A phase II study of 5-day intravenous azacitidine in patients with myelodysplastic syndromes

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The approved 7-day schedule of subcutaneous azacitidine for myelodysplastic syndrome is associated with injection site reactions and bruising and may be inconvenient because of the need for weekend doses. Although pharmacokinetic data with IV azacitidine suggests equivalence, there are no efficacy data published. Patients with all myelodysplastic syndromes (MDS) FAB subtypes were enrolled and received 75 mg/m^2/d of azacitidine by 20-min intravenous infusion for 5 days in every 28 days. Global methylation studies were performed at baseline and prior to Cycle 3. Twenty-five patients were enrolled and 22 were evaluable. Median age was 69.5 years; 9 (41%) patients had lower-risk disease (IPSS Low or Int-1) and 13 (59%) had higher-risk disease (IPSS Int-2 or High). Twenty-seven percent of patients responded (5 CRs and 1 PR). The median time to response was 108 days. The median PFS was 339 days (11.3 months), the median OS was 444 days (14.8 months) and the median duration of response (DOR) was 450 days (15.0 months). Global methylation studies suggest a greater degree of demethylation in responders. This regimen appeared to offer a PR + CR rate and median DOR somewhat similar to what has been reported with the 7-day subcutaneous regimen; however, OS was shorter. Am. J. Hematol. 84:560–564, 2009. © 2009 Wiley-Liss, Inc.

Introduction

Myelodysplastic syndromes (MDS) is comprised of a heterogeneous group of hematopoietic stem cell disorders characterized by dysplastic changes in myeloid, erythroid, and megakaryocytic progenitors and cytopenias affecting one or more lineage [1]. On the basis of most recent SEER data, it is estimated that there were 11,000 new cases of MDS diagnosed in the United States in 2008 in addition to the greater than 25,000 people living with MDS [2].

The past decade has witnessed the approval of effective therapies for MDS. Azacitidine, a DNA hypomethylating agent, was the first drug approved by the FDA for the treatment of all FAB subtypes of MDS based on the results of CALGB 9221 [3]. Although the CALGB 9221 trial suggested an overall survival (OS) advantage for azacitidine over best supportive care (median OS 21 vs. 13 months), AZA-001 clearly demonstrated a survival advantage for azacitidine [4]. This trial enrolled patients with IPSS-High and Intermediate-2 MDS, chronic myelomonocytic leukemia (CMML) and WHO acute myelogenous leukemia (AML) (blast count 20–29%). Patients that received azacitidine survived nearly 10 months longer than those randomized to conventional therapy (24.5 vs. 15 months), which included low-dose cytarabine, induction chemotherapy, or best supportive care.

Azacitidine is relatively insoluble, and, therefore, a large volume of suspension might be necessary to meet target dosing, mandating some patients receive two or three separate injections daily. With the approved dose and schedule of azacitidine (75 mg/m^2/d subcutaneously for 7 days q28) as studied in the CALGB study 9221 and AZA-001, approximately 23% of patients had injection site pain and 14% had bruising [5]. The schedule of administration is potentially inconvenient because it requires weekend dosing. Although these may seem to be trivial issues, they may pose barriers to patient compliance. To address this latter point, Lyons et al. explored various subcutaneous dosing regimens without weekend doses [6].

An alternative option could be an abbreviated, intravenous (IV) schedule of azacitidine. Based on exposure levels obtained after IV administration, the IV regimen has been anticipated to be efficacious; however, prospective efficacy data for IV administration are lacking [7]. To determine the efficacy of an abbreviated course of IV azacitidine, we conducted an open-label, single-arm, single-center phase II study of IV azacitidine given more than 5 days in a 28-day Cycle in patients with MDS.

Results

Patients

Twenty-five patients were enrolled and 22 were evaluable (three patients had AML according to the FAB classification on enrollment bone marrow biopsy review and were removed from the study) (Table I). The median age was 69.5 years (range, 53–79 years). Thirteen patients (59%) were male and nine were female; nearly all patients were Caucasian (95%). The median ECOG performance status at study entry was 1 (range, 0–2). Six patients (27%) had secondary MDS and the median time from diagnosis to start of protocol therapy was 15.5 days (range, 0–1412).

According to the FAB classification, 6 patients had refractory anemia (RA), 2 had CMML, 12 had refractory anemia with excess blasts (RAEB), and 2 had refractory anemia with excess blasts in transformation (RAEB-t). According to the WHO classification 3 patients had RA, 3 patients had

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Conflict of interest: Nothing to report.

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TABLE I. Characteristics of Patients Enrolled

	Number of patients
Patients	22
Median age (range)	69.5 years (17.3–79.6)
Male/female	13/9
ECOG performance status	Median 1
0	4 (18%)
1	15 (68%)
2	2 (9%)
Unknown	1 (5%)
Median duration of MDS at study entry	15.5 days (0–1412 days)
Prior therapy	
Growth factors	7 (32%)
Hydroxyurea	2 (9%)
Transfusion dependence	10 (45%)
Red blood cell	4
Platelet	3
Red blood cell and platelet	3
Secondary MDS	6 (27%)
Prior chemotherapy	2
Prior radiation therapy	3
Both	1
Hematologic parameters at study entry	
Median WBC (range)	2.4 (0.4–83.5)$\times 10^9$/L
Median ANC (range)	772 (0–49,265)$\times 10^6$/L
Median hemoglobin (range)	8.95 (7.8–11.5) g/dL
Median platelet count (range)	45,500 (6,000–341,000)$\times 10^6$/L
FAB classification	
RA	6
CMML	2
RAEB	12
RAEB-t	2
WHO classification	
RA	3
RCMD	3
CMML-1	2
RAEB-1	3
RAEB-2	9
AML	2
Cytogenetics	
Normal	7
Intermediate	4
Complex	11
IPSS	
Low	1
Int-1	8
Int-2	7
High	6
Median bone marrow cellularity (range)	68% (20–90%)

WBC, white blood cell counts; ANC, absolute neutrophil count.

refractory cytopenia with multilineage dysplasia (RCMD), 2 patients had CMML-1, 3 had RAEB-1, 9 had RAEB-2, and 2 had AML. Seven patients had normal cytogenetics, 4 had intermediate risk cytogenetics, and 11 had complex cytogenetics. Median bone marrow cellularity at diagnosis was 68% (range, 20–90%). The median IPSS score was 2 (range, 0–3); by IPSS risk category one patient was Low risk, 8 were Int-1, 7 were Int-2, and 6 were High risk (IPSS scores were calculated in patients with secondary MDS to facilitate comparisons with previous trials). Seven patients had previously received growth factors and 2 had previously received hydroxyurea.

At study entry, the median absolute neutrophil count (ANC) was 0.772 $\times$ 10^9/L (range, 0–49.27 $\times$ 10^9/L); the median platelet count was 45.5 $\times$ 10^9/L (range, 6–341 $\times$ 10^9/L); and the median hemoglobin was 8.95 g/dL (range, 7.8–11.5). Fourteen patients (63%) had an ANC $<1.0 \times 10^9$/L and 18 patients (82%) had an ANC $<1.5 \times 10^9$/L at baseline. With exclusion of the two patients with CMML, these percentages increased to 70 and 90%, respectively. Three patients were both red blood cell (RBC) and platelet transfusion dependent at baseline, four patients were RBC transfusion dependent, and three patients were platelet transfusion dependent.

TABLE II. Efficacy Data for Entire Study Population and Divided into Low Risk (Low and Int-1) Versus High Risk Disease (Int-2 and High)

	All evaluable patients	Low risk	High risk	*P*
Number of patients	22	9	13	
Secondary MDS	6 (27%)	2 (22%)	4 (31%)	
CR	5 (23%)	3 (33%)	2 (15%)	
PR	1 (5%)	0	1 (8%)	
CR + PR	6 (27%)	3 (33%)	3 (23%)	0.655[a]
SD	9 (41%)	4 (44%)	5 (38%)	
PD	2 (9%)	0	2 (15%)	
Early death (<60 days)	5 (23%)	2 (22%)	3 (23%)	
Median PFS	339 days	357 days	302 days	0.053[b]
Median OS	444 days	Not reached	304.5 days	0.027[b]
Median time to response	108 days	109 days	107 days	NS
Median duration of response	450 days	577.5 days	302 days	0.025[b]

[a] Fisher's exact test.

[b] Low risk versus high risk by Log-rank (Mantel-Cox) test.

Responses

All analyses were conducted according to intention-to-treat principals, unless otherwise stated. The median numbers of Cycles administered was 4.5 (range, 1–20). Five patients (23%) achieved a CR, one (5%) achieved a PR, nine had SD (41%), and two had PD (9%) (Table II). No patient had a hematologic improvement (HI) according to the response criteria. The combined clinical benefit rate (PR + CR) was 27%. Two of the four RBC transfusion dependent patients achieved transfusion independence and one of the three platelet transfusion dependent patients achieved transfusion independence. None of the three patients who were both RBC and platelet dependent achieved independence. The median time to best response was 108 days (range, 27–192). No patient with secondary MDS achieved a response. The median PFS and OS were 339 days (11.3 months) (range, 32 days–not reached) and 444 days (14.8 months) (range, 23 days–not reached), respectively (Fig. 1). The 2-year PFS was 14% and the 2-year OS was 49%. The median DOR was 450 days (15 months) (range, 169 days–not reached).

Patients with lower-risk disease (IPSS Low and Int-1) appeared to benefit more from therapy than higher-risk patients (Int-2 and High). Although the clinical benefit rate was not different (33 vs. 23%, $P = 0.655$), patients with low risk disease trended toward having a longer median PFS (357 vs. 302 days, log-rank test $P = 0.053$) and had a longer median DOR (577.5 vs. 302 days, log-rank test $P = 0.025$) and median OS (not reached versus 304.5 days, long-rank test $P = 0.027$). These discrepancies may alternatively be explained by the natural histories of low- versus high-risk MDS. There was no difference in early mortality for patients with low- versus high-risk disease.

There was no statistical difference between the median DOR in patients with CR/PR (450 days; range, 169–not reached) and those with SD (357 days; range, 92–not reached) (log-rank test $P = 0.4210$).

Toxicity

The principal toxicity of azacitidine was myelosuppression (Table III). Eleven patients (50%) required a dose delay for cytopenias; a total of 21 of the 122 Cycles delivered were delayed because of cytopenias (17%). Twelve patients (55%) developed Grade 3 or 4 anemia, 10 (45%) developed Grade 3 or 4 neutropenia, and 4 (18%) developed Grade 3 or 4 thrombocytopenia. Although febrile neutropenia was relatively common (27%), major bleeding was not (5%).

The principle Grade 3 or 4 non-hematologic toxicity was fatigue (5 patients or 23%). Nine percent of patients suf-

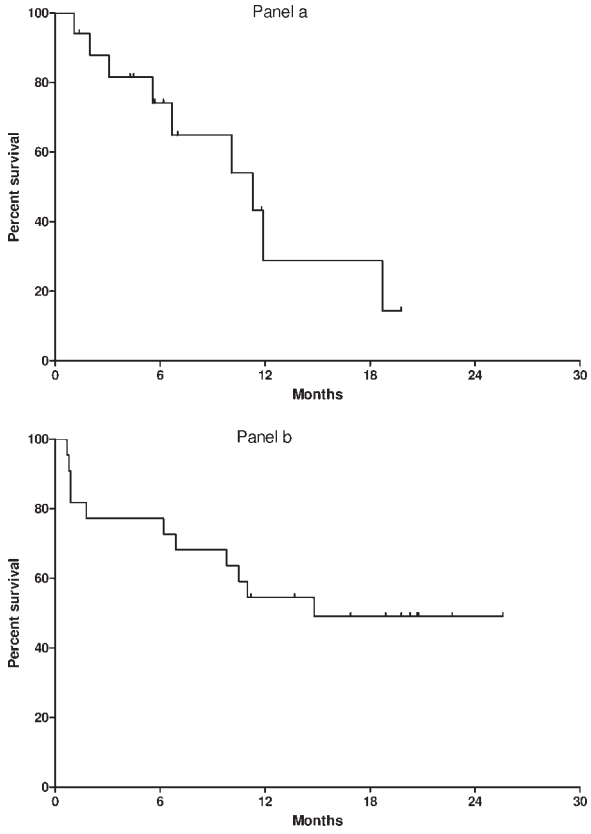

Figure 1. Kaplan-Meier estimates of PFS (panel a) and OS (panel b).

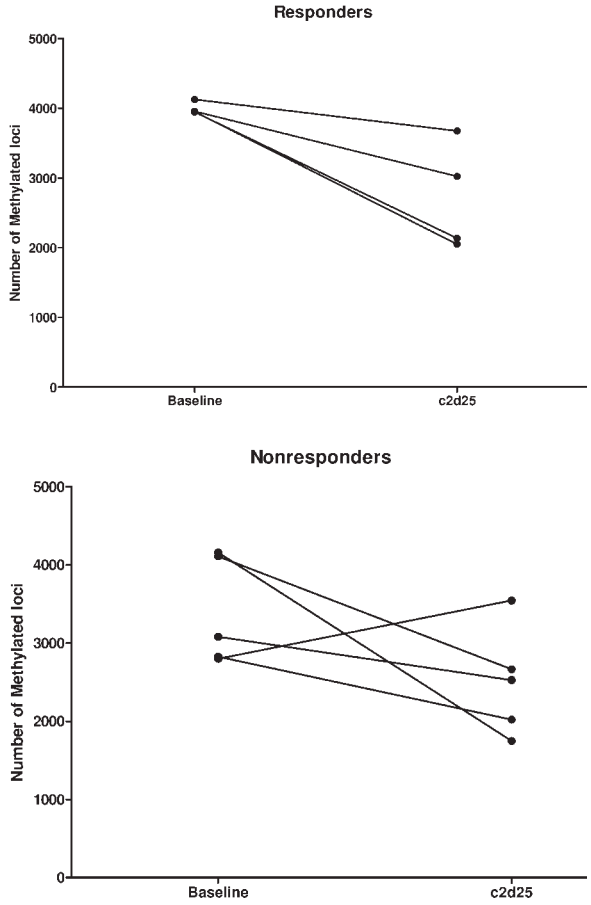

Figure 2. Individual patient changes, divided into responders and non-responders, in number of methylated loci prior to Cycle 3.

TABLE III. Number of Patients Experiencing Grade 3 or 4 Adverse Events

	Frequency
Hematologic	
Anemia	12 (55%)
Neutropenia	10 (45%)
Leukopenia	8 (36%)
Febrile neutropenia	6 (27%)
Thrombocytopenia	4 (18%)
Non-hematologic	
Fatigue	5 (23%)
Cardiac event	4 (18%)
ALT elevation	3 (14%)
AST elevation	2 (9%)
Nausea	2 (9%)
GI bleed	1 (5%)

fered Grade 3 nausea and all patients required prophylactic anti-emetics by Cycle 3 (for Grades 1–3 nausea). Cardiac events as a part of infectious episodes and liver function abnormalities occurred in 18 and 14% of patients, respectively.

Early mortality

Five patients (23%) died during the first 2 Cycles; three patients died of sepsis, one died of pneumonia and one died of a myocardial infarction. No patient received prophylactic antibiotics. The median baseline ANC for the five patients that died in the first 2 Cycles was 63×10^6/L (range, 0–784). This trended toward being lower than the median ANC for the other patients (median 817, range, 7–49,265; *t* test with Welch's correction $P = 0.182$). Excluding the 5 patients that died in <60 days, the median OS for the remaining patients has not been reached with a median follow-up of 444 days.

Global methylation

For all patients with paired samples ($n = 9$), the percentage of methylated loci decreased from baseline (13.8%) to c2d25 (9.8%) (3547 methylated versus 22929 partially or ummethylated loci and 2609 methylated versus 23867 partially or ummethylated loci, respectively) ($P = 0.0027$). The decrease in methylated loci was significant in responders ($n = 4$) (15.1–10.3%) (3993 methylated versus 22479 partially or ummethylated loci and 2365 methylated versus 24113 partially or ummethylated loci, respectively) ($P = 0.0167$) and trended toward significance in non-responders ($n = 5$) (3292 methylated vs. 22319 partially or ummethylated loci and 2873 methylated vs. 23692 partially or ummethylated loci, respectively) (12.8–9.5%) ($P = 0.0747$) (Fig. 2). The percentage of methylated loci at baseline was not significantly different between responders and non-responders ($P = 0.1283$). Baseline methylation was not associated with age ($P = 0.1475$) or high- vs. low-grade MDS ($P = 0.3575$) but trended toward an association with IPSS (fewer methylated loci were associated with a higher IPSS) ($P = 0.0934$). On analysis of paired samples between responders and non-responders, no genes were consistently differentially methylated at baseline. In addition, no consistent patterns of differential methylation were observed with treatment when comparing baseline and follow-up samples.

Discussion

This is the first prospective trial to report on an abbreviated 5-day regimen of IV azacitidine in MDS. The PR + CR rate was 27% according to the modified IWG criteria. Although the small sample size limited our ability to draw definitive conclusions, this is similar to the PR + CR seen

in CALGB 9221 (23%) and AZA-001 (29%), which used comparable response criteria [3,4]. The median time to best response (108 vs. 93 days) and median DOR (15 months in both) were also similar to what was reported in CALGB 9221 [3]. In the current study, patients with Low or Int-1 disease had a significantly longer response duration (577.5 vs. 302 days; log-rank test $P = 0.025$). The median PFS in our study was 339 days (11.3 months), and it trended toward being longer in low-risk patients (357 vs. 302 days, log-rank test $P = 0.053$). This is comparable with the median PFS (9.1 months) reported in CALGB 9221. These observations may reflect either that patients with low-risk disease benefit more from azacitidine or the favorable natural history of lower risk disease relative to higher risk disease. In contrast, the median OS in this study was 444 days (14.8 months), which was shorter than seen in the azacitidine arms of either CALGB 9221 (21 months) or AZA-001 (24.4 months) [3,4].

There is discrepancy between the similar objective response rate, DOR and median PFS between this study and CALGB 9221, but different OS. This is likely explained by the early mortality rate (<60 days) of 23% in the current trial. There was essentially no early mortality in CALGB 9221 or AZA-001 [3,4]. Although the patients enrolled in the current study and CALGB 9221 were similar across most baseline variables, the patients in the current trial had a lower baseline median ANC [3]. The median baseline ANC in the present study was 0.8×10^9/L compared to 1.5×10^9/L in CALGB 9221. Neither study placed restrictions on ANC at enrollment or mandated prophylactic antibiotics. The trials contained a comparable number of patients with CMML. In fact, 82% of our patients had an ANC below the median in the CALGB 9221. This likely significantly contributed to our high (23%) early mortality rate as most deaths were due to infectious complications, and all occurred in patients with baseline ANC's below 1,000. Excluding these patients, the median OS has not been reached. Patients with an ANC $<1.0 \times 10^9$/L at baseline starting therapy with IV azacitidine should be considered for antibacterial prophylaxis.

The principle non-hematologic toxicity we observed was nausea, and all patients required prophylactic anti-emetics by Cycle 3. In the subcutaneous azacitidine, trial nausea was rare with only 5% of patients experiencing a Grade 3 event [3,4]. Other non-hematologic toxicities were similar.

Although anticipated to be limited in scope by the small sample size, the current study prospectively collected samples for genome wide methylation analysis. In contrast to previous studies, which were candidate gene driven, this approach provides the potential for the discovery of novel genes involved in MDS [8–10]. The correlative studies performed in this limited data set demonstrate the feasibility of this approach and provide preliminary evidence of the global methylation changes in patients treated with azacitidine. Studies with larger sample sizes and correlative mRNA expression levels are needed to further define the mechanism of action of azacitidine and to identify critical genes associated with response or resistance. Larger multicenter trials will likely be required to deliver sample sizes sufficient to allow multi-variant analyses for predictors of response.

In this first prospective trial of short course IV azacitidine, we describe a PR + CR rate of 27% and median DOR of 15 months. Although limited by the small sample size, this appears similar to what has been reported in trials that employed a 7-day subcutaneous regimen, although OS was shorter. These results also suggest that in patients with baseline neutropenia, treatment with IV azacitidine without prophylactic antibiotics may result in an unacceptably high rate of early mortality. Prophylactic antibiotics should be considered for patients receiving azacitidine with a baseline ANC $<1.0 \times 10^9$/L. Our study requires confirmation in a larger cohort and should ideally to be compared in a randomized fashion to subcutaneous azacitidine.

Methods

Patient eligibility

The protocol was approved by the Institutional Review Board at Washington University School of Medicine and registered at www.clinicaltrials.gov (NCT00384956). All patients provided written informed consent. Patients 18 years and older with a diagnosis of MDS based on the French-American-British (FAB) classification were eligible [11]. There were no eligibility restrictions based on pretreatment peripheral blood counts and both patients with de novo and secondary disease were allowed. Patients had to have an Eastern Cooperative Oncology Group Performance Status of 2 or less and adequate renal and hepatic function. Patients could not have received any chemotherapy within 4 weeks of study enrollment and must have recovered from any prior treatment-related toxicity. Women of childbearing age were required to have a negative serum pregnancy test prior to initiating therapy, and men were required to be willing not to father a new child while receiving therapy. Patients treated with investigational agents within 30 days of enrollment or hypomethylating agents were not eligible. Further, patients were excluded with ongoing or active infection, congestive heart failure of New York Heart Association Class 3 or 4, unstable angina pectoris, cardiac arrhythmia, or known positive serology for HIV.

Response criteria

Responses were defined according to the modified International Working Group (IWG) (2006) response criteria for myelodysplasia [12]. Briefly, complete remissions (CR) were defined by bone marrow with <5% myeloblasts and 0% peripheral blasts, hemoglobin ≥11g/dL, platelets $\geq 100 \times 10^9$/L, and neutrophils $\geq 1.0 \times 10^9$/L. Residual dysplasia was allowed. Partial remissions (PR) were defined by all of the CR criteria if abnormal before treatment except: bone marrow blasts decreased by ≥50% over pretreatment but still >5%; stable disease (SD) was defined as a failure to achieve a PR but without evidence of progression for >8 weeks; and lineage specific response criteria were erythroid response by a hemoglobin level >1.5g/dL, platelet response by an absolute increase of 30×10^9/L for patients starting with less than 20×10^9/L or at least a 100% increase in patients starting $>20 \times 10^9$/L; and neutrophil response by an increase of 100% and an absolute increase $>0.5 \times 10^9$/L.

Treatment

Patients received azacitidine 75 mg/m^2 given as a 20-min IV infusion once daily on days 1 to 5 of a 28-day Cycle. The dose was increased to 100 mg/m^2 for patients that failed to respond to therapy after 2 Cycles. Patients could continue to receive therapy as long as they responded. Those that achieved a CR were to receive three further Cycles and then move to a maintenance phase where the Cycles would be administered every 56 days. Patients that failed to demonstrate a response within 6 Cycles of therapy were removed from study. Patients were routinely followed with bone marrow biopsies obtained at the end of all even number Cycles or every 8 weeks if on maintenance therapy.

For patients whose baseline absolute neutrophil count was greater than 500 and/or their platelet count was greater than 20,000, a failure to achieve cell count recovery to within 25% of the baseline of the previous cycle resulted in a delay in initiating the next treatment cycle. Doses were not delayed for cytopenias in patients with a baseline ANC <500 or platelet count <20,000. Subsequent doses were reduced by 50% if patients required a >8-week delay between cycles for either blood count recovery or renal dysfunction. Therapy was discontinued if a second 8-week delay was needed.

Routine administration of erythroid growth factors was not permitted during the study, and filgrastim (G-CSF) and sargramostim (GM-CSF) administration was permitted only in patients with febrile neutropenia. The use of prophylactic antimicrobials was left to the discretion of the treating physicians. Routine anti-emetics were not required.

Methylation studies

Bone marrow was harvested at baseline and on Cycle 2 day 25 (c2d25). DNA from unfractionated bone marrow underwent bisulfate conversion and 1500 ng of DNA was hybridized to the Infinium Human Methylation27 Bead Array (Illumina, San Diego, CA). Arrays were processed by the Genome Sequencing Center at Washington University. Data were analyzed using Bead Studio version 3 and GraphPad Prism

version 5. Loci were considered methylated if their average beta value was $\geq$0.80 and unmethylated if their average beta value was $\leq$0.20. Changes in the methylation status of individual loci were considered potentially interesting if the delta average beta was $\geq$0.20 between paired samples. Loci on the X and Y chromosomes were excluded from analyses because of gender imbalances between responders and non-responders.

Statistical design and analysis

Median DOR, median progression free (PFS), and OS were calculated by Kaplan-Meier estimates and compared by the log-rank test. Patients were censored for duration of SD at the time of last follow-up or the start of a subsequent therapy.

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