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# Rituximab plus HyperCVAD alternating with high dose cytarabine and methotrexate for the initial treatment of patients with mantle cell lymphoma, a multicentre trial from Gruppo Italiano Studio Linfomi

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Received 8 August 2011; accepted for publication 3 October 2011

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## Summary

This study investigated the clinical activity and toxicity of R-HCVAD-AM [rituximab plus HyperCVAD (R-HCVAD) alternating with high-dose cytarabine and methotrexate (AM)] in patients with newly diagnosed Mantle Cell Lymphoma (MCL). Patients aged ≤70 years with confirmed MCL received four alternating cycles each of R-HCVAD and AM. Patients who obtained a partial response proceeded to autologous stem cell transplant. Sixty-three patients were enrolled and 60 were fully eligible. Median age was 57 years (22–66); 60%, 33% and 7% were classified at low (L)-, intermediate (I)- or high (H)-risk, respectively, according to the MCL International Prognostic Index (MIPI). Only 22 patients (37%) completed the four cycles and three patients died during therapy. Overall response and complete response rates were 83% and 72% respectively. After a median follow-up of 46 months (range 1–72) the estimated 5-year overall survival (OS) and progression-free survival rates were 73% [95% confidence interval (CI) 59–83%], and 61% (95%CI 45–73%) respectively. MIPI maintained the prognostic value with an estimated 5-year OS of 89%, 80% and 24% for L, I, and H groups respectively ($P < 0{\cdot}001$). This multicentre study confirms that R-HCVAD-AM is an active regimen for the initial treatment of patients with MCL, but is associated with significant toxicity.

**Keywords:** non-Hodgkin lymphoma, clinical trials, chemotherapy, monoclonal antibodies, prognostic factors.

Mantle cell lymphoma (MCL) is an uncommon type of B-cell non-Hodgkin lymphoma (NHL), characterized by the proliferation of small B-lymphocytes with a typical phenotype ($CD20^+$ $CD5^+$ $CD23^-$) and carrying the *t*(11;14) chromosomal translocation. MCL typically affects the elderly population and, although heterogeneous, the clinical course of the disease is generally aggressive with a median survival of around 4 years (Swerdlow & Williams, 2002).

Before the anti-CD20 monoclonal antibody, rituximab, became available, patients with MCL were treated with conventional chemotherapy with disappointing results (Norton *et al*, 1995; Teodorovic *et al*, 1995; Zucca *et al*, 1995;

First published online 7 December 2011
doi:10.1111/j.1365-2141.2011.08958.x

 

Argatoff *et al*, 1997). More encouraging results were achieved with the use of highly active compounds, such as cytarabine (Ara-C), and with consolidation treatment by autologous bone marrow transplant (ABMT) (Vandenberghe *et al*, 2003; Lefrere *et al*, 2004; Dreyling *et al*, 2005; Magni *et al*, 2009; van 't Veer *et al*, 2009). With the advent of rituximab the use of chemoimmunotherapy is suggested as a standard approach to patients with MCL, although its use has somewhat less impressive activity than in other B-cell indolent malignancies (Forstpointner *et al*, 2004; Thieblemont *et al*, 2005; Schulz *et al*, 2007; Geisler *et al*, 2008; Herrmann *et al*, 2009). However, the addition of rituximab to chemotherapy favoured the development of treatment strategies based on intensive chemoimmunotherapy regimens, questioning the need for consolidative ABMT. Romaguera *et al* (2005) published the results of a monocentric Phase II study on 97 patients with advanced MCL who were initially treated with an intensive programme that combined rituximab with HyperCVAD regimen (hyperfractionated cyclophsophamide, vincristine, doxorubicin, dexamethasone) alternating with high dose (HD) Ara-C and HD-methotrexate (R-HCVAD-AM). The 87% complete response (CR) rate and the 82% 3-year overall survival (OS) rate were considered promising results when compared with a previous series of patients that received the same regimen without rituximab and were consolidated with ABMT (Khouri *et al*, 1998).

Here we report the results of a prospective study performed by the Gruppo Italiano Studio Linfomi (GISL), to investigate, in a multicentre setting, the clinical activity and the safety of R-HCVAD-AM regimen as initial treatment for untreated patients with MCL. In our current study ABMT was considered only for patients achieving partial response (PR) with the induction treatment.

## Patients and methods

This study was conducted according to the Good Clinical Practice guidelines and the October 2000 revision of the Declaration of Helsinki. The study protocol was approved by the ethic committees according to local rules. All patients gave their written informed consent to participate before study entry.

To be enrolled in the trial patients were required to have newly diagnosed, previously untreated MCL. MCL diagnosis was histologically determined on tissue biopsy, bone marrow (BM) and/or mononuclear cell suspension. In cases lacking histological diagnosis the presence of *t*(11;14) was mandatory. Patients were also required to be younger than 70 years and have clinical stage II to IV; gastroscopy and colonoscopy at baseline were recommended procedures but were not mandatory for the staging of the disease. Adequate renal, hepatic, and haematological functions were also required, unless alterations were secondary to lymphoma. Patients were required to have negative serology for human deficiency virus, hepatitis B and C viruses; only left ventricular ejection fractions (LVEF) ≥50% were allowed. Clinical and instrumental disease assessment was required before treatment start, after two cycles, and at the end of treatment. BM biopsy was not repeated if negative at diagnosis.

Molecular assessment was limited to BM samples and was centralized to the Molecular Laboratory of the Haematology Division, University of Pisa. The study was limited to polymerase chain reaction (PCR) evaluation of *t*(11;14) chromosomal translocation and, in negative cases, to PCR evaluation of the hypervariable region of the immunoglobulin heavy chain gene (*IGH*). Patients with baseline PCR positivity for *t*(11;14) or *IGH* rearrangement on BM were re-assessed at evaluation of response and during follow-up visits.

High molecular weight DNA was extracted by Genomic DNA Isolation Reagent DNAZOL$^{TM}$ (Gibco BRL, Milan, Italy); after precipitation with ethanol 100%, the DNA was suspended in Tris-EDTA (TE) buffer (pH = 8). After quantitative spectrophotometric evaluation suitable aliquots were utilized for PCR tests.

Two consensus primers were designed for the variable-diverse-joining regions of *IGH*, as previously described (Galimberti *et al*, 1999); the downstream primer was 5′ labelled with 6-carboxyfluorescein (6-FAM) fluorochrome.

PCR-amplified products were resolved by capillary electrophoresis on an ABI Prism 310 Genetic Analyzer (Applied Biosystem, Milan, Italy). PCR assays for qualitative *CCND1*/*IGHJ* rearrangements were performed according to the protocol established by the European network (BIOMED-2 Concerted Action) (van Dongen *et al*, 2003). Every PCR procedure included distilled water instead of DNA as a negative control and DNA carrying monoclonal *IGH* or *CCND1*/*IGHJ* rearrangement as a positive control.

Patients were treated with the four cycles of the R-HCVAD-AM regimen [rituximab plus HyperCVAD (hyperfractionated cyclophsophamide, vincristine, doxorubicin, dexamethasone; R-CVAD) alternating with high dose methotrexate and Ara-C (AM)] for four cycles. Each R-HCVAD-AM cycle consisted of two blocks, called 'A' [i.e. cyclophosphamide 300 mg/m$^2$ (days 1–3), vincristine 1·4 mg/m$^2$ (days 4 and 11, at a maximum dose of 2 mg), doxorubicin 50 mg/m$^2$ (days 4–5), dexamethasone 40 mg/day (days 1–4 and 11–14), plus rituximab 375 mg/m$^2$ (day 1)] and 'B' [i.e. methotrexate (200 mg/m$^2$ bolus infusion day 1 and 800 mg/m$^2$ 24-h continuous infusion day 1), cytarabine 3000 mg/m$^2$ (days 2–3), plus rituximab 375 mg/m$^2$ (day 1)]. Patients older than 60 years could be treated with lower doses of Ara-C (1000 mg/m$^2$ days 2–3) at the physician's discretion and as originally reported. Patients achieving at least a partial response (PR) after cycle 2 continued planned treatment and only patients achieving PR at the end of fourth cycle were to be proceeded with HDC followed by ASCT.

Patients could not be treated concomitantly with other antineoplastic agents but could receive supportive medications, including antibiotics and antiemetics, according to their physician's discretion. Antifungal, antiviral and pneumocystis

(PCP) prophylaxis was mandatory but drug choice was left to the treating physician. The use of Granulocyte colony-stimulating factor (G-CSF) and/or of erythropoiesis-stimulating agents (ESA) was recommended and was administered as per institution practice.

### *Assessment of response, survival, and toxicity*

The main objective of the study was to assess clinical activity of R-HCVAD-AM in a multicentre setting. The primary endpoint was the complete response (CR) rate. Secondary objectives included toxicity and survival. The safety of R-HCVAD-AM was evaluated by assessment of laboratory parameters and adverse events (AEs). All toxic effects were assessed according to the National Cancer Institute's Common Toxicity Criteria and graded on a scale of 0–4. Events were counted both per patient and per cycle. Survival was measured using failure-free survival (FFS), progression-free survival (PFS), and OS. All analyses were carried out on an intention-to-treat (ITT) basis. In addition, an efficacy population was defined considering only patients who received at least one cycle of R-HCVAD-AM and were assessed for response.

Response to treatment was assessed on the basis of clinical, radiological, and pathological criteria, according to modified International Workshop Response Criteria Guidelines (Cheson *et al*, 1999); patients achieving a CR unconfirmed without evidence of disease progression/relapse within the first 3 months of follow-up were recorded as CR. All tests that were positive for lymphoma at time of diagnosis were repeated to define response, including endoscopic procedures. Functional imaging with positron emission tomography was not included among staging and study procedures. FFS was measured from the date of study entry to any treatment failure, including treatment discontinuation for toxicity, any response less than PR, administration of additional chemo- or immuno-therapy in responding patients, shift to BM transplantation (BMT) in CR patients, progression, relapse or death from any cause. Responding patients who did not complete the full four courses of chemotherapy, but received at least one cycle, without showing disease progression or severe toxicity were not counted as failures. PFS was defined as the time from study entry until documented lymphoma progression or relapse or death as a result of lymphoma. OS was defined as the time from study entry to last observation or death from any cause.

### *Statistical analysis*

The number of subjects to be included in the trial was determined using an empirical approach, due to the rarity of the disease. The study was initially designed to accrue 40 subjects in 2 years, but was then amended to enrol 60 evaluable cases assuming a treatment phase duration of 8 months and 2 years of follow-up a total study duration of 56 months was planned. Survival function was estimated by means of Kaplan–Meier method. Comparison between two groups was performed using Mann–Whitney test, if involving continuous covariates, or Fisher's exact if involving categorical covariates. Comparison between more than two groups was performed using the Kruskal–Wallis test for continuous variables or the chi-square test. To evaluate the association between toxicity and cycles of therapy we used a non-parametric test for trend (Cuzick, 1985). Survival curves were compared by means of log-rank test. The MCL prognostic index (MIPI) was computed following the algorithm proposed by Hoster *et al*, 2008. All statistical tests were two-sided with a *P*-value of 5%.

## Results

From April 2005 to March 2010, a total of 63 patients from 15 Italian centres were prospectively recruited into the study. Three patients were excluded from further analysis due to major violations of inclusion criteria including consent withdrawn, unconfirmed diagnosis, and start of a treatment different from R-HCVAD-AM (Fig 1). Demographic and baseline data of the remaining 60 eligible patients are shown in Table I.

Diagnosis of MCL was performed on nodal or extranodal biopsy in 27 and 25 cases (45% and 42%), respectively; in the remaining eight cases, diagnosis was performed on BM biopsy and/or peripheral blood cell suspension. Upper and lower gastrointestinal tracts were evaluated in 45 patients and were positive for MCL in 20 (44%) and 25 (56%) cases respectively. Disease showed a splenic-marrow presentation in 25 cases (42%).

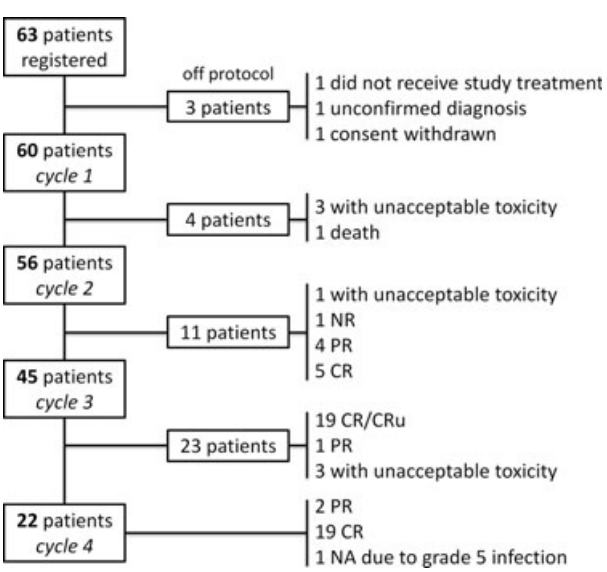


**Fig 1.** Flow chart of patient treatment and outcome in the study. CR, complete response; CRu, unconfirmed complete response; PR, partial response; NR, no response; NA, not assessed.

**Table I.** Characteristics of 60 patients with newly diagnosed MCL treated with R-HCVAD-AM.

| Variable | *N* | *n* (%) |
|---|---|---|
| Age > 60 years | 60 | 21 (35) |
| Median (range) | 57 (29–66) | |
| Male Gender | 60 | 45 (75) |
| PS > 1 | 60 | 1 (2) |
| Stage III–IV | 60 | 56 (93) |
| B Symptoms | 60 | 15 (25) |
| LDH > 1 (iu/l) | 58 | 18 (31) |
| Hb < 100 g/l | 59 | 9 (15) |
| WBC > $10 \times 10^9$/l | 59 | 24 (41) |
| Plt < $100 \times 10^9$/l | 49 | 11 (22) |
| MIPI | 58 | |
| Low | | 35 (60) |
| Intermediate | | 18 (31) |
| High | | 5 (9) |

*N*, Total number of patients; *n*, number of patients; PS, performance status; LDH, lactate dehydrogenase, Hb, haemoglobin; WBC, white blood cell count; Plt, platelet count; MIPI, Mantle Cell Lymphoma International Prognostic Index.

### *Efficacy results*

Among the 60 eligible patients, 45 (75%) completed at least three cycles and 22 (37%) completed all four cycles (Fig 1). The reasons for treatment discontinuation included AEs (18), unsatisfactory response (4), and decision of treating physician (16). Overall, three patients died due to treatment toxicity, one after cycle 1 (sepsis), one after cycle 2 (cardiac failure) and one immediately after cycle 4 (pulmonary aspergillosis).

The overall response rate (ORR) was 83%, including 43 patients achieving a CR and seven patients with PR (Table II). The CR and PR rate was 83% and 14% (ORR 98%) respectively, when nine patients whose response was not assessable due to treatment discontinuation were excluded. Eighteen and 17 patients who achieved a CR after cycle 4 and 3, respectively, were followed by observation, as requested by protocol. Five patients in CR were shifted to BMT after cycle 2 (three patients) or cycle 3 (two patients); one patient in CR after cycle 3 and one patient in CR after cycle 4 proceeded with additional rituximab courses. All these seven cases were counted as treatment failures.

**Table II.** Summary of outcome measures in 60 patients with newly diagnosed MCL treated with R-HCVAD-AM.

| Response | *N* (%) | 95% CI |
|---|---|---|
| CR | 43 (72) | 59–82 |
| PR | 7 (11) | 5–23 |
| No resp. | 10 (17) | 8–28 |
| OS | 3-year (83) | 70–91 |
| | 5-year (73) | 59–83 |
| PFS | 3-year (73) | 58–84 |
| | 5-year (61) | 45–73 |
| FFS | 3-year (52) | 37–65 |
| | 5-year (46) | 33–59 |

*N*, Number of patients; CI, confidence interval; CR, complete response; PR, partial response; No resp., stable disease or response was not assessable due to treatment discontinuation; OS, overall survival; PFS, progression-free survival; FFS, failure-free survival.

The mean calculated dose intensities (DI) of cyclophosphamide, vincristine, doxorubicin, methothrexate, cytarabine and rituximab for the delivered cycles were 98·8%, 100%, 99·6%, 97·4%, 89·6% and 98% respectively. In patients older than 60 years the DI of cytarabine dropped, from 90% at cycle 1, to 73% at cycle 4. Mean DI was also calculated considering the missing cycles for patients that did not complete the full therapy and was 90·4%, 100%, 91·7%, 92·8%, 86·0% and 91·3% for cyclophosphamide, vincristine, doxorubicin, methothrexate, cytarabine and rituximab respectively.

After a median follow-up of 46 months (range 1–72), 28 failures were recorded, including 10 responses less than PR. A total of 17 patients developed progressive disease including four patients who progressed from PR, one patient with stable disease and 12 patients who relapsed from CR. Overall, 15 patients (25%) died: causes of death were lymphoma progression (10), treatment-related events (3), secondary acute myeloid leukaemia (1) and sepsis after salvage therapy with BMT (1). The estimated 2-year OS, PFS and FFS rates were 86% [95% confidence interval (CI) 74–93%], 83% (95%CI 70–90%) and 61% (95% CI 48–72%) respectively. Considering the median follow-up we also estimated the 5-year OS, PFS and FFS rates to be 73% (95% CI 59–83%), 61% (95%CI 45–73%) and 46% (95% CI 33–59%) respectively (Table II; Fig 2).

Considering only the 51 patients who were available for response assessment after R-HCVAD-AM, the estimated OS, PFS and FFS was 74% (95%CI 58–85%), 60% (95%CI 43–74%) and 55% (95%CI 39–68%) respectively.

The MIPI score maintained the prognostic value when applied to the 58 patients enrolled in our trial with available data; the estimated 5-year OS was 89%, 80% and 24% for low, intermediate and high risk score respectively (log-rank $P < 0{\cdot}001$) (Fig 3).

### *Molecular analysis*

The BM sample of 33 patients was tested for the presence of a molecular marker at the time of diagnosis (55%). Of those, 29 were positive (88%), 10 for both $t(11;14)$ and for *IGHV* rearrangement, and 19 for *IGHV* rearrangement only. At the end of therapy 23 patients were assessed for minimal residual disease on BM. Overall, 15 patients achieved a molecular remission (65%). Eleven out of 15 patients with molecular remission also achieved a clinical CR (73%); a CR was obtained in four out of eight patients that did not achieve a molecular remission (50%). Three out of 15 patients in molecular remission (25%) and two patients out of eight without molecular remission (20%) developed progressive disease.

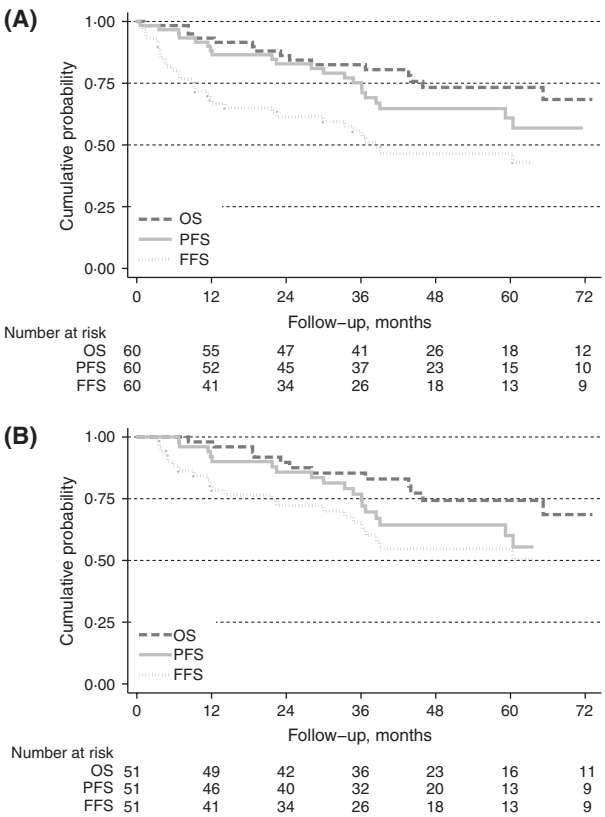


**Fig 2.** Overall survival (OS), progression-free survival (PFS) and failure-free survival (FFS) in 60 patients with newly diagnosed MCL treated with R-HCVAD-AM.

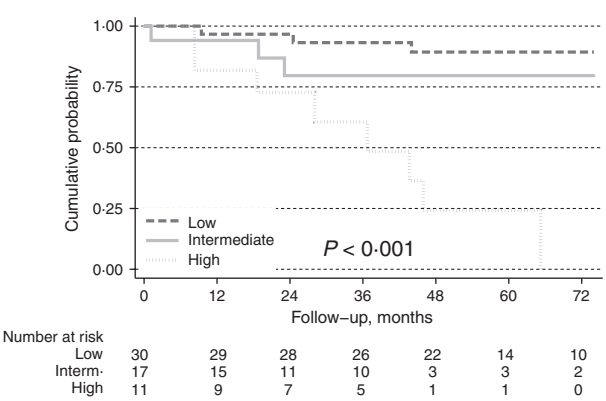


**Fig 3.** Overall survival by MIPI subgroup in 58 patients with newly diagnosed MCL treated with R-HCVAD-AM. MIPI: mantle cell lymphoma international prognostic index.

### *Safety results*

Data on haematological toxicity were available in 54 patients and for 323 chemotherapy administrations (including both HCVAD and HD Ara-C and methotrexate). Neutropenia was the most frequent grade 3–4 event, occurring in 94% of patients (61% of cycles), followed by thrombocytopenia (69% of patients; 45% of cycles), and anaemia (44% of patients; 14% of cycles). Data on infections were available in 48 cases; grade 3–4 events were reported in 50% of patients (10% of cycles); lethal infections were described in two cases (one sepsis after cycle 1 and one aspergillosis after cycle 4). The distribution of haematological toxicity by therapy cycle is reported in Fig 4.

All patients received PCP, antiviral and antifungal prophylaxis. Data on the use of G-CSF was available in 49 patients (82%), who received a total of 216 doses of G-CSF, with a mean number of administrations of 7 d (standard error, 4 d). The use of ESA was reported in 18 patients (30%), with an increased trend from cycle 1 to cycle 4 ($P = 0{\cdot}014$). One or more packed red cell transfusion was required in 34 patients (27%). No difference in the rate of haematological events and infections was observed between elderly (>60 years) and young patients.

Non-haematological grade 3–4 toxicity was described in 13 patients: the most frequent events were cardiac arrhythmia and fever, both occurring in four patients: in particular two patients experienced grade 3 atrial fibrillation, and two had grade 3 sinus bradicardia. Severe stypsis was described in three cases (one had grade 4 paralitic ileum), and thromboembolic events were documented in two patients. Less frequent events occurred only in one patient each and included grade 3 acute pancreatitis, grade 4 skin reaction (epidermolysis), grade 4 peripheral neuropathy and grade 3 infusion reaction. In all but two cases the local physician decided to discontinue per protocol treatment and addressed the patient to an alternative regimen.

### *Prognostic assessment*

Among clinical parameters collected at time of diagnosis, anaemia (Hb < 120 g/l) and leucocytosis (WBC > $10 \times 10^9$/l) were predictive of a poor OS and FFS in univariate analysis. OS, but not FFS, was also influenced by age with patients older than 60 year showing a poorer outcome (2-year OS 92% vs. 76%, for young *versus* elderly patients respectively: $P = 0{\cdot}014$). Interestingly, both age and lactate dehydrogenase were not predictive for survival (data not shown). MIPI was available in 58 patients and was confirmed as a valid prognostic tool for OS (Fig 3).

## Discussion

R-HCVAD-AM was recently proposed as an alternative regimen to ABMT for the initial treatment of patients with MCL following a study at the MD Anderson Cancer Center (MDACC) (Romaguera *et al*, 2005). Currently, the 98% ORR and the 64% 3-year FFS are among the best results ever achieved in patients with MCL without the use of ABMT. When data from the MDACC were initially disseminated we decided to test if these results could be confirmed also in a multicentre setting. Similarly to the MDACC experience, our trial did not allow ABMT except for patients not achieving a CR with induction chemotherapy. In our multicentre trial, R-HCVAD-AM was confirmed as an effective regimen for patients with MCL, achieving an ORR of 83% and a 5-year FFS

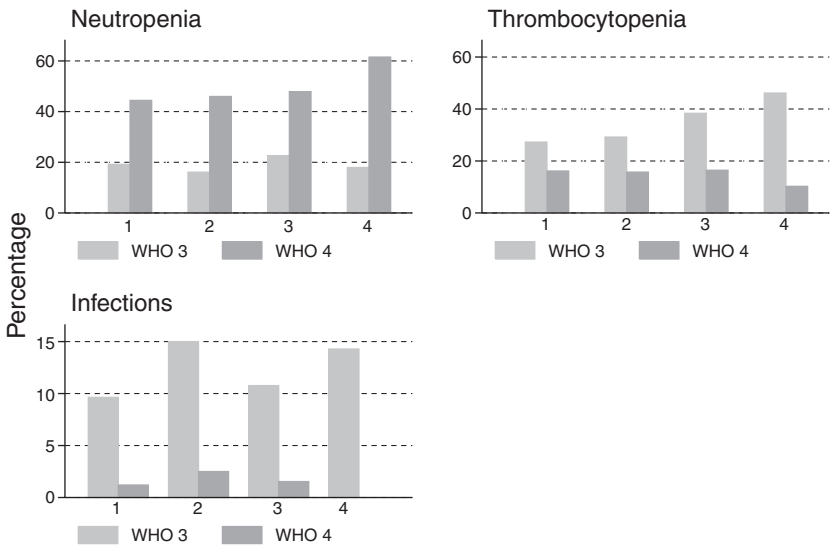


**Fig 4.** Frequency of acute haematological toxicity and infections according to World Health Organization (WHO) classification and R-HCVAD-AM cycle (%).

of 46%. Similarly to what observed with mature data from the MDACC trial, in our study no plateau phase was observed in the PFS curve confirming that even with the addition of rituximab, the use of intensive regimens like HCVAD-AM cannot cure the disease.

These results seem to show that, at least in our hands, this complex regimen was less effective compared with the original report. We tried to compare the two studies to understand why we achieved different results.

Looking at the study design, the same number of cycles and the same drug doses were planned with a main difference in the use of G-CSF that was mandatory in the MDACC trial and was only 'recommended' in our study. Actually, a high number of our patients received prophylaxis for neutropenic fever or infection with G-CSF, but the different strength of the recommendation may have resulted in a higher rate of events.

Compared with the MDACC experience, we observed a higher rate of treatment failures that led investigators to prematurely interrupt the treatment programme. Of note, only 37% of our patients completed the full programme, i.e., approximately half that of the 71% rate of patients that completed all planned cycles in the original report from the MDACC (Romaguera *et al*, 2005). Most patients interrupted planned therapy due to physician decision after the occurrence of a toxic event; in addition, seven patients were considered failures because they proceeded with intensified chemotherapy or maintenance treatment, thus deviating from protocol. All the above considerations may then suggest that a high activity of the regimen can be counterbalanced by a reduced reproducibility when the treatment is tested in a multicentre setting.

Interestingly differences in ORR and FFS did not translate into differences in OS, which remained comparable between this study, the MDACC trial and other patients series treated with rituximab-containing regimens. This suggests that MCL patients who fail initial therapy can be rescued with effective second-line regimens and can now be treated with excellent results if compared with previous series (Dreyling *et al*, 2005; Romaguera *et al*, 2005; Thieblemont *et al*, 2005; Geisler *et al*, 2008; Herrmann *et al*, 2009).

Currently, several different regimens have been assessed in patients with MCL. In particular, results from the MCL2 study from the Nordic group are directly comparable with our experience because the Ara-C dose used is the same and both are multi-institutional studies (Geisler *et al*, 2008). The two studies mainly differed in the use of consolidation BMT, which was not allowed in our study except for patients with less than CR but was allowed and actually used in 90% of patients in the Nordic trial. In a head to head comparison the two studies are similar in terms of OS (73% at 5 years vs. 81% at 4 years, in the current and in the Nordic trial, respectively) with an apparent better PFS in the Nordic trial (61% at 5 years and 66% at 6 years respectively). The good results achieved confirm that the use of high dose Ara-C is needed in MCL patients to improve results of conventional CHOP (cyclophosphamide, doxorubicin, vincristine, prednisolone) or CHOP-like regimens. Whether the difference in PFS is real and can be explained by the use of consolidation BMT need to be further investigated.

Finally, some limitations of our study should be acknowledged. First, the study was also designed to evaluate R-HCVAD-AM in terms of molecular remission. Unfortunately we were not able to provide robust data on this endpoint as a molecular marker at time of diagnosis was identified in half of cases. However a molecular remission was achieved in 65% of cases, 75% of also obtained clinical CR. Further studies are needed to define the prognostic role of molecular remission in this lymphoma subtype, as in FL or other indolent NHL. Second, looking at the patients' characteristics, 60% of them were at low risk when the MIPI was retrospectively calculated and 40% had a splenic-marrow presentation. Both features may

identify a subgroup of patients with a relatively indolent form of MCL but, according to the original study design, presenting features were not considered for treatment decision, and the MIPI was not available at that time: patients were only identified when the local physician requested treatment. Based on the currently available data in these two groups of patients (low-risk MIPI and/or splenic marrow presentation) less intense approaches might be considered.

In conclusion, this multicentre trial confirmed that R-HCVAD-AM is an active regimen for the initial treatment of patients with MCL, but is affected by significant toxicity that may limit its use outside specialist centres. This regimen should be further investigated to define the optimal therapeutic strategy in patients with MCL, taking the availability of new drugs and the role of maintenance therapies into consideration.

## Acknowledgements

This work was supported by grants from the Associazione Angela Serra per la Ricerca sul Cancro, Modena, Italy, and Gr.A.D.E. Onlus (Gruppo Amici Dell'Ematologia-Onlus), Reggio Emilia, Italy.

## Author contributions

Francesco Merli, Stefano Luminari, Francesco Caracciolo, Angelo Michele Carella and Massimo Federico designed the research study; Stefano Luminari analysed the data; Stefano Luminari, Francesco Merli, Elisa Barbolini and Massimo Federico wrote the paper; Francesco Merli, Fiorella Ilariucci, Mario Petrini, Carlo Visco, Achille Ambrosetti, Caterina Stelitano, Francesco Caracciolo, Nicola Di Renzo, Francesco Angrilli, Angelo Michele Carella, Isabella Capodanno, Sara Galimberti performed the research.

All authors approved the submitted version of the manuscript.

## Conflict of interests

The authors have declared no conflict of interests.

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