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Efficacy and safety of darbepoetin alpha in patients with myelodysplastic syndromes: a systematic review and meta-analysis

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Summary

We conducted a systematic review and meta-analysis to estimate the efficacy of darbepoetin alpha (DA) for treatment of myelodysplastic syndrome (MDS)-related anaemia. Eligible studies were prospective, interventional, and reported World Health Organization, French-American-British, or International Prognostic Scoring System (IPSS) criteria. Outcomes included erythroid response rate (primary); haemoglobin response; change in haemoglobin, transfusion status, and quality-of-life (QoL); and safety. Ten studies ($N = 647$) were analysed. Erythroid response rate range was 38–72%; median response duration range was 12–51+ months. Patients with erythropoietin (EPO) <100 iu/l had 35% [95% confidence interval (CI): 22–48%; $P < 0{\cdot}001$) better response than patients with EPO >100 iu/l. Erythropoesis-stimulating agent (ESA)-naïve patients had 17% (95% CI: 3–32%; $P = 0{\cdot}022$) greater response rate than those previously treated with ESA. Nonetheless, previously treated patients had response rates of 25–75%. Higher baseline haemoglobin levels, higher dose, transfusion-independence and low-risk IPSS status were reported by several studies to be associated with better response. QoL, transfusion rates and haemoglobin levels improved with treatment. Hypertension, thromboembolism and progression to acute myeloid leukaemia were reported in 2%, 1% and 1% of patients, respectively. This meta-analysis suggests that DA treatment can be useful for improving erythroid response in MDS patients with anaemia, even among patients previously treated with ESA.

Keywords: darbepoetin alpha, myelodysplastic syndromes, systematic review, meta-analysis.

Myelodysplastic syndromes (MDS) are a group of heterogeneous disorders characterized by one or more peripheral blood cytopenias that originate in dysfunctional clonal bone marrow stem cells with abnormal proliferation and differentiation. The incidence rate of MDS is estimated to be 5·3–13·1 cases per 100 000 people worldwide (Cogle, 2015). Patients with MDS often progress to acute myeloid leukaemia (AML), particularly patients with high-risk MDS (Steensma & Bennett, 2006). Patients are most commonly stratified into risk categories using the International Prognostic Scoring System (IPSS) based on percentage of blasts, number of cytopenias and cytogenetics. The primary objectives of treatment in patients with low- or intermediate-risk MDS are to treat the anaemia resulting from disease, reduce transfusions, improve quality of life (QoL) and prevent progression to AML or other higher-risk disease (Garcia-Manero, 2014).

Anaemia is a major burden for MDS patients and is associated with fatigue, weakness and shortness of breath. The symptoms of anaemia may be temporarily improved by red blood cell (RBC) transfusion, but there are safety concerns associated with repeated transfusions (Greenberg *et al*, 2013). Anaemia leading to transfusion dependency and iron overload is commonly observed in patients diagnosed with MDS. Transfusion dependency and iron overload have been associated with shorter survival and worse clinical outcomes

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among patients with MDS, including cardiac, hepatic, and endocrine dysfunction (Greenberg *et al*, 2013). Prevention of transfusion-related events for MDS patients can have a substantial effect on survival, which varies by risk stratification: median survival in low-risk patients can exceed 3 years and in high-risk patients can be as low as 2 months (Garcia-Manero, 2014).

Erythropoesis-stimulating agents (ESAs) have been used to treat anaemic MDS patients in clinical practice to reduce the risk of RBC transfusion (Sekeres *et al*, 2008). Two retrospective studies (Spiriti *et al*, 2005; Park *et al*, 2010) and one prospective study (Greenberg *et al*, 2009) have suggested that use of ESA rather than transfusions improved the overall survival of MDS patients. Additionally, longer survival times have been reported in MDS patients with ESA therapy than without (Jadersten *et al*, 2008; Park *et al*, 2008).

Previous reviews reported on studies that used short-acting epoetin alpha (EA) to treat MDS-related anaemia (Ross *et al*, 2007; Moyo *et al*, 2008), but these reviews focused on EA and included few studies assessing long-lasting darbepoetin alpha (DA). The number of studies that have assessed DA use has increased (Gabrilove *et al*, 2008; Gotlib *et al*, 2009; Oliva *et al*, 2010; Nilsson-Ehle *et al*, 2011; Villegas *et al*, 2011; Kelaidi *et al*, 2013a; Jang *et al*, 2015). Studies have demonstrated efficacy and a safe profile with few reported adverse events (AEs) (Kelaidi & Fenaux, 2010); however, to our knowledge no meta-analysis or systematic review has been conducted on these DA studies.

Here, we conducted a systematic review and meta-analysis of the use of DA as supportive care to treat anaemia in patients with MDS. The primary objective of this study was to estimate the erythroid response rate in MDS patients receiving DA. We also examined the response rate by baseline erythropoietin levels, DA dose, response measurement timing and transfusion history.

Methods

Literature search and data extraction

The literature search was performed by two professional independent librarians using the following databases: PubMed, EMBASE, MEDLINE, BIOSIS Previews and the Current Contents/all editions for papers published until August 2015. A range of MeSH (Medical Subject Headings) terms and key words were used to search the relevant databases (Data S1). Additional searches of conference proceedings were conducted for the American Society of Hematology (ASH), American Society of Clinical Oncology (ASCO), MDS Foundation, European Haematology Association (EHA), International Symposium on Supportive Care in Cancer (MASCC) and European Society for Medical Oncology (ESMO). Titles and abstracts of identified studies were evaluated to determine if they met eligibility criteria. Full articles were obtained and screened manually for confirmation. A manual check of the reference lists of all accepted papers and recent reviews was also performed to supplement the electronic searches described above. Screening and evaluation were conducted independently by two reviewers with resolution of disagreement by consensus or adjudication by a third reviewer. To avoid review of duplicate reports of the same study, this analysis used the most complete publication, or the most recent of multiple publications using the same patient population.

Publications were extracted if the following inclusion criteria were met: (i) prospective interventional study design of MDS patients treated with DA; (ii) at least 10 adult patients with MDS reporting either World Health Organization (WHO), French-American-British (FAB) criteria or IPSS status; (iii) reported at least one of the following outcomes: number and proportion of patients with erythroid response, haemoglobin change, RBC transfusions (pre- and post-treatment), QoL using validated instruments [e.g., the Functional Assessment of Cancer Therapy (FACT), as pre- and post-treatment scores or change scores], or the utilization of health resources; (iv) publication written in English, or studies reported in languages other than English with the relevant data for the analysis available from the abstract; and (v) published since 1980. Studies were excluded they if they were: (i) animal or *in vitro* studies; (ii) case reports, letters, news, editorials, or reviews; or (iii) study populations of other malignancies.

Data were extracted independently by two reviewers and entered into a pre-designed data extraction form. Disagreement was resolved through discussion until consensus was achieved. Information regarding the study design and outcomes of interest were collected. In the extraction of safety data, a zero number of events was recorded only where there was a clear statement in the original study that a particular event did not occur; when no mention of an event was made, the data were treated as missing and not included in the analyses.

Analytical approach

The primary endpoint of interest of this study was the international Working Group (IWG) 2000 erythroid response rate in MDS patients receiving DA (Cheson *et al*, 2000), as this outcome was more commonly reported than the IWG 2006 response definition (Cheson *et al*, 2006) over the time period of the systematic review. Other endpoints analysed descriptively included: change in the number of units and percentage of RBC transfusions from baseline; time to response; duration of response; change in QoL measures from baseline; and numbers and percentages of AEs.

We evaluated clinical and methodological heterogeneity with respect to the study population [e.g., MDS subtype, baseline clinical characteristics including serum erythropoietin (EPO) level], participant selection method (e.g., inclusion/exclusion criteria), intervention or treatment evaluated

[e.g., DA dosing regimen, addition of granulocyte colony-stimulating factor (G-CSF)], outcome measurements (e.g., definition of erythroid response) and analysis methods. Additional visual inspection of forest plots and statistical methods, such as the I^2 statistic (Higgins & Thompson, 2002) and Cochran's Q statistic (Hedges & Olkin, 1985), were used to assess the heterogeneity across studies and assist in the decision as to how to proceed with data synthesis. Throughout the analyses, estimates were only combined using formal meta-analytical techniques when it was warranted, based on sample size or tests of homogeneity; otherwise results were reported descriptively or graphically only.

Publication bias (the potential for non-significant studies to go unpublished, thereby skewing the published results toward a more favourable conclusion) was evaluated graphically using a funnel plot where proportion of response was compared against within-study variation for each study. If no publication bias exists, a balanced scatter around the true response rate forming a triangular 'funnel' shape is seen, with greater variation for smaller (high-variation) studies and less variation for larger studies. If publication bias exists, there is a tendency for negative studies to be missing (particularly the negative, small, high-variation studies) resulting in an asymmetrical shape.

Meta-analysis

A formal meta-analysis was performed to estimate the erythroid response rate based on studies using IWG 2000 response definitions, although the results from studies with other response definitions were included descriptively. Furthermore, we chose outcomes that were assessed at 12–24 weeks after the initiation of treatment, taking the earliest response reported within that window when responses at multiple time points were reported for the same study. A formal meta-analysis was not performed for other outcomes of interest.

Where combined estimates were warranted, we used random effects models based on a restricted maximum-likelihood estimator (Raudenbush, 2009) for combined estimates and meta-regression. All confidence intervals (CIs) for single studies were based on the asymptotic CI for a single proportion (Newcombe, 1998). For the two-sample subgroup analyses, we used the absolute difference in response as the outcome measure because the response rate difference between the subgroups was the quantity of clinical interest. As a sensitivity analysis, we also compared subgroups using the log odds ratio. All statistical analyses were performed using the package 'metafor' (Viechtbauer, 2010) in the statistical software R (Venables & Smith, 2015).

Results

A total of 173 references were identified, of which 41 references were considered potentially relevant based on the abstracts according to the inclusion and exclusion criteria (Fig 1). Full texts of these articles were reviewed and 10 studies (N = 647 patients) were confirmed to meet the eligibility criteria (Table I).

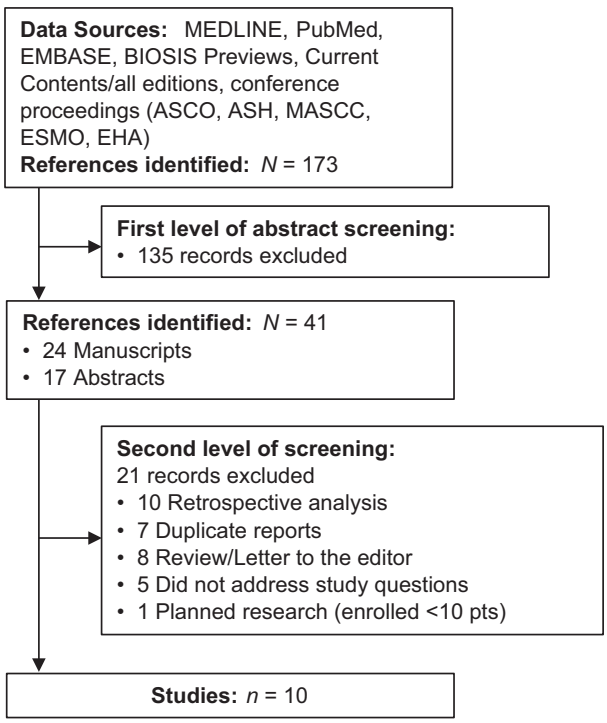

Fig 1. Flow chart of literature search results and study selection. ASCO, American Society of Clinical Oncology; ASH, American Society of Hematology; MASCC, Multinational Association of Supportive Care in Cancer; ESMO, European Society for Medical Oncology; EHA, European Haematology Association.

Clinical and methodological characteristics of published studies

Nine of ten studies that met the inclusion criteria were single-arm phase II studies (Musto *et al*, 2005; Stasi *et al*, 2005; Mannone *et al*, 2006; Gabrilove *et al*, 2008; Gotlib *et al*, 2009; Oliva *et al*, 2010; Nilsson-Ehle *et al*, 2011; Villegas *et al*, 2011; Kelaidi *et al*, 2013a), and one study was a randomized controlled trial (RCT) (Jang *et al*, 2015) that evaluated the response rate across different DA doses. When evaluating the effect of dose on response, the response rates from the three dose arms reported by Jang *et al* (2015) were entered into the analysis separately; for the rest of the analysis, the response rates, safety events and other outcomes from all three arms were combined. Despite the inclusion of a range of WHO or FAB classifications, most studies enrolled patients with low-risk or intermediate-1 IPSS risk only, with a few studies including a small proportion of patients with intermediate-2 IPSS risk (Musto *et al*, 2005; Mannone *et al*, 2006; Gotlib *et al*, 2009; Nilsson-Ehle *et al*, 2011). Most studies clearly stated that patients with other causes of anaemia

Table I. Studies*, baseline clinical characteristics, MDS assessment criteria, and treatment in published studies.

Study (N enrolled/N evaluated)	MDS assessment			Baseline clinical characteristics			Treatment history	
	FAB, *n* (%)	WHO, *n* (%)	IPSS, *n* (%)	Time from diagnosis to treatment, months	Haemoglobin, g/l	Serum EPO, iu/l	ESA	TD†, *n* (%)
Musto *et al* (2005) (37/37)	NA	RA: 11 (29·7) RARS: 3 (8·1) RCMD: 12 (32·4) RCMD-RS: 2 (5·4) RAEB: 8 (21·6) 5q-: 1 (2·7)	Low: 16 (43·2) INT-1: 17 (45·9) INT-2: 4 (10·8)	Mean (SD): 30·4 (43·8) Median: 20 Range: 1–240	Mean (SD): 82 (12) Median: 84 Range: 55–107	Mean (SD): 303·1 (438·7) Median: 109 Range: 19–2000	Eight patients received rEPO for ≥12 weeks	23 (62)
Stasi *et al* (2005) (53/53)	Not stated	RA: 31 (58·5), RCMD: 10 (18·9) RAEB-1: 8 (15·1) RARS: 3 (5·7) RCMD-RS: 1 (1·9)	Low: 29 (54·7) INT-1: 24 (45·3)	Nonresponders: Median: 16 Range: 9–21 Responders: Median: 15·5 Range: 9–21	Median: 79 Range: 68–93	Median: 171 Range: 26–515	No	46 (86·8)
Mannone *et al* (2006) (66/62)	RA: 22 (35·5) RAEB: 18 (29·0) RARS: 20 (32·3) CMML: 2 (3·2)	RA: 11 (17·7) RCMD: 8 (12·9) RARS: 18 (29·0) RCMD-RS: 2 (3·2) RAEB-1: 18 (29·0) 5q-: 3 (4·8) CMML1: 2 (3·2)	Low: 16 (25·8) INT-1: 26 (41·9) INT-2: 8 (12·9) Missing: 12 (19·4)	Median: 10 Range: 0–124	<100 (mean/median not provided)	Median: 67 Range: 6–487	Yes; 13 patients with failure of rEPO treatment before inclusion	41 (66·1)
Gabrilove *et al* (2008) (209/206)	RA: 119 (57·8) RARS: 73 (35·4) RAEB: 13 (6·3) Missing: 1 (0·5)	NA	Low: 138 (67·0), INT-1: 58 (28·2) Missing: 10 (4·9)	NA	Mean (SD): 98 (10) ESA-naïve: 97 (10) ESA-treated: 100 (11)	<100: *n* = 137 100–500: *n* = 43 ≥500: *n* = 21 Missing: *n* = 5 Median: 58·1 Range: 12–4643	ESA-naïve: 144 ESA previously treated: 62	9 (4·4)
Gotlib *et al* (2009) (24/24)	RA: 10 (41·7) RARS: 9 (37·5) CMML: 2 (8·3) RAEB: 3 (12·5)	RCMD: 8 (33·3) RCMD-RS: 9 (37·5) 5q-: 2 (8·3) CMML-1: 2 (8·3) RAEB-1: 3 (12·5)	Low: 12 (50) INT-1: 10 (41·7) INT-2: 2 (8·3)	NA	Mean (SD): 92 (9) Median: 93 Range: 71–108	Mean (SD): 241·5 (505·5) Median: 111 Range: 12–2556	Yes; rEPO-naïve or EPO tx ≤40,000 units/week for ≤4 weeks	16 (66·7)
Oliva *et al* (2010) (41/40)‡	NA	RA: 24 (59) RCMD: 11 (27) RARS: 2 (5) RAEB1: 1 (2) 5q-: 2 (5) MDS-U: 1 (2)	Low: 29 (71) INT-1: 12 (29)	Mean (SD): 25 (28)	Mean (SD): 90 (10)	Mean (SD): 113 (108)	Patients on rEPO within 6 mos before enrollment excluded; 12 patients resistant to prior rEPO alpha at adequate dose	17 (41·5)

Table I. (*Continued*)

Study (N enrolled/N evaluated)	MDS assessment			Baseline clinical characteristics			Treatment history	
	FAB, *n* (%)	WHO, *n* (%)	IPSS, *n* (%)	Time from diagnosis to treatment, months	Haemoglobin, g/l	Serum EPO, iu/l	ESA	TD†, *n* (%)
Nilsson-Ehle *et al* (2011) (36/30)§	NA	RA: 6 (16·7) RARS: 8 (22·2) RCMD: 12 (33·3) RAEB: 5 (13·9) 5q-: 3 (8·3) MDS-U: 1 (2·8) CMML: 1 (2·8)	Low: 14 (38·9) INT-1: 12 (33·3) INT-2: 3 (8·3) Not performed: 7 (19·4)	Median: 3·6 Range: 0–197	Mean (SD): 93 (11·3) Median: 91·5 Range: 74–127	NA	Patients on EPO within 8 weeks prior to study excluded	17 (47·2)
Villegas *et al* (2011) (44/43)	RA: 14 (31·8) RARS: 27 (61·4) RAEB: 3 (6·8)	Not stated	Low: 34 (77·3) INT-1: 10 (22·7)	NA	Mean (SD): 92 (8)	Mean (SD): 120·7 (122·5)	Patients on EPO within 4 weeks before entry excluded	12 (27)
Kelaidi *et al* (2013a) (99/95)	NA	RA: 24 (25) RARS: 31 (33) RCMD: 15 (16) RCMD-RS: 7 (7) 5q-: 2 (2) RAEB-1: 14 (15) CMML: 2 (2)	Low: 51 (54) INT-1: 44 (46)	NA	Median: 92 Range: 62–100	Median: 60 Range: 3–461	Patients on ESA in previous 8 weeks excluded	44 (46)
Jang *et al* (2015) (52/50)	RA: 33 (63·5) RARS: 14 (26·9) RAEB: 5 (9·6)	RARS: 4 (7·7) RCMD: 31 (59·6) RAEB-1: 5 (9·6) 5q-: 2 (3·8) RCUD: 4 (7·7) MDS-U: 6 (11·5)	Low: 9 (17·3) INT-1: 43 (82·7)	NA	Mean (SD): 79·2 (9·1)	Mean (SD): 221 (134)	NA	52 (100)

CMML, chronic myelomonocytic leukaemia; EPO, erythropoietin; ESA, erythropoiesis-stimulating agent; FAB, French-American-British; INT, intermediate; IPSS, International Prognostic Scoring System; MDS, Myelodysplastic syndromes; MDS-U, Myelodysplastic syndrome – unclassifiable; NA, not available; RA, refractory anaemia; RAEB, refractory anaemia with excess blasts; RARS, refractory anaemia with ring sideroblasts; RCMD, refractory cytopenias with multilineage dysplasia; RCMD-RS, refractory cytopenias with multilineage dysplasia and ring sideroblasts; RCUD, refractory cytopenia with unilineage dysplasia; rEPO, recombinant erythropoietin; SD, standard deviation; TD, transfusion dependent; tx, treatment; WHO, World Health Organization.

*All studies were phase II, single-arm, except for Musto *et al* (2005) (pilot study) and Jang *et al* (2015) (randomized controlled trial).

†Transfusion dependence was defined as ≥1 transfusion per month.

‡Based on intent-to-treat sample $N = 41$: information on the WHO or IPSS status of the patient that left the study was not available so percentages cannot be updated for $N = 40$.

§$N = 30$ at 16 weeks. Although the study was a 24-week study and 27 patients completed the study, the study protocol changed after week 16 so that nonresponding patients received transfusions.

were excluded (Stasi *et al*, 2005; Mannone *et al*, 2006; Gabrilove *et al*, 2008; Oliva *et al*, 2010; Nilsson-Ehle *et al*, 2011; Villegas *et al*, 2011; Kelaidi *et al*, 2013a).

Baseline patient clinical characteristics of the original studies are summarized in Table I. All but one of the studies (Mannone *et al*, 2006) reported either mean or median baseline haemoglobin levels (range of median levels: 79–93 g/l; range of mean levels: 79–98 g/l) and all studies except one (Musto *et al*, 2005) stated that patients were at or below a threshold haemoglobin level for inclusion. All studies except one (Nilsson-Ehle *et al*, 2011) reported baseline serum EPO levels (range of median EPO levels: 58·1–171 iu/l; range of mean EPO levels: 113–221·1 iu/l) (Musto *et al*, 2005; Stasi *et al*, 2005; Mannone *et al*, 2006; Gabrilove *et al*, 2008; Gotlib *et al*, 2009; Oliva *et al*, 2010; Villegas *et al*, 2011; Kelaidi *et al*, 2013a; Jang *et al*, 2015). The duration of time from MDS diagnosis to enrolment ranged from 3·6 to 20 months (both mean and median) for the five studies that reported duration (Musto *et al*, 2005; Stasi *et al*, 2005; Mannone *et al*, 2006; Oliva *et al*, 2010; Nilsson-Ehle *et al*, 2011). Most studies either excluded patients with previous ESA exposure (Stasi *et al*, 2005), or excluded patients who had received EPO within 1–6 months of the start of the study (Gotlib *et al*, 2009; Oliva *et al*, 2010; Nilsson-Ehle *et al*, 2011; Villegas *et al*, 2011; Kelaidi *et al*, 2013a). All of the studies included some transfusion-dependent patients (range 4–100%), although the definition of transfusion dependency varied slightly across studies.

Although treatment regimens varied, there were many commonalities (Table II). Seven of the ten studies had an initial fixed-dose regimen that was administered once weekly (QW), with doses ranging from 60 to 300 μg (Musto *et al*, 2005; Stasi *et al*, 2005; Mannone *et al*, 2006; Oliva *et al*, 2010; Nilsson-Ehle *et al*, 2011; Villegas *et al*, 2011; Jang *et al*, 2015). Two studies treated patients with an initial dose of 500 μg every 2 weeks (Q2W) (Kelaidi *et al*, 2013a) or 3 weeks (Q3W) (Gabrilove *et al*, 2008). One study administered a weight-based dose QW (Gotlib *et al*, 2009). Nine of the ten studies increased the initial dose, the frequency of dosing or added G-CSF when minor response or non-response was observed (Stasi *et al*, 2005; Mannone *et al*, 2006; Gabrilove *et al*, 2008; Gotlib *et al*, 2009; Oliva *et al*, 2010; Nilsson-Ehle *et al*, 2011; Villegas *et al*, 2011; Kelaidi *et al*, 2013a; Jang *et al*, 2015). Two studies added G-CSF to the treatment regimen for patients with refractory anaemia with ringed sideroblasts (RARS) from week 1 (Gotlib *et al*, 2009; Nilsson-Ehle *et al*, 2011), and four studies added G-CSF to the regimen after poor response at 8–12 weeks (Mannone *et al*, 2006; Gotlib *et al*, 2009; Villegas *et al*, 2011; Kelaidi *et al*, 2013a). In total, nine studies reported IWG 2000 results at 12–24 weeks, with total treatment periods ranging from 12 to 52 weeks. Five studies reported median follow-up ranging from 9 to 52 months (Stasi *et al*, 2005; Mannone *et al*, 2006; Villegas *et al*, 2011; Kelaidi *et al*, 2013a; Jang *et al*, 2015).

Erythroid response rate and meta-analysis

Nine studies reported response according to IWG 2000 criteria within 12–24 weeks, with response rates ranging from 38% to 72·5% (Musto *et al*, 2005; Stasi *et al*, 2005; Mannone *et al*, 2006; Gabrilove *et al*, 2008; Gotlib *et al*, 2009; Oliva *et al*, 2010; Villegas *et al*, 2011; Kelaidi *et al*, 2013a; Jang *et al*, 2015). The results for response rates are summarized in Table II. Median time to response ranged from 4 to 9 weeks among responders (Musto *et al*, 2005; Mannone *et al*, 2006; Gotlib *et al*, 2009; Kelaidi *et al*, 2013a) and 8 to 9 weeks when both responders and non-responders were included using the Kaplan–Meier estimates of response time (Oliva *et al*, 2010; Villegas *et al*, 2011). Additionally, Gabrilove *et al* (2008) reported a median time to response of 11 weeks for ESA-naïve patients and 31 weeks for patients previously treated with ESAs based on responders and non-responders (Table II). The median duration of response was between 12 and at least 51 months (Gotlib *et al*, 2009; Musto *et al*, 2005; Oliva *et al*, 2010; Stasi *et al*, 2005b).

The response rates are also summarized in Fig 2. The responses were too heterogeneous to be combined ($P < 0·001$) but several subgroups were similar enough to be combined. Based on the six studies that reported results separately by baseline EPO level (Musto *et al*, 2005; Mannone *et al*, 2006; Gabrilove *et al*, 2008; Gotlib *et al*, 2009; Villegas *et al*, 2011; Jang *et al*, 2015), patients with EPO levels <100 iu/l had 38% (95% CI: 26–49%; $P < 0·0001$) better absolute response rate than patients with baseline EPO levels >100 iu/l (Fig 3, Fig S1). Patients with EPO levels <100 iu/l had an average response of 81% (95% CI: 74%–88%). Based on the four studies that reported response by previous ESA treatment (Musto *et al*, 2005; Mannone *et al*, 2006; Gabrilove *et al*, 2008; Oliva *et al*, 2010), patients who were ESA-naïve at baseline had a 17% (95% CI: 3–32%; $P = 0·022$) improved response rate than patients who had been previously treated with an ESA (Fig 4A). Response rates for ESA-naïve patients ranged from 45% to 73% and previously treated patients ranged from 25% to 75% (Musto *et al*, 2005; Mannone *et al*, 2006; Gabrilove *et al*, 2008; Oliva *et al*, 2010), showing that even patients previously exposed to an ESA can exhibit an erythroid response when treated with DA. Additionally, at the study level, higher mean baseline haemoglobin was associated with a greater response rate ($P = 0·020$) (Fig 5, Fig S2). No evidence of publication bias was seen in the funnel plot (Fig S3). Sensitivity analyses of the subgroups using log odds ratio rather than absolute difference in response yielded similar results.

Other trends were found that were suggestive but did not reach statistical significance. Patients who were transfusion-independent at baseline tended to have a 17% (95% CI: −2–36%; $P = 0·073$) better response rate than patients who were transfusion-dependent at baseline (Fig 4B) in the five studies that reported response rates by baseline transfusion status (Musto *et al*, 2005; Stasi *et al*, 2005; Mannone *et al*,

Table II. DA treatment characteristics and erythroid response rates in published studies.

Study	Treatment	Response criteria	Treatment period	Follow-up Median (range)	Response rate assessment, *N* (%)*					Time to response Median (range)	Duration of response
					Week 8	Week 12	Week 16	Week 24	Other		
Musto *et al* (2005)	150 μg QW for ≥12 weeks	IWG 2000‡	12 weeks	NA	NA	15 (41) MaR: 13 (35) MiR: 2 (5)	NA	NA	NA	9 weeks (2–11)	13 patients had stable Hb after 7–22 months
Stasi *et al* (2005)	150 μg QW; increased to 300 μg if no/ suboptimal response by week 12; dose adjusted to maintain Hb 110–130 g/l if Hb >130 g/l	IWG 2000	24 weeks (primary)	9·4 mos	–	20 (38) MaR: 20 (38) MiR: 0 (0)	–	24 (45) MaR: 21 (40) MiR: 3 (6)	–	NA	Median: NE (95% CI: 51–NE)
Mannone *et al* (2006)	300 μg/week for 12 weeks; DA+G-CSF for patients without response after week 12; withheld DA until Hb <130 g/l and maintained Hb 110–130 g/l	IWG 2000	24 weeks	40 weeks (4–84)	NA	44 (71) MaR: 34 (55) MiR: 10 (16)	NA	46 (74) MaR: 35 (56) MiR: 11 (18)	NA	4 weeks (4–12)	NA
Gabrilove *et al* (2008)	500 μg Q3W, increased to 500 μg Q2W at week 7 if no response; dose reduced to 300 μg if increase >100 g/l in any 2-week period; DA withheld for patients with Hb ≥130 g/l until decreased to ≤120 g/l	IWG 2000 (primary) IWG 2006§ (week 53/55 only)	'Test' period: week 13 Treatment period: 27/ 28 weeks Extended period: 52 weeks	NA	NA	NA	NA	NA	13 week: ESA-naïve: 102 (71) MaR: 71 (49) MiR: 31 (22) ESA-treated: 27 (44) MaR: 16 (26) MiR: 11 (18) 53/55 week: ESA-naïve: 107 (74) MaR: 85 (59) MiR: 22 (15) ESA-treated: 31 (50) MaR: 21 (34) MiR: 10(16)	ESA-naïve: 11 weeks (95% CI: 9–15) ESA-treated: 31 weeks (95% CI: 13–NE)	NA

Table II. (*Continued*)

Study	Treatment	Response criteria	Treatment period	Follow-up Median (range)	Response rate assessment, *N* (%)*					Time to response Median (range)	Duration of response
					Week 8	Week 12	Week 16	Week 24	Other		
Gotlib *et al* (2009)	Non-RARS: DA 4·5 μg/kg/week for 6 weeks; if did not achieve Hb >20 g/l rise, increased to 9·0 μg/kg/week for 6 weeks; if no >20 g/l Hb rise added 2·5 μg/kg G-CSF Q2W for 6 weeks RARS: initiated DA 9·0 μg/kg/week for 6 weeks; if no >20 g/l Hb rise then addition of 2·5 μg/kg G-CSF Q2W	IWG 2000 (primary), IWG 2006 (calculated)	Non-RARS: 18 weeks RARS:12 weeks All patients followed for 3 months (maintenance phase if MaR achieved)	NA	NA	IWG 2000: Non-RARS (N = 15): 4 (27) MaR: 4 (27); MiR: 0 (0)	NA	NA	18 week IWG 2000 (N = 24): 16 (67) Mar: 12 (50) MiR: 4 (17) IWG 2006 14 (58)	5 weeks (3–18)	MaR: 12 months (4–19) MiR: 5 months (2–7·5)
Oliva *et al* (2010)	150 μg QW; 300 μg QW for no response after 4 weeks in TF patients and 8 weeks in TD patients; dose escalated to 300 μg for patients without MiR at week 8 for TF or week 16 for TD. 150 μg Q3W if Hb >10 g/l during the first 2 weeks or Hb increased to >120 g/l	IWG 2000 (primary), IWG 2006 (evaluated)	24 weeks	NA	NA	NA	NA	29 (73) TF: 15 (65) TD: 14 (82) IWG 2006 23 (58) TF: 13 (56) TD: 10 (59)		8·0 weeks (95% CI: 7·6–8·4)	Mean: 21·9 weeks (95% CI: 19·7–24·0), median not reached

Table II. (*Continued*)

Study	Treatment	Response criteria	Treatment period	Follow-up Median (range)	Response rate assessment, *N* (%)*					Time to response Median (range)	Duration of response
					Week 8	Week 12	Week 16	Week 24	Other		
Nilsson-Ehle *et al* (2011)	300 μg QW, G-CSF added for patients with TD RARS, and nonresponders from week 9, DA dose interval increased to 14 days, further to 21 days if persistent high Hb, and then stopped until Hb <120 g/l. Pts not reaching Hb 120 g/l after week 16 also received erythrocyte transfusions for ≥8 weeks (study weeks 18–26) to maintain Hb >120 g/l	Non-IWG: Complete response (Hb ≥115 g/l and no transfusion requirement) Partial response (increase of ≥15 g/l or transfusion independence in TD patients.	26 weeks		–	–	20 (56) CR: 11 (31) PR: 9 (25)	–	–	NA	NA
Villegas *et al* (2011)	300 μg QW for 8 weeks; dosing extended to Q2W for patients with MaR, or G-CSF initiated for patients with MiR/no response. At week 16 patients without MaR withdrawn; patients with MaR on treatment at physician's discretion weeks 16–24.	IWG 2000	24 weeks	Median 28 weeks (95% CI: 20–NE)	31 (70) MaR: 21 (48) MiR: 10 (23) TF: 23 (72) MaR: 15 (47) MiR: 8 (25) TD: 8 (67) MaR: 6 (50) MiR: 2 (17)		31 (70) MaR: 27 (61) MiR: 4 (9) TF: 23 (72) MaR: 19 (59) MiR: 4 (13) TD: 8 (67) MaR: 8 (67) MiR: 0 (0)	32 (73) MaR: 27 (61) MiR: 5 (11) TF: 24 (75) MaR: 19 (59) MiR: 5 (16) TD: 8 (67) MaR: 8 (67) MiR: 0 (0)	NA	MaR: 9 weeks (95% CI: 4–16)	NA

Table II. (*Continued*)

Study	Treatment	Response criteria	Treatment period	Follow-up Median (range)	Response rate assessment, *N* (%)*					Time to response Median (range)	Duration of response
					Week 8	Week 12	Week 16	Week 24	Other		
Kelaidi *et al* (2013a)	500 μg Q2W for 12 weeks; G-CSF 300 μg twice weekly added if no response for additional 12 weeks; DA discontinued if no response after 24 weeks; DA doses adjusted to maintain Hb 110–120 g/l in responders	IWG 2006 (primary), IWG 2000 (calculated)	24 weeks	52 months	NA	IWG 2006: 46 (48) IWG 2000: 57 (60) MaR: 39 (41) MiR: 18 (19)	NA	IWG 2006: 53 (56) IWG 2000: 56 (59) MaR: 47 (49) MiR: 9 (9)	NA	5 weeks (1–20)	Median duration not reached (95% CI: 30 months–undefined)
Jang *et al* (2015)†	60, 120, 240 μg for 16 weeks, dose adjustment allowed to 48 weeks with QW, Q2W	Modified IWG 2000**	48 weeks; initial evaluation 16 weeks	316 days (1-600)	NA	NA	29 (58) MaR: 11 (22) MiR: 18 (36)		Some results reported for week 17–48 but timing of responses not specified	NA	NA

CI, confidence interval; CR, complete response; DA, darbepoetin alpha; G-CSF, granulocyte colony-stimulating factor; Hb, haemoglobin; MaR, major response; MiR, minor response; NA, not available; NE, not estimable; PR, partial response; QW, once weekly; Q2W, every 2 weeks; Q3W, every 3 weeks; RARS, refractory anaemia with ring sideroblasts; TD, transfusion-dependent; TF, transfusion-free.

*Response rate estimated based on the evaluable sample size.

†Abstract only.

‡Cheson *et al* (2000).

§Cheson *et al* (2006).

**Modified International Working Group 2000 criteria: MaR: RBC transfusion-free with an increase in Hb ≥1 g/l above baseline; MiR: ≥50% reduction in RBC transfusion compared to baseline.

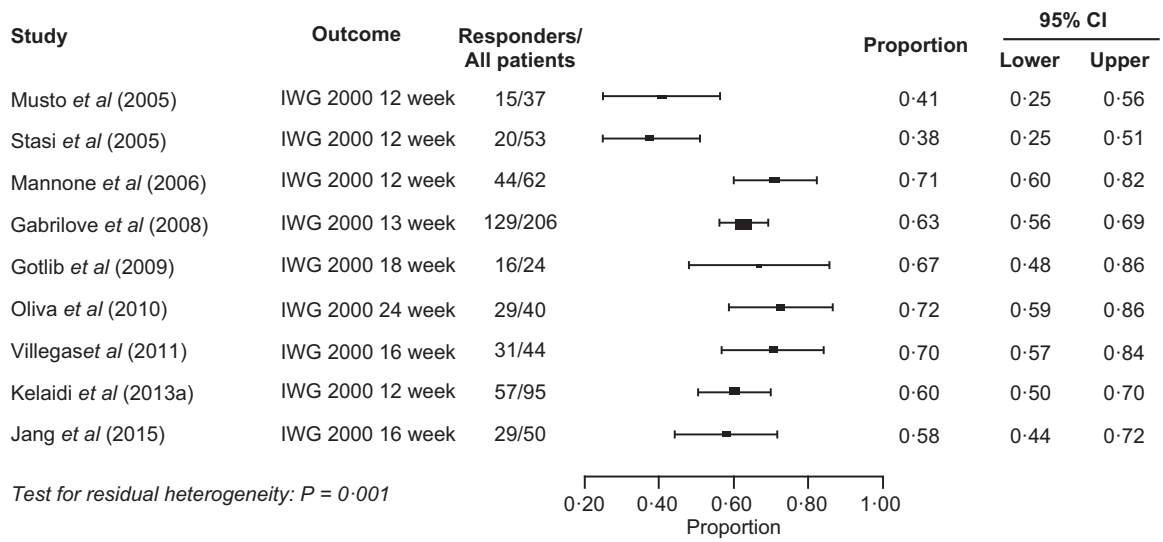

Fig 2. Erythroid response rate reported in studies using IWG 2000 criteria (Cheson *et al*, 2000). Study-level meta-analysis of the proportion of MDS patients treated with darbepoetin alpha who achieved an erythroid response is shown. Squares represent point values, diamonds represent combined estimate point value and confidence interval, and horizontal lines represent 95% confidence intervals. CI, confidence interval.

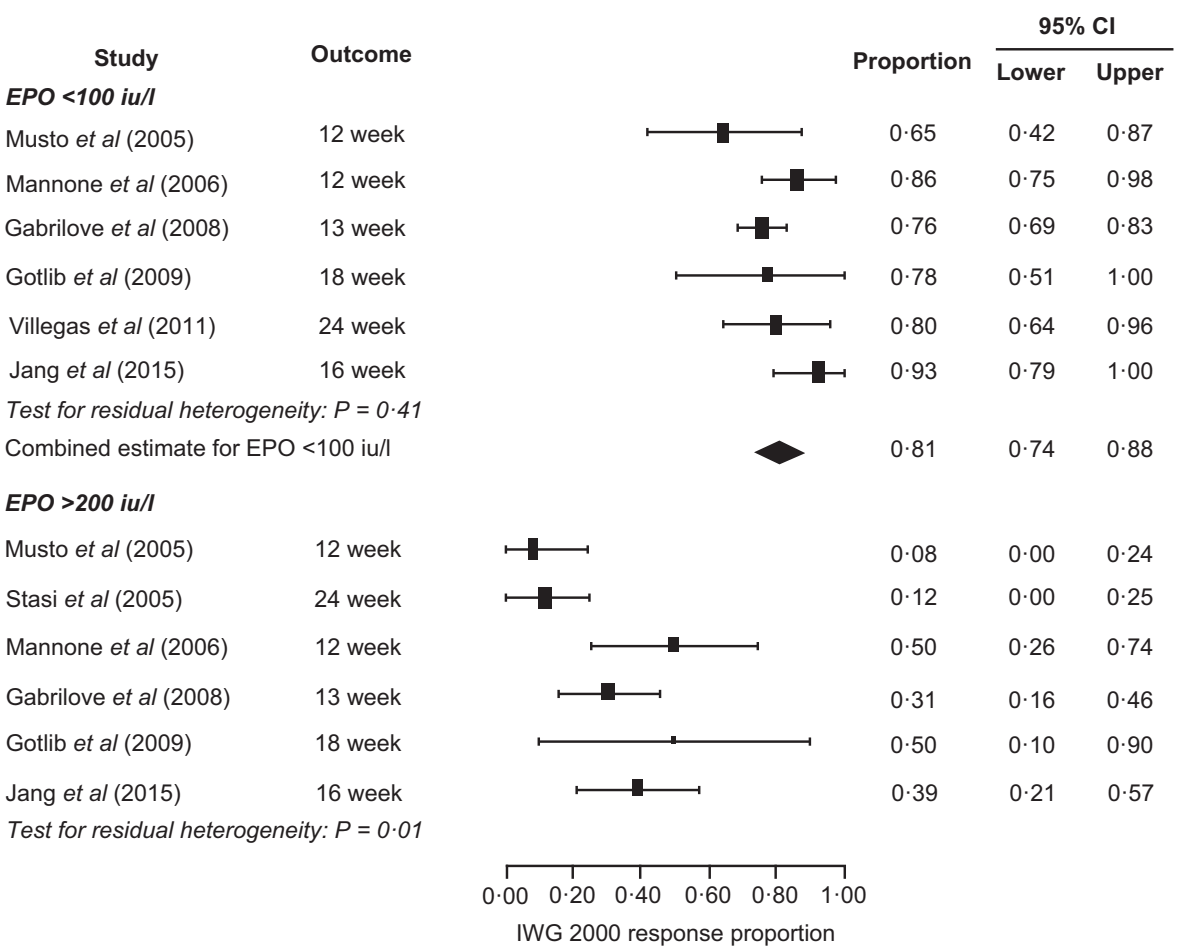

Fig 3. Erythroid response rate by baseline serum EPO level: EPO <100 iu/l compared to EPO >200 iu/l. Study-level meta-analysis of the proportion of MDS patients with erythroid response by serum EPO levels <100 iu/l and >200 iu/l at baseline is shown. Squares represent point values, diamonds represent combined estimate point value and confidence interval, and horizontal lines represent 95% confidence intervals. EPO, erythropoietin; CI, confidence interval.

2006; Gotlib *et al*, 2009; Oliva *et al*, 2010). At the study level, initial mean dose tended to be associated with response: the estimated response for a mean initial dose of 150 μg QW was 57% (95% CI: 49–66%) compared to 64% (95% CI: 55–74%) for 300 μg QW ($P = 0{\cdot}20$) (Fig 6).

The two studies that initiated G-CSF add-on therapy for non-RARS patients before response evaluation (Gotlib *et al*, 2009; Villegas *et al*, 2011) had response rates of 67% and 70% compared to the remaining seven studies, which had response rates ranging from 38% to 72%. Villegas *et al* (2011) added G-CSF for patients with no or minor response after 8 weeks of 300 μg of DA QW. Gotlib *et al* (2009) doubled the weight-based dose of DA from 4·5 μg/kg/week to 9·0 μg/kg/week at 6 weeks for patients who did not achieve an increase in their haemoglobin of greater than 20 g/l; at 12 weeks, G-CSF was added to the regimen for patients who did not achieve an increase in their haemoglobin of greater than 20 g/l.

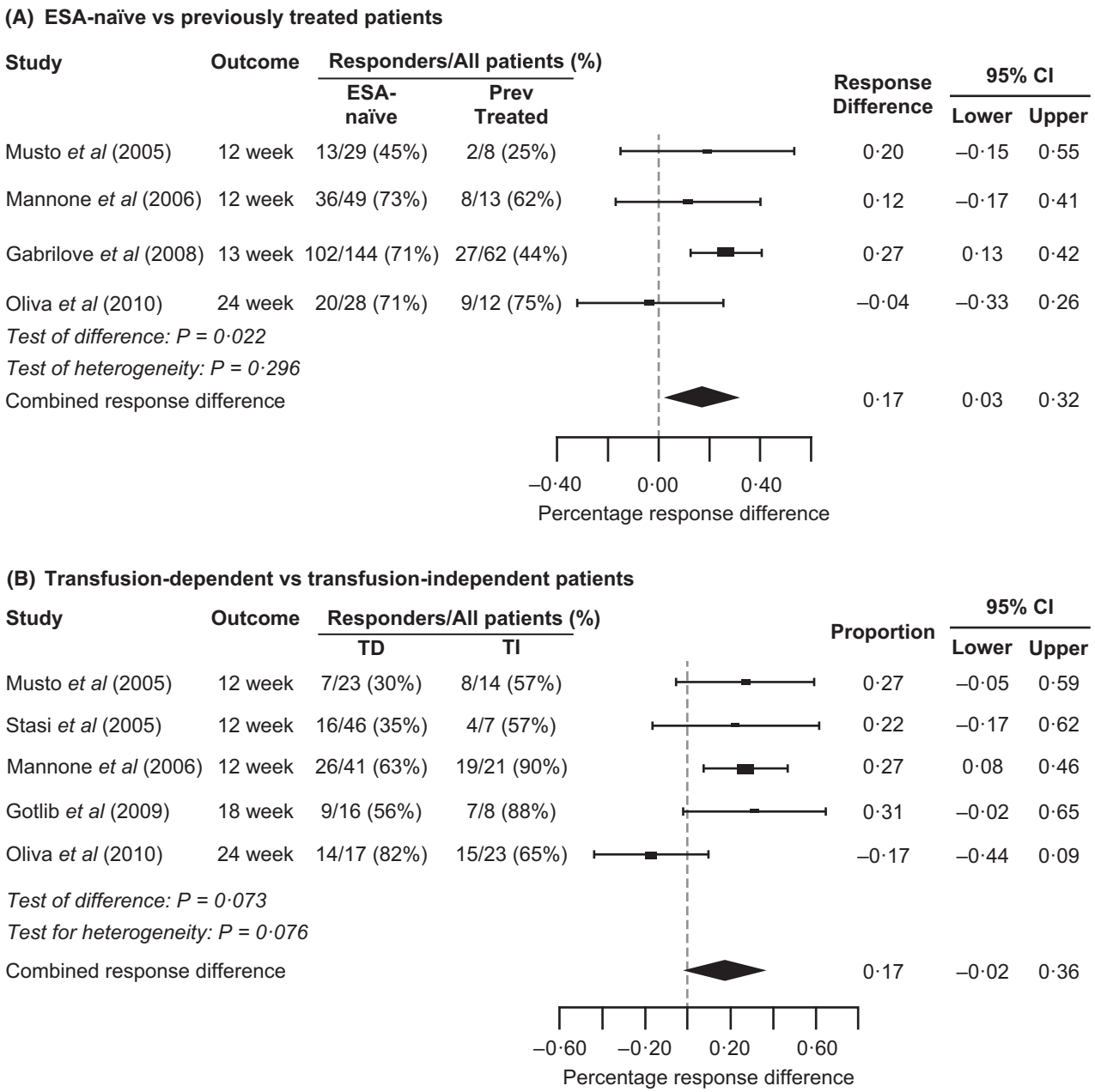

Fig 4. Erythroid response rates based on transfusion dependence/independence and previous ESA exposure. Study-level meta-analyses of the erythroid response rates in MDS patients who were (A) ESA-naïve vs previously treated patients and (B) transfusion-dependent and transfusion-independent are shown. Squares represent point values, diamonds represent combined estimate point value and confidence interval, and horizontal lines represent 95% confidence intervals. CI, confidence interval; TD, transfusion-dependent; TI, transfusion-independent.

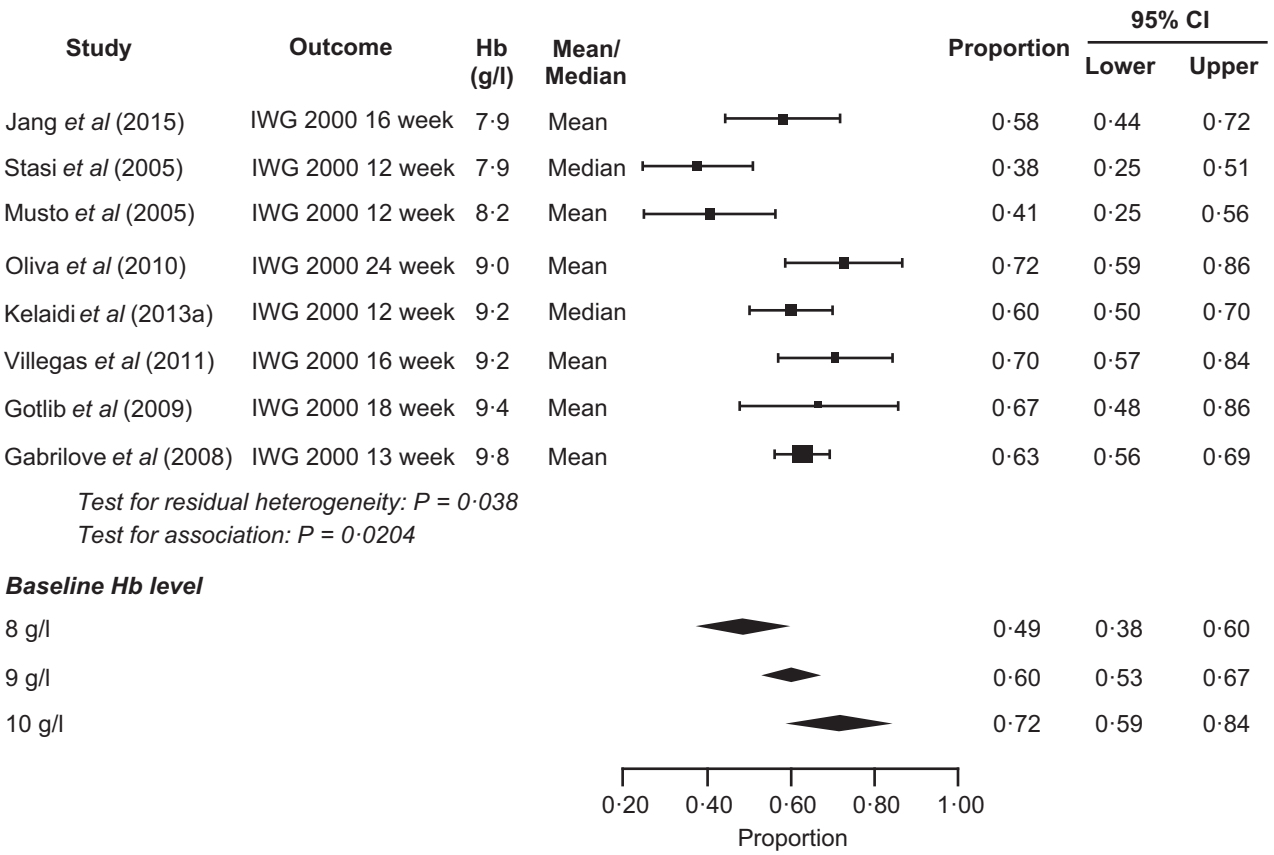

Fig 5. Erythroid response rate by baseline mean or median haemoglobin concentration. Study-level meta-analysis of the erythroid response rates in MDS patients by mean or median haemoglobin level is shown. Squares represent point values, diamonds represent combined estimate point value and confidence interval, and horizontal lines represent 95% confidence intervals. CI, confidence interval; Hb, haemoglobin.

Qualitative results

Predictors of response. Several studies identified significant potential predictors of response. These results are summarized in Table III. The most commonly identified predictor of response was low EPO level at baseline, which was reported in seven studies (Musto *et al*, 2005; Stasi *et al*, 2005; Mannone *et al*, 2006; Gotlib *et al*, 2009; Villegas *et al*, 2011; Kelaidi *et al*, 2013b; Jang *et al*, 2015). This result was confirmed in the current meta-analysis. Five studies identified transfusion independence as a predictor of response (Musto *et al*, 2005; Stasi *et al*, 2005; Mannone *et al*, 2006; Gotlib *et al*, 2009; Kelaidi *et al*, 2013a). In addition to baseline EPO and transfusion-dependent status at baseline, the most commonly identified predictors of response were: low IPSS score (Gabrilove *et al*, 2008; Gotlib *et al*, 2009; Kelaidi *et al*, 2013b); baseline haemoglobin (Oliva *et al*, 2010; Kelaidi *et al*, 2013b; Jang *et al*, 2015); no excess of blasts in bone marrow (Musto *et al*, 2005; Kelaidi *et al*, 2013b); iron status at baseline (Gabrilove *et al*, 2008; Jang *et al*, 2015); WHO classification (Kelaidi *et al*, 2013b); karyotype (Gotlib *et al*, 2009); hypoplastic bone marrow (Musto *et al*, 2005); and ESA-naïve at baseline (Gabrilove *et al*, 2008).

Transfusion independence. Table S1 summarizes transfusion independence and the risk of transfusion, with transfusion requirements generally decreasing after treatment with DA. The decrease in transfusion rate was quantified in several ways. Nilsson-Ehle *et al* (2011) reported that of 11 patients who were transfusion-dependent at baseline, 5 became transfusion-independent by the end of the study; and Jang *et al* (2015) reported that 14 previously transfusion-dependent patients became transfusion-independent during the study. Villegas *et al* (2011) reported the transfusion rate decreased from 27·3% at baseline to 0% at 24 weeks, and Gabrilove *et al* (2008) reported that their overall transfusion rate for the 52-week treatment period was 28% for ESA-naïve patients and 42% for patients previously treated with an ESA. In terms of numbers of units, Musto *et al* (2005) reported that the transfusion requirement decreased from 2·1 units per person per month at baseline to 1·6 units per person per month at 12 weeks. A further two studies reported a significant decrease in transfusion requirements from baseline (Stasi *et al*, 2005; Nilsson-Ehle *et al*, 2011).

Quality of life. QoL was measured using several metrics in six of the studies, with most studies reporting that responders

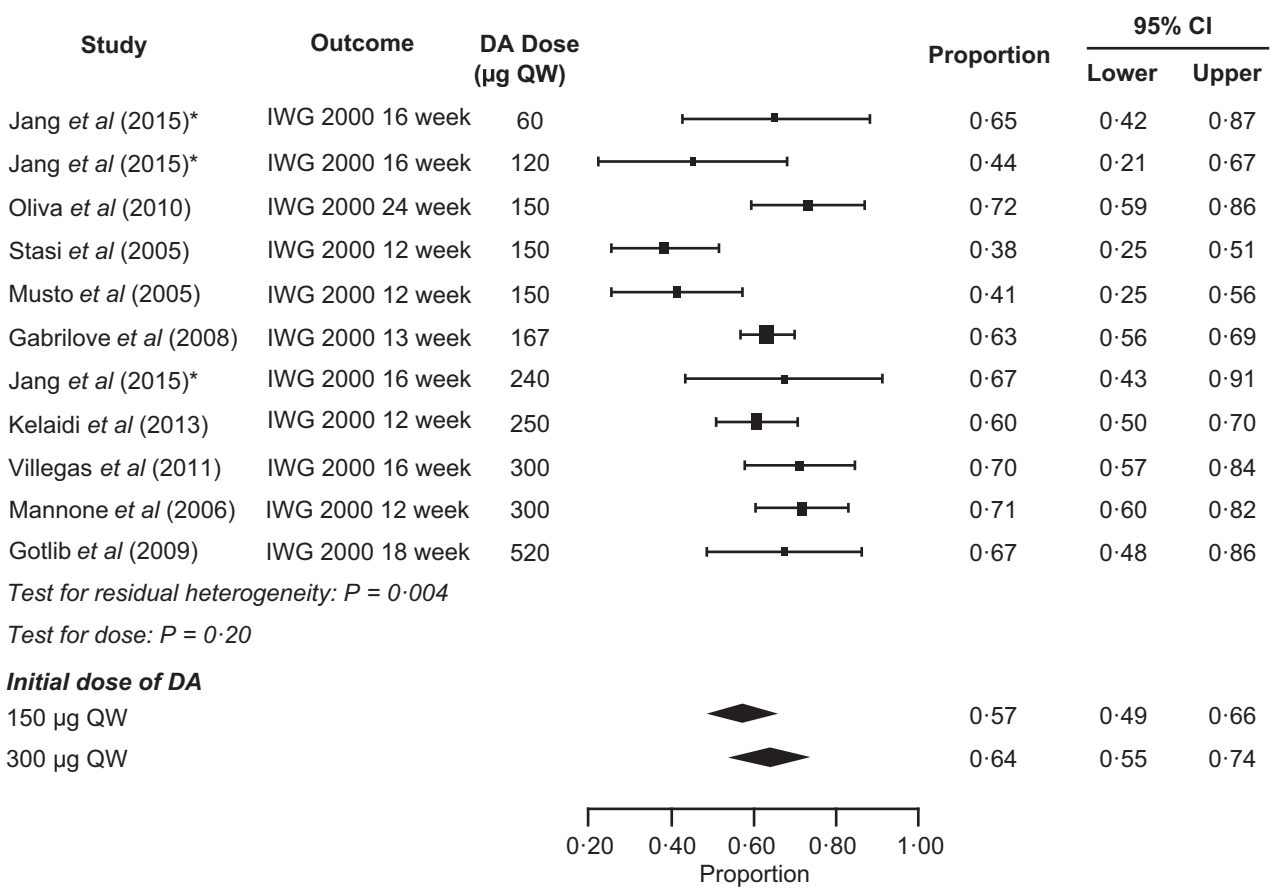

Fig 6. Erythroid response rate by mean initial dose of DA. Study-level meta-analysis of erythroid response rates in MDS patients by DA dose is shown. Squares represent point values, diamonds represent combined estimate point value and confidence interval, and horizontal lines represent 95% confidence intervals. *Jang 2015 study was a three-arm randomized controlled trial with three different dose levels. CI, confidence interval; DA, darbepoetin alpha; QW, once weekly; mcg, μg.

showed significant improvement in QoL (Table S2). For example, two studies reported that responders had a difference of between +12 and +17 points in the FACT-Anaemia (FACT-An) compared to non-responders (Stasi *et al*, 2005; Kelaidi *et al*, 2013a), and a difference of between +2·4 to +5·6 points between responders and non-responders for the FACT-Fatigue (FACT-F) has been reported (Gabrilove *et al*, 2008; Villegas *et al*, 2011). An increase in the FACT-F of ≥3 points has been reported to be clinically significant (Cella *et al*, 2002). Increased QoL was correlated with increased haemoglobin levels in four of the six studies that reported QoL (Stasi *et al*, 2005; Gabrilove *et al*, 2008; Oliva *et al*, 2010; Villegas *et al*, 2011).

Safety. Reported AEs across the ten studies were collated and are summarized in Table IV. A total of 127 AEs were reported across the 647 patients in the ten studies, including 37 deaths (5·7%). Progression to AML was reported in eight cases (1·2%) and disease progression was reported in three cases (0·5%). The most common AEs were neoplasm (3·2%), hypertension (3·2%) and stroke (1·4%).

Discussion

This up-to-date analysis comprehensively examined the published literature evaluating the efficacy and safety of DA for treating anaemia in patients with MDS. This systematic review found that patients treated with DA exhibited high haemoglobin response (38–72%) and a median duration of response ranging from 12 to at least 51 months. This compares favourably to ESAs; the most recent meta-analysis on ESA treatment in patients with MDS reported an overall response rate of 57·6% in patients receiving ESA monotherapy (Moyo *et al*, 2008), and in another large study, median response to ESA therapy was approximately 2 years (Park *et al*, 2008).

It is important to identify patients for whom ESA treatment may be most efficacious. The meta-analysis of studies that reported results stratified by baseline EPO levels indicated that patients with lower EPO levels (<100 iu/l) at baseline had an overall response of 81%, and on average 38% better response than patients with higher baseline EPO levels (>100 iu/l). However, EPO level is a continuous variable, with lower baseline

Table III. Findings on predictors of erythroid response in published studies.

	Studies that reported statistical positive findings*	
Potential predictor	Univariate	Multivariate
WHO classification	Kelaidi *et al* (2013a)†	
IPSS	Gabrilove *et al* (2008); Kelaidi *et al* (2013b); Gotlib *et al* (2009)	
Karyotype (cytogenetic risk)	Gotlib *et al* (2009)	
Hypoplastic bone marrow		Musto *et al* (2005)
ESA-naïve vs ESA-prior treated	Gabrilove *et al* (2008)	
Iron status/baseline ferritin	Kelaidi *et al* (2013a); Jang *et al* (2015)	
Baseline Hb	Kelaidi *et al* (2013a)†; Oliva *et al* (2010); Jang *et al* (2015)	
Baseline serum EPO	Musto *et al* (2005); Stasi *et al* (2005); Mannone *et al* (2006); Gabrilove *et al* (2008); Villegas *et al* (2011); Kelaidi *et al* (2013b)†; Jang *et al* (2015)	Musto *et al* (2005); Stasi *et al* (2005); Kelaidi *et al* (2013b)†
No excess of blast in bone marrow	Kelaidi *et al* (2013a); Musto *et al* (2005)	Musto *et al* (2005)
Transfusion independence	Musto *et al* (2005); Stasi *et al* (2005); Mannone *et al* (2006); Gotlib *et al* (2009); Kelaidi *et al* (2013a)†	Musto *et al* (2005)

EPO, erythropoetin; ESA, erythropoiesis-stimulating agent; Hb, haemoglobin; IPSS, International Prognostic Scoring System; WHO, World Health Organization.

*P-value <0·05 considered significant.

†Based on International Working Group 2006 definition of response (Cheson *et al*, 2006).

EPO levels tending to be correlated with higher erythroid response rates. We also identified prior ESA treatment as a significant risk factor for poor response: in studies that reported results for ESA-naïve and previously treated patients separately, ESA-naïve patients had a 17% better response rate than previously treated patients. Although the response rate for ESA-naïve patients was greater than for previously treated patients, 25% to 75% of patients who had been previously treated with an ESA did respond to the long-acting DA in the four studies that reported results by ESA exposure. Where possible, we reported results separately for ESA-naïve patients and those who had been previously treated with ESAs.

We also observed a positive association between baseline haemoglobin and response rate: higher baseline levels were correlated with improved responses. It is likely that higher endogenous EPO level (e.g., patients with refractory anaemia), or lower haemoglobin level may indicate relative non-responsiveness of bone marrow. This may also suggest that early initiation of ESA treatment, before haemoglobin decreases to very low levels, may increase the likelihood of response. However, this result should be interpreted with caution as the study-level mean or median baseline haemoglobin concentration may mask important information at the patient level.

Other non-significant results were suggestive. Higher dosing regimens of DA tended to correlate with improved erythroid response, but further studies are needed. Another trend we observed was between transfusion dependence at baseline and response: in the studies that reported results stratified by transfusion dependence, transfusion-independent patients tended to have 17% higher response rate on average.

In the qualitative review, several studies reported a significant association between IPSS and G-CSF and response. However, most of the significant predictors of response were identified based on unadjusted estimates (univariate) rather than adjusted for other factors (multivariate). For IPSS, the vast majority of patients in the studies were classified as either low-risk or intermediate-1 risk, and this would make detecting a trend between IPSS risk and response difficult. Indeed, in general, the results from the meta-regression analysis should be interpreted cautiously because of lack of individual data. More well-designed prospective studies are needed to investigate simultaneously the effects of these factors and their interactions on response.

Five studies that examined the transfusion requirement (risk) after treatment consistently suggested that the transfusion requirements were significantly decreased after DA treatment compared to baseline. In addition, in the six studies reporting QoL using similar validated instruments, it was suggested that QoL significantly improved during treatment and was correlated with improved haemoglobin levels. However, the possibility of a confounding factor responsible for both QoL improvement and response cannot be ruled out.

As for the safety of DA, selected AEs were generally reported in less than 5% of treated patients in the published studies, mostly in single-arm trials. As a result, it is not possible to determine whether the AEs observed in these single-arm trials occurred at a rate that differs from the background rate. Results from an on-going RCT should provide more insights on the efficacy and safety of DA for treating anaemia among patients with MDS.

Several limitations should be acknowledged. First, due to the amount of variation, the response rates could not be combined into a single meta-analytic estimate. The variation in response rates may be due to the heterogeneous nature of the study populations and various treatment regimens. For

Table IV. Safety of DA in patients with MDS.

AE reporting	*N* (*n* = 647 patients) *n* (%)	N of studies	Comment
Total number of reported AEs	127		Only includes 'AE of interest' from Gabrilove *et al* (2008)
Total all-cause death	37/647 (5·7)		
Cardiovascular/Pulmonary/Cerebrovascular			
Hypertension	21 (3·2)	2 (Mannone *et al*, 2006; Gotlib *et al*, 2009); 6 (Gabrilove *et al*, 2008; Kelaidi *et al*, 2013a); 6 (Jang *et al*, 2015)	1 case related to treatment (Gabrilove *et al*, 2008)
Thromboembolism	7 (1·1)	3 (Gabrilove *et al*, 2008; Nilsson-Ehle *et al*, 2011; Villegas *et al*, 2011)	2 cases not related to treatment (Gabrilove *et al*, 2008)
Pulmonary embolism	2 (0·3)	2 (Nilsson-Ehle *et al*, 2011; Kelaidi *et al*, 2013a)	1 fatal, one nonfatal
Stroke	9 (1·4)	2 (Gabrilove *et al*, 2008; Kelaidi *et al*, 2013a)	1 fatal (Kelaidi *et al* (2013a); 7 cases unknown if related to treatment (Gabrilove *et al*, 2008)
Haematological			
Thrombocytopenia	5 (0·8)	1 (Gotlib *et al*, 2009)	4 cases unknown if related to treatment
Iron deficiency	2 (0·3)	1 (Villegas *et al*, 2011)	
Progressed disease	3 (0·5)	2 (Gotlib *et al*, 2009; Nilsson-Ehle *et al*, 2011); 1 (Kelaidi *et al*, 2013a)	1 case progression not reported
Hyperleucocytosis	1 (0·2)	1	
Progression to AML	8 (1·2)	6 (Gabrilove *et al*, 2008) 2 (Jang *et al*, 2015); 2 (Kelaidi *et al*, 2013a); 1 (Mannone *et al*, 2006); 4 (Gotlib *et al*, 2009)	6 cases unknown if related to treatment (Gabrilove *et al*, 2008)
Renal dysfunction			
Renal failure	1 (0·2)	1 (Mannone *et al*, 2006)	
Creatinine elevation	1 (0·2)	1 (Gotlib *et al*, 2009)	
Proteinuria	1 (0·2)	1 (Gotlib *et al*, 2009)	
Other			
Neoplasm	21 (3·2)	1 (Gabrilove *et al*, 2008)	1 case unknown if related to treatment
Bone pain	3 (0·5)	1 (Gotlib *et al*, 2009)	
Coma	1 (0·2)	1 (Kelaidi *et al*, 2013a)	1 case unknown if related to treatment
Filgrastim-related arthralgia/myalgia	4 (0·6)	2 (Nilsson-Ehle *et al*, 2011; Kelaidi *et al*, 2013a)	
Flu	1 (0·2)	1 (Villegas *et al*, 2011)	
Headache	1 (0·2)	1 (Villegas *et al*, 2011)	
Immune system disorder	1 (0·1)	1 (Gabrilove *et al*, 2008)	1 case unknown if related to treatment
Injection site reaction	5 (0·8)	2 (Stasi *et al*, 2005; Kelaidi *et al*, 2013a)	
Rash	3 (0·5)	1 (Kelaidi *et al*, 2013a)	
Seizure	1 (0·2)	1 (Gabrilove *et al*, 2008)	1 case unknown if related to treatment

AE, adverse event; AML, acute myeloid leukaemia; DA, darbepoetin alpha.

example, studies reported differences in DA initiation and maintenance doses, prior ESA treatment, and rates of transfusion dependence, among other factors. Second, the total number of DA studies in MDS is relatively small, and studies are mostly single-arm. However, it is known that, without intervention, patients are likely to deteriorate. Furthermore, a review of trials that compared ESA treatment to non-growth factor therapy found that patients with ESA treatment do significantly better with respect to overall survival and progression-free survival (Golshayan *et al*, 2007). Third, although meta-analysis has the ability to improve the power of small or inconclusive studies, it cannot improve the quality of design and reporting of the original studies. For instance, data regarding duration of follow-up and duration of response were limited. Fourth, the lack of primary source data from the original studies was a limitation as ecological bias may influence results. Thus, the results from our analyses should be interpreted with caution. Lastly, each study reported a different set of AEs and the reporting varied; a conclusive safety profile from the studies cannot be drawn. Thus, the safety data in this review serve to provide insight on the range of AEs reported in the literature, rather than to

establish the safety evidence for use of ESAs in patients with MDS.

In summary, the present review suggests that treating low- to intermediate-risk MDS patients with DA provides a clinical benefit for an otherwise debilitating chronic anaemia. Further studies are required to better identify predictors of the erythroid response, and therefore to better identify patients for whom ESA treatment may have a better opportunity of success. More RCTs are required to understand the long-term benefit and safety of DA in patients with MDS with an appropriate risk profile.

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Author contributions

S. Park contributed to the conception and design of the study, analysis and interpretation of the data, and drafting of the manuscript. P. Fenaux contributed to the conception and design of the study, acquisition of data, and analysis and interpretation of the data. P. Greenberg contributed to the conception and design of the study and the analysis and interpretation of the data. B. Mehta contributed to the conception and design of the study and analysis and interpretation of the data. F. Callaghan contributed to the conception and design of the study, acquisition of data, and analysis and interpretation of the data. C. Kim contributed to the acquisition of data and analysis and interpretation of the data. D. Tomita contributed to the analysis and interpretation of the data. H. Xu contributed to the conception and design of the study, acquisition of data, and analysis and interpretation of the data. All authors contributed to the drafting of the manuscript, critical revisions of the manuscript for important intellectual content, and have approved the draft for publication.

Conflict-of-interest disclosures

S. Park has received grant/research support from Novartis International AG and Hospira, Inc., and has been a consultant to Hospira, Inc. and Celgene Corp. P. Fenaux and P. Greenberg have no conflicts to declare. B. Mehta, F. Callaghan, C. Kim, D. Tomita, and H. Xu are employees and shareholders of Amgen Inc.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Data S1. MeSH (Medical Subject Headings) terms and key words used in the systematic literature search.

Fig S1. Erythroid response rate difference for baseline EPO level: EPO <100 iu/L compared to EPO >100 IU/L. Study-level meta-analysis of the difference between erythroid response rates in MDS patients with baseline serum EPO levels <100 IU/L vs. >200 iu/L is shown. EPO, erythropoietin; CI, confidence interval.

Fig S2. Erythroid response rate by baseline hemoglobin levels. The proportion of patients achieving erythroid response by baseline hemoglobin level is shown for each study. Hb, hemoglobin; QW, once weekly.

Fig S3. Funnel plot for all studies. The proportion of response is compared against within-study variation for each study; the symmetrical shape of the funnel suggested no evidence of publication bias. [a]Study with non-IWG 2000 criteria assessment.

Table S1. Transfusion independence and risk of transfusion in anemic MDS patients receiving DA in published studies

Table S2. Change in quality of life among patients with MDS receiving DA in published studies

References

Cella, D., Lai, J.S., Chang, C.H., Peterman, A. & Slavin, M. (2002) Fatigue in cancer patients compared with fatigue in the general United States population. *Cancer*, **94**, 528–538.

Cheson, B.D., Bennett, J.M., Kantarjian, H., Pinto, A., Schiffer, C.A., Nimer, S.D., Löwenberg, B., Beran, M., deWitte, T.M., Stone, R.M., Mittelman, M., Sanz, G.F., Wijermans, P.W., Gore, S. & Greenberg, P.L.; World Health Organization (WHO) international working group. (2000) Report of an international working group to standardize response criteria for myelodysplastic syndromes. *Blood*, **96**, 3671–3674.

Cheson, B.D., Greenberg, P.L., Bennett, J.M., Lowenberg, B., Wijermans, P.W., Nimer, S.D., Pinto, A., Beran, M., de Witte, T.M., Stone, R.M., Mittelman, M., Sanz, G.F., Gore, S.D., Schiffer, C.A. & Kantarjian, H. (2006) Clinical application and proposal for modification of the International Working Group (IWG) response criteria in myelodysplasia. *Blood*, **108**, 419–425.

Cogle, C.R. (2015) Incidence and burden of the Myelodysplastic syndromes. *Currrent Hematologic Malignancy Reports*, **10**, 272–281.

Gabrilove, J., Paquette, R., Lyons, R.M., Mushtaq, C., Sekeres, M.A., Tomita, D. & Dreiling, L. (2008) Phase 2, single-arm trial to evaluate the effectiveness of darbepoetin alfa for correcting anaemia in patients with myelodysplastic syndromes. *British Journal of Haematology*, **142**, 379–393.

Garcia-Manero, G. (2014) Myelodysplastic syndromes: 2014 update on diagnosis, risk-stratification, and management. *American Journal of Hematology*, **89**, 97–108.

Golshayan, A.R., Jin, T., Maciejewski, J., Fu, A.Z., Bershadsky, B., Kattan, M.W., Kalaycio, M.E. & Sekeres, M.A. (2007) Efficacy of growth factors compared to other therapies for low-risk myelodysplastic syndromes. *British Journal of Haematology*, **137**, 125–132.

Gotlib, J., Lavori, P., Quesada, S., Stein, R.S., Shahnia, S. & Greenberg, P.L. (2009) A Phase II intra-patient dose-escalation trial of weight-based darbepoetin alfa with or without granulocyte-colony stimulating factor in myelodysplastic syndromes. *American Journal of Hematology*, **84**, 15–20.

Greenberg, P.L., Sun, Z., Miller, K.B., Bennett, J.M., Tallman, M.S., Dewald, G., Paietta, E., van der Jagt, R., Houston, J., Thomas, M.L., Cella, D. & Rowe, J.M. (2009) Treatment of myelodysplastic syndrome patients with erythropoietin with or without granulocyte colony-stimulating factor: results of a prospective randomized phase 3 trial by the Eastern Cooperative Oncology Group (E1996). *Blood*, **114**, 2393–2400.

Greenberg, P.L., Attar, E., Bennett, J.M., Bloomfield, C.D., Borate, U., De Castro, C.M., Deeg, H.J., Frankfurt, O., Gaensler, K., Garcia-Manero, G., Gore, S.D., Head, D., Komrokji, R., Maness, L.J., Millenson, M., O'Donnell, M.R., Shami, P.J., Stein, B.L., Stone, R.M., Thompson, J.E., Westervelt, P., Wheeler, B., Shead, D.A. & Naganuma, M. (2013) Myelodysplastic syndromes: clinical practice guidelines in oncology. *Journal of the National Comprehensive Cancer Network*, **11**, 838–874.

Hedges, L.V. & Olkin, L. (1985) Statistical Methods for Meta-Analysis, 1st edn. Academic Press, Waltham, MA.

Higgins, J.P. & Thompson, S.G. (2002) Quantifying heterogeneity in a meta-analysis. *Statistics in Medicine*, **21**, 1539–1558.

Jadersten, M., Malcovati, L., Dybedal, I., Della Porta, M.G., Invernizzi, R., Montgomery, S.M., Pascutto, C., Porwit, A., Cazzola, M. & Hellstrom-Lindberg, E. (2008) Erythropoietin and granulocyte-colony stimulating factor treatment associated with improved survival in myelodysplastic syndrome. *Journal of Clinical Oncology*, **26**, 3607–3613.

Jang, J.H., Harada, H., Shibayama, H., Shimazaki, R., Kim, H.J., Sawada, K. & Mitani, K. (2015) A randomized controlled trial comparing darbepoetin alfa doses in red blood cell transfusion-dependent patients with low- or intermediate-1 risk myelodysplastic syndromes. *International Journal of Hematology*, **102**, 401–412.

Kelaidi, C. & Fenaux, P. (2010) Darbepoetin alfa in anemia of myelodysplastic syndromes: present and beyond. *Expert Opinion on Biological Therapy*, **10**, 605–614.

Kelaidi, C., Beyne-Rauzy, O., Braun, T., Sapena, R., Cougoul, P., Ades, L., Pillard, F., Lamberto, C., Charniot, J.C., Guerci, A., Choufi, B., Stamatoullas, A., Slama, B., De Renzis, B., Ame, S., Damaj, G., Boyer, F., Chaury, M.P., Legros, L., Cheze, S., Testu, A., Gyan, E., Bene, M.C., Rose, C., Dreyfus, F. & Fenaux, P. (2013a) High response rate and improved exercise capacity and quality of life with a new regimen of darbepoetin alfa with or without filgrastim in lower-risk myelodysplastic syndromes: a phase II study by the GFM. *Annals of Hematology*, **92**, 621–631.

Kelaidi, C., Park, S., Sapena, R., Beyne-Rauzy, O., Coiteux, V., Vey, N., Stamatoullas, A., Choufi, B., Delaunay, J., Gourin, M.P., Cheze, S., Ravoet, C., Ferrant, A., Escoffre-Barbe, M., Aljassem, L., Raffoux, E., Itzykson, R., Ades, L., Dreyfus, F. & Fenaux, P. (2013b) Long-term outcome of anemic lower-risk myelodysplastic syndromes without 5q deletion refractory to or relapsing after erythropoiesis-stimulating agents. *Leukemia*, **27**, 1283–1290.

Mannone, L., Gardin, C., Quarre, M. C., Bernard, J. F., Vassilieff, D., Ades, L., Park, S., Vaultier, S., Hamza, F., Beyne-rauzy, M. O., Cheze, S., Giraudier, S., Agape, P., Legros, L., Voillat, L., Dreyfus, F. & Fenaux, P., Groupe Francais des Myelodysplasies. (2006) High-dose darbepoetin alpha in the treatment of anaemia of lower risk myelodysplastic syndrome results of a phase II study. *British Journal of Haematology*, **133**, 513–519.

Moyo, V., Lefebvre, P., Duh, M.S., Yektashenas, B. & Mundle, S. (2008) Erythropoiesis-stimulating agents in the treatment of anemia in myelodysplastic syndromes: a meta-analysis. *Annals of Hematology*, **87**, 527–536.

Musto, P., Lanza, F., Balleari, E., Grossi, A., Falcone, A., Sanpaolo, G., Bodenizza, C., Scalzulli, P.R., La Sala, A., Campioni, D., Ghio, R., Cascavilla, N. & Carella, A.M. (2005) Darbepoetin alpha for the treatment of anaemia in low-intermediate risk myelodysplastic syndromes. *British Journal of Haematology*, **128**, 204–209.

Newcombe, R.G. (1998) Two-sided confidence intervals for the single proportion: comparison of seven methods. *Statistics in Medicine*, **17**, 857–872.

Nilsson-Ehle, H., Birgegard, G., Samuelsson, J., Antunovic, P., Astermark, J., Garelius, H., Engstrom, L.M., Kjeldsen, L., Nilsson, L., Olsson, A., Skov-Holm, M., Wallvik, J., Gulbrandsen, N. & Hellstrom-Lindberg, E. (2011) Quality of life, physical function and MRI T2* in elderly low-risk MDS patients treated to a haemoglobin level of >/=120 g/L with darbepoetin alfa +/- filgrastim or erythrocyte transfusions. *European Journal of Haematology*, **87**, 244–252.

Oliva, E.N., Nobile, F., Alimena, G., Specchia, G., Danova, M., Rovati, B., Ronco, F., Impera, S., Risitano, A., Alati, C., Breccia, M., Carmosino, I., Vincelli, I. & Latagliata, R. (2010) Darbepoetin alfa for the treatment of anemia associated with myelodysplastic syndromes: efficacy and quality of life. *Leukemia and Lymphoma*, **51**, 1007–1014.

Park, S., Grabar, S., Kelaidi, C., Beyne-Rauzy, O., Picard, F., Bardet, V., Coiteux, V., Leroux, G., Lepelley, P., Daniel, M. T., Cheze, S., Mahe, B., Ferrant, A., Ravoet, C., Escoffre-Barbe, M., Ades, L., Vey, N., Aljassem, L., Stamatoullas, A., Mannone, L., Dombret, H., Bourgeois, K., Greenberg, P., Fenaux, P. & Dreyfus, F., for the GFM group (Groupe Francophone des Myélodysplasies). (2008) Predictive factors of response and survival in myelodysplastic syndrome treated with erythropoietin and G-CSF: the GFM experience. *Blood*, **111**, 574–582.

Park, S., Kelaidi, C., Sapena, R., Vassilieff, D., Beyne-Rauzy, O., Coiteux, V., Vey, N., Ravoet, C., Cheze, S., Rose, C., Legros, L., Stamatoullas, A., Escoffre-Barbe, M., Guerci, A., Chaury, M.P., Fenaux, P. & Dreyfus, F. (2010) Early introduction of ESA in low risk MDS patients may delay the need for RBC transfusion: a retrospective analysis on 112 patients. *Leukemia Research*, **34**, 1430–1436.

Raudenbush, S. W. (2009) Analyzing effect sizes: random effects models. In: The Handbook of Research Synthesis and Meta-Analysis (ed. by H. Cooper, L. V. Hedges & J. C. Valentine), pp. 295–315. 2nd edn. Russel Sage Foundation, New York.

Ross, S.D., Allen, I.E., Probst, C.A., Sercus, B., Crean, S.M. & Ranganathan, G. (2007) Efficacy and safety of erythropoiesis-stimulating proteins in myelodysplastic syndrome: a systematic review and meta-analysis. *Oncologist*, **12**, 1264–1273.

Sekeres, M.A., Schoonen, W.M., Kantarjian, H., List, A., Fryzek, J., Paquette, R. & Maciejewski, J.P. (2008) Characteristics of US patients with myelodysplastic syndromes: results of six cross-sectional physician surveys. *Journal of the National Cancer Institute*, **100**, 1542–1551.

Spiriti, M.A., Latagliata, R., Niscola, P., Cortelezzi, A., Francesconi, M., Ferrari, D., Volpe, E., Clavio, M., Grossi, A., Reyes, M.T., Musto, P., Mitra, M.E., Azzara, A., Pagnini, D., D'Arena, G., Spadano, A., Balleari, E., Pecorari, P., Capochiani, E., De Biasi, E., Perego, D., Monarca, B., Pisani, F., Scaramella, G. & Petti, M.C. (2005) Impact of a new dosing regimen of epoetin alfa on quality of life and anemia in patients with low-risk myelodysplastic syndrome. *Annals of Hematology*, **84**, 167–176.

Stasi, R., Abruzzese, E., Lanzetta, G., Terzoli, E. & Amadori, S. (2005) Darbepoetin alfa for the treatment of anemic patients with low- and intermediate-1-risk myelodysplastic syndromes. *Annals of Oncology*, **16**, 1921–1927.

Steensma, D.P. & Bennett, J.M. (2006) The myelodysplastic syndromes: diagnosis and treatment. *Mayo Clinic Proceedings*, **81**, 104–130.

Venables, W. N. & Smith, D. M., R Core Team. (2015) An introduction to R. Notes on R: A Programming Environment for Data Analysis and Graphics, Version 3.2.2. Available at: https://cran.r-project.org/doc/manuals/r-release/R-intro.pdf. Accessed 4 November 2015.

Viechtbauer, W. (2010) Conducting meta-analyses in R with the metafor package. *Journal of Statistical Software*, **36**, 1–48.

Villegas, A., Arrizabalaga, B., Fernandez-Lago, C., Castro, M., Mayans, J.R., Gonzalez-Porras, J.R., Duarte, R.F., Remacha, A.F., Luno, E. & Gasquet, J.A. (2011) Darbepoetin alfa for anemia in patients with low or intermediate-1 risk myelodysplastic syndromes and positive predictive factors of response. *Current Medical Research and Opinion*, **27**, 951–960.