-negativity is assessed in the peripheral blood 6
10 months after completing alemtuzumab, 18/47 participants (38%) reached this target
11 (Table 2). PFS and OS was significantly better in the MRD-negative participants at 6
12 months compared to those who were MRD-positive (p-values 0.010 and 0.029
13 respectively) (figures 4A and 4B).

14
15
16
17
18
19
20
21
22
23
24
25
26
27
28 Fifteen participants were MRD-negative at registration to the trial, 4 of whom became
29 MRD-positive and were eligible for trial treatment, and the remaining 11 were
30 followed up for relapse within the monitoring investigation. As of August 2015, the
31 median follow-up from previous treatment in these 11 participants was 7 years, with
32 one clinical progression at approximately 6 years after treatment and 2 deaths, both
33 approximately 5 years after treatment. Ten of the 15 participants who were MRD-
34 negative at registration have relapsed to become MRD-positive or died. The median
35 duration of MRD-negativity is 59.4 months (95%CI: 24.7,not reached), which was not
36 significantly different to those patients who converted to MRD negativity when
37 assessed 6 months after alemtuzumab consolidation, although the numbers for
38 comparison are small (hazard ratio 1.57 (95%CI: 0.62,3.97)), figure 4C.

53 **Subgroup Analyses**

54
55
56 Nine participants went on to receive 11 or 12 weeks of therapy of which 6(67%)
57 became MRD-negative by the end of treatment, but only 1(11%) remained MRD-
58
59
60

1
2
3 negative at 6 months post-alemtuzumab. This suggests that there is no advantage to
4
5 extend the therapy beyond 6 weeks.
6

7
8 Nine participants had previously received rituximab; all became MRD-negative
9
10 following therapy, and 2 (22%) remained MRD-negative after 6 months. None of
11
12 these participants suffered an unacceptable toxicity.
13

14
15 The MRD responses in participants who had received one (20/23(87%)) or multiple
16
17 prior therapies (19/24(79%)) were comparable. After 6 months these response rates
18
19 became 10/23(44%) and 8/24(33%) respectively. **There was a trend towards more**
20
21 **participants who had received multiple prior therapies suffering an**
22
23 **unacceptable toxicity (7/24(29%) vs 3/23(13%)), and having a worse median**
24
25 **PFS and OS (PFS 71 vs 45 months; OS not reached vs 60 months). Since the**
26
27 **subgroup analyses are exploratory, it was not planned to assess significance**
28
29 **in the differences between the subgroups, but the trends seen are expected**
30
31 **due to the nature of the disease and treatment.**
32
33

34 35 Safety

36
37 As of August 2015, 15 participants in the main trial (31.9%) have died. One death,
38
39 caused by parainfluenza pneumonitis, was considered directly related to treatment.
40
41 Two other deaths were the outcome of SAEs, although not directly related to
42
43 treatment: myelodysplasia related infection and EBV driven lymphoproliferative
44
45 disorder. Other causes of death were: 4 CLL related infections; 3 overwhelming
46
47 tumour burden; 2 transformed disease; 1 Crohn's Disease; 1 pulmonary embolism; 1
48
49 multiple myeloma. 22 SAEs were reported from 17(36.2%) participants, with
50
51 19suspected to be related to alemtuzumab, of which 2 were unexpected (SUSARs):
52
53 the parainfluenza infection resulting in death and an EBV related diffuse large B-cell
54
55 lymphoma (DLBCL) leading to upper gastrointestinal haemorrhage. SAEs and AEs
56
57
58
59
60

1
2
3 are summarised in Table 2. Eleven events from 10 participants were considered
4 unacceptable as per protocol definition. Four were considered highly unacceptable: 1
5 pneumocystis pneumonia; 1 parainfluenza related death and 2 EBV related
6 transformed diseases. Seven other events were easily manageable but considered
7 unacceptable due to requirement of intravenous antibiotics (n=5), grade 4
8 thrombocytopenia (n=1) and grade 4 neutropenia not responding to G-CSF (n=1).
9
10 414 AEs were reported with 339 suspected to be alemtuzumab related, the most
11 common being rashes(13%), cytopenia(13%), fatigue(8%), non-specific respiratory
12 symptoms(8%) and fever(5%). Twenty-one(44.7%) of the treated participants had a
13 positive CMV PCR during alemtuzumab; 19(90.5%) occurred within the first 3 weeks
14 of treatment. 30 participants received growth factor support, before treatment(n=3),
15 while on treatment(n=12) or after treatment(n=15). The range of G-CSF support
16 varied widely from pre-treatment to 27 months post treatment, with median
17 duration 11 months (range 0–67 months).
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33

34 **Of the 12 participants receiving more than 6 weeks of treatment, 4 SAEs**
35 **related to alemtuzumab were reported from 3 (25%) participants. Of these, only**
36 **2 were reported after their 6th cycle. Of the 11 events that were deemed**
37 **unacceptable, only 1 was from 1 participant who received 12 cycles (at cycle**
38 **12). The other 10 were within the first 6 cycles. This may imply that most of the**
39 **toxicity happens within the first 6 weeks of treatment itself.**
40
41
42
43
44
45
46
47

48 **Pharmacokinetics study**

49
50 Serum alemtuzumab concentration was analysed in 5 participants. Lympholytic level
51 was reached within 3 weeks (when the 1st post dose sample was tested) in all
52 participants, unlike refractory patients treated with alemtuzumab in whom it took an
53 average of 6 weeks to reach a concentration of 1µg/mL . The mean highest
54
55
56
57
58
59
60

1
2
3 concentration in individual participants was 8.24 μ g/mL(range2.70 μ g/mL-
4
5 11.97 μ g/mL), similar to the refractory patient group. The drug was detected in
6
7 plasma up to 11 weeks after finishing treatment.
8
9

10 **Discussion**

11
12 Alemtuzumab consolidation is efficient, with 83% of participants attaining MRD-
13
14 negativity at the end of treatment. This high rate of MRD-negativity was not
15
16 sustained, and 54% of MRD-negative participants relapsed at the MRD level in the
17
18 peripheral blood by 6 months. At this time the MRD relapse curve flattened,
19
20 suggesting that the 18 participants who remained MRD-negative in their peripheral
21
22 blood at 6 months had a sustained MRD-negative response. Although a number of
23
24 participants relapsed quickly at the MRD level, this did not translate into a fast
25
26 clinical progression or death. Of the 47 treated participants, the 5 year PFS and OS
27
28 rates were 53% and 72% respectively.
29
30
31

32
33 Faster rate of MRD relapse within the first 6 months after treatment compared to
34
35 beyond this period is probably explained by the redistribution of malignant cells
36
37 between compartments. It is well described that alemtuzumab clears the disease in
38
39 blood and marrow much more effectively than nodal disease. It seems likely that
40
41 residual disease in lymph nodes redistributes to the blood and marrow within the first
42
43 6 months after completing alemtuzumab suggesting that this is not a true relapse,
44
45 but a redistribution of resistant disease. The participants who relapse beyond this
46
47 initial 6 month period represent a true progression.
48
49

50
51 This study also demonstrates that patients benefiting from alemtuzumab
52
53 consolidation tend to respond within the first 6 weeks of treatment. In addition,
54
55 peripheral blood MRD level at 6 months after consolidation is a truer indicator of
56
57 persistent disease at MRD level than a marrow at the end of treatment, which would
58
59
60

1
2
3 be an unnecessary investigation as it is unlikely to change the future disease
4
5 management.

6
7 Participants who attained MRD-negativity have a better PFS and OS compared to
8
9 those who remained MRD-positive. Previously, CALGB-10101, an alemtuzumab
10
11 consolidation trial, showed no statistically significant difference in 2-years PFS and
12
13 OS between consolidated and non-consolidated groups(PFS 76%vs68% p=0.35;OS
14
15 84%vs88% p=1.0), but here the consolidation treatment was given only to
16
17 participants in PR or SD, but not MRD-positive CRs(Lin *et al*, 2010). GermanCLL4B,
18
19 however, randomised just 21 participants but showed a significant difference
20
21 between participants who were consolidated vs not consolidated, with 3-year PFS
22
23 81.8% vs 30.0% (p=0.004)⁵, with participants receiving alemtuzumab having
24
25 pronounced reductions in MRD levels. **Long term follow up data from the German**
26
27 **CLL8 trial has also shown that low ($< 10^{-4}$) level MRD was associated with**
28
29 **significantly better PFS and OS compared to intermediate ($\geq 10^{-4}$ to $<10^{-2}$) and**
30
31 **high ($\geq 10^{-2}$) level MRD(Böttcher *et al*, 2012). Although MRD negativity is not**
32
33 necessary to improve outcomes with certain therapies, such as B-cell receptor
34
35 inhibitors, achieving MRD negativity is associated with a better outcome in all
36
37 scenarios that have been studied and as one goal of future therapy is to move to a
38
39 defined shorter duration of therapy MRD eradication will be an end-point that can be
40
41 used to decide when to stop novel therapies such as ibrutinib or venetoclax. **In this**
42
43 **setting, as far as we know, the remission will be sustained only if the treatment**
44
45 **is continued, but it will be useful to know whether a combination or**
46
47 **consolidation with monoclonal antibodies will shorten the duration of therapy.**
48
49 **This may have implication both in terms of toxicity of long term use as well as**
50
51 **the cost of treatment. Alemtuzumab is not the right choice in this situation,**
52
53
54
55
56
57
58
59
60

1
2
3 **due to its toxic profile and non availability for commercial use, but this trial**
4
5 **substantiates our understanding that attaining MRD negativity even with**
6
7 **consolidation therapy can improve the survival outcome in CLL patients.**
8

9
10 Further analysis has shown that the MRD status at the end of treatment is not the
11 accurate determinant of long-term PFS and OS ($p=0.602$ and $p=0.780$ respectively),
12
13 but it is the MRD status 6 months after the treatment. The 5-year PFS and OS of
14
15 MRD-negative and positive participants at 6 months are 77.8% vs 39.3% ($p=0.010$)
16
17 and 88.9% vs 63.9% ($p=0.029$) respectively. Their progression is similar to the
18
19 participants who were MRD-negative at the entry of trial (HR=1.57 (95%CI:
20
21 0.62,3.97)). **Meaningful interpretations of subgroup analysis for various**
22
23 **prognostic factors including genetic factors were not possible due to small**
24
25 **numbers in each subgroup.**
26
27

28
29 Treatment is associated with substantial but manageable toxicity. Delaying the
30
31 treatment to at least 6 months after the immediate prior treatment means that
32
33 patients entering trial had a relative high proportion of normal haematological and
34
35 other laboratory parameters. In addition, limiting the duration of treatment to 6 weeks
36
37 reduces the chance of alemtuzumab being toxic to other CD52+ve targets like CD4+
38
39 T-cells. The pharmacokinetic study supports this shorter duration of treatment as the
40
41 lympholytic dose is reached much earlier, as the disease burden is much smaller
42
43 when compared to patients with clinical disease. Similarly patients who had more
44
45 than one line of previous therapy experienced higher rate of unacceptable toxicity
46
47 and had shorter PFS and OS. These suggest the need for careful patient selection
48
49 and possibly consider consolidation at an earlier stage in the treatment line of CLL.
50
51 In summary, the CLL207 trial is strongly suggestive that that consolidation of MRD-
52
53 positive to MRD-negative remissions after completing conventional chemotherapy
54
55
56
57
58
59
60

1
2
3 leads to a large improvement in outcome. However alemtuzumab in this setting
4
5 carries a significant toxicity and it would be preferable to use a more specific
6
7 monoclonal antibody that did not deplete T-cells. On the basis of the very promising
8
9 sustained remissions seen in CLL207 we have commenced a randomised Phase III
10
11 consolidation trial, the NCRI GALACTIC Trial, of the anti-CD20 antibody,
12
13 obinutuzumab, which appears to result in MRD-negative remissions without the
14
15 immune suppressive problems associated with alemtuzumab.
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

For Peer Review

Acknowledgments: The study was supported by Genzyme, UK National Cancer Research Institute and Clinical Trials Research Unit of University of Leeds

References

- Böttcher, S., Ritgen, M., Fischer, K., Stilgenbauer, S., Busch, R.M., Fingerle-Rowson, G., Fink, A.M., Bühler, A., Zenz, T., Wenger, M.K., Mendila, M., Wendtner, C.-M., Eichhorst, B.F., Döhner, H., Hallek, M.J. & Kneba, M. (2012) 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*, **30**, 980–988.
- Bryant, J. & Day, R. (1995) Incorporating toxicity considerations into the design of two-stage phase II clinical trials. *Biometrics*, **51**, 1372–1383.
- Cheson, B.D., Bennett, J.M., Grever, M., Kay, N., Keating, M.J., O'Brien, S. & Rai, K.R. (1996) National Cancer Institute-sponsored Working Group guidelines for chronic lymphocytic leukemia: revised guidelines for diagnosis and treatment. *Blood*, **87**, 4990–4997.
- Hale, G., Rebello, P., Brettman, L.R., Fegan, C., Kennedy, B., Kimby, E., Leach, M., Lundin, J., Mellstedt, H., Moreton, P., Rawstron, A.C., Waldmann, H., Osterborg, A. & Hillmen, P. (2004) Blood concentrations of alemtuzumab and antiglobulin responses in patients with chronic lymphocytic leukemia following intravenous or subcutaneous routes of administration. *Blood*, **104**, 948–955.
- Hallek, M., Cheson, B.D., Catovsky, D., Caligaris-Cappio, F., Dighiero, G., Dohner, H., Hillmen, P., Keating, M.J., Montserrat, E., Rai, K.R. & Kipps, T.J. (2008) Guidelines for the diagnosis and treatment of chronic lymphocytic leukemia: a report from the International Workshop on Chronic Lymphocytic Leukemia updating the National Cancer Institute-Working Group 1996 guidelines. *Blood*, **111**, 5446–5456.
- Kwok, M., Rawstron, A.C., Varghese, A. & Hillmen, P. (2009) Minimal Residual Disease Is a Predictor for Progression-Free and Overall Survival in Chronic Lymphocytic Leukemia (CLL) That Is Independent of the Type or Line of Therapy. *ASH Annual Meeting Abstracts*, **114**, 540.
- Lin, T.S., Donohue, K.A., Byrd, J.C., Lucas, M.S., Hoke, E.E., Bengtson, E.M., Rai, K.R., Atkins, J.N., Link, B.K. & Larson, R.A. (2010) Consolidation therapy with subcutaneous alemtuzumab after fludarabine and rituximab induction therapy for previously untreated chronic lymphocytic leukemia: final analysis of CALGB 10101. *Journal of clinical oncology: official journal of the American Society of Clinical Oncology*, **28**, 4500–4506.

- 1
2
3 Moreton, P., Kennedy, B., Lucas, G., Leach, M., Rassam, S.M.B., Haynes, A., Tighe,
4 J., Oscier, D., Fegan, C., Rawstron, A. & Hillmen, P. (2005) Eradication of
5 minimal residual disease in B-cell chronic lymphocytic leukemia after
6 alemtuzumab therapy is associated with prolonged survival. *Journal of clinical
7 oncology: official journal of the American Society of Clinical Oncology*, **23**,
8 2971–2979.
9
- 10 Rawstron, A.C., Villamor, N., Ritgen, M., Böttcher, S., Ghia, P., Zehnder, J.L.,
11 Lozanski, G., Colomer, D., Moreno, C., Geuna, M., Evans, P.A.S., Natkunam,
12 Y., Coutre, S.E., Avery, E.D., Rassenti, L.Z., Kipps, T.J., Caligaris-Cappio, F.,
13 Kneba, M., Byrd, J.C., Hallek, M.J., et al (2007) International standardized
14 approach for flow cytometric residual disease monitoring in chronic
15 lymphocytic leukaemia. *Leukemia: official journal of the Leukemia Society of
16 America, Leukemia Research Fund, U.K.*, **21**, 956–964.
17
- 18
19 Rebello, P., Cwynarski, K., Varughese, M., Eades, A., Apperley, J.F. & Hale, G.
20 (2001) Pharmacokinetics of CAMPATH-1H in BMT patients. *Cytotherapy*, **3**,
21 261–267.
22
- 23
24 Schulz, K.F., Altman, D.G., Moher, D. & CONSORT Group (2010) CONSORT 2010
25 statement: updated guidelines for reporting parallel group randomised trials.
26 *BMJ (Clinical research ed.)*, **340**, c332.
27
- 28 Schweighofer, C.D., Ritgen, M., Eichhorst, B.F., Busch, R., Abenhardt, W., Kneba,
29 M., Hallek, M. & Wendtner, C.-M. (2009) Consolidation with alemtuzumab
30 improves progression-free survival in patients with chronic lymphocytic
31 leukaemia (CLL) in first remission: long-term follow-up of a randomized phase
32 III trial of the German CLL Study Group (GCLLSG). *British Journal of
33 Haematology*, **144**, 95–98.
34
- 35 Strati, P., Keating, M.J., O'Brien, S.M., Burger, J., Ferrajoli, A., Jain, N., Tambaro,
36 F.P., Estrov, Z., Jorgensen, J., Challagundla, P., Faderl, S.H. & Wierda, W.G.
37 (2014) Eradication of bone marrow minimal residual disease may prompt
38 early treatment discontinuation in CLL. *Blood*, **123**, 3727–3732.
39
- 40
41 Wendtner, C.-M., Ritgen, M., Schweighofer, C.D., Fingerle-Rowson, G., Campe, H.,
42 Jäger, G., Eichhorst, B., Busch, R., Diem, H., Engert, A., Stilgenbauer, S.,
43 Döhner, H., Kneba, M., Emmerich, B. & Hallek, M. (2004) Consolidation with
44 alemtuzumab in patients with chronic lymphocytic leukemia (CLL) in first
45 remission--experience on safety and efficacy within a randomized multicenter
46 phase III trial of the German CLL Study Group (GCLLSG). *Leukemia: official
47 journal of the Leukemia Society of America, Leukemia Research Fund, U.K.*,
48 **18**, 1093–1101.
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9 **Figure Legends**

10
11 Figure 1: Study flow diagram

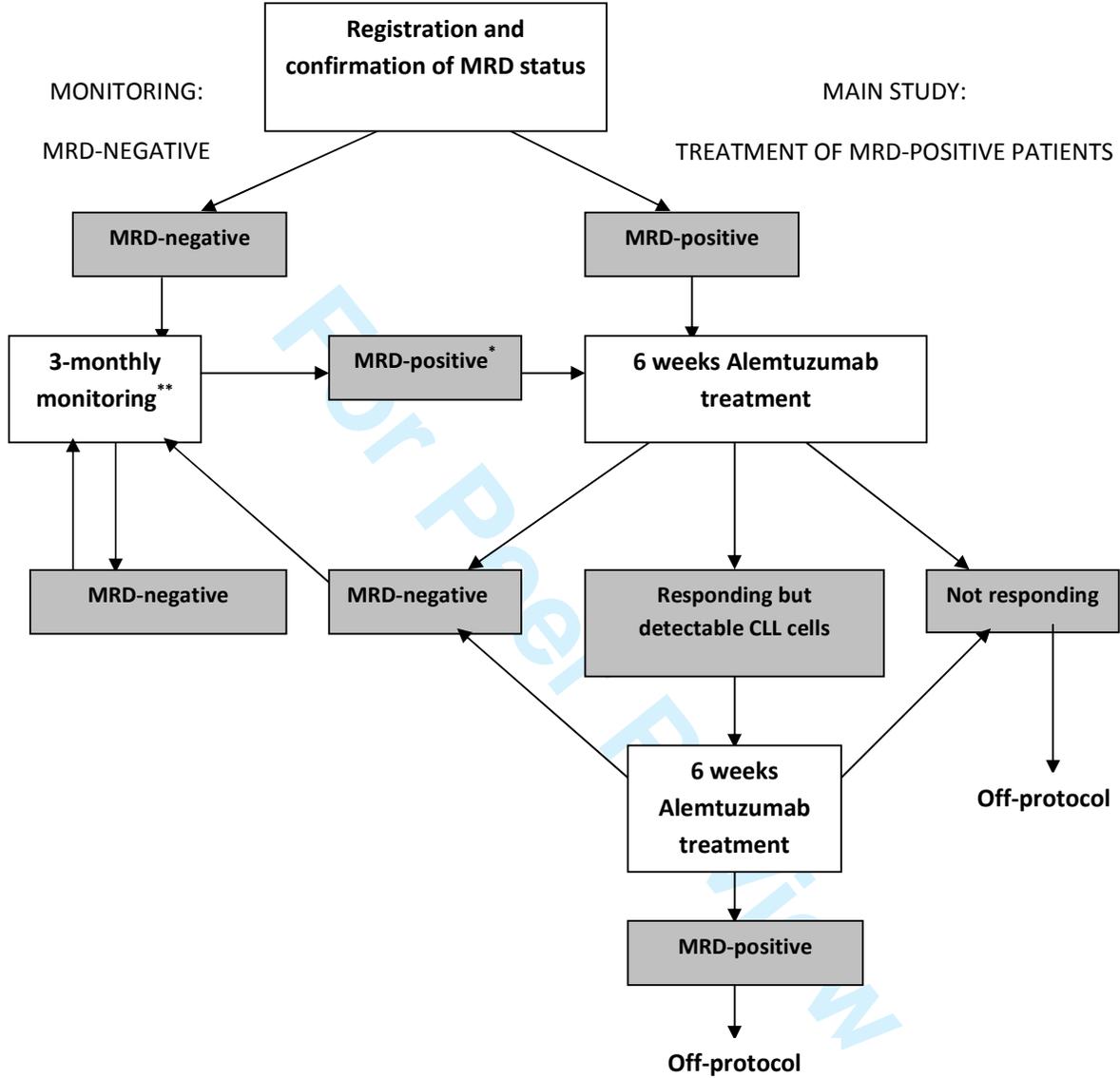
12
13
14 Figure 2: CONSORT flow diagram

15
16
17 Figure 3: (A) Progression-free survival and (B) overall survival of the treated
18 population (C) MRD relapse in participants who were MRD-negative at end of
19 therapy
20
21
22
23

24 Figure 4: (A) Progression-free Survival and (B) overall survival by MRD status at 6
25 months post treatment (C) survival outcomes for MRD relapse in participants who
26 were MRD-negative at end of therapy compared to those who became MRD-
27 negative 6 months after consolidation
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

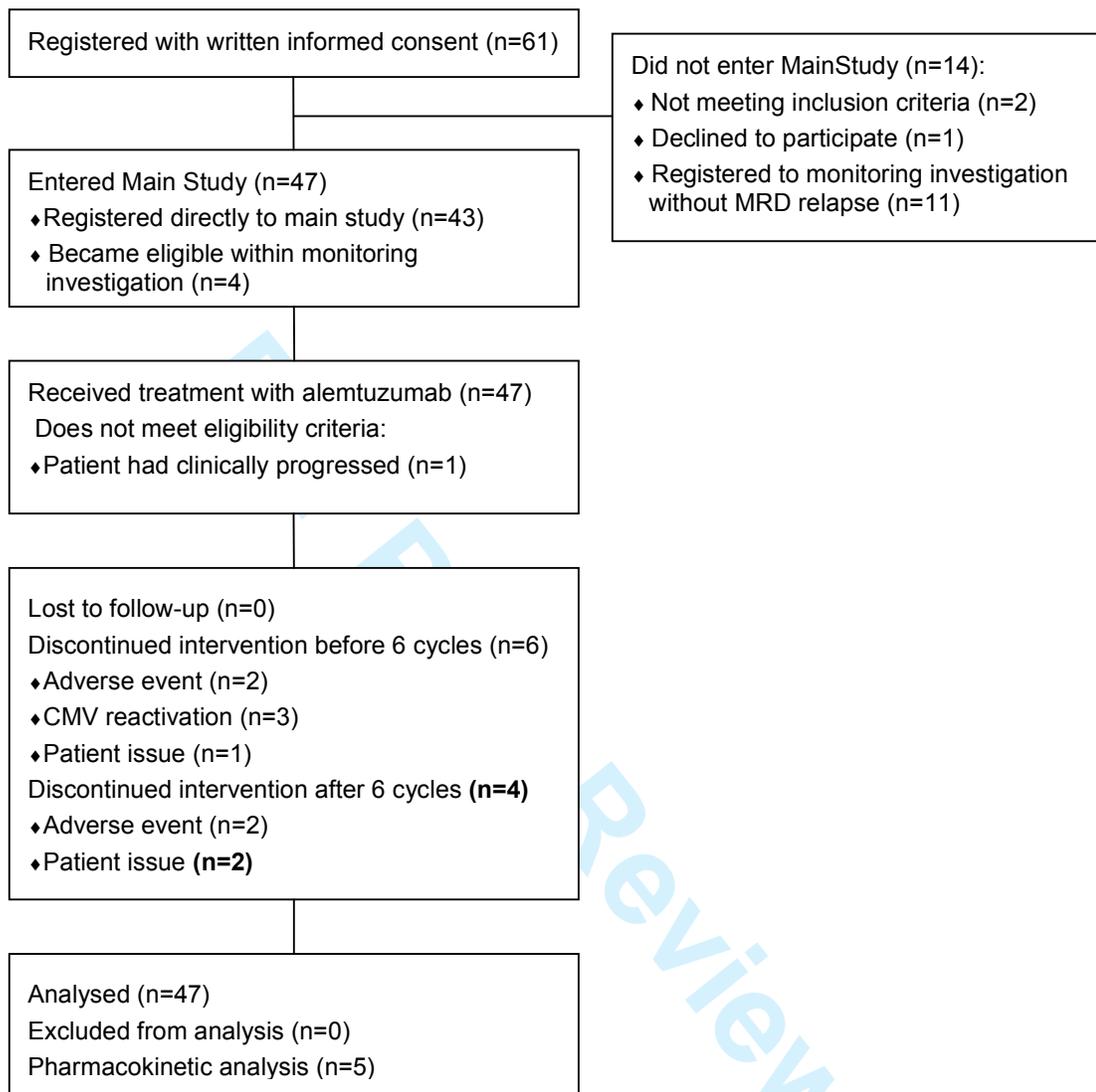
Figure 1



* Participants previously entering the main study must have had a minimum of 6 months since treatment Alemtuzumab

** Until end of study

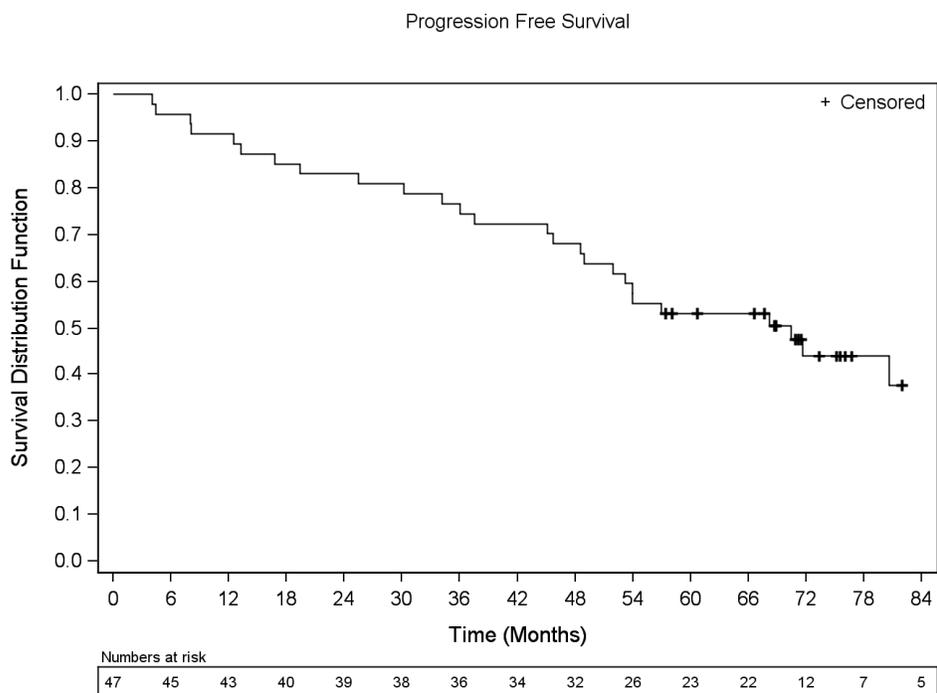
Figure 2



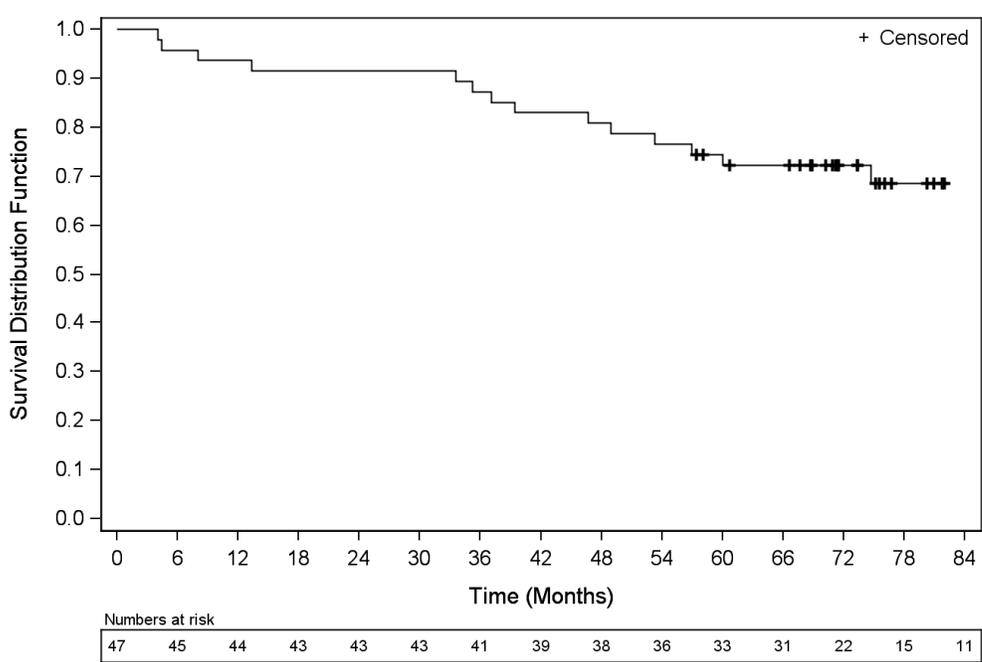
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 3

A

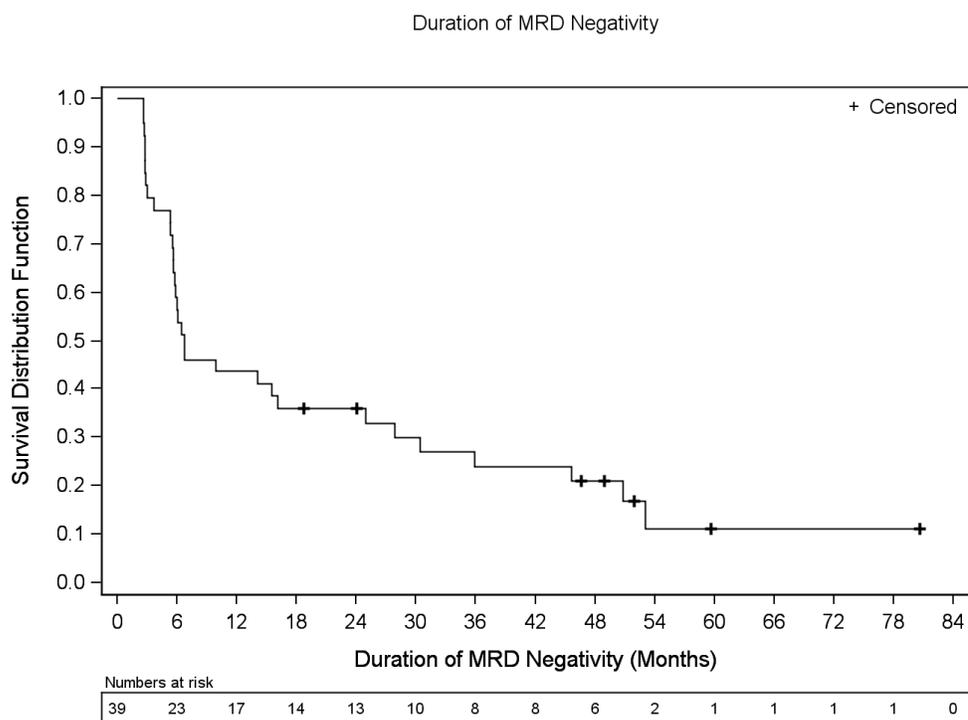


Overall Survival



B

c



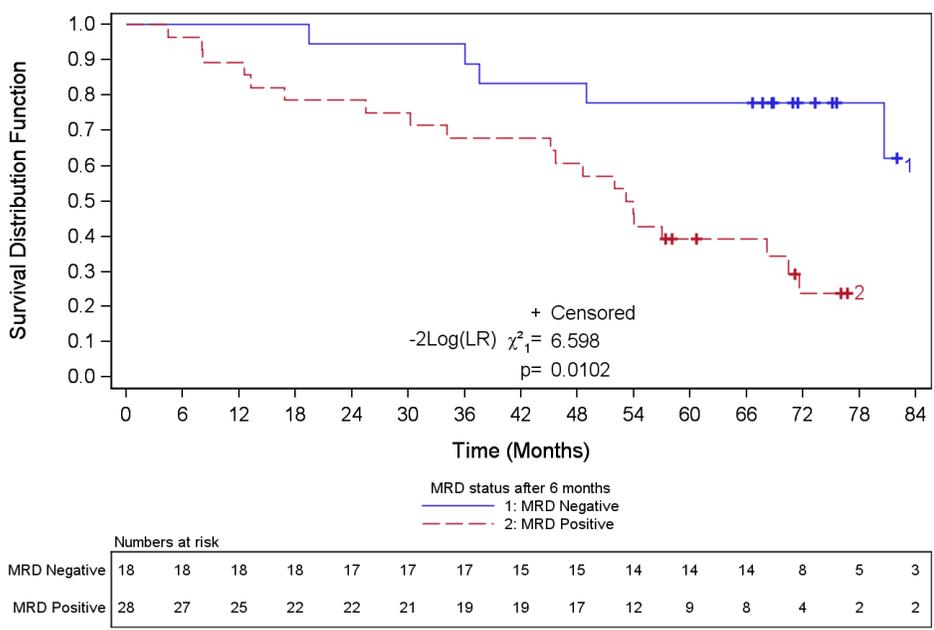
Peer Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

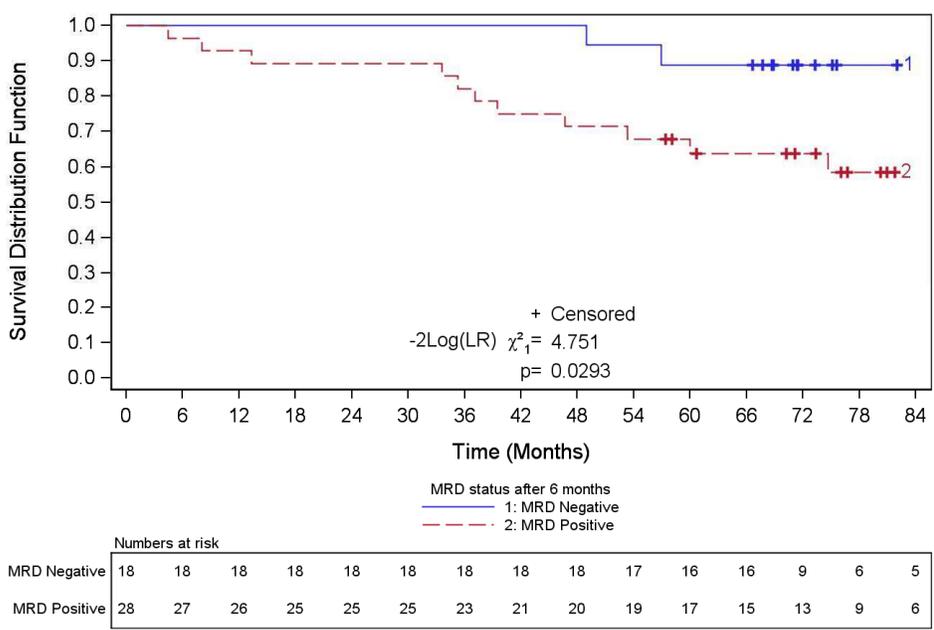
Figure 4
A

Progression Free Survival by MRD 6 months after treatment end



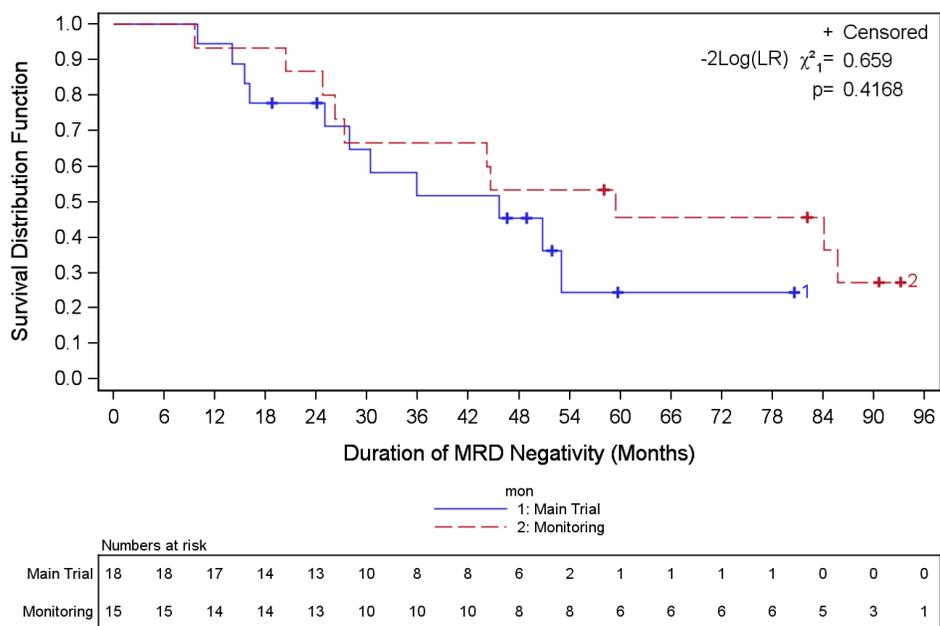
B

Overall Survival by MRD 6 months after treatment end



C

Duration of MRD Negativity in Monitoring Investigation and Main Trial Patients



er Review

Table 1 Baseline Characteristics

	Main Study (Treated Population)	Monitoring Investigation (No Consolidation Treatment)	Total
Total	47	11	58
Male	35 (74.5%)	8 (72.7%)	43 (74.1%)
Age: median (range)	58.0 (40–77)	60.0 (44-76)	58.5 (40-77)
Disease Stage (BINETS criteria)			
Stage A	39 (83.0%)	5 (45.5%)	44 (75.9%)
Stage B	2 (4.3%)	2 (18.2%)	4 (6.9%)
Stage C	3 (6.4%)	0 (0.0%)	3 (5.2%)
Number of Previous Lines of Therapy			
1	23 (48.9%)	8 (72.7%)	31 (53.4%)
2	14 (29.8%)	3 (27.3%)	17 (29.3%)
3-4*	10 (21.3%)	0 (0.0%)	10 (17.2%)
NCI Response to Previous Treatment			
Complete Response	21 (44.7%)	8 (72.7%)	29 (50.0%)
Partial Response	26 (55.3%)	2 (18.2%)	28 (48.3%)
Prior Fludarabine Treatment	46 (97.9%)	10 (90.9%)	56 (96.6%)
Prior Rituximab Treatment	9 (19.1%)	2 (18.2%)	11 (19.0%)

* The eligibility criteria were limited to 3 previous lines of therapy after two participants had already entered with 4.

Table 2 Treatment Duration, Efficacy and Safety Summaries

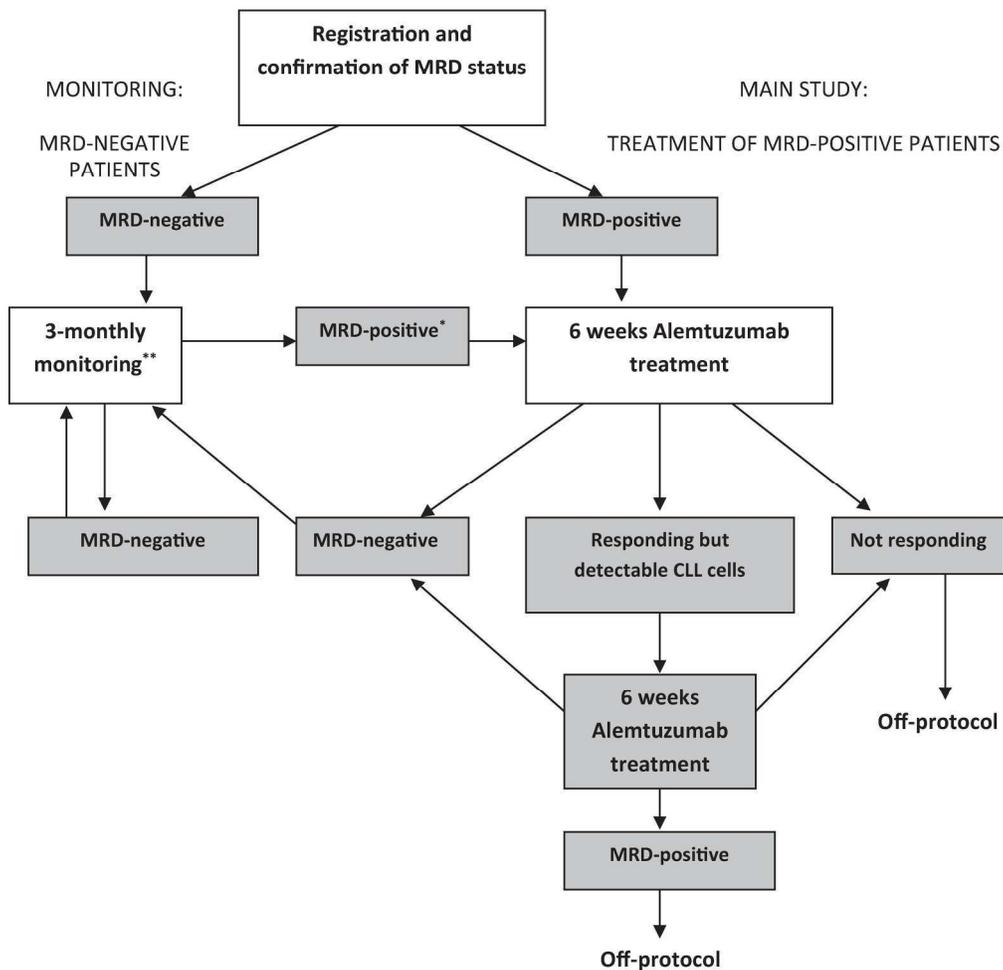
	Main Study (Treated Population)
Total	47
Treatment Received	
6 weeks	41(87.2%)
3 weeks	2 (4.3%) (1 AE (non-specific), 1 CMV reactivation)
4 weeks	3 (6.4%) (1 AE (Chronic Obstructive Pulmonary Disease); 2 CMV reactivation)
5 weeks	1 (2.1%) (participant choice)
Eligible to continue beyond 6 weeks	
completed 12 weeks	8 (66.6%)
6 weeks	3 (25%) (1 cytopenia; 1 AE (non-specific); 1 participant choice)
completed 11 weeks	1 (8.3%) (participant choice)
MRD response following therapy (Efficacy Primary Endpoint)	
MRD-Negative [95% CI]	39 (83.0%) [69.2%, 92.4%]
MRD-Positive	7 (14.9%)
Missing*	1 (2.1%)
Unacceptable Treatment Related Toxicity (Safety Primary Endpoint)	
Yes [95% CI]	10 (21.3%) [10.7%, 35.7%]
No	37 (78.7%)
Overall Response	
Achieved at least PR [95% CI]	43 (91.5%) [79.6%, 97.6%]
Complete Remission (CR)	27 (57.4%)
Partial Remission (PR)	7 (14.9%)
At least PR (Undeterminable CR)	9 (19.1%)
Did not achieve PR	3 (6.4%)
Stable Disease (SD)	3 (6.4%)
Progressive Disease (PD)	0 (0.0%)
Missing*	1 (2.1%)
MRD response 6 months after treatment end	
MRD-Negative	18 (38.3%)
MRDPositive	28 (59.6%)
Missing*	1 (2.1%)
SAE Summaries	

		Main Study (Treated Population)
	Any participants with SAEs reported	17 (36.2%)
	Total number of SAEs reported:	22
	Suspected, unexpected	2 (9.1%) (1 parainfluenza related death, 1 EBV related DLBCL)
	Suspected, expected	17 (77.3%)
	Not suspected	3 (13.6%)
	No SAEs reported	30 (63.8%)

* The participant was too ill to have their sample taken.

For Peer Review

Figure 1



* Participants previously entering the main study must have had a minimum of 6 months since treatment Alemtuzumab

** Until end of study

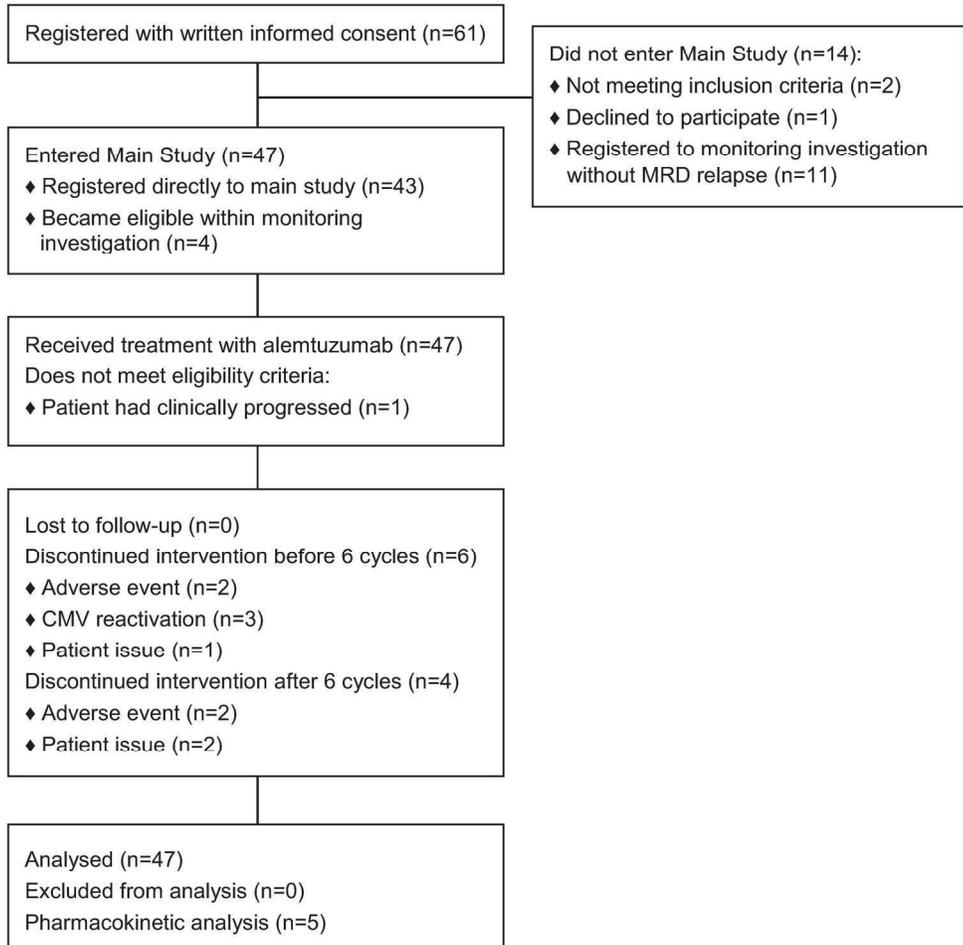
Study flow diagram

189x217mm (300 x 300 DPI)

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Figure 2

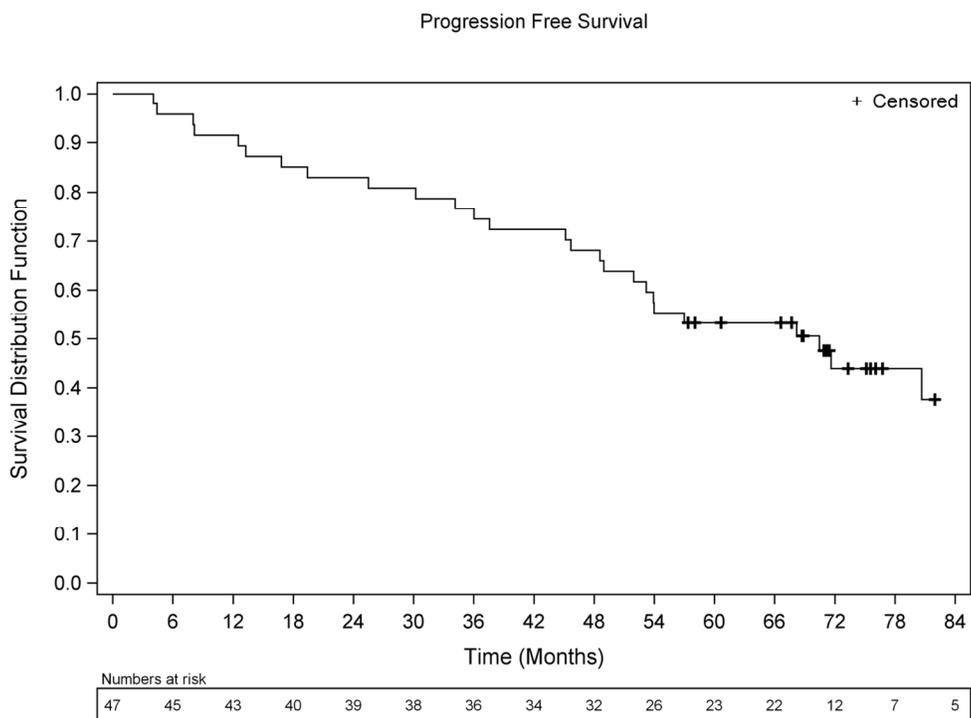


CONSORT flow diagram

162x162mm (300 x 300 DPI)



1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

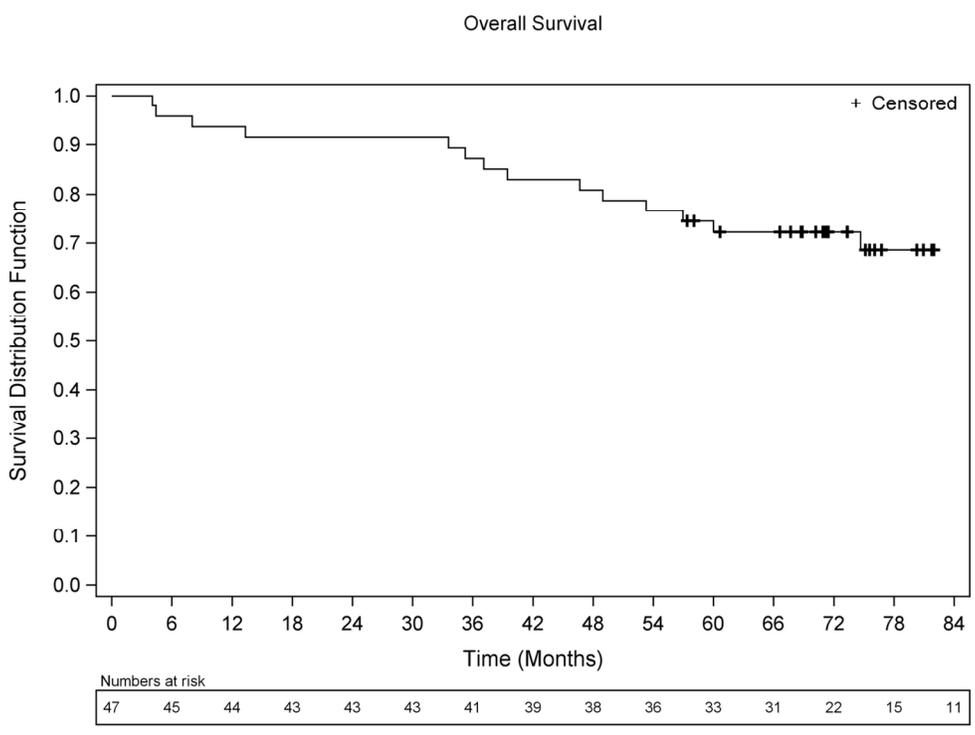


(A) Progression-free survival and (B) overall survival of the treated population (C) MRD relapse in participants who were MRD-negative at end of therapy

127x95mm (300 x 300 DPI)

Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

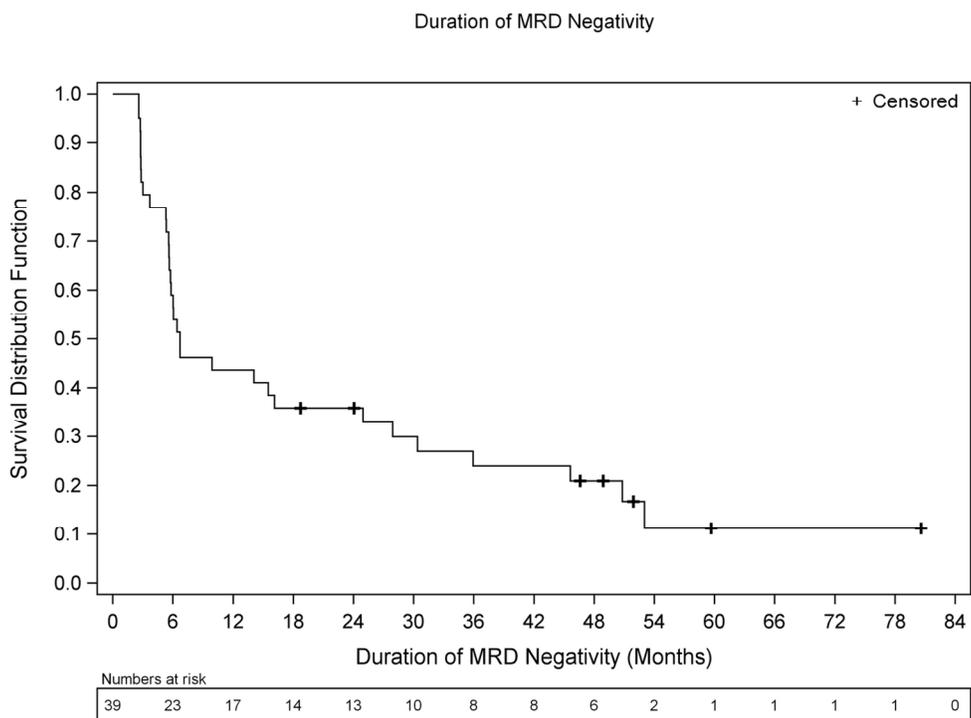


(A) Progression-free survival and (B) overall survival of the treated population (C) MRD relapse in participants who were MRD-negative at end of therapy

127x95mm (300 x 300 DPI)

Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60



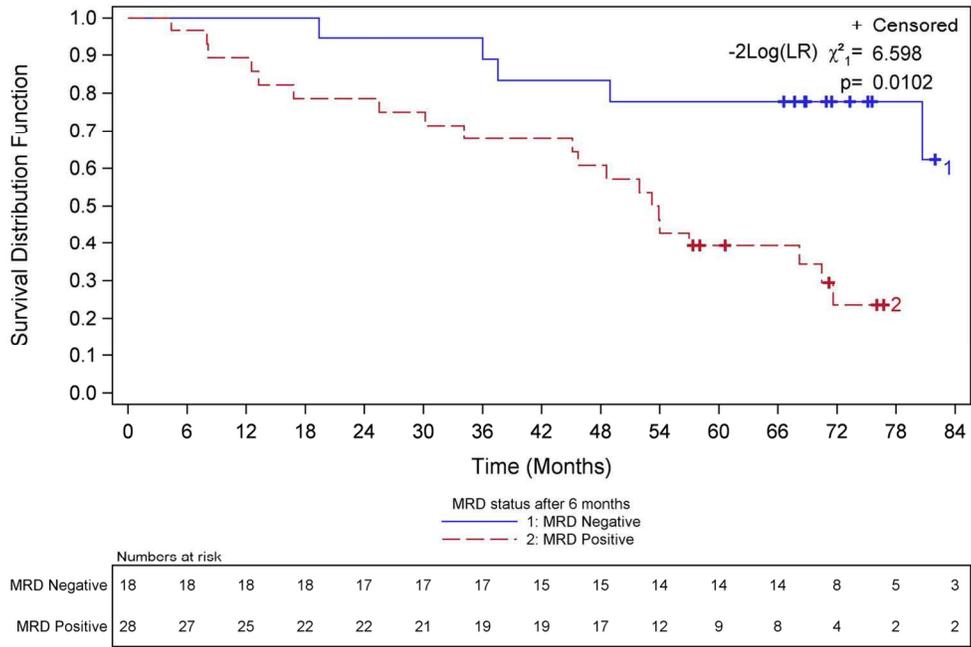
(A) Progression-free survival and (B) overall survival of the treated population (C) MRD relapse in participants who were MRD-negative at end of therapy

127x95mm (300 x 300 DPI)

Review

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Progression Free Survival by MRD 6 months after treatment end



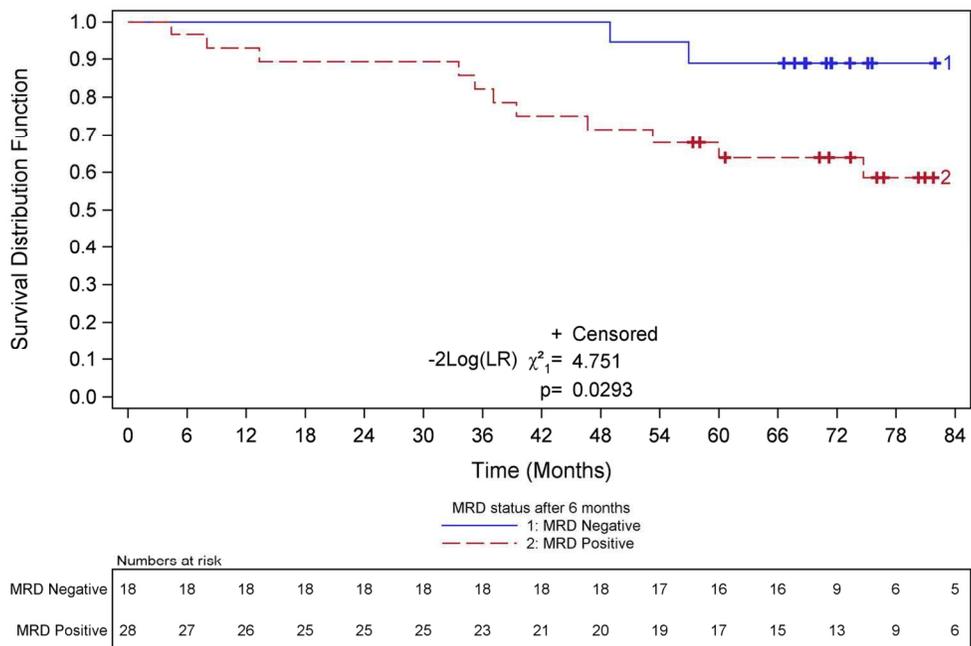
(A) Progression-free Survival and (B) overall survival by MRD status at 6 months post treatment (C) survival outcomes for MRD relapse in participants who were MRD-negative at end of therapy compared to those who became MRD-negative 6 months after consolidation

127x95mm (300 x 300 DPI)

view

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Overall Survival by MRD 6 months after treatment end



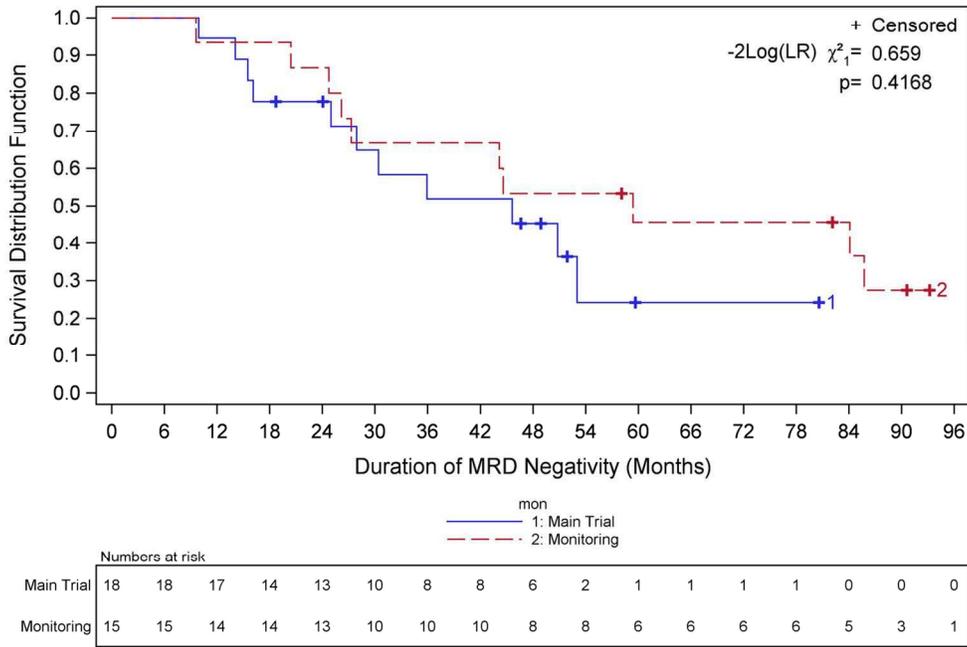
(A) Progression-free Survival and (B) overall survival by MRD status at 6 months post treatment (C) survival outcomes for MRD relapse in participants who were MRD-negative at end of therapy compared to those who became MRD-negative 6 months after consolidation

127x95mm (300 x 300 DPI)

view

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46
47
48
49
50
51
52
53
54
55
56
57
58
59
60

Duration of MRD Negativity in Monitoring Investigation and Main Trial Patients



(A) Progression-free Survival and (B) overall survival by MRD status at 6 months post treatment (C) survival outcomes for MRD relapse in participants who were MRD-negative at end of therapy compared to those who became MRD-negative 6 months after consolidation

127x95mm (300 x 300 DPI)

view