A phase II, multicentre trial of decitabine in higher-risk chronic myelomonocytic leukemia

Decitabine in higher-risk chronic myelomonocytic leukemia: results from a phase 2 Italian study

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Conflicts of interest: **[information to be collected from each author – please complete separate request for information sheet as sent in e-mail]**

Running title: Decitabine in chronic myelomonocytic leukemia

Key words: Chronic myelomonocytic leukemia
CMML-scoring systems
Decitabine
Hypomethylating agents
MDS/MPN
Somatic mutations

Abstract

Chronic myelomonocytic leukemia (CMML) is a complex clonal hematological disorder classified among myelodysplastic/myeloproliferative neoplasms (MDS/MPNs). Prognosis is poor and there is a lack of effective treatments. The hypomethylating agent decitabine has shown activity against MDS and elderly acute myeloid leukemia, but there is little data focusing specifically on its efficacy in CMML. In this prospective, phase 2 Italian study, CMML patients received i.v. decitabine 20 mg/m^2/day on Days 1 to 5 of a 28-day treatment cycle. Response was evaluated after 4 and 6 cycles; patients responding at the end of 6 cycles could continue treatment with decitabine. Forty-three patents were enrolled; >50% were high-risk according to four CMML-specific scoring systems. In the intent-to-treat population (n = 42), the overall response rate after 6 cycles was 47.6%, with 7 complete responses (16.6%), 8 marrow responses (19%), 1 partial response (2.4%) and 4 hematological improvements (9.5%). After a median follow-up of 51.5 months (range: 44.4-57.2), median overall survival was 17 months, with responders having a significantly longer survival than non-responders (P=0.02). Grade 3/4 anemia, neutropenia and thrombocytopenia occurred in 28.6%, 50% and 38% of patients, respectively. Decitabine appears to be an effective and well tolerated treatment for patients with high-risk CMML.

Word count (abstract): 199 (*max 200*)

Word count (full paper, excluding references): 2613 (*max 4000*)

Number of tables/figures: 7 *(max 8)*

INTRODUCTION

Chronic myelomonocytic leukemia (CMML) is a complex clonal hematological disorder that is classified by the World Health Organization (WHO) among myelodysplastic/myeloproliferative neoplasms (MDS/MPNs).[1] The 2016 revision to the WHO classification of tumours of the hematopoietic and lymphoid tissues describes three categories of CMML based on blast count:[2,3] CMML-0 (<2% peripheral blasts and <5% bone marrow blasts), CMML-1 (2–4% peripheral blasts and/or 5–9% bone marrow blasts) and CMML-2 (5–19% peripheral blasts, 10–19% bone marrow blasts and/or presence of Auer rods). Before this revision, patients with <2% peripheral blasts and <5% bone marrow blasts were included in the CMML-1 category.[4]

Diagnosis can be difficult, requiring a combination of morphologic, histopathologic and cytogenetic approaches.[5] The WHO diagnostic criteria for CMML are:[2,3] persistent monocytosis ≥1 x 10^9/L; no Philadelphia chromosome or *BCR-ABL1* fusion gene; exclusion of primary myelofibrosis, polycythemia vera and essential thrombocytothemia; no *PDGFRA*, *PDGFRB* or *FGFR1* rearrangements or *PCM1-JAK2* fusions if eosinophilia present; <20% blasts in peripheral blood and bone marrow; dysplasia in one or more myeloid lineages. If myelodysplasia is absent or minimal, a diagnosis of CMML can still be made if a cytogenetic abnormality is present in the hematopoietic stem cell, or if monocytosis has persisted for more than three months with all other possible causes excluded.

Significant heterogeneity makes prognosis in CMML difficult to estimate, but in general it is poor. Commonly used for MDS, the original and revised International Prognostic Scoring System (IPSS)[6,7] are not suitable for CMML because they exclude patients with proliferative disease. Newer prognostic models (such as the CMML-specific prognostic scoring system (CPSS),[8,9] Groupe Francophone de Myelodysplasies (GFM) model,[10] and the Mayo Molecular Model[11]) take cytogenetics and somatic mutations into account. Very recently, an integrated prognostic scoring

system has been proposed that takes clinical parameters, cytogenetics and somatic mutations into account.[12]

The only potentially curative treatment option for CMML is hematopoietic stem cell transplant (HSCT), but this is not suitable for many patients because of their age and comorbidities. There are currently no prospective data on the benefits and risks of HSCT in CMML. Management usually focuses on supportive care and cytoreductive therapy, depending on whether the disease is dysplastic or myeloproliferative.[13] Hydroxyurea is currently a mainstay therapy for proliferative disease.[14]

The hypomethylating agents (HMAs) azacitidine and decitabine have been shown to be active in MDS patients in randomized phase 3 trials.[15-17] However, the numbers of CMML patients in these trials were limited, and their results were not reported separately. In two retrospective analyses of decitabine, overall response rates (ORRs) ranged from 26 – 68%, and 2-year survival from 25 – 48%.[18,19] In a prospective phase 2 study in which 39 CMML patients received 20 mg/m^2 decitabine per day on days 1 – 5 of 28-day cycles, the ORR was 38% and 2-year survival was 48%.[20]

The European Medicines Agency has approved azacitidine for the treatment of non-proliferative CMML (white blood cell (WBC) count <12,000), but HMAs are not currently a licensed option for treating proliferative forms. In Italy, several national societies recommend that patients with myelodysplastic-type CMML and ≥10% bone marrow blasts should be managed with supportive therapy in combination with HMAs.[13] Alongside the lack of specific treatment options, CMML-specific response criteria were not used in any clinical trials, having only been recently developed by Savona *et al*.[21]

Here, we report the results of a prospective phase 2 study that assessed the efficacy and safety of decitabine in Italian CMML patients.

METHODS

Study design and patients

This was an open-label, phase 2 study carried out at 15 centres across Italy between April 2010 and October 2011. Patients aged ≥18 years with a diagnosis of CMML according to WHO criteria,[4] an Eastern Cooperative Oncology Group (ECOG) performance status ≥2, and a life expectancy ≥6 months were eligible to enter the study. Patients with a WBC count ≥12 000/mm^3 were required to have IPSS intermediate-2 risk. Those with a WBC count <12 000/mm^3 had to have at least two of the following: blast cells >5% in bone marrow; a cytogenetic abnormality other than t(5;12) (q33;p13); anaemia (i.e. Hb <10 g/dl); thrombocytopenia (i.e. platelets <100 000/mm^3); splenomegaly (>5 cm below the costal margin); extramedullary localization. Patients with a myeloproliferative or myelodysplastic syndrome other than CMML, and those who had acute blastic transformation of CMML with bone marrow blast cells >20% were excluded. Other exclusion criteria included eligibility for allogenic stem cell transplant with an identified donor; CMML with t(5:12) or *PDGFBR* rearrangement; intensive chemotherapy in the last 3 months; previous treatment with a HMA.

Patients received i.v. decitabine (Dacogen®; Janssen Pharmaceutica NV, Beerse, Belgium) 20 mg/m^2/day on Days 1 to 5 of a 28-day treatment cycle. Discontinuation was allowed at the patient's request, or if they experienced progression with blastic transformation, grade 3/4 toxicity according to National Cancer Institute criteria (except cytopenia), or other changes in their condition that the investigator felt warranted removal of the patient from the study.

After a minimum of 4 treatment cycles, patients were assessed for response to treatment. Responders were defined as patients who achieved hematological improvement (HI) or better according to International Working Group 2006 criteria;[22] these patients continued treatment for a further 2 cycles. Minor responders and patients with stable disease were allowed to continue in the study at the investigator's discretion. Patients with progressive disease were discontinued from the

study. Patients who completed all 6 treatment cycles were eligible to receive maintenance treatment with decitabine. After completion of, or discontinuation from, the study, patients were followed-up every 4 months.

The study was carried out in accordance with the Declaration of Helsinki. All patients provided written informed consent and all participating trial sites gained approval from the relevant local ethics committee. This study is registered on ClinicalTrials.gov (NCT01251627).

Objectives and outcome measures

The primary aim of the study was to assess the efficacy of decitabine in the treatment of CMML. The primary outcome measure was the ORR, defined as the proportion of patients achieving a complete response (CR), marrow CR (mCR), partial response (PR) or HI. Secondary outcome measures included: overall survival (OS); event-free survival (EFS); duration of response; the number of blood and platelet transfusions; the number of days of hospitalization; safety.

Somatic mutations

Bone marrow samples were collected before treatment and DNA was extracted from unsorted mononuclear cells. The methods used for mutational sequencing have been published previously.[23] Briefly, target regions (exons plus splice junctions) were captured and 500 ng of DNA from each sample was quantified and sequenced using paired-end sequencing. Sequences were aligned to the human genome and the Genome Analysis Toolkit[24] was used to perform further local indel alignment and base-quality score recalibration, and to generate single nucleotide variation and indel calls. Variants with functional consequence on genes were annotated and their presence identified in dbSNP 137, the 1000 Genomes Project, ESP6500 (the National Heart, Lung and Blood Institute GO Exome Sequencing Project), and COSMIC 67.

Statistical analyses

Conventional treatments for CMML (hydroxyurea or etoposide) give ORRs of no more than 15%. This study was designed to detect a clinically relevant 20% increase in ORR with decitabine (i.e. from 15% to 35%) with 85% power and a significance level of 0.05. The planned sample size for this single-stage Fleming-A'Hern phase 2 design was 39 patients. Achievement of OR by ≥11 patients after 6 cycles was to be considered sufficient to justify further investigation. To take into account losses to follow-up for time-to-event endpoints, the sample size was increased by 10%, and 43 patients were enrolled.

Primary efficacy and safety data were analyzed for the intent-to-treat (ITT) population, i.e. all patients who received at least one dose of decitabine. Discrete variables were summarized by frequency and percentage. Continuous variables were summarized by mean and standard deviation (SD) or median and interquartile range (IQR).

OS was defined as the time from enrolment to death from any cause or last follow-up evaluation. EFS was defined as the time from enrolment to progression, transformation to acute myeloid leukemia (AML), or death from any cause. Duration of response was defined as the time from clinical response to progression, transformation to AML or death from any cause. These time-to-event endpoints were analyzed using the Kaplan-Meier method.

Adverse events (AEs) were reported by type and grade according to the Common Terminology Criteria for Adverse Events (version 3.0).

RESULTS

Patients

Between April 2010 and October 2011, 43 patients were enrolled at 15 sites across Italy. The ITT population included 42 patients; their baseline characteristics are shown in Table 1. Most patients were male (71.4%) and two-thirds had proliferative CMML. Between 76% and 93% of patients were high- or intermediate-risk (depending on the prognostic scoring system; Table 1), and approximately one-third had an *ASXL1* mutation.

Figure 1 shows the flow of patients through the study. The median number of treatment cycles was 6 (range: 1–34). Twenty-six patients (62%) received all 6 cycles; the most common reasons for discontinuation were treatment failure (n = 9) and death (n = 5).

Somatic mutations

The results of the analysis of the most frequent somatic mutation found in CMML were not possible in all cases in this study, and have already been published previously in a study in which a subset of the CMML patients treated with decitabine was analysed for methylation pattern, gene expression profile and presence of somatic mutations[23]. The incidence and type of mutation at diagnosis is presented in Table 1. The most frequent mutations as expected were those of SRSF2 (45.2%), TET2 (38.1%) and ASXL1 (35.7%).

There was no correlation between the presence of a single mutation and pattern of response to decitabine.

Response rates

In the ITT population, the ORR was 47.6% (Table 2). Patients with CMML-1 had a higher ORR than those with CMML-2 (53.8% *vs.* 37.5%, respectively). The ORR was also higher in patients with dysplastic CMML (64.3%) than in those with proliferative CMML (39.3%).

For the ITT population, the binominal proportion (95% CI) of ORR after 6 cycles was 0.40 (0.25-0.56). The lower limit of the CI was superior to the null hypothesis (i.e. that ORR = 0.15) and the *P*-value was <0.0001, indicating that decitabine is significantly more effective than standard therapy. **[Please advise if this paragraph is to remain. If so, do the data need updating – this was taken from the original results document from December 2015].**

Survival and progression

The median duration of follow-up was 51.5 months (range: 44.4–57.2). Median OS was 17 months (Figure 2A). The 1-year, 2-year and 3-year OS rates were 66.7%, 33.3% and 28.6%, respectively. Patients who responded to treatment had a significantly longer OS than those who did not (*P* = 0.02; Figure 2B). Median EFS was 8 months (Figure 3). The most common event was death, which occurred in 36 patients (85.7%). Thirty-two patients (76.2%) progressed and 24 (57.1%) had transformation to AML. The 1-year, 2-year and 3-year EFS rates were 35.7%, 21.4% and 19.1%, respectively. The median duration of response was 10 months (Figure 4). At 1-year, 52.6% of responders were still responding to treatment; the corresponding figures at 2 and 3 years were 42.1% and 26.3%, respectively.

Transfusions and hospitalizations

At baseline, 18 patients (42.9%) required transfusions. During the treatment period, transfusions were carried out during 117 cycles; 39 patients needed at least one transfusion. During follow-up, 21 patients needed at least one transfusion.

During the treatment period, 9 patients had a total of 24 scheduled hospital admissions. The median length of hospitalization was 6.5 (range 1.0-31.0) days. Five patients each had one unscheduled hospital admission; the median length of their hospital stay was 7.0 (range 1.0-21.0) days. During follow-up, 10 patients had a total of 21 hospital admissions.

Safety

The most common AEs were haematological: thrombocytopenia, anemia and neutropenia (Table 3). In 50% of cases, anemia was grade 3 or 4. More than three-quarters of thrombocytopenia cases, and over 80% of neutropenia cases were grade 3 or 4. The most common non-hematological AEs were gastrointestinal; all of these were grade 1 or 2. Two patients had grade 5 AEs: one cardiac event and one bleeding event. Thirty-six patients died: 5 during the six-month study period and 31 during follow-up. In 29 of these patients, MDS was the cause of death.

DISCUSSION

CMML is a disease that is difficult to diagnose and has a poor prognosis. There are currently no effective treatments for patients who are unsuitable for HSCT. Dysplastic and proliferative forms of CMML are likely to require different treatment approaches. Current recommendations are to treat dysplastic CMML with supportive care plus azacitidine, and proliferative CMML with cytoreductive therapy to control proliferation and reduce organomegaly.[13]

In our study, decitabine induced a response in approximately half of patients, with responders having a survival advantage over non-responders. Although patients with CMML-2 and those with proliferative disease had lower response rates than those with CMML-1 or dysplastic disease, the results in these subgroups were encouraging. Decitabine was well tolerated in our elderly cohort; the incidence and type of AEs were as expected.

The ORR in our study is slightly higher than that of 38% reported in a previous study in 39 CMML patients conducted by the GFM.[20] Whereas we found CMML-1 patients to be more likely to respond to treatment, the GFM CMML study showed the opposite, with 50% of CMML-2 patients responding, compared with 17.6% of CMML-1 patients. Median OS was similar in our study and the GFM CMML study, but 2-year OS was lower in our study (33.3% vs. 48%).

Previous studies in mixed cohorts of patients with MDS and related malignancies have linked increased response to HMAs to mutations in *TET2*[25,26] (particularly when *ASXL1* is not mutated[26]) and *DNTM3A*.[25] However, in a cohort of 40 patients from the present study, we found that no somatic mutation, including *ASXL1,* was predictive of response to decitabine in CMML.[23] Likewise, the GFM CMML study also found no association between response to decitabine and mutational status.[20] This may indicate a difference in the impact of mutational status between patients with CMML and those with other myeloid malignancies. In addition, the studies showing an association between somatic mutations and response included patients who received azacitidine, as well as patients who received decitabine.[25,26]

Although somatic mutations did not differentiate responders from non-responders in our cohort, we found a pattern of 167 differentially methylated regions of DNA that was predictive of response.[23] Using this, we developed an epigenetic classifier that can accurately predict response to decitabine at the time of diagnosis. It can take several cycles of treatment before it becomes apparent whether the patient will respond or not; this classifier would allow potential non-responders to be identified early and put onto an alternative treatment, rather than having to endure months of fruitless treatment with decitabine.

Many of the patients in our cohort were high risk according to the prognostic scoring systems used, so we could not determine whether high- and low-risk patients have a differential sensitivity to decitabine. The median OS was 17 months, which compares favourably with best supportive care and hydroxyurea.[9,10] Such et al used a cohort of patients receiving best supportive care when developing the CPSS prognostic scoring system. Patients who fell into the high-risk category had a median OS of 5-9 months.[9] Patients classed as high-risk according to the GFM prognostic scoring system had a median OS of 14.4 months.[10] Most patients in this latter study were receiving best supportive care, but hydroxyurea and HMAs were also used. High-risk patients according to the Mayo Molecular Model had a median OS of 16 months; the authors do not report what treatment(s) the patients were receiving.[11].

Decitabine appears to be an effective treatment for patients with high-risk CMML, including those with proliferative disease. Further research is needed to determine whether there is a difference in response between low- and high-risk patients. Owing to the rarity of CMML, large, specific trials can be difficult to conduct. However, we are currently conducting (within the guidance of the European MDS Studies Coordination Office) an international, randomized, phase 3 trial comparing decitabine (±hydroxyurea) with hydroxyurea in patients with advanced proliferative CMML (ClinicalTrials.gov identifier: NCT 02214407). The results of this trial will provide further important insights into the

efficacy of decitabine as a treatment for CMML, particularly in patients with proliferative disease, for whom treatment with HMAs is currently not a licensed option.

ACKNOWLEDGEMENTS

This study was funded by the Fondazione Italiana Sindromi Mielodisplastiche Onlus (FISMOnlus). Under direction of the authors, medical writing services for early versions of the manuscript were provided by Dr Joanna Todd of Rocket Science Medical Communications Limited. Medical writing services were funded by the FISMOnlus.

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FIGURE LEGENDS

Figure 1. Patient disposition. Flow of patients from enrolment to time of analysis.

Figure 2. Overall survival. Kaplan-Meier curves showing overall survival in (A) the ITT population and (B) responders *vs.* non-responders. Vertical lines denote censored patients.

Figure 3. Event-free survival. Kaplan-Meier curves showing event-free survival in the ITT population. Vertical lines denote censored patients.

Figure 4. Duration of response. Kaplan-Meier curves showing duration of response. Vertical lines denote censored patients.

362 **Table 1.** Baseline characteristics (ITT population)

Patients, n	42	
Median age, years (range)	71.5	(42-84)
Male/female, n (%)	30/12	(71.4/28.6)
CMML-1/CMML-2[a], n (%)	26/16	(61.9/38.1)
dCMML/pCMML, n (%)	14/28	(33.3/66.7)
Hb g/dl, median (IQR)	9.8	(9.1-11.0)
AMC x 10^9/l, median (IQR)	3.39	(2.23-7.25)
WBC x 10^9/l, median (IQR)	18.6	(13.9-28.1)
PLT x 10^9/l, median (IQR)	54.5	(34.0-75.0)
Bone marrow blasts %, median (IQR)	6.0	(3-12)
Cytogenetics, n (%)		
Altered	12	(28.6)
Normal	28	(66.7)
Not evaluable	2	(4.7)
Splenomegaly, n (%)	22	(52.4)
Hepatomegaly, n (%)	19	(45.2)
Lymphadenomegaly, n (%)	6	(14.3)
Marrow fibrosis, n (%)	13	(30.9)
ASXL1, n (%)		
Mutated	15	(35.7)
Not evaluable	4	(9.5)
SRSF2, n (%)		
Mutated	19	(45.2)
Not evaluable	5	(11.9)
TET2, n (%)		
Mutated	16	(38.1)
Not evaluable	5	(11.9)
P53, n (%)		
Mutated	3	(7.1)
Not evaluable	5	(11.9)

MMM prognostic risk categories, n (%)		
High risk	13	(30.9)
Int-2	14	(33.3)
Int-1	10	(23.8)
Not evaluable	5	(12.0)
CPSS prognostic risk categories, n (%)		
High risk	3	(7.1)
Int-2	20	(47.6)
Int-1	15	(35.7)
Low	2	(4.8)
Not evaluable	2	(4.8)
Mayo prognostic risk categories, n (%)		
High risk	26	(61.9)
Int	13	(30.6)
Low	3	(7.1)
GFM prognostic risk categories, n (%)		
High	14	(33.3)
Int	18	(42.9)
Low	10	(23.8)

Abbreviations: AMC, absolute monocyte count; CMML, chronic monomyelocytic leukemia; dCMML, dysplastic CMML; CPSS, CMML-specific prognostic scoring system; GFM, Groupe Francophone de Myelodysplasies; Hb, hemoglobin; IQR, interquartile range; MMM, Mayo Molecular Model; PLT, platelet; pCMML, proliferative CMML; WBC, white blood cells. Percentages may not total 100 owing to rounding. [a]Defined according to the 2008 edition of the WHO classification of tumours of the hematopoietic and lymphoid tissues.

Table 2. Overall clinical response (end of cycle 6 or at early withdrawal)

	Number (%) of patients									
	ITT (n = 42)		CMML-1[a] (n = 26)		CMML-2[a] (n = 16)		dCMML (n = 14)		pCMML (n = 28)	
ORR	20	(47.6)	15	(57.6)	5	(31.25)	9	(64.3)	11	(39.3)
CR	7	(16.6)	5	(19.2)	2	(12.5)	3	(21.4)	4	(14.3)
mCR	8	(19.0)	6	(23.1)	2	(12.5)	4	(28.6)	4	(14.3)
PR	1	(2.4)	0	(0.0)	1	(6.2)	0	(0.0)	1	(3.5)
HI	4	(9.5)	4	(15.3)	0	(0.0)	2	(14.2)	2	(7.2)
SD	9	(21.4)	4	(15.3)	5	(31.3)	0	(0.0)	9	(32.1)
PD	13	(31.0)	7	(26.9)	6	(37.5)	5	(35.7)	8	(28.6)

Abbreviations: CR, complete response; dCMML, dysplastic CMML; HI, haematological improvement; ORR, overall response rate; mCR, marrow CR; pCMML, proliferative CMML; PD, progressive disease; PR, partial remission; SD, stable disease. Percentages may not total 100 owing to rounding.

[a]Defined according to the 2008 edition of the WHO classification of tumours of the hematopoietic and lymphoid tissues.

372 **Table 3.** Adverse events (ITT population; n = 42)

	Number (%) of patients		
	Any grade	*Grade 3*	*Grade 4-5*
Anemia	24 (57.1)	11 (26.2)	1 (2.4)
Thrombocytopenia	27 (64.3)	4 (9.5)	17 (63.1)
Neutropenia	19 (45.2)	7 (18.7)	9 (21.4)
Cardiac	2 (4.8)	-	1[a] (2.4)
Neurological	1 (2.4)	-	-
Gastrointestinal	10 (23.8)	-	-
Hepatic	2 (4.8)	1 (2.4)	-
Documented infection	6 (14.3)	2 (4.8)	1 (2.4)
Bleeding	9 (21.4)	-	1[a] (2.4)
Febrile neutropenia	2 (4.8)	2 (4.8)	-
Other	8 (19.0)	1 (2.4)	-

373 [a]Grade 5 event

374 **Figure 1.** Patient disposition

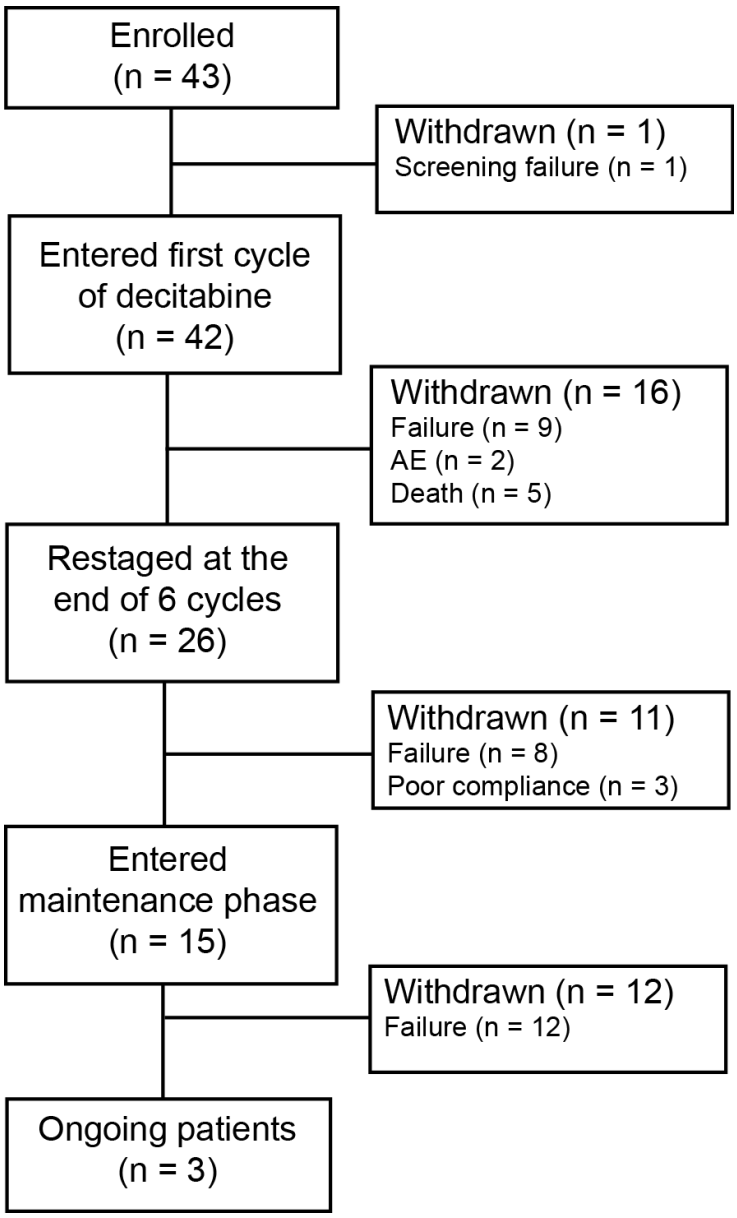

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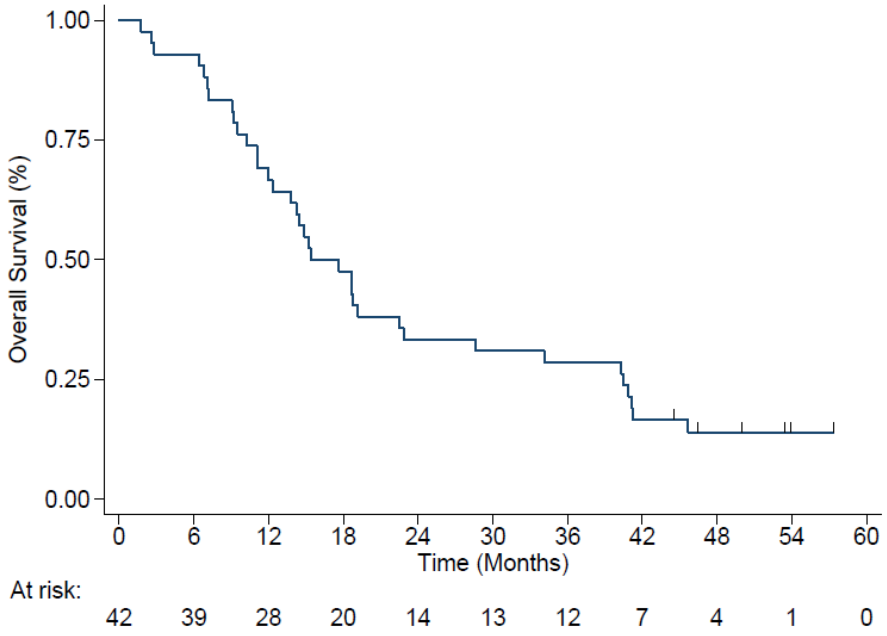
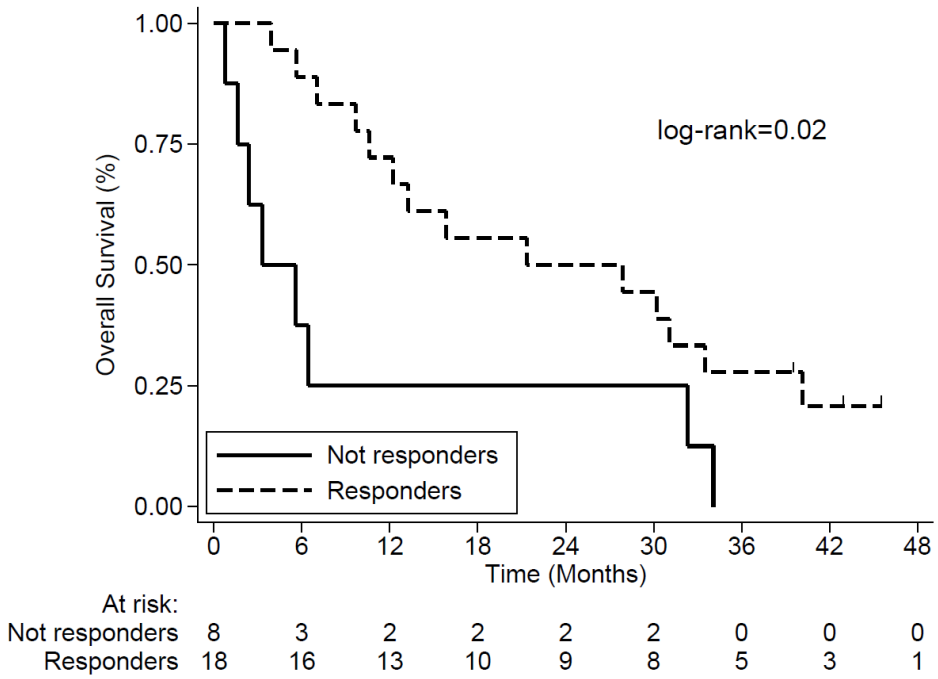
Figure 2. Overall survival.

A



B



402 **Figure 3.** Event-free survival.

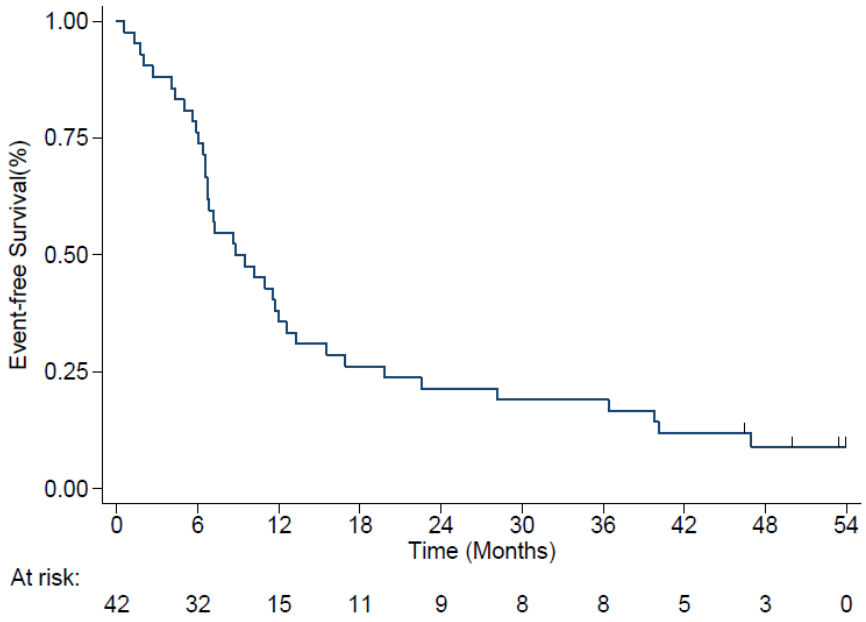


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Figure 4. Duration of response.