Check for updates

blood advances

American Society of Hematology
2021 L Street NW, Suite 900,
Washington, DC 20036
Phone: 202-776-0544 | Fax 202-776-0545
bloodadvances@hematology.org

Long term follow-up of Rituximab plus Bendamustine and Cytarabine (R-BAC) in elderly patients with newly diagnosed MCL

Tracking no: ADV-2023-009744R2

Maria Chiara Tisi (Hematology, San Bortolo Hospital, Vicenza, Italy, Italy) Riccardo Moia (Università del Piemonte Orientale, Italy) Caterina Patti (Azienda Ospedali Riuniti Villa Sofia-Cervello,, Italy) Andrea Evangelista (Azienda Ospedaliero-Universitaria Città della Salute e della Scienza di Torino, Italy) Simone Ferrero (Department of Molecular Biotechnologies and Health Sciences, University of Torino, Italy) Michele Spina (National Cancer Institute - Aviano, Italy) Monica Tani (Hematology, Ospedale di Ravenna, Italy) Barbara Botto (Hematology, Citta' della salute e della scienza University Hospital, Italy) Melania Celli (Ospedale degli Infermi di Rimini, Italy) BENEDETTA PUCCINI (AOU CAREGGI, Italy) Emanuele Cencini (Hematology, Azienda Ospedaliera Universitaria Senese & University of Siena, Italy) Alice Di Rocco (Hematology, University of Rome, Italy) Claudio Chini (Oncology ASST-settelaghi, Varese, Italy, Italy) Chiara Ghiggi (Ematologia e terapie cellulari. IRCCS Ospedale Policlinico San Martino. Genova, Italy) Renato Zambello (Padua University School of Medicine, Italy) Manuela Zanni (Antonio e Biagio e Cesare Arrigo Hospital, Italy) Roberta Sciarra (Fondazione IRCCS Policlinico San Matteo, Italy) Riccardo Bruna (Università del Piemonte Orientale, Italy) Martina Ferrante (Department of Molecular Biotechnologies and Health Sciences, University of Torino, Italy) Stefano Pileri (European Institute of Oncology, Milan, Italy) Francesca Maria Quaglia (Hematology Unit, Azienda Ospedaliera Universitaria Integrata Verona, Italy) Caterina Stelitano (G.O.M. Bianchi Melacrino Morelli, Italy) Alessandro Re (Spedali Civili di Brescia, Italy) Stefano Volpetti (Hematology Department, Santa Maria della Misericordia Hospital - ASUFC, Italy) Vittorio Ruggero Zilioli (ASST Grande Ospedale Metropolitano Niguarda, Italy) Annalisa Arcari (Guglielmo da Saliceto Hospital, Piacenza, Italy, Italy) Francesco Merli (Hematology, AUSL-IRCCS, Reggio Emilia, Italy, Italy) Carlo Visco (University of Verona, Italy)

Abstract:
The combination of rituximab, bendamustine, and low dose cytarabine (R-BAC) has been studied in a phase 2 prospective multicenter study from the Fondazione Italiana Linfomi (FIL RBAC500). In 57 previously untreated elderly patients with mantle cell lymphoma (MCL), R-BAC was associated with complete remission rate of 91%, and 2-years progression free survival (PFS) of 81% (95%CI 68-89). Here, we report the long-term survival outcome, late toxicities, and results of minimal residual disease (MRD) evaluation. After a median follow-up of 86 months (57-107), the median overall survival (OS) and progression-free survival (PFS) were not reached. The 7-years PFS and OS rates were 55% (95%CI 41-67), and 63% (95%CI 49-74), respectively. Responding patients (n=53) had a 7-years PFS of 59% (95%CI 44-71), with no relapse or progression registered after the 6th year. At multivariate analysis blastoid/pleomorphic morphology was the strongest adverse predictive factor for PFS (p=0.04). Patients with an end of treatment negative minimal residual disease (MRD) had better, but not significant, outcomes for both PFS and OS than MRD positive patients (p=0,148 and p=0,162, respectively). There was no signal of late toxicity or increase of secondary malignancies during the prolonged follow-up. In conclusion, R-BAC, which was not followed by maintenance therapy, showed sustained efficacy over time in elderly patients with MCL. Survival outcomes compare favorably with other immuno-chemotherapy regimens (with or without maintenance), including combinations of BTK-inhibitors upfront.

Conflict of interest: COI declared - see note

Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

COI notes: MCT: AB: Incyte, BMS, Gilead Science, Novartis; Speakers Bureau: Incyte, Gilead Science, Novartis, Janssen; CP: advisory board (takeda, Incyte, Abbvie, Astrazeneca, Janssen);SF: Research funding: Janssen, Morphosys, Gilead, Beigene, Consultancy: EusaPharma, Janssen, Sandoz, Abbvie, Advisory Board: EusaPharma, Janssen, Clinigen, Incyte, Italfarmaco, Speakers Honoraria: Janssen, EusaPharma, Servier, Gentili; ADR: advisory board (Takeda, Jannsen); Consultant (Incyte, Gilead); SP: advisory board for Celgene, NanoString, Roche, Beigene; FMQ: Advisor role for AstraZeneca and Janssen; speaker for Janssen; consultant for Sandoz; AR: advisory board (Takeda, Incyte, Italfarmaco), consultant (Takeda, Servier); SV: advisory board Abbvie; VRZ: AB --> Gentili, Gilead, MSD, Servier, Takeda, Presentations --> Janssen, Takeda, Consultant --> Roche, Travel expenses, accomodations: Janssen, Takeda; AA: Advisory board, speaker's bureau, travel expenses: Janssen, Abbvie, Gilead, Novartis, Gentili, Servier FM: advisory board Roche, Gilead, Incyte, MSD, Takeda; CV: Consultancy: AbbVie, BMS, Incyte, Roche, Pfizer, Janssen, Kyowa Kirin, Gentili, Beigene; Speakers Bureau: AbbVie, BMS, Astra Zeneca, Servier, Incyte, Roche, Pfizer, Novartis, Gentili, Janssen, Kite-Gilead, Beigene; Research funding: Janssen; all other authors declare no conflicts of interest.

Preprint server: No;

Author contributions and disclosures: CV and MCT conceived and wrote the paper. All authors were involved in patient recruitment, data collection, and database assembly, and approved the final version of the report. CV, MCT, SFe and AE, analyzed and interpreted the data. SFe and MF analyzed minimal residual disease and SAP performed the central pathological review.

Non-author contributions and disclosures: No;

Agreement to Share Publication-Related Data and Data Sharing Statement: I agree to share publication-related data by emails to the corresponding author

Clinical trial registration information (if any): EUDRA-CT, number 2011-005739-23, and ClinicalTrials.gov, number NCT01662050.

Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

Regular Article

Title: Long term follow-up of Rituximab plus Bendamustine and Cytarabine (R-BAC) in elderly patients with newly diagnosed MCL

Short Title: R-BAC in MCL patients: long term outcome

AUTHORS: Maria Chiara Tisi[1], Riccardo Moia[2], Caterina Patti[3], Andrea Evangelista[4], Simone Ferrero[5], Michele Spina[6], Monica Tani[7], Barbara Botto[8], Melania Celli[9], Benedetta Puccini[10], Emanuele Cencini[11], Alice Di Rocco[12], Claudio Chini[13], Chiara Ghiggi[14], Renato Zambello[15], Manuela Zanni[16], Roberta Sciarra[17], Riccardo Bruna[2], Martina Ferrante[18], Stefano Alessandro Pileri[19], Francesca Maria Quaglia[20], Caterina Stelitano[21], Alessandro Re[22], Stefano Volpetti[23], Vittorio Ruggero Zilioli[24], Annalisa Arcari[25], Francesco Merli[26], Carlo Visco[20]

Affiliation

1 Hematology Unit, San Bortolo Hospital, A.U.L.S.S. 8 "Berica", Vicenza, Italy
2 Division of Hematology, Department of Translational Medicine, Università del Piemonte Orientale and AOU Maggiore della Carità, 28100 Novara, Italy
3 Oncohematology Azienda Ospedali Riuniti Villa Sofia- V. Cervello Palermo
4 Unit of Clinical Epidemiology, Azienda Ospedaliera Universitaria Città della Salute e della Scienza and CPO Piemonte, Turin, Italy
5 Hematology 1 U, Department of Molecular Biotechnologies and Health Sciences, University of Torino, Torino, Italy/AOU "Città della Salute e della Scienza di Torino", Torino, Italy
6 Division of Medical Oncology and Immune-related Tumors, Centro di Riferimento Oncologico di Aviano (CRO) IRCCS, Aviano (PN), Italy
7 Hematology, Ospedale di Ravenna, Ravenna, Italy
8 Hematology, Citta' della salute e della scienza University Hospital, Torino, Italy
9 Hematology, Ospedale degli Infermi, Rimini, Italy
10 Department of Hematology, Careggi Hospital and University of Florence, Italy
11 UOC Ematologia, Azienda Ospedaliera Universitaria Senese & University of Siena
12 Department of Translational and Precision Medicine; 'Sapienza' University of Rome
13 Oncology ASST-settelaghi, Varese, Italy
14 U.O Ematologia e Terapie Cellulari, IRCCS Ospedale Policlinico San Martino, Genova
15 Hematology, University of Padova, Padova, Italy
16 Hematology, SS Antonio e Biagio e Cesare Arrigo Hospital, Alessandria, Italy
17 Division of Hematology, Fondazione IRCCS Policlinico San Matteo, 27100 Pavia, Italy
18 Hematology 1 U, Department of Molecular Biotechnologies and Health Sciences, University of Torino, Torino, Italy
19 Division of Haematopathology, IEO, European Institute of Oncology IRCCS, Milan, Italy
20 Department of Engineering for Innovation Medicine, Section of Hematology, University of Verona, Verona, Italy
21 Grande Ospedale Metropolitano (GOM), Bianchi Melacrino Morelli, Ematologia Reggio Calabria
22 Hematology, Spedali Civili, Brescia, Italy
23 Hematology Department, Santa Maria della Misericordia Hospital - ASUFC, Udine, Italy
24 Hematology, ASST Grande Ospedale Metropolitano Niguarda, Milan, Italy
25 Hematology Unit, Guglielmo da Saliceto Hospital, Piacenza, Italy
26 Hematology, AUSL/IRCCS Reggio Emilia

Corresponding author:
Carlo Visco
Department of Engineering for Innovation Medicine, Section of Hematology,
University of Verona, Verona, Italy
Address: Piazzale L. Scuro 10A, 37129 Verona

Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

e-mail: carlo.visco@univr.it
Tel: 045 8124797
Fax: 045 8125660

For data sharing, contact the corresponding author: carlovisco@hotmail.com.

Abstract Word Count: 227
Text Word Count: 3016
Number of figures: 4
Number of tables: 1
Reference count: 26

Supplementary figures: 3
Supplementary tables: 1

Key Points:

1. R-BAC was associated with high rate of sustained remissions in elderly patients with MCL

2. After 7-years of follow-up, median overall survival and progression-free survival were not reached, with no signal of late toxicity

Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

Abstract

The combination of rituximab, bendamustine, and low dose cytarabine (R-BAC) has been studied in a phase 2 prospective multicenter study from the Fondazione Italiana Linfomi (FIL RBAC500). In 57 previously untreated elderly patients with mantle cell lymphoma (MCL), R-BAC was associated with complete remission rate of 91%, and 2-years progression free survival (PFS) of 81% (95%CI 68-89). Here, we report the long-term survival outcome, late toxicities, and results of minimal residual disease (MRD) evaluation.

After a median follow-up of 86 months (57-107), the median overall survival (OS) and progression-free survival (PFS) were not reached. The 7-years PFS and OS rates were 55% (95%CI 41-67), and 63% (95%CI 49-74), respectively. Responding patients (n=53) had a 7-years PFS of 59% (95%CI 44-71), with no relapse or progression registered after the 6th year. At multivariate analysis blastoid/pleomorphic morphology was the strongest adverse predictive factor for PFS (p=0.04).

Patients with an end of treatment negative minimal residual disease (MRD) had better, but not significant, outcomes for both PFS and OS than MRD positive patients (p=0,148 and p=0,162, respectively). There was no signal of late toxicity or increase of secondary malignancies during the prolonged follow-up.

In conclusion, R-BAC, which was not followed by maintenance therapy, showed sustained efficacy over time in elderly patients with MCL. Survival outcomes compare favorably with other immuno-chemotherapy regimens (with or without maintenance), including combinations of BTK-inhibitors upfront. This study is registered with EudraCT 2011-005739-23, and ClinicalTrials.gov NCT01662050.

INTRODUCTION

Mantle cell lymphoma (MCL) is an aggressive histotype of non-Hodgkin lymphoma (NHL), characterized by continuous relapses over time, and no standard front-line therapy. Therapeutic choices for transplant ineligible patients are represented by R-CHOP (rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisolone), or bendamustine and rituximab (BR)[1-3], both followed by rituximab maintenance[4,5].

As compared to R-CHOP, BR has currently been recommended as the preferred first-line regimen in contemporary clinical practice guidelines[6]. The R-BAC regimen, which is based on the addition of intermediate dose cytarabine to BR[7], has also been included in clinical guidelines[6,8]. This combination has been supported by preclinical studies showing that bendamustine and cytarabine have distinct and synergistic mechanism of action, especially when administered sequentially[9]. Between 2012 and 2014, the Fondazione Italiana Linfomi (FIL) conducted a phase 2 multicentre trial (RBAC500)[7], analyzing the efficacy and safety of the R-BAC regimen (rituximab, bendamustine and intermediate dose cytarabine) in patients with MCL not eligible to autologous transplant. R-BAC was not followed by rituximab maintenance. The primary analysis of the study, with a median follow-up of 35 months, reported high CR rate (91%) with 2-years overall survival (OS) of 86% (95%CI 74-93), and progression-free survival (PFS) of 81% (68-89). Despite this relevant antitumoral activity, R-BAC was more toxic than BR, particularly in terms of hemato-toxicity between cycles. A recent real-life report confirmed that R-BAC was significantly more effective than BR, but more toxic, with doses that were frequently reduced to spare hemato-toxicity[10]. Finally, the phase 3 randomized SHINE study has shown that the addition of

Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

ibrutinib to BR, as compared to placebo, followed by double maintenance, significantly improved PFS of elderly patients with MCL[11]. With this in mind, we performed a long-term analysis on the efficacy and toxicity end-points of the RBAC500 trial.

METHODS

Study design and participants

RBAC500 was a multicenter, single arm, phase 2 study, recruiting previously untreated patients with confirmed histological diagnosis of MCL. The study involved 29 FIL centers. To be included patients had to be older than 65 years and fit according to the comprehensive geriatric assessment, or aged 60–65 years if they were ineligible for high-dose chemotherapy plus autologous stem-cell transplantation, fit or unfit according to the comprehensive geriatric assessment (for the modified comprehensive geriatric assessment, see appendix p.2 of the original report)[7]. The diagnostic criteria of MCL included positivity for cyclin D1 and SOX11 expression [mandatory in patients who were cyclin D1 expression or t (11;14) negative]. Patients with in-situ MCL or non-nodal leukemic disease were excluded; for the complete list of inclusion and exclusion criteria see the original report[7].

The study was conducted in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. Ethics approval was granted by the institutional review board at each participating institution, and all patients provided written informed consent.

This study was registered with EUDRA-CT, number 2011-005739-23, and ClinicalTrials.gov, number NCT01662050.

Procedures

Baseline assessment included bone marrow biopsy, tumor staging with contrast-enhanced CT, and PET. Paraffin blocks of the diagnostic specimen were collected for central pathological review, which were performed by expert hematopathologists (SAP), according to criteria of the WHO Classification[12]. All patients received RBAC500 (rituximab 375 mg/m² on day 1; bendamustine 70 mg/m² over 30–60 min on days 2 and 3; cytarabine 500 mg/m² over a 2 h infusion starting 2 h after bendamustine on days 2–4; all administered intravenously) every 4 weeks for up to six cycles. No patient received rituximab maintenance. Prophylaxis and toxicity management were described in the original paper[7], with granulocyte colony-stimulating factor (G-CSF) that was mandatory following each cycle. Patients who did not respond to the first two cycles were discontinued from the study. Response assessment was according to Lugano criteria[13]. Follow-up including clinical evaluation, laboratory tests, and CT scan was performed every 3 months for the first year after end of treatment, and then every 6 months for 2 more years. After the third year, patients were followed up with visits according to the local hematologists. It was demanded that patients were seen at least 2 times per year for clinical examination and blood tests. CT-scan was mandatory for any clinical suspicion of relapse or appearance of adenopathies or organomegaly or alterations of the blood exams. Queries were sent to the centers twice a year by FIL offices to update the state of disease and inform on late toxicities or secondary malignancies (SM).

Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

Centralized assessment of minimal residual disease (MRD) was performed in the EuroMRD standardized laboratory of the University of Torino (Torino, Italy) at diagnosis, before cycle 3, and at 1 month, 6 months, 12 months, and 24 months after the end of treatment. We used ASO-droplet digital polymerase chain reaction (ddPCR) analysis[14] to assess MRD in bone marrow (BM) and peripheral blood (PB) samples, using patient-specific primers and consensus probes to detect the immunoglobulin heavy-chain (IGH) variable region gene rearrangement or the IGH::BCL1 product of the t (11;14) translocation. The sensitivity of MRD detection by ddPCR was 2E-05, allowing the detection of a single event among 75000 cells analyzed. For survival analysis a positive sample in at least one of the tissues analyzed (either BM or PB) defined MRD positivity.

Primary efficacy and safety outcomes were reported in the original paper. For the purpose of the present paper we estimated long-term OS and PFS as primary efficacy outcomes. Toxicity was analyzed in terms of secondary malignancies, or any other toxicity reported by investigators during the follow-up period.

Statistical analysis

Demographics and clinical patient characteristics were summarized with descriptive statistics. PFS and OS were estimated using the Kaplan–Meier method and the groups were compared using log-rank test. Overall survival from the progression of disease - POD (OS-2), was defined as time from POD until death due to any cause. Multivariable analysis was performed using Cox regression models. Toxicity, completed treatment rates and treatment response rates were compared with the chi-square or Fisher's test. Cumulative incidence of SM was estimated using the method proposed by Gooley et al.[15], considering death from any cause as a competing event.

RESULTS

Demographic features, cycles delivery, dose reductions

Between May 2012 and Feb 2014, 57 patients from 29 centers were consecutively enrolled in the RBAC500 trial. The main characteristics of these patients, whose median age was 71 years (range, 61 to 79), were described in the original report[7]. Briefly, 25 patients (44%) had high risk MIPI, 16 (31%) had ki67 $\geq$ 30%, and 14 (25%) had blastoid/pleomorphic morphology. No patient had non-nodal leukaemic disease, as for inclusion criteria. Overall, 54 (95%) received at least four cycles of RBAC500, and 38 (67%) had six cycles, with a median of 6·0 (range 2–6) cycles per patient (for distribution of patients and treatment see figure 1, "Trial Profile" from the original report[7]). The majority of patients (75%) had dose reductions of the R-BAC regimen as per-protocol, which mostly consisted in avoiding the 3rd day of cytarabine.

Efficacy outcomes

As previously described, all responding patients achieved CR (52, 91%). Two patients (4%) had progressive disease during induction, while 3 patients (5%) were considered not responders because of early treatment interruption due to toxicity.

Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

At the time of the present report, after a median follow-up of 86 months (range 57-107), 35 patients (61%) are still alive, while 22 patients died (39%). The median OS and PFS have not been reached (Figure 1A-1B). The 7-years PFS and OS rates were 55% (95%CI 41-67) and 63% (95%CI 49-74), respectively. The 7-years duration of response (DOR) of the 52 responding patients was 59% (95%CI 44-71, Figure 1C).

At univariate analysis, adverse predictive factors affecting PFS were high risk MIPI ($p=0.04$), Ki67$\geq$30% ($p<0.001$), blastoid/pleomorphic morphology ($p<0.001$), (Figure 2A, 2B, 2C, respectively), and failure to achieve CR at the end of treatment ($p<0.001$). Patients who had 4 cycles had similar PFS than those completing full treatment (6 cycles), as no difference was observed between patients having dose reductions as per-protocol along cycles ($p=0.2$ for both).

At multivariate analysis, after computing variables that were significant at univariate analysis, morphology (blastoid/pleomorphic variant) resulted the strongest risk factor for PFS (HR 3.12, $p=0.04$, 95%CI 1.05-9.28), Table 1.

Long term side effects, SM and cause of deaths

There were 22 registered deaths during the study period. The majority of them (17, 77%) were due to lymphoma progression, while remaining five (23%) were due to other causes (two to SM, two to sepsis and one to a septic shock in secondary acute myeloid leukemia). No other late toxicity was addressed as possibly related to the induction treatment.

During follow-up, six patients developed SM: 1 prostate cancer, 1 bladder cancer, 2 head and neck cancer (1 larynx, 1 thyroid), 1 lung cancer and 1 secondary acute myeloid leukemia. The cumulative incidence of SM at 7 years was 11.2% (95% CI 4.5-21.3), and the median time to SM was 68 months (range 55-91), Figure S1.

Salvage therapy

Of the 25 patients that were refractory or relapsed, three (12%) did not receive any further treatment due to rapid deterioration of clinical status. Six (24%) had ibrutinib monotherapy as second line, of whom four responded (3 still in CR). The remaining 16 patients received miscellaneous treatment, (7 had R-CHOP or R-CHOP-like, 3 had BR, 1 had high dose-methotrexate for central nervous system relapse, 2 had lenalidomide, and 3 patients received radiation therapy).

Overall, OS from time of first relapse (OS-2) was 8.9 months (range 0-61.9 months) (Figure 3A). The median OS-2 for patients treated with ibrutinib was longer, albeit not significantly, than for patients treated with other approaches [2y OS-2 63% (95%CI 14-89) vs 36% (95% CI 11-63), $p=0.16$; Figure 3B].

Furthermore, patients that were refractory to induction therapy or who experienced progression of disease (POD) within 24 months (POD-24, n=10), had significantly inferior OS-2 than patients with late-POD [22% (95% CI 3-51) vs 53% (95% CI 21-78), $p=0.02$, figure 3C].

Minimal residual disease

Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

Of 57 patients, 45 (79%) had a molecular marker (29 IGH rearrangement, eight IGH::BCL1 target and eight had both). Follow-up DNA samples from BM and/or PB of 31 patients (28 IGH and 3 BCL1) were available for MRD analysis. For this report, samples were reanalyzed by highly sensitive, standardized ASO-ddPCR, in addition to previously reported nested PCR results[14].

No significant differences in baseline characteristics of analyzed vs not analyzed patients were observed, except for BM involvement (p=0.003, Table S1), as no differences were noted for PFS and OS rates [7 years PFS and OS 40% (95%CI 14-68) vs 59% (95%CI 42-72), p=0.38, and 67% (95%CI 34-86 vs 62% (95%CI 46-74), p=0.95, respectively].

After two cycles of RBAC500 (REST1), 20/28 patients (71%) scored MRD negative in BM and 23/28 (82%) in the PB. At the end of treatment (REST2), 22/27 (81.5%) scored MRD negative in BM, and 22/26 (85%) in the PB. Of the 20 patients with available follow-up samples at one-year after the end of treatment (REST4), 16 (80%) were still MRD negative in the BM and 18/20 (90%) in the PB. In the following time-points the number of negative patients slightly decreases over time, as showed in Figure 4.

In a landmark analysis starting from response at the end of treatment, MRD negative patients had better, but not significant, outcomes for both PFS and OS than MRD positive patients [7-year PFS 65% (95% CI 42-81) vs 40% (95% CI 5-75), p=0.148]; 7-year OS, 65% (95%CI 42-81) vs 40% (95% CI 5-75), p=0.162], (Figure 1D, 1E).

Further details on patients' behavior according to MRD analysis at different time-points are showed in the supplements (Figure S2A-S2B and S3A-S3B).

DISCUSSION

We report on the long-term follow-up of the RBAC500 phase 2 trial, showing that this regimen achieved its goal of maintained activity over time, with median PFS and OS figures that were not yet reached after prolonged follow-up. These results, that were achieved without maintenance therapy, support the use of RBAC in elderly patients with MCL, even when judicious dose reductions are implemented in order to avoid hemato-toxicity.

As for previous observations, blastoid/pleomorphic morphology was the most relevant independent predictor of adverse survival. Unfortunately, at the time of the study conception, no study of TP53 function was planned, which limits our knowledge on the activity of R-BAC in TP53 mutated patients, and represents a limitation of this study. Indeed, the FIL VR-BAC trial (NCT03567876), which has completed recruitment approximately one year ago[16], will fill this gap soon.

The results obtained with R-BAC compare favorably with other immuno-chemotherapy regimens (with or without maintenance) in similar populations. The VR-CAP frontline therapy was tested in a randomized trial against R-CHOP[17], showing a median PFS of 24·7 months, but prolonged median OS of 90·7 months. In 2020 Kluin-Nelemans et al. reported on the long-term update of the phase III European Mantle Cell Lymphoma Network Elderly trial, where median OS was 6.4 years. Maintenance therapy with rituximab following response to R-CHOP was associated with a median PFS of 5.4 years[18]. In the STiL study

Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

patients treated with BR had a median PFS of three years. Our trial has apparently similar population to that included in the STiL study[2], with median age of 70 (64.5–74) and 71 (67–75), respectively, but was associated with a 3-years PFS of 76%[7]. Similarly, in the Bright study, median PFS after long-term follow up for the BR arm approximated 48 months[19]. A direct comparison between BR and R-BAC has been performed in a real-life study using a propensity score to match patients for characteristics at presentation and reduce selection bias. This study[10] showed that patients treated with R-BAC had 2-year PFS of 87% ± 3% as compared to 64% ± 7% for BR (p = 0.001). In terms of toxicity, R-BAC was associated with significantly more pronounced grade 3–4 thrombocytopenia than BR (50% vs. 17%), but the efficacy of R-BAC was preserved when doses were reduced. Overall, the present trial, and real-life experience in patients treated with R-BAC consistently report similar efficacy results, but significantly reduced toxicity, when the regimen is administered at attenuated doses, in a 2-day fashion, or with flat doses of bendamustine and cytarabine (i.e. 100-500 mg total dose, respectively, in 2 consecutive days, skipping the 3rd day of cytarabine)[7,10]. In clinical practice, after a first cycle we generally administer at full dose, we recommend to adopt similar dose reductions, with patients who are unfit, or older than 75, that may take advantage of the 100-500 regimen.

The SHINE trial[11] is a large randomized international trial reporting on the association of ibrutinib, a Bruton's tyrosine kinase inhibitor, in combination with BR, followed by rituximab maintenance, in elderly patients with previously untreated mantle-cell lymphoma. This study established the superiority of the ibrutinib containing arm, together with BR and maintenance rituximab, in terms of PFS in respect to BR plus maintenance but with placebo. With a median follow-up of 84.7 months (very similar to that of the present study), the median PFS in the ibrutinib containing winner arm was 80.6 months, which resembles our observation with the R-BAC regimen, where cytarabine is added to BR instead of ibrutinib, but no maintenance is administered. Overall, the demographic characteristics of the population analyzed in the SHINE trial was comparable to that described in the present study [(median age 71 y for both, advanced Ann Arbor Stage in 89.3% and 91%, high risk MIPI in 35.6% and 31%, respectively, but much higher prevalence of blastoid/pleomorphic variants in RBAC 500 trial (25% vs 7.3%, respectively)]. Seven-years OS was 55% in the ibrutinib group of the SHINE, as compared to 63% in the RBAC500. We acknowledge that comparisons between different studies usually hide pitfalls, but still we describe a PFS curve for R-BAC that is largely similar to the PFS achieved by BR+ibrutinib+maintenance rituximab and ibrutinib in the SHINE trial. Furthermore, the avoidance of maintenance may gain importance in the era of COVID-19, where concern has been raised on infectious complications for patients that are long-term maintained with anti-CD20 antibodies[20].

R-BAC was associated with high MRD negativity at the end of induction, with 81.5% of analyzed patients scoring negative in the BM, and 85% in the PB. This percentage is in line with MRD results of intensive chemo-immunotherapy regimens usually offered to younger patients[21,22].

The cumulative incidence of SM (including skin cancers) at 7 years in our cohort was 11.2% (95% CI 4.5-21.3), and the median time to SM was 68 months (range 55-91), with three patients (5%) that died during follow-up due to SM. One patient developed secondary leukemia. This is in line with the SHINE study[11], where SM (also including skin cancers) were observed in 20.8% and 18.8%, respectively, in the two arms, and 3.8% of deaths were related to SM. In the VR-CAP versus R-CHOP study[17] the reported rate of SM was lower (4.1%, ten patients in each group), and mortality was 1.6%. In younger patients treated with Rituximab-HyperCVAD, a 5% rate of secondary myelodysplasia was reported[23] It is difficult

Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

to drive conclusions on the risk of developing SM according to different regimens, but we acknowledge that this topic deserves specific studies in MCL.

In the present study, OS from the time of progression of disease - POD (OS-2) was 8.9 months. Patients that were refractory to induction therapy or who experienced POD-24, n=25, had significantly inferior 2y OS-2 than patients with late-POD [22% (95% CI 3-51) vs 53% (95% CI 21-78), p=0.02, Figure 3C, confirming observations from others both in younger and elderly patients[24-26]. Indeed, ibrutinib was not available in Italy at the time of relapse for most patients included in this study, and this explains why only a minority of patients were treated at relapse with this compound.

In conclusion, we have reported long-term outcome of elderly patients with MCL treated upfront with R-BAC in the prospective RBAC500 FIL trial. Responses were durable without maintenance therapy, and compared favorably with modern examples of combination therapy. This regimen was not associated to unexpected long-term toxicities. With a median PFS and OS exceeding 50% after 7-years this regimen has significantly impacted on the life-expectancy of elderly patients with MCL, a disease that a couple of decades ago was still associated with a median OS of less than 3 years in similar populations.

Acknowledgements:
We thank all patients who participated in this trial. We thank the Fondazione Italiana Linfomi secretary and particularly Elisa Masiera, Claudia Peracchio and Antonella Ferranti. We thank Emilia Elizbieta Florea and Anna Bordin for scientific and administrative contribution to the work, and AIL section of Verona. We thank Valentina Tabanelli for contributing to the central pathological review. This research was supported by Progetto di Ricerca Sanitaria Finalizzata 2009 grant (RF-2009-1469205) and 2010 grant (RF-2010-2307262); AO S Maurizio, Bolzano/Bozen, Italy; Fondi di Ricerca Locale, Università degli Studi di Torino, Italy; Fondazione Neoplasie Del Sangue, Torino, Italy; and Fondazione Cassa di Risparmio di Torino (project code 2015.1044), Torino, Italy.

Authorship Contributions:
CV and MCT conceived and wrote the paper. All authors were involved in patient recruitment, data collection, and database assembly, and approved the final version of the report. CV, MCT, SFe and AE, analyzed and interpreted the data. SFe and MF analyzed minimal residual disease and SAP performed the central pathological review.

Disclosure of Conflicts of Interest
MCT: Advisory board (AB): Incyte, BMS, Gilead Science, Novartis; Speakers Bureau: Incyte, Gilead Science, Novartis, Janssen; CP: advisory board (takeda, Incyte, Abbvie, Astrazeneca, Janssen);SF: Research funding: Janssen, Morphosys, Gilead, Beigene, Consultancy: EusaPharma, Janssen, Sandoz, Abbvie, Advisory Board: EusaPharma, Janssen, Clinigen, Incyte, Italfarmaco, Speakers Honoraria: Janssen, EusaPharma, Servier, Gentili; ADR: advisory board (Takeda, Jannsen); Consultant (Incyte, Gilead); SP: advisory board for Celgene, NanoString, Roche, Beigene; FMQ: Advisor role for AstraZeneca and Janssen; speaker for Janssen; consultant for Sandoz; AR: advisory board (Takeda, Incyte, Italfarmaco), consultant (Takeda, Servier); SV: advisory board Abbvie; VRZ: AB --> Gentili, Gilead, MSD, Servier, Takeda, Presentations --> Janssen, Takeda, Consultant --> Roche, Travel expenses, accomodations: Janssen, Takeda; AA: Advisory board, speaker's bureau, travel expenses: Janssen, Abbvie, Gilead, Novartis, Gentili, Servier. FM: advisory board Roche, Gilead, Incyte, MSD, Takeda; CV: Consultancy: AbbVie, BMS, Incyte, Roche, Pfizer, Janssen, Kyowa Kirin, Gentili, Beigene; Speakers Bureau: AbbVie, BMS, Astra Zeneca, Servier, Incyte, Roche, Pfizer, Novartis, Gentili, Janssen, Kite-Gilead, Beigene; Research funding: Janssen; all other authors declare no conflicts of interest.

References:

1. Herrmann A, Hoster E, Zwingers T, et al. Improvement of overall survival in advanced stage mantle cell lymphoma. J Clin Oncol. 2009;27:511–518.

Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

2. Rummel MJ, Niederle N, Maschmeyer G, et al. Bendamustine plus rituximab versus CHOP plus rituximab as first-line treatment for patients with indolent and mantle-cell lymphomas: an open-label, multicentre, randomised, phase 3 non-inferiority trial. Lancet. 2013;381:1203–1210.
3. Flinn IW, van der Jagt R, Kahl BS, et al. Randomized trial of bendamustine–rituximab or R-CHOP/R-CVP in first-line treatment of indolent NHL or MCL: the BRIGHT study. Blood. 2014;123:2944–2952.
4. Kluin-Nelemans HC, Hoster E, Hermine O, et al. Treatment of older patients with mantle-cell lymphoma. N Engl J Med. 2012;367: 520–531.
5. Martin P, Cohen JB, Wang M, et al. Transplantation and Maintenance Rituximab in Patients With Previously Untreated Mantle Cell Lymphoma: Results From Large Real-World Cohorts [published online ahead of print 28 June 2022]. J Clin Oncol. doi: 10.1200/JCO.21.02698.
6. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: B-cell lymphoma; version 5.2022.
7. Visco C, Chiappella A, Nassi L, et al. Rituximab, bendamustine, and low-dose cytarabine as induction therapy in elderly patients with mantle cell lymphoma: a multicentre, phase 2 trial from Fondazione Italiana Linfomi. Lancet Haematol. 2017;4(1):15-23.
8. Dreyling M, Campo E, Hermine O, et al. Newly diagnosed and relapsed mantle cell lymphoma: ESMO Clinical Practice Guidelines for diagnosis, treatment and follow-up. Ann Oncol. 2017;1;28(suppl_4):iv62-iv71.
9. Visco C, Castegnaro S, Chieregato K, et al. The cytotoxic effects of bendamustine in combination with cytarabine in mantle cell lymphoma. Blood Cells Mol Dis. 2012;48:68-75.
10. Bega G, Olivieri J, Riva M, et al. Rituximab and Bendamustine (BR) Compared with Rituximab, Bendamustine, and Cytarabine (R-BAC) in Previously Untreated Elderly Patients with Mantle Cell Lymphoma. Cancers (Basel). 2021;3;13(23):6089.
11. Wang ML, Jurczak W, Jerkeman M, et al. Ibrutinib plus Bendamustine and Rituximab in Untreated Mantle-Cell Lymphoma. N Engl J Med. 2022;386(26):2482-2494.
12. Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th Edition, Volume 2. IARC Press, Lyon; 2008.
13. Cheson BD, Pfi stner B, Juweid ME, et al. Revised response criteria for malignant lymphoma. J Clin Oncol. 2007;25:579–586.
14. Drandi D, Alcantara M, Benmaad I, et al. Droplet Digital PCR Quantification of Mantle Cell Lymphoma Follow-up Samples From Four Prospective Trials of the European MCL Network. Hemasphere. 2020;3;4(2):e347.
15. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. Stat Med. 1999;18:695–706.
16. Visco C, Tabanelli V, Peracchio C, et al. Rituximab, Bendamustine and Cytarabine Followed By Venetoclax (V-RBAC) in High-Risk Elderly Patients with Mantle Cell Lymphoma. Blood. 2021;138 (Supplement 1):2427. Abstract 623
17. Robak T, Jin J, Pylypenko H, et al. Frontline bortezomib, rituximab, cyclophosphamide, doxorubicin, and prednisone (VR-CAP) versus rituximab, cyclophosphamide, doxorubicin, vincristine, and prednisone (R-CHOP) in transplantation-ineligible patients with newly diagnosed mantle cell lymphoma: final overall survival results of a randomised, open-label, phase 3 study. Lancet Oncol. 2018; 19(11):1449-1458.
18. Kluin-Nelemans HC, Hoster E, Hermine O, et al. Treatment of Older Patients With Mantle Cell Lymphoma (MCL): Long-Term Follow-Up of the Randomized European MCL Elderly Trial. J Clin Oncol. 2020;20;38(3):248-256.
19. Flinn IW, van der Jagt R, Kahl B. First-Line Treatment of Patients With Indolent Non-Hodgkin Lymphoma or Mantle-Cell Lymphoma With Bendamustine Plus Rituximab Versus R-CHOP or R-CVP: Results of the BRIGHT 5-Year Follow-Up Study. J Clin Oncol. 2019;20;37:984-991.

Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

20. Passamonti F, Cattaneo C, Arcaini L, et al. Clinical characteristics and risk factors associated with COVID-19 severity in patients with haematological malignancies in Italy: a retrospective, multicentre, cohort study. Lancet Haematol. 2020;7(10):e737-e745.
21. Hermine O, Hoster E, Walewski J, et al. Addition of high-dose cytarabine to immunochemotherapy before autologous stem-cell transplantation in patients aged 65 years or younger with mantle cell lymphoma (MCL Younger): a randomised, open-label, phase 3 trial of the European Mantle Cell Lymphoma Network. Lancet. 2016;388:565-575.
22. Ferrero S, Grimaldi D, Genuardi E, et al. Punctual and kinetic MRD analysis from the Fondazione Italiana Linfomi MCL0208 phase 3 trial in mantle cell lymphoma. Blood. 2022;140:1378-1389.
23. Romaguera JE, Fayad LE, Feng L, et al. Ten-year follow-up after intense chemoimmunotherapy with Rituximab-HyperCVAD alternating with Rituximab-high dose methotrexate/cytarabine (R-MA) and without stem cell transplantation in patients with untreated aggressive mantle cell lymphoma. Br J Haematol. 2010;150:200-208.
24. Visco C, Tisi MC, Evangelista A, et al. Time to progression of mantle cell lymphoma after high-dose cytarabine-based regimens defines patients risk for death. Br J Haematol. 2019;185:940-944.
25. Visco C, Di Rocco A, Evangelista A, et al. Outcomes in first relapsed-refractory younger patients with mantle cell lymphoma: results from the MANTLE-FIRST study. Leukemia. 2021;35:787-795.
26. Bond DA, Switchenko JM, Villa D, et al. Early relapse identifies MCL patients with inferior survival after intensive or less intensive frontline therapy. Blood Adv. 2021;14;5:5179-5189.

Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

Tables:

Table 1: Multivariate analysis

Variable	HR	P>|z|	[95% C.I.]
Morphology (classic vs others)	3.12	**0.041**	1.05-9.28
MIPI High Risk	2.14	0.096	0.87-5.24
Ki-67 ≥ 30%	2.53	0.085	0.88-7.25

C.I.: Confidence Interval
MIPI: Mantle Cell Lymphoma International Prognostic Index

Figure Legends:

Figure 1. Survival curves at a median follow-up of 86 months, and MRD evaluation. (A) Progression-free survival (PFS), all patients [7-y PFS 55% (95%CI 41-67)]; **(B)** Overall survival (OS), all patients [7-y OS 63% (95%CI 49-74)]; **(C)** Duration of response (DOR), for the 52 responding patients [7-y DOR 59% (95%CI 44-71)]; **(D)** PFS [7-y PFS 65% vs 40%, P=.14] and **(E)** OS [7-y OS 65% vs 45%, P=.16] according to MRD at the end of the treatment (blue curve=positive; yellow curve=negative).

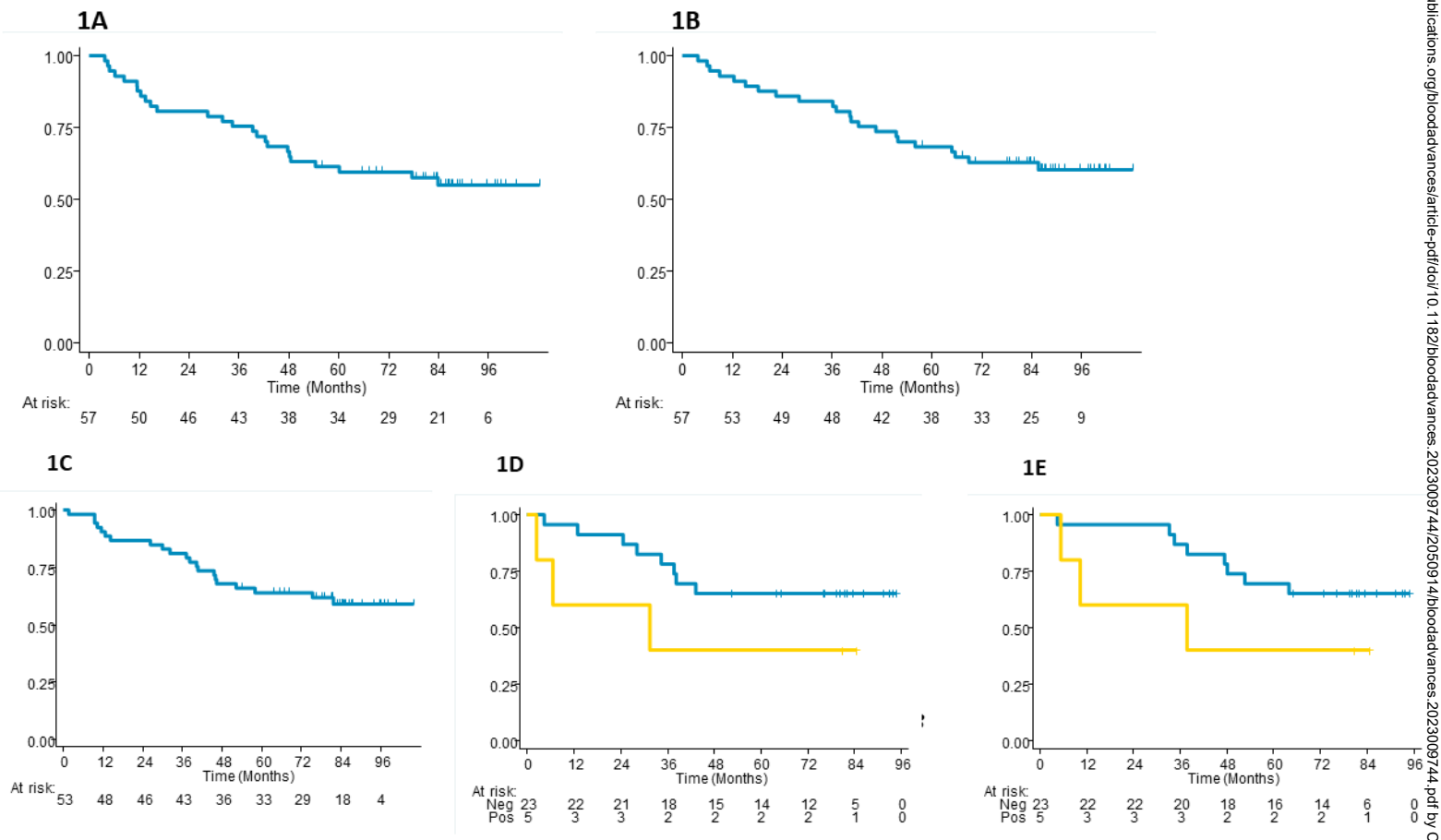
Figure 2: Survival curves for progression-free survival (PFS) according to (A) MIPI score, **(B)** Ki67 value, **(C)** morphological variant, **(D)** or risk group defined as follows: low risk (Ki67<30% and classical morphological variant); high risk group (Ki67 ≥30% and/or blastoid/pleomorphic morphological variant).

Figure 3: Overall survival from time of first progression of disease (OS-2). (A) OS-2 in all 25 relapsed/refractory patients, **(B)** OS-2 in patients who had Ibrutinib as second line (n=6) vs other relapsed and treated patients (n=16), **(C)** POD-24 in 25 relapsed/refractory patients: early- versus late-POD.

Figure 4: Molecular responses evaluated on BM and PB during subsequential time-points.

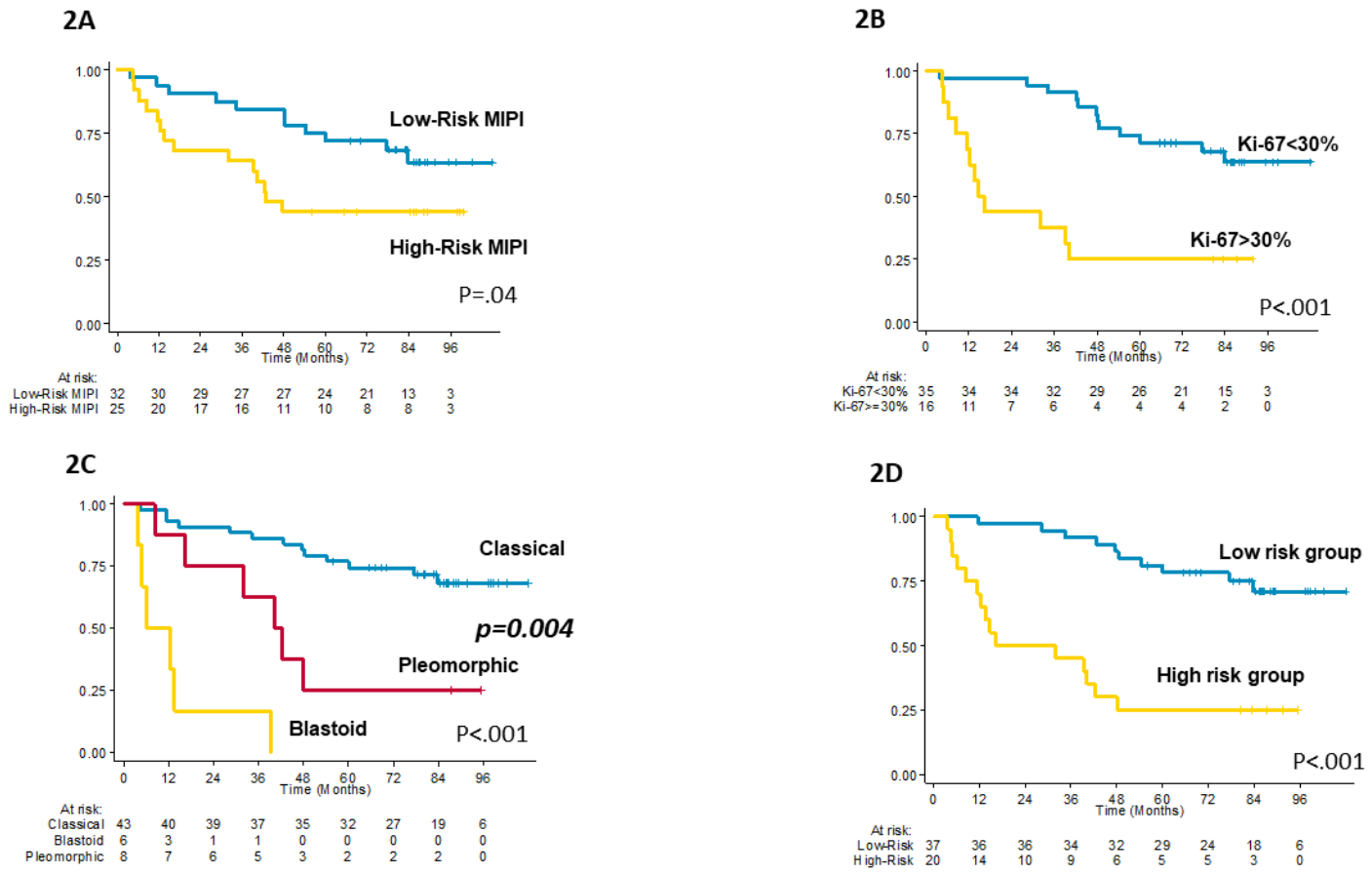
Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

Figure 1



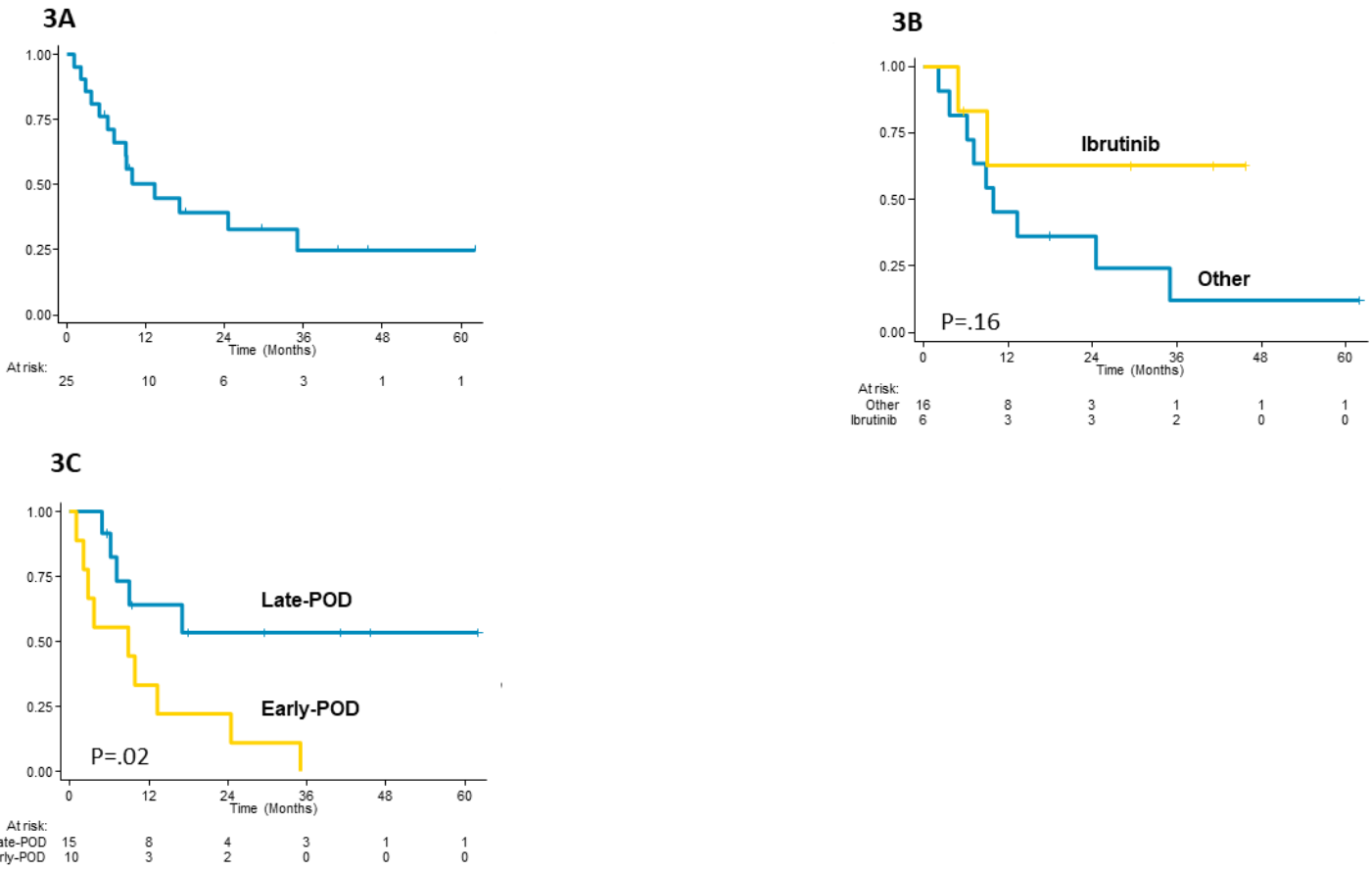
Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

Figure 2

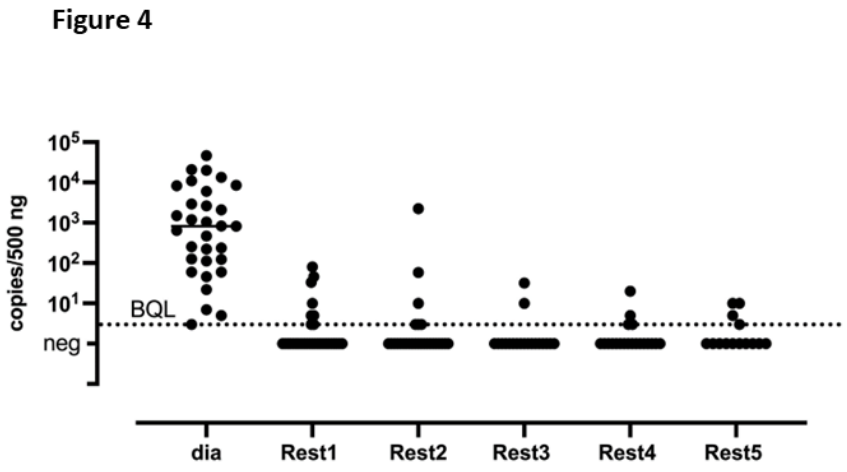


Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023

Figure 3



Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023



Downloaded from http://ashpublications.org/bloodadvances/article-pdf/doi/10.1182/bloodadvances.2023009744/2050914/bloodadvances.2023009744.pdf by Chiara Masucci on 25 May 2023