Response and Resistance to Cladribine in Patients with Advanced Systemic Mastocytosis: A Registry-Based Analysis

Johannes Lübke[1], Nicole Naumann[1], Georgia Metzgeroth[1], Sebastian Kreil[1], Timo Brand[1], Hans-Peter Horny[2], Karl Sotlar[3], Nicholas C.P. Cross[4], Alice Fabarius[1], Peter Valent[5,6], Wolf-Karsten Hofmann[1], Andreas Reiter[1], Juliana Schwaab[1]

[1] Department of Hematology and Oncology, University Hospital Mannheim, Heidelberg University, Mannheim, Germany
[2] Department of Pathology, Ludwig-Maximilians-University, Munich, Germany
[3] Department of Pathology, Paracelsus Medical University of Salzburg, Salzburg, Austria
[4] Faculty of Medicine, University of Southampton, Southampton, United Kingdom
[5] Department of Internal Medicine I, Division of Hematology & Hemostaseology, Medical University of Vienna, Austria
[6] Ludwig Boltzmann Institute for Hematology and Oncology, Medical University of Vienna, Austria

AR and JS contributed equally.

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Corresponding author:
Prof. Andreas Reiter, M.D.
Department of Hematology and Oncology
University Hospital Mannheim
Theodor-Kutzer-Ufer 1-3
68167 Mannheim
Germany
Tel.: +49-621-383-4214
Fax: +49-621-383-4201
e-mail: andreas.reiter@medma.uni-heidelberg.de

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ABSTRACT

We sought to evaluate the efficacy of the purine analogue cladribine in 79 patients with advanced systemic mastocytosis (AdvSM) using data from the 'German Registry on Disorders of Eosinophils and Mast Cells (GREM)'. The overall response rate according to modified Valent criteria (46 evaluable patients) for first- (1L) and second-line (2L) cladribine treatment was 41% (12/29) and 35% (6/17, *P*=0.690), respectively, and the median overall survival (OS, all patients evaluable) was 1.9 years (n=48) and 1.2 years (n=31; *P*=0.311). Univariate and multivariable analyses of baseline and on-treatment parameters identified diagnosis of mast cell leukemia (hazard ratio [HR] 3.5, 95% confidence interval [CI, 1.3-9.1], *P*=0.012), eosinophilia ≥1.5 x 10^9/L (HR 2.9 [CI 1.4-6.2], *P*=0.006) and <3 cycles of cladribine (HR 0.4 [CI 0.2-0.8], *P*=0.008) as independent adverse prognostic parameters for OS. There was no impact of other laboratory (anemia, thrombocytopenia, serum tryptase) or genetic markers (mutations in *SRSF2*, *ASXL1* or *RUNX1*) on OS. In consequence, none of the recently established prognostic scoring systems (MARS, IPSM, MAPS or GPSM) was predictive for OS. Modified Valent criteria were superior to a single factor-based response assessment (HR 2.9 [CI 1.3-6.6], *P*=0.026). In conclusion, cladribine is effective in 1L and 2L treatment of AdvSM. Mast cell leukemia, eosinophilia, application of <3 cycles and a lack of response are adverse prognostic markers.

INTRODUCTION

Systemic mastocytosis (SM) is a rare myeloid neoplasm characterized by multifocal accumulation of neoplastic mast cells (MC) in the bone marrow (BM), visceral organs and skin.[1-4] Advanced systemic mastocytosis (AdvSM) comprises aggressive SM (ASM), SM with an associated hematologic neoplasm (AHN), and MC leukemia (MCL). SM phenotype driver is an acquired somatic point mutation in *KIT* at codon D816V (*KIT* D816V) found in >90% of AdvSM patients.[5,6] In addition, 60-80% of patients harbor additional somatic mutations, e.g. in *SRSF2*, *ASXL1*, *RUNX1* (S/A/R gene panel), *NRAS*, or *DNMT3A*, which are important parameters for combined clinico-genetic prognostic risk scoring systems (e.g., Mutation-Adjusted Risk Score, MARS; Mayo Alliance Prognostic System, MAPS; Global Prognostic Score for SM, GPSM).[7-12]

The development of novel targeted drugs, e.g., the multikinase inhibitor midostaurin[13-15] and the *KIT* D816V inhibitor avapritinib[16,17], has extended the therapeutic options for patients with AdvSM, which were previously based on the off-label use of the purine analogue cladribine[18-22]. However, recent data on response rates and variably on leukemia-free (LFS), event-free- (EFS) and overall survival (OS) meanwhile favor the use of midostaurin and avapritinib.[23-26] Notwithstanding, cladribine will remain a relevant treatment option beyond first-line treatment due to intolerance, resistance and progression on KIT inhibitors.[23,27,28] No predictive markers have yet been established for response, resistance and survival in cladribine-treated AdvSM patients[18-22], a gap which we aimed to fill by analysis of a comprehensive cohort of 79 cladribine-treated patients enrolled within the ‘German Registry on Disorders of Eosinophils and Mast Cells’ (GREM).

PATIENTS AND METHODS

Study population

All cladribine-treated patients (n=79) from the GREM which were diagnosed between 2003 and 2021 were selected for this project, which is an updated and more detailed analysis of a comparative study between midostaurin and cladribine.[23] The diagnosis of SM was established according to the World Health Organization classification.[1,29-31] All BM biopsies were evaluated by reference pathologists (H.-P.H., K.S.) of the European Competence Network on Mastocytosis (ECNM).[32] The study design adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review board of the Medical Faculty of Mannheim, Heidelberg University, Germany. Written informed consent was provided by all patients.

Treatment

The number of patients allowed separation of first- (1L) and second-line (2L) treatment. Prior treatment included midostaurin while subsequent treatment approaches included (individually or sequentially) midostaurin, avapritinib, acute myeloid leukemia-like intensive chemotherapy and, rarely, allogeneic stem cell transplantation. Treatment options with a potentially low disease-modifying impact (e.g. interferon-alpha) or solely directed towards AHN (e.g. hydroxyurea, azacytidine) were not considered as 1L- or 2L-treatment.

Gene mutation analyses

Quantitative assessment of the *KIT* D816V expressed allele burden (EAB) was performed by allele-specific quantitative real-time reverse-transcriptase polymerase chain reaction (RT-qPCR) analysis on RNA/complementary DNA as previously described.[33] NGS analyses on DNA were performed through library preparation by the

Access Array Technology (Fluidigm, San Francisco, CA) and sequencing on the MiSeq Instrument (Illumina, San Diego, CA). Gene mutations were annotated using the reference sequence of the Ensembl Transcript ID (Ensembl release 85: July 2016).

Prognostic scoring systems

The predictive value and clinical utility of several recently established prognostic scoring systems (MARS, International Prognostic Scoring System for AdvSM [IPSM-AdvSM], MAPS, and GPSM) was conducted according to published criteria.[7,11,12,34] Similarities and differences between the scores are given elsewhere.[11,30]

Response assessment

Response assessment according to modified Valent criteria[21] included regular monitoring of C-findings, serum tryptase and a BM biopsy within 2 months after the last applied course of cladribine. The reasons for not using the more recently established International Working Group-Myeloproliferative Neoplasms Research Treatment-ECNM (IWG-MRT-ECNM) criteria included: (i) the retrospective nature of our analysis did not allow to adequately address the complex IWG-MRT-ECNM criteria, (ii) the modified Valent response criteria were commonly used for response assessment of cladribine in prior studies. Molecular response was defined as *KIT* D816V expressed allele burden reduction ≥25% within 2 months after the last course.[7,23,33,35]

Statistical analyses

All statistical analyses considering clinical, laboratory and molecular parameters were obtained at the time of diagnosis/first referral to our center (initial parameters),

treatment initiation with cladribine (baseline parameters) and at multiple time points during treatment (including time point for response assessment). The Mann-Whitney *U*-test was used to compare continuous variables and medians of distributions. Fisher's exact test was used for categorical variables. We retrospectively analyzed the OS (time of diagnosis/treatment initiation to the date of death/last visit) by using the Kaplan-Meier method with log-rank test for group comparisons/visualizations. Disease progression was defined as a shift to a more aggressive AdvSM subtype (secondary MCL or secondary acute myeloid leukemia [AML]). Duration of treatment was defined as the duration from initiation of cladribine to discontinuation for any reason. For the estimation of hazard ratios (HRs) and multivariable analysis, the Cox proportional hazard regression model was used. All variables that showed prognostic significance in univariate analyses were included in multivariable analyses. The first multivariable analysis was performed in an unmodified cohort of patients irrespective of prior or following treatment approaches (midostaurin, avapritinib, intensive chemotherapy and allogeneic stem cell transplantation); the second multivariable analysis was performed in a modified cohort in which patients with prior or following treatment approaches were either excluded or censored at the time of initiation of the next treatment line. *P* values of <0.05 (two-sided) were considered as significant. Data management and statistical analyses were performed with SPSS (SPSS version 20.0; IBM Corporation, Armonk, NY) and GraphPad Prism software (version 8, GraphPad, La Jolla, CA, USA).

RESULTS

Therapeutic modalities

Cladribine was used at a dose of 0.14 mg/kg/day subcutaneously or intravenously on days 1-5 of a 28-day course. For both 1L- (n=48, 61%) and 2L-treatment (n=31, 39%),

a median number of 3 cycles (range 1-6 and 1-8, respectively) was applied over a median of 3.3 (range 0.1-16.0) and 3.0 months (range 0.1-28.5), respectively (*P*=0.612; **Table 1**). Three or more cycles were applied in 32/79 (41%) patients (1L, n=21, 44%; 2L, n=11, 35%). The main reasons for dose reduction, e.g. application only on days 1-3 or extension of intervals, was prolonged myelosuppression (15/79, 19%).

Comparison of baseline characteristics

Compared to 1L-treatment, patients on 2L-treatment presented with a higher frequency of anemia (61% vs. 35%, *P*=0.039), a higher percentage of BM MC infiltration (58% vs. 40%, *P*=0.023) and a higher median serum tryptase level (448 vs. 199µg/L, *P*=0.018). No significant differences were observed regarding median time from diagnosis (2.2 vs. 2.6 years, *P*=0.821) and median time from start of treatment (0.8 vs. 1.5 years, *P*=0.186; **Table 1, Appendix Table 2**).

Evaluation of on-treatment and outcome parameters

According to modified Valent criteria, the overall response rate (ORR) on cladribine in 46/79 (58%) evaluable patients was 18/46 (39%) with a complete remission (CR) in 0/46, a major remission (MR) in 10/46 (22%), and a partial remission (PR) in 8/46 (17%) patients. Comparisons between the patient cohorts with and without available response assessment revealed balanced subgroups (**Appendix Table 1**). There was no difference between 1L- (12/29, 41%) and 2L-treatment (6/17, 35%; *P*=0.690). Any response (MR + PR) vs. no response was associated with improved median OS (3.4 vs. 1.5 years, *P*=0.021; **Figure 2A**) and was independent of 1L- (3.5 vs. 1.5 years, *P*=0.060) or 2L- (3.2 vs. 1.2 years, *P*=0.023) treatment **(Figures 2B-C**). The use of ≥3 cycles was associated with an improved ORR (14/25, 56% vs. 4/21, 19% responder;

P=0.011) and median OS (2.8 vs. 1.2 years, *P*=0.038). The median OS (1.9 vs. 1.2 years, *P*=0.311) was not different between 1L- and 2L-treatment (**Figure 1A**, **Table 1**). The median percentage change from baseline to response assessment of serum tryptase, BM MC infiltration and *KIT* D816V EAB was -29% (range -97% to 75%), 11% (range -94% to 233%) and -1% (range -100% to 1669%; **Figure 3**), respectively. The median percentage change was significantly higher in responders vs. non-responders according to modified Valent criteria (serum tryptase -46% vs. -28%, BM MC infiltration -50% vs. 0% and *KIT* D816V EAB -41% vs. 0%; *P*<0.05).

Risk stratification according to recently established prognostic scoring systems

MARS[7] and the IPSM-AdvSM[34] were recently validated for up-front midostaurin risk-stratification.[23] Both risk scores were assessed for stratification at time of diagnosis (all patients) and at time of initiation of 1L- or 2L-treatment. At diagnosis, median OS according to MARS (n=69 evaluable) was 1.5, 2.1, and 1.9 years in low- (n=16, 23%), intermediate- (n=11, 16%) and high-risk patients (n=42, 61%, *P*=0.270), respectively. Median OS according to IPSM-AdvSM (n=71 evaluable) was 1.3, 2.5, and 1.2 years in AdvSM-1/2 (n=16, 23%), AdvSM-3 (n=36, 50%), and AdvSM-4 patients (n=19, 27%, *P*=0.053; **Figure 1B-C**), respectively. Data were not different when applied at start of 1L- (*P*=0.592, *P*=0.769) or 2L-treatment (*P*=0.125, *P*=0.054). Of note, neither MAPS (*P*=0.358) nor GPSM (*P*=0.127) were able to predict OS on cladribine (**Appendix Figure 1**).

Univariate and multivariable analyses

Univariate and multivariable analyses of baseline parameters from all 79 patients identified diagnosis of MCL (hazard ratio [HR] 3.5, 95% confidence interval [CI, 1.3-9.1], *P*=0.012), eosinophilia ≥ 1.5 x 10^9/L (HR 2.9 [CI 1.4-6.2], *P*=0.006) and application

of <3 cycles cladribine (HR 0.4 [CI 0.2-0.8], *P*=0.008) as independent adverse prognostic parameters for OS (**Figure 4-5, Appendix Figure 2, Appendix Table 3**). Outcome on cladribine was independent of the presence of one or more additional somatic mutations in the S/A/R gene panel (HR 0.6 [CI 0.2-2.0], *P*=0.412). In univariate analysis, modified Valent criteria were superior (HR 2.9 [CI 1.3-6.6], *P*=0.026; **Figure 6; Appendix Table 4**) to a single factor-based response assessment, e.g. BM MC infiltration, serum tryptase or *KIT* D816V EAB.

DISCUSSION

In historical cohorts of up to a maximum of 32 AdvSM patients,[18,19,21] the ORR on cladribine according to (modified) Valent criteria[21,36] ranged between 50% and 100%.[20] Further interpretation on the impact of treatment with cladribine on progression-free (PFS), relapse-free (RFS), event-free (EFS), leukemia-free (LFS) and overall survival is limited because (i) most reports did not clearly differentiate between ISM and AdvSM, (ii) no report separated between 1L- and 2L-treatment and (iii) the definitions of PFS/RFS/EFS/LFS were not consistent between studies. In a registry-based cross-assessment, we recently reported an ORR (modified Valent criteria) of 35% in midostaurin-treated and 40% in cladribine-treated patients.[23] Notwithstanding, the OS on cladribine was significantly inferior to midostaurin in both 1L- and 2L-treatment cohorts. In the current report, we sought to provide a more detailed analysis on response rates on cladribine in 1L- and 2L-treatment, biomarkers indicating response and resistance, and the association between ORR and OS.

Multivariable analysis identified hypereosinophilia (>1.5x10^9/l), as marker of an AHN, diagnosis of MCL, and application <3 cycles as adverse prognostic markers. This

confirms a recent report from the Mayo Clinic registry on 22 cladribine-treated AdvSM patients indicating a diagnosis of an AHN (in addition to older age and absence of *KIT* D816V) as adverse prognostic markers for survival and is also in line with a previous publication on the poor prognostic impact of eosinophilia in SM.[18,37] Recent data also revealed that midostaurin was superior to cladribine in controlling AHN-associated myeloproliferation.[23] The application of ≥3 cycles was further associated with a higher ORR.

In a minority of patients (<10%), cladribine was used for bridging the interval to the start of the midostaurin trial in 2009 and at later time points, it was used in a few patients for more rapid MC debulking with subsequent pre-planned switch to midostaurin. Although myelosuppression became apparent in approximately 20% of patients, infectious complications were not noted as reasons for treatment discontinuation. In contrast to midostaurin, OS on cladribine was not influenced by cytopenias prior to treatment or additional somatic mutations in the *S*/*A*/*R* gene panel. Consequently, none of the prognostic scoring systems (MARS, IPSM, MAPS, GPSM) was predictive for OS. The reasons for this observation are unknown but may be explained at least in part by the fact that the scores more effectively identify low-risk patients on targeted treatment with midostaurin[23,27] or avapritinb[26] than on conventional chemotherapy with cladribine.

In contrast to the recent report from the Mayo Clinic, possibly due to the higher number of patients in our study, any response according to modified Valent criteria in 1L- but also 2L-treatment was associated with improved OS, thus confirming the usefulness of response assessment for guiding further treatment strategies. The data were underscored by the predictive superiority of modified Valent criteria versus a single

factor-based response assessment. Although 2L patients presented with a higher disease burden, response and survival were not statistically different from 1L patients.

Recently reported propensity score weighted analyses on LFS/EFS and OS revealed superiority of midostaurin over cladribine and of avapritinib over best available treatment including midostaurin and cladribine.[23-25] However, we conclude that (i) cladribine remains a relevant option within the AdvSM treatment algorithm; its application in 1L-, 2L- or 3L-line locally depends on the approval status of midostaurin and avapritinib; (ii) the presence of an AHN (leukocytosis, eosinophilia), application of <3 cycles and lack of response according to modified Valent criteria are adverse prognostic markers, and (iii) commonly used prognostic models for AdvSM are of limited value because of high mortality in low- and intermediate-risk patients.

The genetic and clinical complexity of AdvSM requires further prospective clinical trials to study the effects of KIT inhibitors in combination with simultaneous or intermittent use of other anti-neoplastic drugs, e.g. cladribine or hypomethylating agents. Such an approach may counteract the potential outgrowth of *KIT* D816V negative or multimutated subclones.[38] For patients with progression into secondary MCL or secondary AML, AML-like chemotherapy with or without subsequent allogeneic stem cell transplantation remains the most reasonable and potentially curative treatment options.

COMPLIANCE WITH ETHICAL STANDARDS

Disclosure of potential conflicts of interest

Disclosures of conflict of interest: H.-P.H. served as a consultant for Novartis and Blueprint. P.V. received a research grant from Blueprint and Celgene, served as a consultant in a midostaurin trial with Novartis, and received consultancy honoraria from Blueprint, Deciphera, Novartis, Celgene and Pfizer. A.R. was a member of the Study Steering Committee (SSC) for the global trial of midostaurin in advanced systemic mastocytosis (AdvSM) (Novartis), the Response Adjudication Committee (RAC) for studies of avapritinib in AdvSM (Blueprint Medicines), and the SSC for the phase II trial of ripretinib in AdvSM (Deciphera Pharmaceuticals); has received funding for the conduct of these trials; and has received honoraria and reimbursement of travel expenses from Novartis, Blueprint Medicines and Deciphera Pharmaceuticals. J.S. has served as a member in the advisory board of Blueprint for studies of avapritinib in indolent SM and received honoraria from Novartis.

Research involving human participants

The study design adhered to the tenets of the Declaration of Helsinki and was approved by the institutional review board of the Medical Faculty of Mannheim, Heidelberg University, Germany.

Informed consent

Written informed consent was provided by all patients.

DATA AVAILABILITY STATEMENT

The data sets used and/or analyzed during the current study are available from the corresponding author (A.R.) on reasonable request.

AUTHORSHIP CONTRIBUTIONS

Conception and design: JL, AR, JS

Financial support: JS, AR

Administrative support: WKH, AR, JS

Provision of study materials or patients: JL, NN, GM, SK, AF, WKH, AR, JS

Collection and assembly of data: JL

Data analysis and interpretation: JL

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

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FIGURE LEGENDS

Figure 1. Kaplan-Meier estimates of overall survival according to (A) the first- and second-line use of cladribine, (B) the Mutation-Adjusted Risk Score (MARS) and (C) the International Prognostic Scoring System for Advanced Systemic Mastocytosis (IPSM-AdvSM).

Figure 2. Best percentage change of (A) serum tryptase, (B) bone marrow masto cell infiltration and (C) *KIT* D816V expressed allele burden. The dashed line displays the median change. The triangle indicates percentage change >60%.

Figure 3. (A) Kaplan-Meier estimates of overall survival in cladribine treated patients stratified according to the modified Valent response categories. Respective analyses were performed for cladribine in first- (B) and second-line (C) use.

Figure 4. Kaplan-Meier estimates of overall survival in cladribine treated patients with ≥/< 3 cycles.

Figure 5. Univariate and multivariable analysis of baseline parameters (entire cohort). Abbreviations: Eos, eosinophils; CMML chronic myelomonocytic leukemia; Hb, hemoglobin; HES/CEL, hypereosinophilic syndrome/chronic eosinophilic leukemia; MC, mast cell; MCL, mast cell leukemia; MDS/MPNu, myelodysplastic/myeloproliferative neoplasms unclassifiable; Plt, platelets; *S/A/R, SRSF2/ASXL1/RUNX1*; Wbc, white blood cells.

Figure 6. Univariate analysis of on-treatment parameters. *Cheson criteria for transfusion were considered if necessary. #or normalization. Abbreviations: AP, alkaline phosphatase; BM, Bone marrow; CI, confidence interval; Eos, eosinophilia; Hb, hemoglobin; MC, mast cell; Mono, monocytosis; N, normalization; HR, Hazard ratio; MC, mast cell; Plt, platelets; R, response.

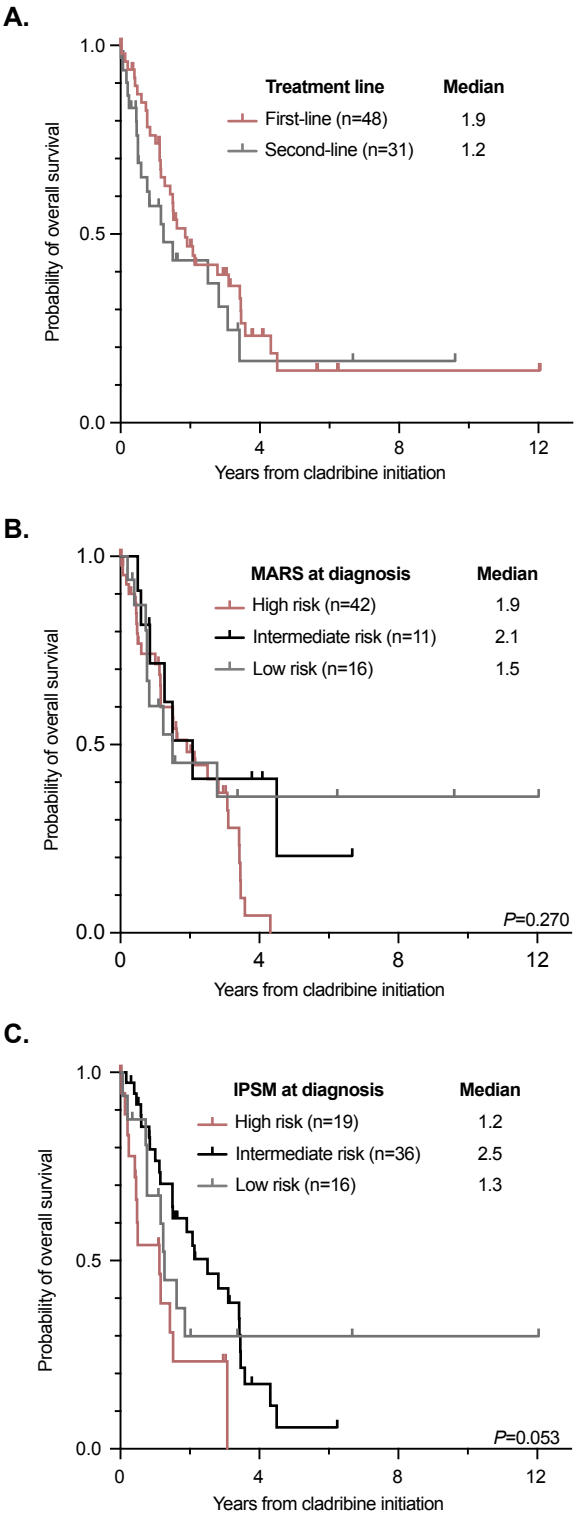
TABLES

Table 1: Demographic and disease characteristics of 79 cladribine treated stratified according first- and second-line treatment

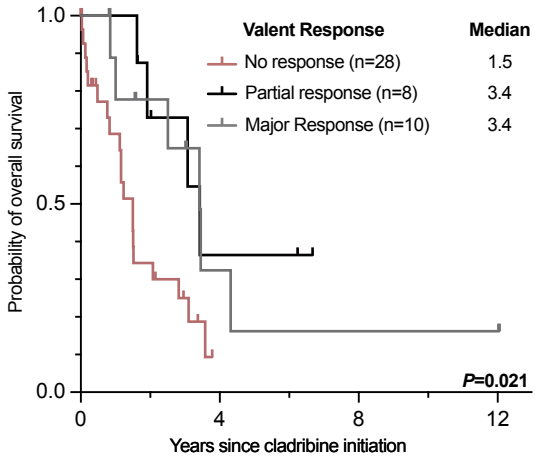
	All	First-line	Second-line	*P*
Number of patients at baseline, *n* (%)	79	48 (61)	31 (39)	
Age in years at treatment initiation; median (range)	68 (27-87)	69 (27-81)	66 (48-87)	0.770
Male, *n* (%)	53 (79)	32 (48)	21 (68)	0.921
Diagnosis				
ASM, *n* (%)	9 (11)	7 (15)	2 (7)	0.267
SM-AHN, *n* (%)	56 (71)	35 (73)	21 (68)	0.621
MCL±AHN, *n* (%)	14 (18)	6 (13)	8 (26)	0.130
C-findings				
Hemoglobin, g/dL; median (range)	10 (7-15)	11 (7-13)	9 (7-15)	0.124
Platelets, $x10^9$/L; median (range)	99 (12-630)	105 (12-630)	87 (25-388)	0.254
ANC, $x10^9$/L; median (range)	5 (0-65)	6 (1-65)	4 (0-62)	0.648
Alkaline phosphatase, U/L; median (range)	270 (45-1736)	242 (45-1736)	300 (63-919)	0.580
Albumin level, g/L; median (range)	34 (15-48)	34 (21-44)	34 (15-48)	0.709
Other relevant parameters				
Leukocytes, $x10^9$/L; median (range)	9.8 (1.3-14.2)	10.4 (1.3-10.4)	9.0 (2.6-14.2)	0.799
Monocytes, $x10^9$/L; median (range)	0.9 (0.0-18.5)	1.1 (0.0-17.9)	0.9 (0-18.5)	0.862
Eosinophils, $x10^9$/L; median (range)	0.5 (0.0-68.3)	0.5 (0.0-1.4)	0.3 (0.0-68.3)	0.254
MC-infiltration in BM biopsy, %; median (range)	45 (3-100)	40 (5-100)	58 (3-90)	0.023
Serum tryptase level, µg/L; median (range)	215 (23-1200)	199 (23-1150)	448 (54-1200)	0.018
Splenomegaly, *n* (%)	64 (94)	41 (91)	23 (100)	0.141
KIT D816V EAB in PB, %, median (range)	35 (0-80)	35 (0-61)	37 (0-80)	0.409
MARS score at diagnosis, *n* (%)	69 (87)	40 (83)	29 (94)	
Low-risk, *n* (%)	16 (23)	10 (25)	6 (21)	0.675
Intermediate-risk, *n* (%)	11 (16)	6 (15)	5 (17)	0.802
High-risk, *n* (%)	42 (61)	24 (60)	18 (62)	0.862
Treatment and outcome				
Follow-up, years since diagnosis; median (range)	2.5 (0.1-17.0)	2.6 (0.1-17.0)	2.2 (0.1-16.4)	0.821
Follow-up, years since 1st cycle; median (range)	1.2 (0.0-12.0)	1.5 (0.0-12-0)	0.8 (0.0-9.6)	0.186
Years to treatment since diagnosis; median (range)	0.7 (0.0-11.0)	0.5 (0.0-10.1)	1.0 (0.1-8.8)	0.083
Years of treatment duration; median (range)	0.3 (0.0-2.4)	0.3 (0.0-1.3)	0.3 (0.0-2.4)	0.612
Number of cladribine cycles, median (range)	3 (1-8)	3 (1-6)	3 (1-8)	0.743
Cycles per months, median (range)	1.0 (0.4-4.8)	1.0 (0.4-4.0)	1.0 (0.7-4.8)	0.848
Deaths, *n* (%)	53 (67)	34 (71)	19 (61)	0.378
Median OS, years (95% CI)	1.5 (1.0-2.0)	1.9 (1.1-2.6)	1.2 (0.3-2.1)	0.311

ANC, absolute neutrophil count; ASM, aggressive systemic mastocytosis; BM, bone marrow; CI, confidence interval; EAB, expressed allele burden; MARS, mutation-adjusted risk score; MC, mast cell; MCL±AHN, mast cell leukemia with/without an associated hematologic neoplasm; NR, monocytosis non-response; OS, overall survival; PB, peripheral blood; R, monocytosis response; SM-AHN, systemic mastocytosis with an associated hematological neoplasm.

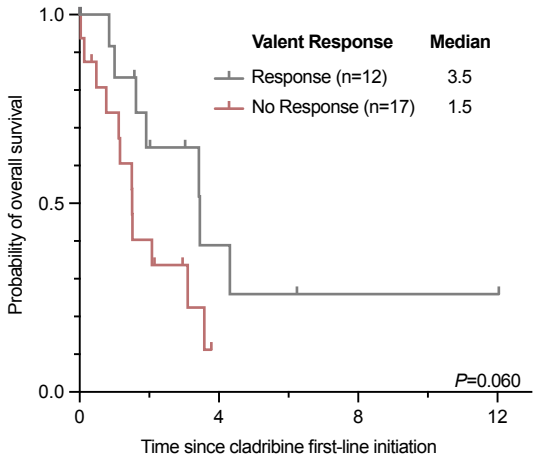
An expanded version of this table is given as Appendix Table 2.


A.
1.0
0.5
0.0
Probability of overall survival
Treatment line
Median
First-line (n=48)
1.9
Second-line (n=31)
1.2
0
4
8
12
Years from cladribine initiation
B.
1.0
0.5
0.0
Probability of overall survival
MARS at diagnosis
Median
High risk (n=42)
1.9
Intermediate risk (n=11)
2.1
Low risk (n=16)
1.5
P=0.270
0
4
8
12
Years from cladribine initiation
C.
1.0
0.5
0.0
Probability of overall survival
IPSM at diagnosis
Median
High risk (n=19)
1.2
Intermediate risk (n=36)
2.5
Low risk (n=16)
1.3
P=0.053
0
4
8
12
Years from cladribine initiation

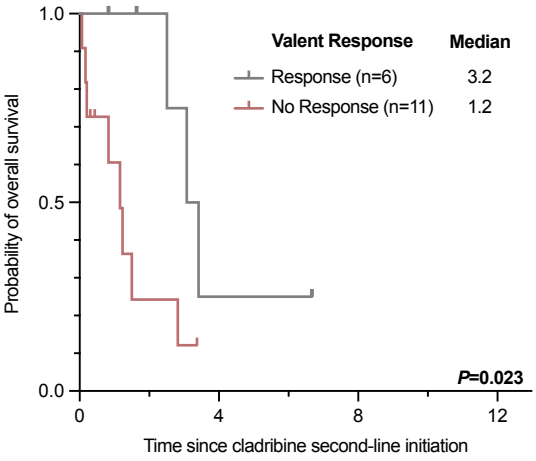
A.

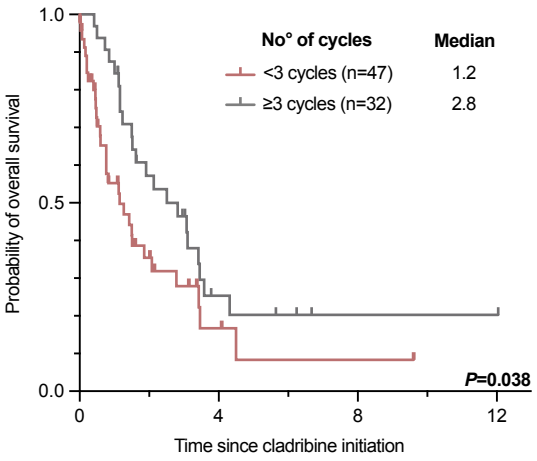

Valent Response
Median
No response (n=28)
1.5
Partial response (n=8)
3.4
Major Response (n=10)
3.4
Probability of overall survival
1.0
0.5
0.0
P=0.021
0
4
8
12
Years since cladribine initiation

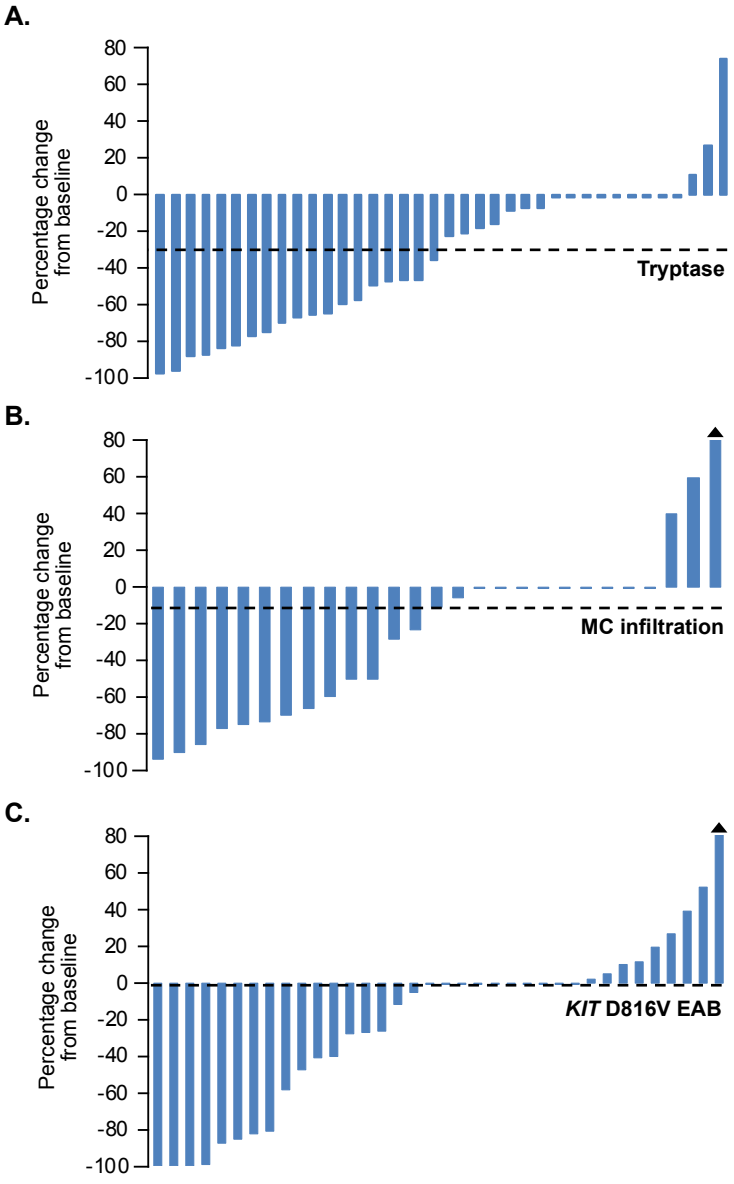
B.

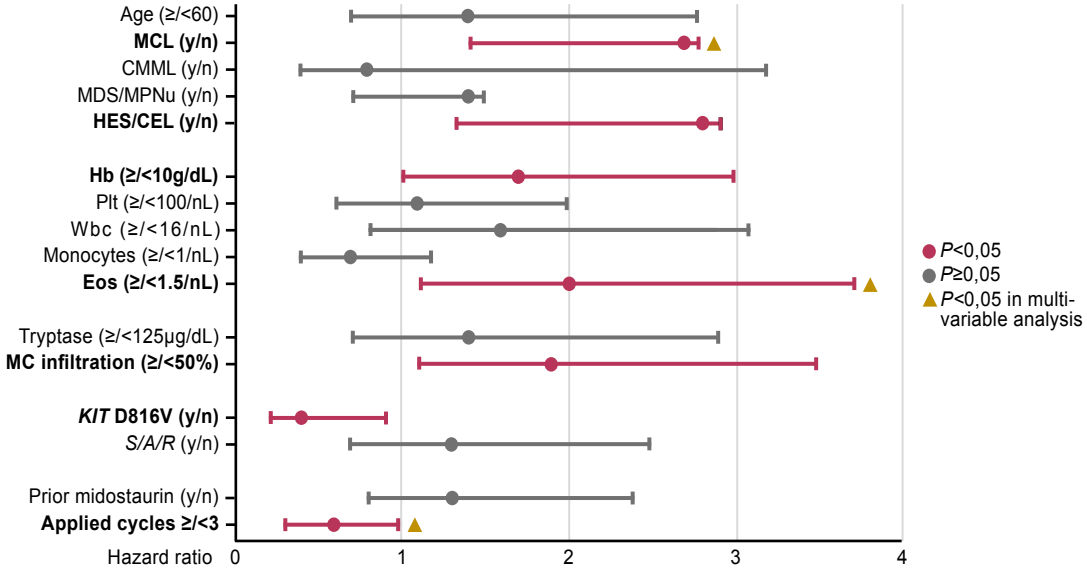

Valent Response
Median
Response (n=12)
3.5
No Response (n=17)
1.5
Probability of overall survival
1.0
0.5
0.0
P=0.060
0
4
8
12
Time since cladribine first-line initiation

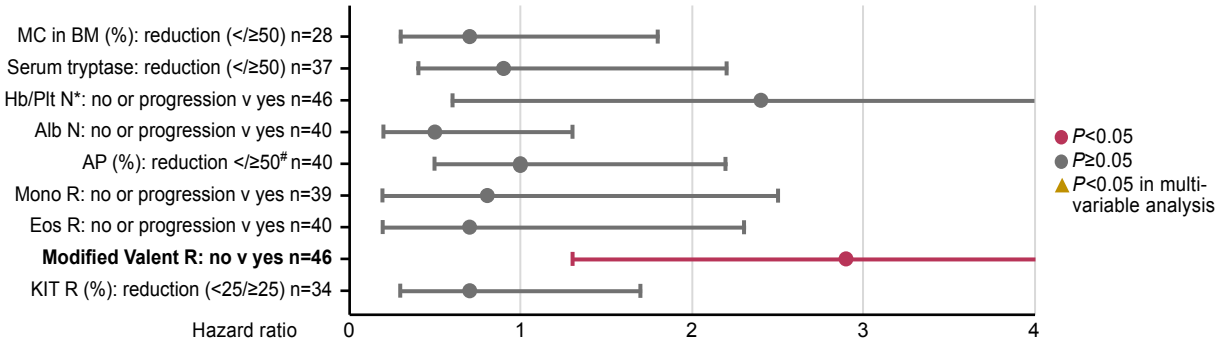
C.


Valent Response
Median
Response (n=6)
3.2
No Response (n=11)
1.2
Probability of overall survival
1.0
0.5
0.0
P=0.023
0
4
8
12
Time since cladribine second-line initiation


No° of cycles
Median
<3 cycles (n=47)
1.2
≥3 cycles (n=32)
2.8
Probability of overall survival
1.0
0.5
0.0
P=0.038
0
4
8
12
Time since cladribine initiation


A.
Percentage change from baseline
80
60
40
20
0
-20
-40
-60
-80
-100
Tryptase
B.
Percentage change from baseline
MC infiltration
C.
Percentage change from baseline
KIT D816V EAB


Age (≥/<60)
MCL (y/n)
CMML (y/n)
MDS/MPNu (y/n)
HES/CEL (y/n)
Hb (≥/<10g/dL)
Plt (≥/<100/nL)
Wbc (≥/<16/nL)
Monocytes (≥/<1/nL)
Eos (≥/<1.5/nL)
Tryptase (≥/<125µg/dL)
MC infiltration (≥/<50%)
KIT D816V (y/n)
S/A/R (y/n)
Prior midostaurin (y/n)
Applied cycles ≥/<3
Hazard ratio
0
1
2
3
4
P<0,05
P≥0,05
P<0,05 in multi-variable analysis


MC in BM (%): reduction (</≥50) n=28
Serum tryptase: reduction (</≥50) n=37
Hb/Plt N*: no or progression v yes n=46
Alb N: no or progression v yes n=40
AP (%): reduction </≥50# n=40
Mono R: no or progression v yes n=39
Eos R: no or progression v yes n=40
Modified Valent R: no v yes n=46
KIT R (%): reduction (<25/≥25) n=34
Hazard ratio
0
1
2
3
4
P<0.05
P≥0.05
P<0.05 in multi-variable analysis

APPENDIX

FIGURE LEGENDS

Figure 1. Kaplan-Meier estimates of overall survival according to (A) the Mayo Alliance Prognostic System (MAPS) and (B) the Global Prognostic Score for Systemic Mastocytosis (GPSM).

Figure 2. Univariate and multivariable analysis of baseline parameters from a modified cohort after exclusion of patients with prior treatment (n=31) and censoring patients at start of subsequent treatment, as potentially confounding treatment-associated parameters, revealed leukocytosis ≥16 x 10^9/L (HR 5.0, 95% confidence interval [CI 1.2-21.0], *P*=0.026) and eosinophilia ≥1.5 x 10^9/L (HR 3.0 [CI 1.0-8.8], *P*=0.048) as adverse prognostic markers for OS. Abbreviations: Eos, eosinophils; CMML chronic myelomonocytic leukemia; Hb, hemoglobin; HES/CEL, hypereosinophilic syndrome/chronic eosinophilic leukemia; MC, mast cell; MCL, mast cell leukemia; MDS/MPNu, myelodysplastic/myeloproliferative neoplasms unclassifiable; Plt, platelets; *S/A/R, SRSF2/ASXL1/RUNX1*; Wbc, white blood cells.

TABLES

Table 1: Demographic and disease characteristics cladribine treated patients stratified according to availability for response assessment

	Response assessment available	Response assessment not available	*P*
Number of patients at baseline, *n* (%)	46 (58)	33 (42)	
Age in years at treatment initiation; median (range)	69 (27-87)	67 (45-84)	0.207
Male, *n* (%)	33 (72)	20 (61)	0.299
Diagnosis			
ASM, *n* (%)	3 (7)	6 (18)	0.108
SM-AHN, *n* (%)	35 (76)	21 (64)	0.230
MCL±AHN, *n* (%)	8 (17)	6 (18)	0.928
C-findings			
Hemoglobin, g/dL; median (range)	11 (7-15)	10 (7-13)	0.496
<10g/dL, *n* (%)	18 (40)	12 (50)	0.425
Platelets, $x10^9$/L; median (range)	99 (12-312)	97 (25-630)	0.263
<$100x10^9$/L, *n* (%)	23 (51)	13 (54)	0.809
ANC, $x10^9$/L; median (range)	5 (0-65)	6 (2-62)	0.453
<$1x10^9$/L, *n* (%)	2 (4)	0 (0)	0.315
Alkaline phosphatase, U/L; median (range)	300 (67-1464)	180 (45-1736)	0.485
>150U/L, *n* (%)	40 (89)	16 (67)	0.025
Albumin level, g/L; median (range)	33 (15-44)	36 (25-48)	0.047
<34g/L, *n* (%)	25 (56)	5 (29)	0.066
Weight loss (>10 % over last 6 months), *n* (%)	33 (83)	12 (80)	0.831
Other relevant findings			
Leukocytes, $x10^9$/L; median (range)	9.1 (2.6-104.4)	10.8 (1.3-142.2)	0.222
Monocytes, $x10^9$/L; median (range)	1.0 (0.0-5.2)	0.9 (0.0-18.5)	0.133
>$1x10^9$/L, *n* (%)	21 (48)	10 (44)	0.741
Eosinophils, $x10^9$/L; median (range)	0.4 (0.0-9.3)	0.8 (0.0-68.3)	0.152
>$1.5x10^9$/L, *n* (%)	12 (27)	9 (39)	0.293
MC-infiltration in BM biopsy, %; median (range)	50 (5-100)	45 (3-90)	0.474
Serum tryptase level, µg/L; median (range)	221 (24-1200)	200 (23-1150)	0.406
Serum tryptase level, >100µg/L, *n* (%)	40 (89)	15 (71)	0.076
Serum tryptase level, >200µg/L, *n* (%)	24 (53)	12 (57)	0.772
Serum tryptase level, >400µg/L, *n* (%)	17 (38)	5 (24)	0.262
Splenomegaly, *n* (%)	42 (94)	22 (96)	0.700
Hepatomegaly, *n* (%)	29 (69)	14 (67)	0.848
Lymphadenopathy, *n* (%)	35 (80)	14 (67)	0.260
KIT D816V EAB in PB, %, median (range)	35 (0-80)	37 (0-55)	0.811
MARS score at diagnosis, *n* (%)			
Low-risk, *n* (%)	7 (16)	8 (33)	0.089
Intermediate-risk, *n* (%)	11 (24)	6 (25)	0.959
High-risk, *n* (%)	27 (60)	10 (42)	0.146
Treatment and outcome			
Follow-up, years since diagnosis; median (range)	2.6 (0.1-17.0)	2.3 (0.2-16.5)	0.500
Follow-up, years since 1st cycle; median (range)	1.6(0.0-12.0)	1.1 (0.0-9.6)	0.306
Years to treatment since diagnosis; median (range)	0.7 (0.0-11.0)	0.8 (0.1-6.8)	0.948
Years of treatment duration; median (range)	0.3 (0.0-2.4)	0.3 (0.0-0.8)	0.154
Number of cladribine cycles, median (range)	4 (1-8)	2 (1-5)	<0.001

Cycles per months, median (range)	0.99 (0.4-4.8)	1.1 (0.7-3.3)	0.326
Deaths, *n* (%)	30 (65)	23 (70)	0.676
Median OS, years (95% CI)	2.1 (0.7-3.5)	1.2 (0.4-1.9)	0.249

ANC, absolute neutrophil count; ASM, aggressive systemic mastocytosis; BM, bone marrow; CI, confidence interval; EAB, expressed allele burden; MARS, mutation-adjusted risk score; MC, mast cell; MCL±AHN, mast cell leukemia with/without an associated hematologic neoplasm; NR, monocytosis non-response; OS, overall survival; PB, peripheral blood; R, monocytosis response; SM-AHN, systemic mastocytosis with an associated hematological neoplasm.

Table 2: Demographic and disease characteristics of 79 cladribine treated stratified according first- and second-line.

	All	**First-line**	**Second-line**	***P***
Number of patients at baseline, *n* (%)	79	48 (61)	31 (39)	
Age in years at treatment initiation; median (range)	68 (27-87)	69 (27-81)	66 (48-87)	0.770
Male, *n* (%)	53 (79)	32 (48)	21 (68)	0.921
Diagnosis				
ASM, *n* (%)	9 (11)	7 (15)	2 (7)	0.267
SM-AHN, *n* (%)	56 (71)	35 (73)	21 (68)	0.621
MCL±AHN, *n* (%)	14 (18)	6 (13)	8 (26)	0.130
C-findings				
Hemoglobin, g/dL; median (range)	10 (7-15)	11 (7-13)	9 (7-15)	0.124
<10g/dL, *n* (%)	30 (44)	16 (35)	14 (61)	0.039
Platelets, x10^9/L; median (range)	99 (12-630)	105 (12-630)	87 (25-388)	0.254
<100x10^9/L, *n* (%)	36 (52)	23 (50)	13 (57)	0.609
ANC, x10^9/L; median (range)	5 (0-65)	6 (1-65)	4 (0-62)	0.648
<1x10^9/L, *n* (%)	2 (3)	1 (2)	1 (4)	0.636
Alkaline phosphatase, U/L; median (range)	270 (45-1736)	242 (45-1736)	300 (63-919)	0.580
>150U/L, *n* (%)	56 (81)	37 (80)	19 (83)	0.828
Albumin level, g/L; median (range)	34 (15-48)	34 (21-44)	34 (15-48)	0.709
<34g/L, *n* (%)	30 (48)	19 (49)	11 (48)	0.946
Weight loss (>10 % over last 6 months), *n* (%)	45 (82)	28 (80)	17 (85)	0.644
Other relevant findings				
Leukocytes, x10^9/L; median (range)	9.8 (1.3-14.2)	10.4 (1.3-10.4)	9.0 (2.6-14.2)	0.799
Monocytes, x10^9/L; median (range)	0.9 (0.0-18.5)	1.1 (0.0-17.9)	0.9 (0-18.5)	0.862
>1x10^9/L, *n* (%)	31 (46)	23 (52)	8 (35)	0.173
Eosinophils, x10^9/L; median (range)	0.5 (0.0-68.3)	0.5 (0.0-1.4)	0.3 (0.0-68.3)	0.254
>1.5x10^9/L, *n* (%)	21 (31)	14 (31)	7 (30)	0.955
MC-infiltration in BM biopsy, %; median (range)	45 (3-100)	40 (5-100)	58 (3-90)	0.023
Serum tryptase level, µg/L; median (range)	215 (23-1200)	199 (23-1150)	448 (54-1200)	0.018
Serum tryptase level, >100µg/L, *n* (%)	55 (83)	36 (82)	19 (86)	0.640
Splenomegaly, *n* (%)	64 (94)	41 (91)	23 (100)	0.141
Hepatomegaly, *n* (%)	43 (68)	27 (68)	16 (70)	0.865
Lymphadenopathy, *n* (%)	49 (75)	33 (77)	19 (73)	0.722
KIT D816V EAB in PB, %, median (range)	35 (0-80)	35 (0-61)	37 (0-80)	0.409
MARS score at diagnosis, *n* (%)	69 (87)	40 (83)	29 (94)	
Low-risk, *n* (%)	16 (23)	10 (25)	6 (21)	0.675
Intermediate-risk, *n* (%)	11 (16)	6 (15)	5 (17)	0.802
High-risk, *n* (%)	42 (61)	24 (60)	18 (62)	0.862
Treatment and outcome				
Follow-up, years since diagnosis; median (range)	2.5 (0.1-17.0)	2.6 (0.1-17.0)	2.2 (0.1-16.4)	0.821

Follow-up, years since 1st cycle; median (range)	1.2 (0.0-12.0)	1.5 (0.0-12-0)	0.8 (0.0-9.6)	0.186
Years to treatment since diagnosis; median (range)	0.7 (0.0-11.0)	0.5 (0.0-10.1)	1.0 (0.1-8.8)	0.083
Years of treatment duration; median (range)	0.3 (0.0-2.4)	0.3 (0.0-1.3)	0.3 (0.0-2.4)	0.612
Number of cladribine cycles, median (range)	3 (1-8)	3 (1-6)	3 (1-8)	0.743
Cycles per months, median (range)	1.0 (0.4-4.8)	1.0 (0.4-4.0)	1.0 (0.7-4.8)	0.848
Deaths, *n* (%)	53 (67)	34 (71)	19 (61)	0.378
Median OS, years (95% CI)	1.5 (1.0-2.0)	1.9 (1.1-2.6)	1.2 (0.3-2.1)	0.311

ANC, absolute neutrophil count; ASM, aggressive systemic mastocytosis; BM, bone marrow; CI, confidence interval; EAB, expressed allele burden; MARS, mutation-adjusted risk score; MC, mast cell; MCL±AHN, mast cell leukemia with/without an associated hematologic neoplasm; NR, monocytosis non-response; OS, overall survival; PB, peripheral blood; R, monocytosis response; SM-AHN, systemic mastocytosis with an associated hematological neoplasm.

Table 3: Demographic and disease characteristics of 79 cladribine treated stratified according to applied cycles

	All	**≥3 cycles**	**<3 cycles**	***P***
Number of patients at baseline, *n* (%)	79	32 (41)	47 (59)	
Age in years at treatment initiation; median (range)	68 (27-87)	69 (45-81)	68 (27-87)	0.979
Male, *n* (%)	53 (79)	22 (69)	31 (66)	0.795
Diagnosis				
ASM, *n* (%)	9 (11)	3 (9)	6 (13)	0.641
SM-AHN, *n* (%)	56 (71)	25 (78)	31 (66)	0.243
MCL±AHN, *n* (%)	14 (18)	4 (13)	10 (21)	0.316
C-findings				
Hemoglobin, g/dL; median (range)	10 (7-15)	11 (8-15)	10 (7-13)	0.273
<10g/dL, *n* (%)	30 (44)	12 (40)	18 (46)	0.609
Platelets, x10^9/L; median (range)	99 (12-630)	105 (26-312)	96 (12-630)	0.432
<100x10^9/L, *n* (%)	36 (52)	15 (50)	21 (54)	0.751
ANC, x10^9/L; median (range)	5 (0-65)	5 (1-65)	6 (0-62)	0.928
<1x10^9/L, *n* (%)	2 (3)	1 (3)	1 (3)	0.880
Alkaline phosphatase, U/L; median (range)	270 (45-1736)	328 (82-1464)	205 (45-1736)	0.098
>150U/L, *n* (%)	56 (81)	26 (87)	30 (77)	0.305
Albumin level, g/L; median (range)	34 (15-48)	35 (15-44)	33 (21-48)	0.666
<34g/L, *n* (%)	30 (48)	12 (41)	18 (55)	0.301
Weight loss (>10 % over last 6 months), *n* (%)	45 (82)	21 (78)	24 (86)	0.446
Other relevant findings				
Leukocytes, x10^9/L; median (range)	9.8 (1.3-14.2)	9.4 (2.6-10.4)	10.0 (1.3-4.2)	0.902
Monocytes, x10^9/L; median (range)	0.9 (0.0-18.5)	1.1 (0.0-7.1)	0.7 (0.0-18.5)	0.454
>1x10^9/L, *n* (%)	31 (46)	17 (57)	14 (38)	0.124
Eosinophils, x10^9/L; median (range)	0.5 (0.0-68.3)	0.5 (0.0-35.1)	0.4 (0.0-68.3)	0.725
>1.5x10^9/L, *n* (%)	21 (31)	6 (20)	11 (29)	0.398
MC-infiltration in BM biopsy, %; median (range)	45 (3-100)	50 (3-90)	40 (3-90)	0.276
Serum tryptase level, µg/L; median (range)	215 (23-1200)	271 (43-1200)	188 (23-1118)	0.486
Serum tryptase level, >100µg/L, *n* (%)	55 (83)	28 (93)	27 (75)	0.047
Serum tryptase level, >200µg/L, *n* (%)	36 (55)	20 (67)	16 (44)	0.071
Serum tryptase level, >400µg/L, *n* (%)	21 (34)	11 (37)	10 (32)	0.717
Splenomegaly, *n* (%)	64 (94)	28 (93)	36 (95)	0.807
Hepatomegaly, *n* (%)	43 (68)	20 (69)	19 (66)	0.780
Lymphadenopathy, *n* (%)	49 (75)	24 (83)	21 (68)	0.180
KIT D816V EAB in PB, %, median (range)	35 (0-80)	37 (0-80)	35 (0-72)	0.370

MARS score at diagnosis, *n* (%)	69 (87)	30 (43)	39 (57)	
Low-risk, *n* (%)	16 (23)	4 (13)	12 (31)	0.089
Intermediate-risk, *n* (%)	11 (16)	5 (17)	6 (15)	0.885
High-risk, *n* (%)	42 (61)	21 (70)	21 (54)	0.173
Treatment and outcome				
Follow-up, years since diagnosis; median (range)	2.5 (0.1-17.0)	3.4 (0.5-17.0)	1.9 (0.1-16.5)	0.270
Follow-up, years since 1st cycle; median (range)	1.2 (0.0-12.0)	2.0 (0.4-12.0)	0.8 (0.0-9.6)	0.007
Years to treatment since diagnosis; median (range)	0.7 (0.0-11.0)	0.7 (0.0-5.0)	0.8 (0.0-11.0)	0.221
Years of treatment duration; median (range)	0.3 (0.0-2.4)	0.4 (0.3-2.4)	0.2 (0.0-0.8)	<0.001
Number of cladribine cycles, median (range)	3 (1-8)	5 (4-8)	2 (1-3)	<0.001
Cycles per months, median (range)	1.0 (0.4-4.8)	1.0 (0.8-4.8)	1.0 (0.4-3.3)	0.242
Deaths, *n* (%)	53 (67)	22 (69)	31 (66)	0.795
Median OS, years (95% CI)	1.5 (1.0-2.0)	2.8 (1.4-4.2)	1.2 (0.3-2.0)	0.038

ANC, absolute neutrophil count; ASM, aggressive systemic mastocytosis; BM, bone marrow; CI, confidence interval; EAB, expressed allele burden; MARS, mutation-adjusted risk score; MC, mast cell; MCL±AHN, mast cell leukemia with/without an associated hematologic neoplasm; NR, monocytosis non-response; OS, overall survival; PB, peripheral blood; R, monocytosis response; SM-AHN, systemic mastocytosis with an associated hematological neoplasm.

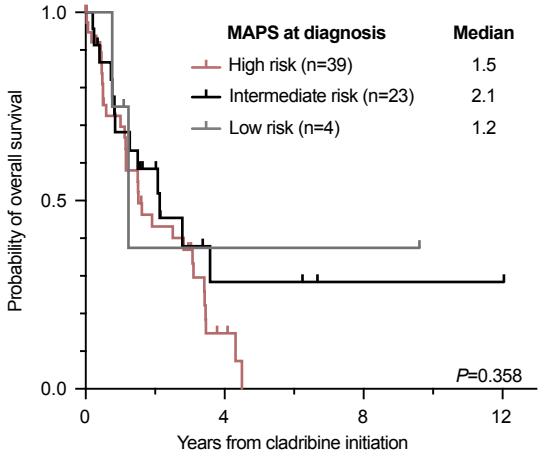
Table 4: Demographic and disease characteristics of 46 cladribine treated stratified according to response status

	Responder	**Non-Responder**	***P***
Number of patients at baseline, *n* (%)	18 (39)	28 (61)	
Age in years at treatment initiation; median (range)	68 (49-77)	69 (27-87)	0.404
Male, *n* (%)	12 (67)	21 (75)	0.540
Diagnosis			
ASM, *n* (%)	3 (17)	0 (0)	0.026
SM-AHN, *n* (%)	14 (78)	21 (75)	0.829
MCL±AHN, *n* (%)	1 (6)	7 (25)	0.090
C-findings			
Hemoglobin, g/dL; median (range)	11 (8-12)	10 (7-15)	0.747
<10g/dL, *n* (%)	7 (41)	11 (39)	0.900
Platelets, x10^9/L; median (range)	114 (37-312)	82 (12-297)	0.274
<100x10^9/L, *n* (%)	8 (47)	15 (54)	0.672
ANC, x10^9/L; median (range)	6 (1-28)	5 (0-65)	0.490
<1x10^9/L, *n* (%)	0 (0)	2 (7)	0.260
Alkaline phosphatase, U/L; median (range)	261 (67-1028)	346 (117-1464)	0.571
>150U/L, *n* (%)	14 (82)	26 (93)	0.277
Albumin level, g/L; median (range)	33 (22-44)	32 (15-42)	0.631
<34g/L, *n* (%)	10 (59)	15 (54)	0.731
Weight loss (>10 % over last 6 months), *n* (%)	14 (88)	19 (79)	0.497
Other relevant findings			
Leukocytes, x10^9/L; median (range)	9.6 (2.6-39.3)	9.0 (2.6-104.4)	0.332
Monocytes, x10^9/L; median (range)	1.0 (0.0-3.7)	0.9 (0.1-5.2)	0.208
>1x10^9/L, *n* (%)	9 (53)	12 (44)	0.583
Eosinophils, x10^9/L; median (range)	0.3 (0.0-2.1)	0.4 (0.0-9.2)	0.081
>1.5x10^9/L, *n* (%)	4 (24)	8 (29)	0.711
MC-infiltration in BM biopsy, %; median (range)	50 (10-100)	50 (5-90)	0.535
Serum tryptase level, µg/L; median (range)	220 (24-1200)	246 (54-1118)	0.858

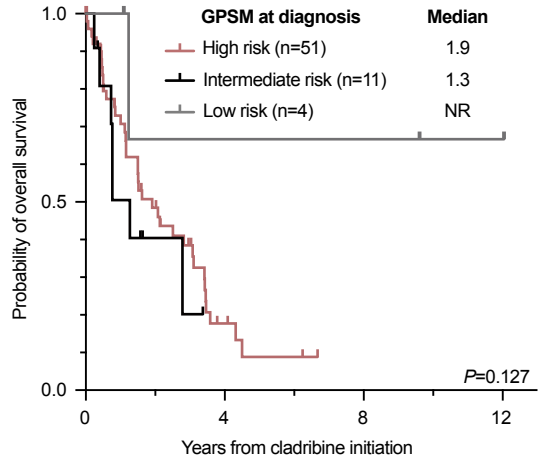
Serum tryptase level, >100µg/L, *n* (%)	15 (88)	25 (89)	0.913
Serum tryptase level, >200µg/L, *n* (%)	9 (53)	15 (54)	0.967
Serum tryptase level, >400µg/L, *n* (%)	6 (35)	11 (39)	0.789
Splenomegaly, *n* (%)	16 (94)	26 (93)	0.870
Hepatomegaly, *n* (%)	11 (65)	18 (72)	0.616
Lymphadenopathy, *n* (%)	13 (77)	22 (82)	0.689
KIT D816V EAB in PB, %, median (range)	29 (0-56)	40 (2-80)	0.405
MARS score at diagnosis, *n* (%)			
Low-risk, *n* (%)	12 (67)	7 (26)	0.007
Intermediate-risk, *n* (%)	3 (17)	3 (11)	0.591
High-risk, *n* (%)	12 (67)	17 (63)	0.799
Treatment and outcome			
Follow-up, years since diagnosis; median (range)	4.0 (1.2-17.0)	2.0 (0.1-12.2)	0.100
Follow-up, years since 1st cycle; median (range)	2.8 (0.8-12.0)	1.1 (0.0-3.8)	0.010
Years to treatment since diagnosis; median (range)	0.8 (0.0-5.0)	0.7 (0.0-11.0)	0.876
Years of treatment duration; median (range)	0.4 (0.1-2.4)	0.3 (0.0-0.6)	0.030
Number of cladribine cycles, median (range)	1.0 (0.5-4.8)	1.0 (0.4-2.5)	0.151
Cycles per months, median (range)	5.5 (1.0-6.0)	3.0 (1.0-8.0)	0.020
Deaths, *n* (%)	10 (56)	20 (71)	0.270
Median OS, years (95% CI)	3.4 (2.9-4.0)	1.5 (1.0-2.0)	0.006

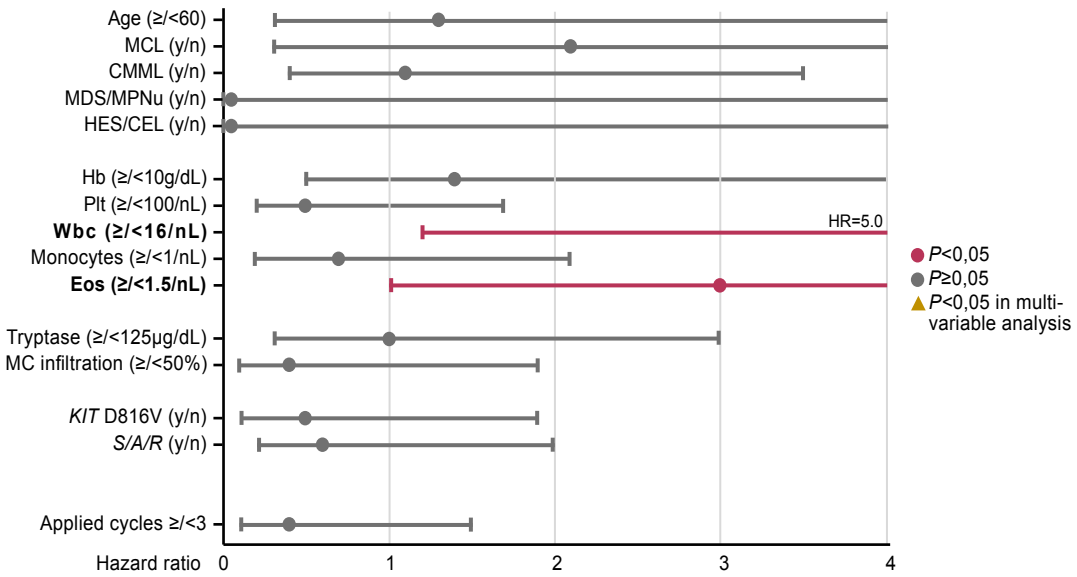
ANC, absolute neutrophil count; ASM, aggressive systemic mastocytosis; BM, bone marrow; CI, confidence interval; EAB, expressed allele burden; MARS, mutation-adjusted risk score; MC, mast cell; MCL±AHN, mast cell leukemia with/without an associated hematologic neoplasm; NR, monocytosis non-response; OS, overall survival; PB, peripheral blood; R, monocytosis response; SM-AHN, systemic mastocytosis with an associated hematological neoplasm.

A.


MAPS at diagnosis
Median
High risk (n=39)
1.5
Intermediate risk (n=23)
2.1
Low risk (n=4)
1.2
Probability of overall survival
1.0
0.5
0.0
P=0.358
0
4
8
12
Years from cladribine initiation

B.


GPSM at diagnosis
Median
High risk (n=51)
1.9
Intermediate risk (n=11)
1.3
Low risk (n=4)
NR
Probability of overall survival
1.0
0.5
0.0
P=0.127
0
4
8
12
Years from cladribine initiation


Age (≥/<60)
MCL (y/n)
CMML (y/n)
MDS/MPNu (y/n)
HES/CEL (y/n)
Hb (≥/<10g/dL)
Plt (≥/<100/nL)
Wbc (≥/<16/nL)
Monocytes (≥/<1/nL)
Eos (≥/<1.5/nL)
Tryptase (≥/<125µg/dL)
MC infiltration (≥/<50%)
KIT D816V (y/n)
S/A/R (y/n)
Applied cycles ≥/<3
Hazard ratio
0
1
2
3
4
HR=5.0
P<0,05
P≥0,05
P<0,05 in multi-variable analysis