ASXL1 Mutations with Serum EPO Levels Predict a Poor Response to Darbepoetin Alfa in Lower-Risk MDS: W-JHS MDS01 Trial

Yasuyoshi Morita
Kindai University
Yasuhito Nannya
Kyoto University
Motoshi Ichikawa
Dokkyo Medical University
Hitoshi Hanamoto
Nara Hospital Kindai University
Hirohiko Shibayama
Osaka University
Yoshinobu Maeda
Okayama University Hospital
Tomoko Hata
Nagasaki University
Toshihiro Miyamoto
Kyushu University
Hiroshi Kawabata
Kanazawa Medical University
Kazuto Takeuchi
Ehime University
Junji Kishimoto
Kyushu University
Hiroko Tanaka
University of Tokyo
Satoru Miyano
University of Tokyo
Itaru Matsumura
Kindai University
Seishi Ogawa
Kyoto University
Koichi Akashi

Kyushu University
Yuzuru Kanakura
Osaka University
Kinuko Mitani (mitanik@dokkyomed.ac.jp)
Dokkyo Medical University

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ASXL1 mutations with serum EPO levels predict a poor response to darbepoetin alfa in lower-risk MDS: W-JHS MDS01 trial

Yasuyoshi Morita,[1] Yasuhito Nannya,[2] Motoshi Ichikawa,[3] Hitoshi Hanamoto,[4] Hirohiko Shibayama,[5] Yoshinobu Maeda,[6] Tomoko Hata,[7] Toshihiro Miyamoto,[8] Hiroshi Kawabata,[9] Kazuto Takeuchi,[10] Hiroko Tanaka,[11] Junji Kishimoto,[12] Satoru Miyano,[11] Itaru Matsumura,[1] Seishi Ogawa,[2] Koichi Akashi,[8]Yuzuru Kanakura,[5] and Kinuko Mitani[3*]

[1]Divison of Hematology and Rheumatology, Department of Internal Medicine, Kindai University Faculty of Medicine, Osaka-Sayama, Osaka, Japan; [2]Departmesnt of Pathology and Tumor Biology, Kyoto University, Kyoto, Japan; [3]Department of Hematology and Oncology, Dokkyo Medical University, Tochigi, Japan; [4]Department of Hematology, Faculty of Medicine, Nara Hospital Kindai University, Nara, Japan; [5]Department of Hematology and Oncology, Graduate School of Medicine, Osaka University, Osaka, Japan; [6]Department of Hematology and Oncology, Okayama University Hospital, Okayama, Japan.; [7]Department of Hematology, Atomic Bomb Disease Institute, Nagasaki University, Nagasaki, Japan; [8]Department of Medicine and Biosystemic Science, Kyushu University Graduate School of Medical Science, Fukuoka, Japan; [9] Department of Hematology and Immunology, Kanazawa Medical University, Uchinada-machi, Ishikawa, Japan; [10]Department of Hematology, Clinical Immunology and Infectious Diseases, Ehime University Graduate School of Medicine, Ehime, Japan; [11]Human Genome Center, Institute of Medical Science, The University of Tokyo, Tokyo Japan; [12]Department of Research and Development of Next Generation Medicine, Kyusyu University, Fukuoka, Japan

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Corresponding author: Kinuko Mitani, M.D, Ph.D.
Department of Hematology and Oncology, Dokkyo Medical University
880, Kitakobayashi, Mibu-machi, Shimotsuga-gun, Tochigi, 321-0293, Japan
E-mail address: mitanik@dokkyomed.ac.jp (K. Mitani)
Phone: +81-282-86-1111 ex.2744 , Fax: +81-282-86-5630

Abstract

Darbepoetin alfa (DA) is often used in treating anemia of lower-risk (IPSS low or int-1) myelodysplastic syndromes (MDS). However, whether mutations can predict effectiveness of DA has not been examined. The present study aimed to determine gene mutations for predicting therapeutic effect of DA. Primary endpoint was correlation between the presence of highly frequent (≥10%) mutations and hematological improvement erythroid according to IWG criteria 2006 by DA (240 μg/week) until week 16. Included were 79 patients (age 29-90, median 77.0 years; 52 [65.8%] male). Frequently (≥10%) mutated genes were *SF3B1* (24 cases, 30.4%), *TET2* (20, 25.3%), *SRSF2* (10, 12.7%), *ASXL1* (9, 11.4%), and *DNMT3A* (8, 10.1%). Overall response rate to DA was 70.9%. Multivariable analysis including baseline erythropoietin levels and red blood cell transfusion volumes as variables revealed that erythropoietin levels and mutations of *ASXL1* gene were significantly associated with worse response (odds ratio 0.146, 95% confidence interval 0.042-0.503; p=0.0023, odds ratio 0.175, 95% confidence interval 0.033-0.928; p=0.0406, respectively). This study indicated that anemic patients who show higher erythropoietin levels and harbor *ASXL1* gene mutations may have poor response to DA. The alternative strategies are needed for the treatment of anemia in this population. Trial registration number and date of registration: UMIN000022185 & 09/05/2016.

Introduction

Myelodysplastic syndromes (MDS) are clonal stem cell disorders characterized by ineffective hematopoiesis, and occasionally progress to acute myelogenous leukemia (AML) [1, 2]. The pathogenesis of MDS is thought to be a multistep process involving two or more genetic alterations that cause clonal proliferation of an abnormal stem cell[3, 4]. Our understanding of the molecular pathogenesis of MDS has improved in recent years, mainly through the identification of major mutational targets[5-9]. The majority of patients with lower-risk MDS of International Prognostic Scoring System (IPSS) [10] low or intermediate-1 risk have symptoms of anemia due to ineffective erythropoiesis[11], and need therapeutic intervention, including red blood cell (RBC) transfusion. Although RBC transfusions can temporarily reduce anemic symptoms, frequent transfusions usually lead to iron overload, which is associated with reduced survival and lower quality of life[12].

On the other hand, development of erythropoiesis in the fetal liver and adult bone marrow is regulated by the hormone erythropoietin (EPO) [13]. Therefore, recombinant human EPO (rHuEPO) and other erythropoiesis-stimulating agents (ESAs) are used for the treatment of MDS-related anemia[14]. In Japan, darbepoetin alfa (DA), which is a re-engineered form of EPO, has been approved for clinical practice. Around 50% of the patients respond to EPO ± granulocyte-colony stimulating factors (G-CSF), and the median duration of response is 2 years[15, 16]. DA has also been reported to show an overall response rate of about 60% when employed for the treatment of anemia in lower-risk MDS[17].

Prediction of response to ESAs in anemic MDS patients is often based on clinical biomarkers such as volume of RBC transfusion, serum EPO levels, ferritin, and IPSS/IPSS-R[18], and the use of Nordic score combined with serum EPO levels and transfusion volume has also been proposed[19]. However, the relationship between molecular pathogenesis of MDS and responsiveness to DA has not yet been well studied. Here we report the results of the West Japan Hematology Study Group (W-JHS) MDS01 trial, to determine gene mutations that predict effectiveness of DA in treating anemia of lower-risk (low or int-1 in IPSS[10] risk categories) MDS.

Methods

Patient eligibility

Patients with lower-risk MDS (low or int-1 in IPSS[10] category) were registered in the W-JHS MDS01 study (UMIN000022185) between February 2016 and May 2019 at 36 institutions in Japan. Other eligibility criteria were as follows: newly diagnosed patients with definite MDS based on diagnostic criteria of FAB classification[20] and the 4th edition of World Health Organization (WHO) classification[21]; patients having anemia associated with MDS, and of age 16 years or older. Patients with present or past medical record of myocardial infarction,

pulmonary infarction, cerebral infarction or similar disorders or risk of thromboembolism, uncontrollable hypertension, medical history of DA or other EPO formulation, severe (requiring hospital care or judged by investigators) or uncontrollable complication, and those judged inappropriate for study participation due to complication of mental disease or psychiatric symptom and cognitive disorder were excluded. This study is registered at the University Hospital Medical Information Network Clinical Trials Registry on 09/05/2016, with ID No. 000022185.

Procedure

DA at a dose of 240 μg per body was administered once weekly and followed for 16 weeks. Analysis was performed to confirm whether the presence of specific gene mutation with a frequency of ≥10% affects efficacy of DA. Peripheral blood sample of the subjects was collected before administration of DA, and genomic DNA was extracted. The presence of gene mutations was then analyzed, mainly on highly frequent gene mutations (i.e., of genes *SF3B1, TET2, SFRS2, ASXL1, DNMT3A, RUNX1,* and *U2AF1*) of a panel of 376 genes in a previous report[6] using a next-generation sequencing method. In brief, 376 known target genes in MDS were examined for mutations in 79 patients from the cohort, using massively parallel sequencing (Illumina, Inc., San Diego, CA, USA) of SureSelect (Agilent Technologies Inc., Santa Clara, CA, USA)-captured target sequences. All sequencing data were analyzed using our in-house pipeline, through which highly probable oncogenic mutations were called by eliminating sequencing/mapping errors and known/possible SNPs based on the available databases and frequencies of variant reads.

Assessment of response

The primary endpoint was correlation between highly frequent gene mutations (≥10%) and hematological improvement-erythroid (HI-E) according to the International Working Group (IWG) criteria 2006[22] at week 16 after the initiation of DA treatment. Secondary endpoints were major and minor responses to DA at week 16 after the initiation of treatment in blood transfusion-dependent subjects, HI-E in blood transfusion-independent subjects, variety and frequency of gene mutations observed in all subjects, correlation between decreased blood cell lineages (erythrocytes, leukocytes, and platelets) and types of gene mutations, mortality (overall survival, OS) and progression to AML (progression-free survival, PFS) from 16 weeks to 1 year after the initiation of treatment, and correlation between highly frequent gene mutations and interval to achievement of the first HI-E according to IWG criteria 2006[22].

IWG criteria 2006 (HI-E) were used, defined as either hemoglobin increased by 1.5 g/dL or more compared to pre-treatment (<11.0 g/dL) or RBC transfusion volume/8 weeks decreased by more than 4 units in RBC transfusion-dependent subjects. In Japan, one unit of red blood cell preparation is produced from 200 ml of whole blood. As for transfusion-dependent subjects, major

response was defined as no need for RBC transfusion (withdrawal from RBC transfusion dependence) for more than 56 consecutive days, and increase in the highest Hb concentration during the withdrawal period by at least 1.0 g/dL compared to the baseline Hb concentration, while minor response was defined as ≥50% decrease in RBC transfusion volume over 56 consecutive days compared to baseline transfusion volume. For AML progression, progression to AML and subsequent death were stated as events, and the subjects without confirmed progression to AML were censored at the date of last known survival. For overall survival (OS), death from any cause was considered an event, and for the survival cases, the study was terminated at the date of the last known survival.

Assessment of safety

All adverse events (AEs) were recorded in subjects who received DA at least once from the first administration to day 29 of the last cycle, and classified according to the Common Terminology Criteria for Adverse Events Version 4.0[23]. For the subjects who dropped out before completion of the study, AEs were monitored for two weeks after the last administration.

Statistical analysis

Odds ratios between the gene mutations and the outcomes were estimated using univariate and multivariate logistic regression models. The multivariate analysis was adjusted for baseline EPO levels (low: <100, high: ≥100 mIU/mL) and RBC transfusion volumes (low: <1 unit, high: ≥1 unit/month) as the explanatory variables.

The survival curves were estimated using Kaplan-Meier methods, and the confidence intervals for the median survival time (MST) and the annual survival rate were calculated using Brookmeyer and Crowley's method and Greenwood's formula, respectively, and comparisons between survival curves were performed using the stratified log-rank test adjusting baseline EPO levels. In all analyses, $p<0.05$ (two-sided) was considered statistically significant. Statistical analysis was performed using SAS Ver. 9.4.

Ethics

This study was conducted in compliance with the Act on the Protection of Personal Information (Act No. 57 of May 30, 2003), the Declaration of Helsinki (October 2013, translated by the Japanese Medical Association in the revised version of Fortaleza), the Clinical Research Act (Act No. 16 of 2017), the Ordinance for Enforcement of the Clinical Research Act (Ordinance No. 17 of the Ministry of Health, Labour and Welfare of 2018), and the Ethical Guidelines for Human Genome/Gene Analysis Research (February 28, 2017).

The protocol and an explanatory document regarding the protocol provided to patients were approved by the Ethics Review Committee of each participating institution (Dokkyo Medical University Hospital Ethics Committee, Kyushu University Hospital Ethics Committee, Osaka City University Hospital Institutional Review Board, Kindai University Faculty of Medicine Genetic Ethic committee, Nagasaki University Hospital Clinical Research Ethics Committee, Kagawa University Hospital Ethics Committee, University Hospital, Kyoto Prefectural University of Medicine Ethics Committee, Shimane University Hospital Ethics Committee, National Hospital Organization Nagasaki Medical Center Ethics Committee, Okayama City General Medical Center Ethics Committee, Hiroshima University Hospital Ethics Committee, Kanazawa University Hospital Ethics Committee for Human Genome/Gene Analysis Research, Kindai University Nara Institutional Review Board, National Hospital Organization Kyushu Cancer Center Ethics Committee, Toyonaka municipal hospital Ethics Committee, National Hospital Organization Kyushu Medical Center Ethics Committee, Kagoshima University Hospital Clinical Research Ethics Committee, Japan Community Health Care Organization, Kyoto Kuramaguchi Medical Center Ethics Committee, Chihaya Hospital Ethics Committee, Fukuoka Red Cross Hospital Ethics Committee, Japanese Red Cross, Kyoto Daini Hospital Ethics Committee, Yokohama City University Hospital Ethics Committee for Medical and Health Research Involving Human Subjects, Osaka University Hospital Ethics Committee for observational study, Okayama University Hospital Clinical Research Review Board, National Hospital Organization Okayama Medical Center Clinical Research Review Board, University of Fukui Medical Research Ethics Committee, Wakayama Medical University Hospital Ethics Committee, Tohoku University Hospital Clinical Research Ethics Committee, Hamanomachi Hospital Clinical Research Review Board, Hokkaido University Hospital Clinical Research Review Board, Toyama Red Cross Hospital Ethics Committee, Matsuyama Red Cross Hospital Ethics Committee, Hiroshima Red Cross Hospital & Atomic-bomb Survivors Hospital Ethics Committee, Faculty of Medicine, University of Miyazaki Hospital NPO Clinical Research Network Fukuoka Certified Review Board). Prior to subject enrollment, the content of the study was explained to the patients using the explanatory document, and written informed consent was obtained from all participants. If a patient was under 20 years of age, written informed consent of the patient and his or her guardian was obtained.

Results

Subjects

A total of 85 patients underwent enrollment screening. Of these, 79 subjects were included in the full analysis set (FAS), after excluding 4 ineligible patients and 2 patients who withdrew their consent before the start of protocol treatment. The median (range) follow-up for FAS was 374

(44-1094) days. Baseline characteristics of the 79 subjects are shown in Table 1. Median (range) age was 77.0 (29-90) years; 52 males (65.8%) and 27 females (34.2%) were included in the study. The number of transfusion-dependent cases was 15 (19.0%).

Outcomes

Rate of overall response (achievement of HI-E according to IWG criteria 2006) was 70.9% (60.0% in transfusion-dependent cases, and 73.4% in non-transfusion-dependent cases) (Table 2). Major/minor responses were observed in 46.7% and 60.0% of the RBC transfusion-dependent subjects (n=15).

In the evaluation of the relationship between mutated genes and the response rate by univariate analysis in FAS, highly frequent (10% or more) gene mutations were found in *SF3B1* (24 cases, 30.4%), *TET2* (20 cases, 25.3%), *SRSF2 (*10 cases, 12.7%), *ASXL1* (9 cases, 11.4%), and *DNMT3A* (8 cases, 10.1%) (Table 3)(Supplementary Fig. S1 online). The univariate logistic regression analysis showed no significant association between these mutations with a frequency of ≥10% and therapeutic efficacy of DA. The same results were obtained when the analysis was limited to RBC non-transfusion-dependent subjects (n=64).

In the multivariable analysis including baseline EPO levels and RBC transfusion volumes as variables, mutation of *ASXL1* gene as well as baseline EPO levels was identified to independently predict poor response to DA with statistical significance (odds ratio 0.180, 95% CI 0.035-0.928, p=0.040 for *ASXL1* mutation, odds ratio 0.146, 95% confidence interval 0.042-0.503; p=0.0023 for EPO levels) (Table 4). Transfusion volumes were not detected as predictive factors, possibly because that major part of our cohort was transfusion-independent. Response rates in subjects with low EPO (<100 mIU/mL) + *ASXL1* mutation(-) (n=35), low EPO + *ASXL1* mutation(+)(n=5), high EPO (≥100 mIU/mL) + *ASXL1* mutation(-),(n=35) and high EPO + *ASXL1* mutation(+) (n=4) were 88.6%, 80.0%, 60.0% and 0%, respectively. The result of chi-squared test showed that the four groups were significant different in terms of response (p=0.0006).

Correlation between decreased cell lineages and gene mutations

The analysis of correlation between decreased cell lineages (erythrocytes, leukocytes, and platelets) and types of gene mutations suggested that mutation of *SF3B1* gene was significantly associated with lower erythrocyte levels (odds ratio and 95% CI, 2.782, 1.020-7.591, p=0.046), and higher WBC (odds ratio and 95% CI, 0.296, 0.106-0.829, p=0.021) and platelet (odds ratio and 95% CI, 0.177, 0.057-0.548, p=0.003) counts. In addition, *SRSF2* mutation was significantly associated with higher erythrocyte level (odds ratio and 95% CI, 0.091, 0.011-0.755, p=0.026). There was no mutation that was correlated with Hb levels.

PFS and OS

Progression to AML was observed in 24 subjects from week 0 to year 1, and in 10 subjects from week 16 to year 1. A total of 23 subjects died between week 0 and year 1, and 9 subjects died between week 16 and year 1.

PFS and OS at from week 16 to year 1 after the initiation of treatment are shown on the 58 subjects who continued treatment without progression to AML until week 16 in Fig. 1 and 2. PFS (95% CI) at year 1 was 81.7% (68.6-89.7%) with MST (95% CI) 37.7 months (29.5 months−not reached), and OS was 83.5% (70.7-91.1%) with MST (95% CI) not reached (30.9 months−not reached).

Time to achievement of the first HI-E

Time to achievement of the first HI-E from the initiation of treatment is shown in Fig. 3. Of the 79 subjects who were included in FAS, 56 subjects achieved the HI-E according to IWG criteria 2006, and the median time to achievement (95% CI) was 7.1 weeks (6.1 – 10.1 weeks).

Correlation between highly frequent gene mutations and interval to achievement of the first HI-E

After adjustment for the baseline EPO levels (cut-off, 100 mIU/mL), although none of the highly frequent gene mutations had significant association with the time to achievement of the first HI-E according to IWG criteria 2006, mutations of the following 4 genes had a tendency of earlier achievement: *SF3B1* (MST of mutation(+)/ mutation(-), 6.1 weeks / 8.1 weeks, p=0.4118), *TET2* (5.1 weeks / 8.0 weeks, p=0.3186), *SRSF2* (5.2 weeks / 8.0 weeks, p=0.8219), *DNMT3A* (6.1 weeks / 7.1 weeks, p=0.9868), while mutation of *ASXL1* gene showed the opposite trend: (not reached / 6.7 weeks, p=0.1649).

Safety

Grade 3/4 adverse events observed during the study period were anemia in 33 subjects (41.8%), neutrophil count decreased in 24 (30.4%), platelet count decreased in 18 (23.4%), white blood cell count decreased in 16 (20.3%), lymphocyte count decreased in 15 (19.0%), hyperglycemia in 3 (5.9%), hypoalbuminemia in 2 (3.1%), and aspartate aminotransferase increased in 1 (1.3%).

Discussion

In this study focusing on DA-eligible lower-risk patients, gene mutations frequently observed in MDS were found in *SF3B1* (30.4%), *TET2* (25.3%), *SRSF2* (12.7%), *ASXL1* (11.4%), and *DNMT3A* (10.1%), which is consistent with a previous report[6]. The response rate of DA up to 16 weeks was 70.9% based on the IWG criteria (60.0% in transfusion-dependent cases, and 73.4%

in non-transfusion-dependent cases). Regarding the association between gene mutations at a frequency of 10% or more and the response rate of DA, the univariate analysis showed no significant association between the mutations and therapeutic efficacy of DA. Multivariable analysis that included serum EPO levels and RBC transfusion volumes revealed that the presence of *ASXL1* gene mutations and higher EPO levels (≥100 mIU/mL) were an independent factor that predicts for poor response to DA. In a previous meta-analysis, it had been shown that the serum EPO level <100 IU/L is a biomarker for effectiveness of DA[24]. Similarly, in our study, DA therapy was effective in subjects with low EPO levels (＜100 mIU/mL), irrespective of the absence (response rate, 88.6%) or presence (response rate, 80.0%) of *ASXL1* mutations. However, it is interesting to note that about 60% of the subjects with serum EPO levels ≥100mIU/mL and without *ASXL1* mutations responded to DA therapy in our cohort. Even in patients with higher levels of EPO, there may be a chance to recover from anemia by DA therapy if they do not possess *ASXL1* mutations. One-year PFS and OS rates was 81.7% (95% CI: 68.6-89.7%) and 83.5% (70.7-91.1%), respectively. Considering that our cohort included 5 subjects with RAEB-1 according to the WHO classification, the PFS and OS were not worse than expected.

DA is an ESA in which sustainable serum concentration has been obtained by substituting 5 amino acids in EPO[25, 26]. Like EPO, DA binds to erythropoietin receptors and thereby promotes erythropoiesis in early and late erythroid progenitor cells in the bone marrow[25, 27]. When injected subcutaneously once a week, sufficient serum levels are maintained for the treatment of anemia[26]. Nine studies performed in patients with MDS reported response to DA according to IWG 2000 criteria, with response rates ranging from 38% to 72.5% within 12-24 weeks[24]. Similarly, in the present study, overall response rate to DA at week 16 was 70.9%, according to IWG criteria 2006. There was a tendency that non-transfusion-dependent subjects had better response to DA than transfusion-dependent subjects, with ORR of 73.4% and 60.0%, respectively. The earlier commencement of DA could enable avoidance of transfusion dependency in anemic patients with lower-risk MDS.

Importantly, by using multivariable analysis that included serum EPO level and RBC transfusion volumes as variables, we showed that the presence of *ASXL1* mutations besides EPO level was significantly associated with poor response to DA among the highly frequent (>10%) gene mutations observed in this study, which were those in *SF3B1, TET2, SRSF2, ASXL1,* and *DNMT3A*. Because MDS are heterogenous not only in morphology but also in molecular pathogenesis, the other types of gene mutations do not occur with sufficient frequency to evaluate their predictive value on the DA treatment. Another important result of this study was that *ASXL1* gene mutations were also correlated with possible prolonged interval to achievement of the first HI-E. *ASXL1* mutations are known genetic factors that predict unfavorable clinical courses[28].

Considering a balance between cost and benefit, DA should perhaps not be applied to patients with lower-risk MDS showing higher level of EPO and carrying *ASXL1* mutations.

ASXL1 is an epigenetics-regulating gene that supports the functions of polycomb complex PRC1 and represses the expression of oncogenes and other genes through methylation of K4 in histone H3[29]. It has also been shown that mutant *ASXL1* disrupts the function of PRC1 and causes derepression of expression in target genes[30], which possibly leads to the development of myeloid malignancies, including MDS[31]. The candidate target genes for the derepression include *HOXA9* and *MIR125A*[32]. While *HOXA9* is a known oncogene in hematopoietic tumors of the myeloid lineage[33], *MIR125A* is suggested to impair hematopoietic cell differentiation[34]. Mutations in the *ASXL1* gene have been described in roughly 10% of MDS and 17% of AML cases[35]. Clinical studies demonstrated that *ASXL1* mutation was related to progression to AML in MDS patients[36], and was associated with poor response to chemotherapy in patients with MDS or AML[37]. Unfortunately, in the present study, the precise mechanism in regard to poor response to DA could not be identified for *ASXL1*-mutated subjects. Shi *et al.* reported that *ASXL1* loss impairs erythroid development and hinders erythroid differentiation, and could be of prognostic value for MDS/MPN patients[38]. Their report showed that ineffective erythropoiesis of MDS may have occurred as a result of *ASXL1* mutation, which could induce refractoriness to DA. From another point of view, Raimbault *et al.* reported that the low expressions of CD117/c-KIT$^+$ in lower-risk MDS erythroid precursors was correlated with ESA failure[39]. It would be interesting to analyze association between *ASXL1* gene mutations and expression levels of CD117/c-KIT$^+$ in a future study.

The main purpose of therapy in lower-risk MDS patients is to support cytopenia in the peripheral blood, and it may include blood cell transfusion or cytokine therapies such as ESA and G-CSF. Luspatercept, a recombinant fusion protein binding TGF-β superfamily ligands, is a novel treatment for anemia in lower-risk MDS patients, especially those with increased ring sideroblasts and/or *SF3B1* mutations[40]. Hypomethylating agents such as azacytidine are sometimes employed for the treatment of cytopenia, including anemia in lower-risk MDS, based on the balance between merits (restoration of blood cell counts) and demerits (bone marrow suppression), and have been suggested to be effective for those with *TET2* mutations[41-43].

This study provided the first evidence in the cytokine therapy stimulating erythropoiesis that the existence of some gene mutations (*ASXL1* mutations) may be associated with response to specific treatments (DA). Kosmider *et al.* have reported that having >2 somatic mutations was associated with lower HI-E in the ESA treatments for lower risk MDS, but that individual mutations of the frequently mutated genes had no significant impact on HI-E. Patients in our cohort were consistently treated with DA at a dose of 240 μg/week, while those in their cohort with EPO or DA at various doses with or without G-CSF. This may have caused different conclusions. In our

opinion, when patients of lower-risk MDS with higher EPO levels have *ASXL1* mutations and do not have *SF3B1* mutations, hypomethylating agents may be considered as the first-line therapy for anemia. Even in cytokine therapies for anemia, molecular stratification needs to be established to determine its application in the near future.

Data availability

All genetic data generated or analysed during this study are included in this published article and Supplementary Fig. S1.

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Authorship Contribution

Contribution: M.I., H.K., H.S., Y.Morita., Y.Maeda., K.T., T.M., T.H., and K.M. designed the study; M.I., H.K., H.S., Y.Morita., Y.Maeda., K.T., T.M., T.H., and H.H. collected clinical data; Y.N., Y.T., S.M., and S.O. performed sample preparation and sequencing; M.I., H.K., H.S., Y.Morita., Y.Maeda., K.T., T.M., T.H., Y.N., H.H., I.M., S.O., K.A., Y.K., and K.M. analyzed data; J.K, performed statistical analysis; M.I., H.K., H.S., Y.Morita., Y.Maeda., K.T., T.M., T.H., Y.N. Y.T. S.M. J.K.,Y.N., S.O., and K.M. wrote the manuscript; and all of the authors reviewed and approved the final manuscript.

Additional information

Competing interests statement

YN (Otsuka Pharmaceutical Co., Ltd.), HK (Celgene Corporation), SM (Fujitsu Limited), SO (Chordia Therapeutics Inc., Kan Research Institute, Inc., Otsuka Pharmaceutical Co., Ltd.), and KA (Sumitomo Dainippon Pharma Co., Ltd., Kyowa Kirin Co., Ltd.) are consultants for each company. SO has an ownership interests (including stock options) in a start-up company, the stock of which is not publicly traded for RegCell Co., Ltd., Asahi Genomics Co., Ltd., and Chordia Therapeutics, Inc. YM (Eisai Co., Ltd.), HS (Astellas Pharma Inc., Teijin Ltd., Shionogi Co., Ltd, Taiho, Pharmaceutical Co., Ltd., Eisai Co., Ltd., Celgene Corporation, Ono Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co., Ltd., MSD K.K., Sumitomo Dainippon Pharma Co., Ltd., Nippon Shinyaku Co., Ltd, Daiichi Sankyo Co., Ltd.), YM (Kyowa Kirin Co., Ltd.), SM (DeNA Life Science Inc.), IM (Chugai Pharmaceutical Co., Ltd., Eisai Co., Ltd., Ono Pharmaceutical Co., Ltd., Kyowa Kirin Co., Ltd., Shionogi Co., Ltd., Sumitomo Dainippon Pharma Co., Ltd., Asahi Kasei Pharma Corporation, Takeda Pharmaceutical Co., Ltd., Nippon Shinyaku Co., Ltd., Pfizer Japan Inc., Taiho Pharmaceutical Co., Ltd., Mitsubishi Tanabe Pharma Corporation, Nihon Pharmaceutical Co., Ltd., Novartis Pharma K.K., Daiichi Sankyo Co., Ltd., MSD K.K., Japan Blood Products Organization, Otsuka Pharmaceutical Co., Ltd.,

Mundipharma K.K., AbbVie GK, Sanofi K.K., Ayumi Pharmaceutical Corporation, Eli Lilly Japan K.K.), SO (Sumitomo Dainippon Pharma Co., Ltd., Chordia Therapeutics Inc., Otsuka Pharmaceutical Co., Ltd., Eisai Co., Ltd.) , KA (Celgene Corporation, Kyowa Kirin Co., Ltd, Astellas Pharma Inc., Shionogi Co., Ltd., Asahi Kasei Pharma Corporation , Chugai Pharmaceutical Co., Ltd, Bristol-Myers Squibb Co.), KM (Novartis Pharma K.K., Kyowa Kirin Co., Ltd., Chugai Pharmaceutical Co., Ltd., Takeda Pharmaceutical Co., Ltd.) have received the research funding of each companies. YM (Kyowa Kirin Co., Ltd.), MI (Novartis Pharma K.K., Takeda Pharmaceutical Co., Ltd.), HS (Takeda Pharmaceutical Co., Ltd., Novartis Pharma K.K., Celgene Corporation, Janssen Pharmaceutical K.K., Chugai Pharmaceutical Co., Ltd., Kyowa Kirin Co., Ltd., Ono Pharmaceutical Co., Ltd, Eisai Co., Ltd., Nippon Shinyaku Co., Ltd., Otsuka Pharmaceutical Co., Ltd., Daiichi Sankyo Co., Ltd., Bristol-Myers Squibb Co., Pfizer Japan Inc., Fujimoto Pharmaceutical Corporation), YM (Kyowa Kirin Co., Ltd), TM (Bristol-Myers Squibb Co., Otsuka Pharmaceutical Co., Ltd., MSD K.K., Astellas Pharma Inc., Amgen Astellas Biopharma K.K., Celgene Corporation, AbbVie GK, Takeda Pharmaceutical Co., Ltd.), HK (Kyowa Kirin Co., Ltd., Sanofi K.K., Novartis Pharma K.K., Chugai Pharmaceutical Co., Ltd.), SM (Ministry of Health, Labour and Welfare, Japan Science and Technology Agency, National Institutes of Biomedical Innovation, Health and Nutrition), IM (Pfizer Japan Inc., Novartis Pharma K.K., Daiichi Sankyo Co., Ltd., Astellas Pharma Inc., Otsuka Pharmaceutical Co., Ltd., Bristol-Myers Squibb Co.) have directly received honoraria from an entity. Seishi Ogawa (Qiagen K.K.) has received patents and royalties. KM (Celgene Corporation, Kyowa Kirin Co., Ltd.) has received fee of expert testimony. HS (Chugai Pharmaceutical Co., Ltd., Ono Pharmaceutical Co., Ltd., AbbVie GK, AstraZeneca), and SM (Uehara Foundation, Nakagani Foundation, Otsuka Foundation, Okawa Foundation) are assigned as an entity's board of directors, speakers bureau, or its advisory committees. HH, TH, KT, HT, JK and YK have no COI to disclose.

Tables

Table 1. Baseline characteristics of the subjects

Males/females, n (%)	52/27 (65.8/34.2)
Median age, years (range)	77.0 (29-90)
WHO classification (the 4th edition), n (%)	
RCUD	23 (29.1)
RARS	12 (15.2)
RCMD	37 (46.8)
RAEB-1	5 (6.3)
RAEB-2	0 (0.0)
MDS-U	1 (1.3)
MDS with isolated del (5q)	1 (1.3)
IPSS, n (%)	
Low	29 (36.7)
Int-1	50 (63.3)
Int-2	0 (0.0)
High	0 (0.0)
Transfusion dependency, n (%)	
No	64 (81.0)
Yes	15 (19.0)
EPO, n (%)	
<100	40 (50.6)
≧100	39 (49.4)

WHO, World Health Organization; RCUD, refractory cytopenia with unilineage dysplasia; RARS, refractory anemia with ring sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RAEB-1, refractory anemia with excess blasts-1, RAEB-2, refractory anemia with excess blasts-2; MDS-U, myelodysplastic syndromes-unclassified; IPSS, International Prognostic Scoring System; EPO, erythropoietin.

Table 2. Rate of overall response to DA at week 16

	N=79	HI-E %	95% CI	
			Lower	Upper
IWG criteria				
All subjects	79	70.9	60.1	79.7
Transfusion independent	64	73.4	61.5	82.7
Transfusion dependent	15	60.0	35.7	80.2
Major response				
Transfusion dependent	15	46.7	24.8	69.9
Minor response				
Transfusion dependent	15	60.0	35.7	80.2

HI-E, hematological improvement-erythroid; DA, darbepoetin alfa; IWG, International Working Group.

Table 3. Association between gene mutation and response to DA at week 16: Univariate analysis

Gene	N (%)	Odds ratio	95% CI		P value
SF3B1	24 (30.4%)	2.639	0.788	8.839	0.116
TET2	20 (25.3%)	2.906	0.761	11.104	0.119
SRSF2	10 (12.7%)	1.750	0.342	8.951	0.502
ASXL1	9 (11.4%)	0.277	0.067	1.145	0.076
DNMT3A	8 (10.1%)	1.260	0.235	6.757	0.787

DA, darbepoetin alfa

Table 4. Association between achievement of HI-E and gene mutation combined with EPO level and transfusion volume: Multivariate analysis

Variable	Odds Ratio	95%CI		P value
SF3B1（mutation(+) / mutation(-)）	2.776	0.767	10.048	0.1197
Baseline EPO (mIU/mL)（100+ / <100）	0.170	0.053	0.546	0.0029
Baseline TF（umit/month）（1+ / <1）	0.797	0.257	2.468	0.6936
TET2（mutation(+) / mutation(-)）	2.361	0.573	9.732	0.2344
Baseline EPO (mIU/mL)（100+ / <100）	0.188	0.059	0.602	0.0049
Baseline TF（umit/month）（1+ / <1）	0.799	0.259	2.465	0.6959
SRSF2（mutation(+) / mutation(-)）	1.226	0.212	7.099	0.8202
Baseline EPO(mIU/mL)（100+ / <100）	0.177	0.055	0.563	0.0034
Baseline TF（umit/month）（1+ / <1）	0.818	0.269	2.491	0.7237
ASXL1（mutation(+) / mutation(-)）	**0.175**	**0.033**	**0.928**	**0.0406**
Baseline EPO(mIU/mL)（100+ / <100）	0.146	0.042	0.503	0.0023
Baseline TF（umit/month）（1+ / <1）	0.766	0.238	2.460	0.6537
DNMT3A（mutation(+) / mutation(-)）	1.093	0.166	7.193	0.9262
Baseline EPO (mIU/mL)（100+ / <100）	0.175	0.055	0.554	0.0031
Baseline TF（umit/month）（1+ / <1）	0.808	0.258	2.535	0.7154

HI-E, hematological improvement-erythroid; EPO, erythropoietin; TF, transfusion; CI, confidence interval.

Figure legends

Figure 1. Progression free survival to AML. Data in 58 subjects who continued treatment without progression to acute myelogenous leukaemia till week 16. Initial date of reckoning: 16 weeks after start of treatment.

Figure 2. Overall survival. Data in 58 subjects who continued treatment without progression to acute myelogenous leukaemia till week 16. Initial date of reckoning: 16 weeks after start of treatment.

Figure 3. Time to achievement of the 1st HI-E.

Data in full analysis set (N=79). Initial date of reckoning: After start of administration.

Figures

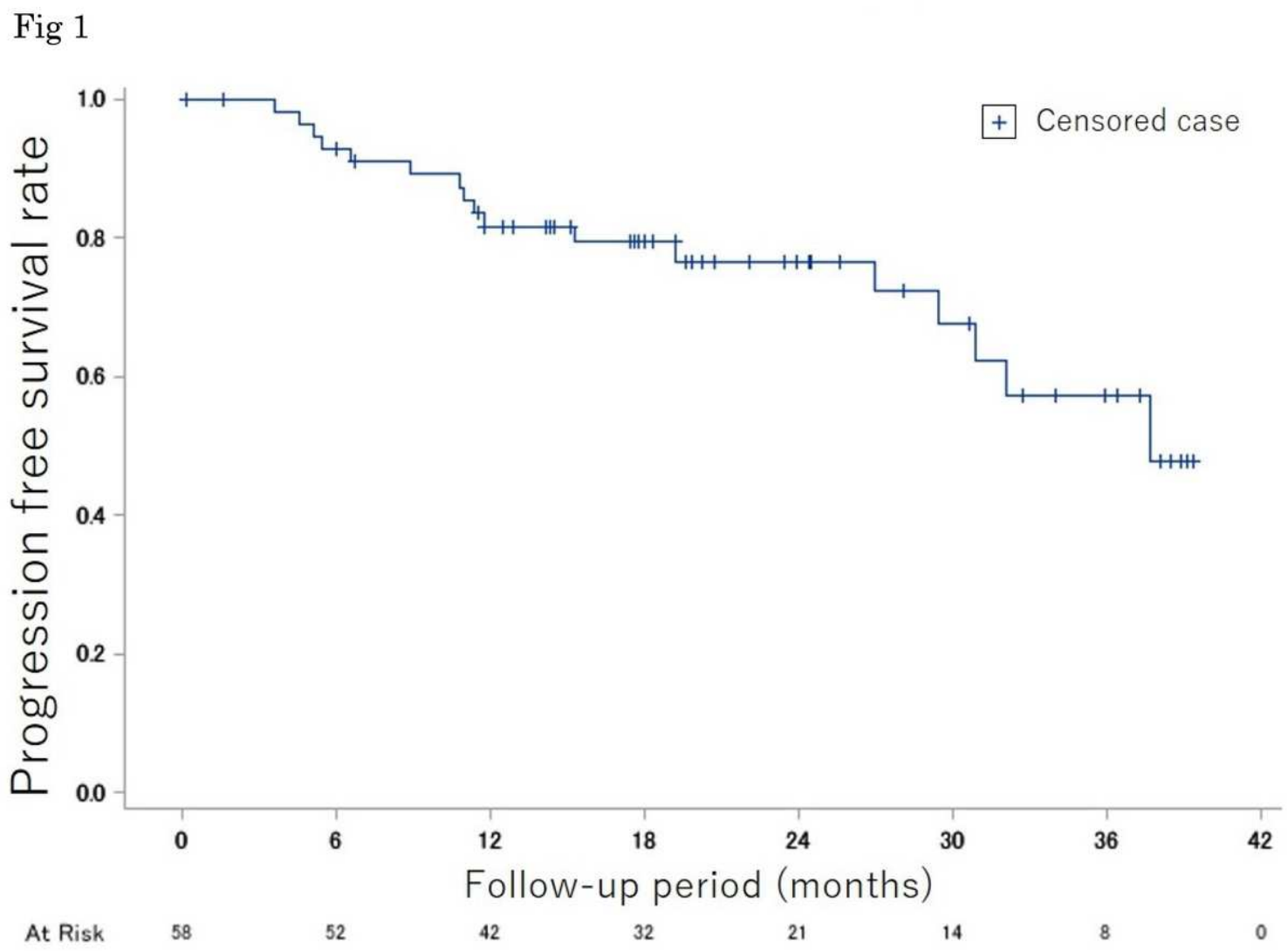

Figure 1

Progression free survival to AML. Data in 58 subjects who continued treatment without progression to acute myelogenous leukaemia till week 16. Initial date of reckoning: 16 weeks after start of treatment.

Fig 2

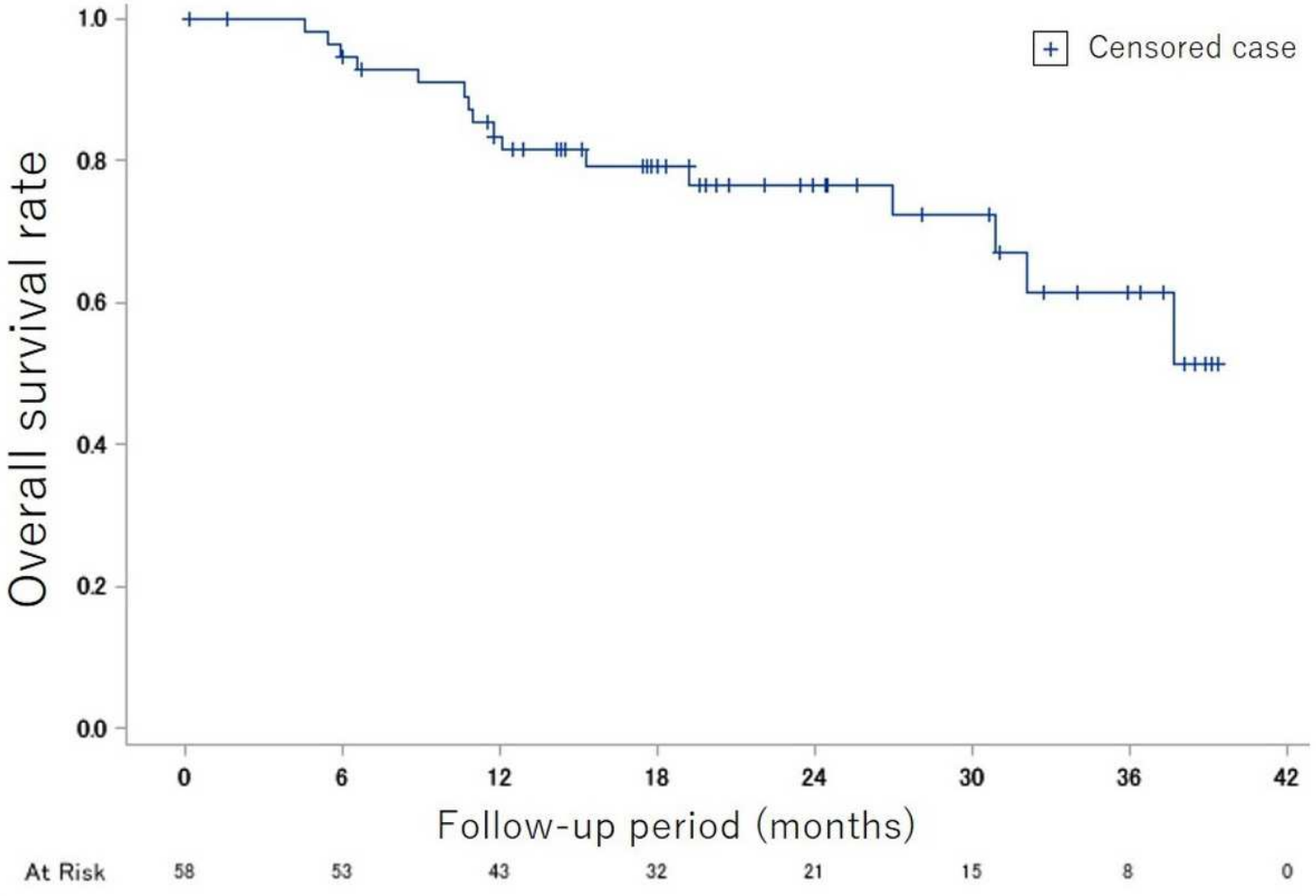

Figure 2

Overall survival. Data in 58 subjects who continued treatment without progression to acute myelogenous leukaemia till week 16. Initial date of reckoning: 16 weeks after start of treatment

Fig 3

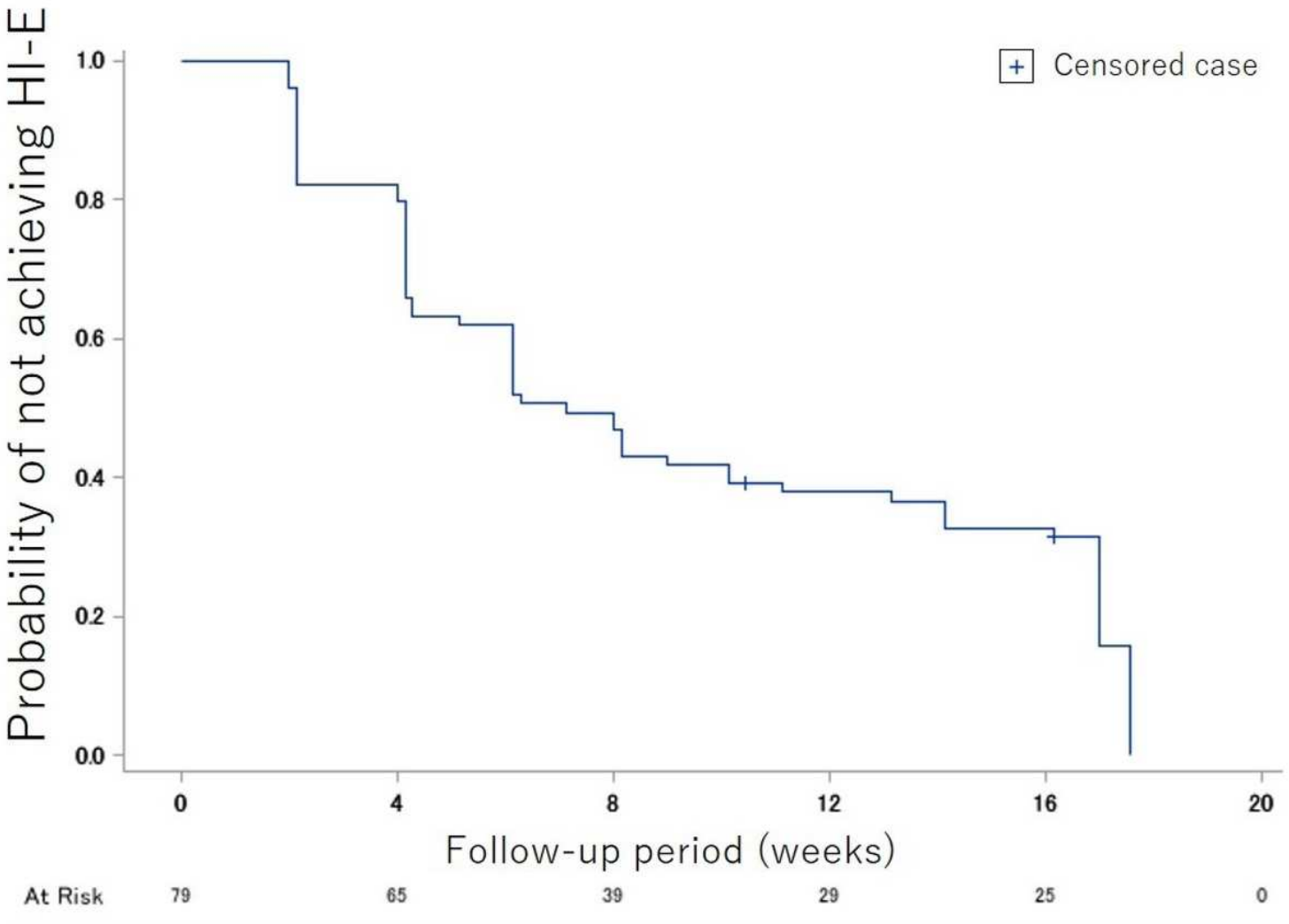

Figure 3

Time to achievement of the 1st HI-E. Data in full analysis set (N=79). Initial date of reckoning: After start of administration.

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