



**Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review**

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Manuscripts

1 Subject: Transplantation for SAA

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4 1 Stem cell transplantation of matched sibling donors compared with immunosuppressive ther-  
5 2 apy for acquired severe aplastic anemia – a Cochrane Systematic Review\*

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24 14 Cochrane Systematic Reviews are regularly updated as new evidence emerges and in response  
25 15 to feedback, and the CDSR should be consulted for the most recent version of the review.

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39 21 **Keywords**

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42 22 severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosup-  
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1 Subject: Transplantation for SAA  
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4 **27 Abstract**  
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8 Objectives: Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of  
9 this Cochrane review was to evaluate the effectiveness and adverse events of first-line  
10 allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors compared  
11 to first-line immunosuppressive therapy.  
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17 Setting: Specialised stem cell transplantations units in primary care hospitals  
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20 Participants: We included 302 participants with newly diagnosed acquired severe aplastic  
21 anemia. The age ranged from early childhood to young adulthood. We excluded studies on  
22 participants with secondary aplastic anemia.  
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28 Interventions: We included allogeneic haematopoietic stem cell transplantation as the test  
29 intervention harvested from any source of matched sibling donor and serving as a first-line  
30 therapy. We included immunosuppressive therapy as comparator with either antithymocyte/-  
31 antilymphocyte globulin or ciclosporin or a combination of the two.  
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38 Primary and secondary outcome measures planned and finally measured: The primary  
39 outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft  
40 failure, graft-versus-host disease, no response to immunosuppressive therapy, relapse after  
41 initial successful treatment, secondary clonal disease or malignancies, health-related quality  
42 of life, and performance scores.  
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49 Results: We identified three prospective non-randomized controlled trials with a study design  
50 that was consistent with the principle of 'Mendelian randomization' in allocating patients to  
51 treatment groups. All studies had a high risk of bias due to the study design and were  
52 conducted more than 15 years. The pooled hazard ratio for overall mortality for the  
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3 49 transplanted group versus the not transplanted group was 0.95 (95% confidence interval 0.43  
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5 50 to 2.12, P = 0.90).  
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8 51 Conclusions: There are insufficient and biased data that do not allow any firm conclusions to  
9  
10 52 be made about the comparative effectiveness of first-line allogeneic hematopoietic stem cell  
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12 53 transplantation of HLA-matched sibling donors and first-line immunosuppressive therapy of  
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14 54 patients with acquired severe aplastic anemia.  
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### 21 56 **Strengths and limitations of this study**

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24 57 • We conducted a comprehensive literature search and strictly adhered to the projected  
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26 58 methodology.
- 27  
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29 59 • We restricted the study design to randomized controlled trials and prospective non-  
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31 60 randomized controlled trials and the studies had to be compatible with 'Mendelian  
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33 61 Randomization' to avoid excess risk of bias.
- 34  
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36 62 • The included data are too scarce and too biased to allow any conclusion on the com-  
37  
38 63 parative effectiveness of MSD-HSCT and IST.
- 39  
40 64 • The included data were collected 15 to more than 30 years ago. Thus, the results may  
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42 65 not be applicable to current modern standard care.  
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## 67 **Introduction**

68 Acquired severe aplastic anemia (SAA) is a rare [1] and potentially fatal disease which is  
69 characterized by hypocellular bone marrow and pancytopenia; it mainly affects young adults.

70 The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2].

71 The underlying pathophysiology is thought to be an aberrant immune response involving the

72 T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are

73 severe infections, bleeding, and exhaustion and patients may experience paleness, weakness,

74 fatigue, and shortness of breath.

75 According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of

76 the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic

77 stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen

78 (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly

79 diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the

80 conditioning regimen may lead to severe non-hematological organ toxicities. First-line

81 ciclosporin and/or antithymocyte or antilymphocyte globulin denoted as first-line

82 immunosuppressive therapy (IST) is indicated for patients where no MSD is available, which

83 can be expected for 70% of patients with SAA [3]. Some patients do not respond well or show

84 no response at all. Frequent transfusions increase the risk of adverse events such as iron

85 overload and early death. If a diagnosis of SAA is established at an early patient age, then it is

86 crucial to know which treatment promises more benefit and less harm in the long run. We

87 aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT compared to

88 IST in patients with SAA.

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3 **89 Methods**

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7 90 This article is based on a Cochrane Systematic Review published in The Cochrane Library  
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9 91 [5]. Publication of this work is in agreement with the policy of The Cochrane Collaboration  
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11 92 [6]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA  
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13 93 statement, adhered to its principles and conformed to its checklist [7].

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17 **94 Study inclusion criteria**

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20 95 We included randomized controlled trials (RCTs) and prospective non-randomized controlled  
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22 96 trials as long as the study design was consistent with the principle of 'Mendelian  
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24 97 randomization' in allocating patients to treatment groups. We required a minimum of 80% of  
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26 98 relevant patients per group and we set a minimum sample size of five participants per group.  
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28 99 We set no limits on language, year of publication, or year of treatment. We included  
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30 100 participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age  
31  
32 101 limits for participants. We excluded studies on participants with secondary aplastic anemia.  
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34 102 We included HSCT as the test intervention harvested from any source of MSD and serving as  
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36 103 a first-line therapy [8]. That means, no other HSCT or IST has been offered to the patients  
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38 104 before. We included IST as comparator with either antithymocyte/antilymphocyte globulin or  
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40 105 ciclosporin or a combination of the two [8]. The primary outcome was overall mortality.  
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42 106 Secondary outcomes were treatment-related mortality, graft failure, graft-versus-host disease,  
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44 107 no response to IST, relapse after initial successful treatment, secondary clonal disease or  
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46 108 malignancies, health-related quality of life, and performance scores.  
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52 **109 Search strategy and selection of studies**

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54 110 We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ov-  
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56 111 id), and Cochrane Library CENTRAL (Wiley) including articles published from inception to  
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3 112 22 April 2013. The corresponding search strategies are depicted in the original Cochrane Re-  
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5 113 view [5]. We retrieved all titles and abstracts by electronic searching and downloaded them to  
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7 114 the reference management database EndNote Version X3 [9]. Two authors assessed the eligi-  
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9 115 bility of retrieved papers independently. We considered studies written in languages other  
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11 116 than English. We judged studies to be prospective if an explicit statement was reported or  
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13 117 there were clues suggesting a prospective design (e.g. prior approval of treatment, informed  
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15 118 consent). We judged studies to be retrospective if an explicit statement was reported or it was  
16  
17 119 implied by description that data were reviewed from an existing source. We regarded each of  
18  
19 120 the following items as an indication of a retrospective design: registry reports and reviewing  
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21 121 of medical records. Gray 1991 and Wheatley 2004 described the potential of 'Mendelian ran-  
22  
23 122 domization' to minimize bias when comparing MSD-HSCT with an alternative therapy [10  
24  
25 123 11]. We judged studies as consistent with the principle of 'Mendelian randomization' if all  
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27 124 transplant donors were clearly siblings and if the allocation of patients to treatment groups  
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29 125 was not based on age. We regarded studies as not consistent with the principle of 'Mendelian  
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31 126 randomization' if age was not balanced between groups, indicating that age played a role in  
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33 127 group assignment. Example for imbalance: distribution of age categories was statistically not  
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35 128 comparable (P value less than 0.05).  
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#### 42 **Assessment of risk of bias in included studies**

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45 130 Two review authors independently assessed the risk of bias in the included studies using six  
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47 131 criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk  
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49 132 of bias [12]: blinding of outcome assessment, complete outcome data such as missing data,  
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51 133 selective reporting such as not reporting pre-specified outcomes, and other sources of bias  
52  
53 134 such as bias related to the specific study design and competing interest. We extended the  
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55 135 Cochrane tool for assessing risk of bias with two additional criteria that are specific to the  
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3 136 inclusion criteria for the present review and critical for confidence in results: comparable  
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5 137 baseline characteristics and concurrent control. We applied The Cochrane Collaboration's  
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7 138 criteria for judging risk of bias [13].  
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10 139 **Data synthesis**

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13 140 One review author entered the data into Review Manager [14]. Another review author  
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15 141 checked the entered data. We synthesized data on mortality (MSD-HSCT versus IST) by  
16  
17 142 using the hazard ratio (HR) for time-to-event data as the primary effect measure with a  
18  
19 143 random-effects model. If the hazard ratio was not directly given in the publication, we  
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21 144 estimated hazard ratios according to methods proposed by [15] and [16].  
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## 145 **Results**

### 146 **Search results**

147 We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure**  
148 **1**). Bayever 1984 [17] and Gratwohl 1981 [18] reported their results in a single original arti-  
149 cle, respectively. Führer 1998 reported five publications including one original article [19], a  
150 follow up article [20], one protocol [21], and two abstracts [22 23]. We did not identify any  
151 RCTs.

### 152 **Characteristics of included articles**

153 The main study, patients and interventions characteristics are shown in **Table 1**. The patients  
154 were treated and observed between 1976 and 1997. Thus, the reported data were collected  
155 more than 15 years ago. Median follow up was not reported. Median age, fraction of males,  
156 and median days of time interval between diagnosis and begin of treatment were roughly  
157 comparable between the treatment groups within each study. The age ranged from early  
158 childhood to young adulthood. In the study by Führer 1998, all patients were less than 17  
159 years old by definition of the inclusion criteria [19]. Bone marrow was used as source for all  
160 transplants. All three included studies had a high risk of bias due to the study design (**Table**  
161 **2**).

### 162 **Effects of intervention**

163 The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence inter-  
164 val of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data  
165 from all three included studies, overall mortality was not statistically significantly different  
166 between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT

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3 167 group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary out-  
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5 168 comes including treatment-related mortality after MSD-HSCT, graft failure after MSD-  
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7 169 HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse  
8  
9 170 after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies,  
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11 171 Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and  
12  
13 172 Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST.  
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15 173 Health-related quality of life questionnaires were not used in any of the included studies. Ba-  
16  
17 174 yever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and  
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19 175 less than half of the patients in the IST group had a Karnofsky Performance Status of higher  
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21 176 than 70%.  
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## 177 **Discussion**

### 178 **Interpretation of main results**

179 We identified three prospective, non-randomized controlled trials [17-19] including 302 par-  
180 ticipants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found  
181 insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST.  
182 Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in  
183 the IST group and in a meta-analysis, overall mortality was not statistically significantly dif-  
184 ferent between the treatment groups. Treatment-related mortality in the MSD-HSCT group  
185 was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the  
186 death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplanted  
187 patients. More than half of patients in one study did not respond to IST. Relapse affected  
188 up to one in eight patients after IST in one study. Secondary clonal disease or malignancies  
189 were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone  
190 marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance  
191 of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of stud-  
192 ies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at  
193 three to five years [24]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT  
194 to patients older than 40 years of age who are without significant co-morbidities [25]. The  
195 results of the studies included in the present systematic review appear to roughly match the  
196 recent estimates reported by others.

### 197 **Recent therapeutic improvement**

198 Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for  
199 IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

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2  
3 200 ment of overall survival in the group of matched related donor transplants but not in the IST  
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5 201 group [26]. Several factors may have contributed to recent improvements in HSCT, such as  
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7 202 detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Con-  
8  
9 203 ference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used  
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11 204 as the source of stem cells and that the upper age limit should be 50 years and that the combi-  
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13 205 nation of antithymocyte globulin and ciclosporin remains the gold standard for immunosup-  
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15 206 pressive therapy [27]. Scheinberg 2012 provided an overview and update of various treatment  
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17 207 options for severe aplastic anemia including immunosuppressive therapy and transplantation  
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21 208 [28].  
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### 23 24 209 **Strengths and limitations**

25  
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27 210 One of the strengths of this review is the broadness of the search strategy such that study re-  
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29 211 trieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled  
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31 212 trials and prospective non-randomized controlled trials that were compatible with 'Mendelian  
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33 213 Randomization' to avoid excess risk of bias. Nevertheless, the included data are too scarce and  
34  
35 214 too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST.  
36  
37 215 The rates of adverse events, such as treatment-related mortality, graft failure, no response to  
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39 216 IST, and GVHD, are unusually high, which may be explained by the age of the studies (start-  
40  
41 217 ing in 1976). All data were collected about 15 up to more than 30 years ago. Thus, the results  
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43 218 may not be applicable to current modern standard care. Use of 'Mendelian randomization' is  
44  
45 219 no guarantee that bias is minimized and Nitsch 2006 described the limits to causal inference  
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47 220 based on 'Mendelian randomization' [29].  
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### 51 52 221 **Conclusions**

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55 222 There are insufficient and biased data that do not allow any firm conclusions to be made about  
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57 223 the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the  
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3 224 early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with  
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5 225 IST should also be made aware that the disease may recur after initial successful treatment,  
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7 226 and that life-threatening late clonal and malignant disease after IST may occur in a higher  
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9 227 percentage compared to HSCT.  
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14 233 **Ethics statement**

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17 234 An ethics statement was not required for this work.

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19  
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21  
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23  
24 237 in study design, data collection and analysis, decision to publish, or preparation of the manu-

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26 238 script.

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28 239 **Conflict of Interest Statement**

29  
30 240 No authors have any competing interests.

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1 Subject: Transplantation for SAA

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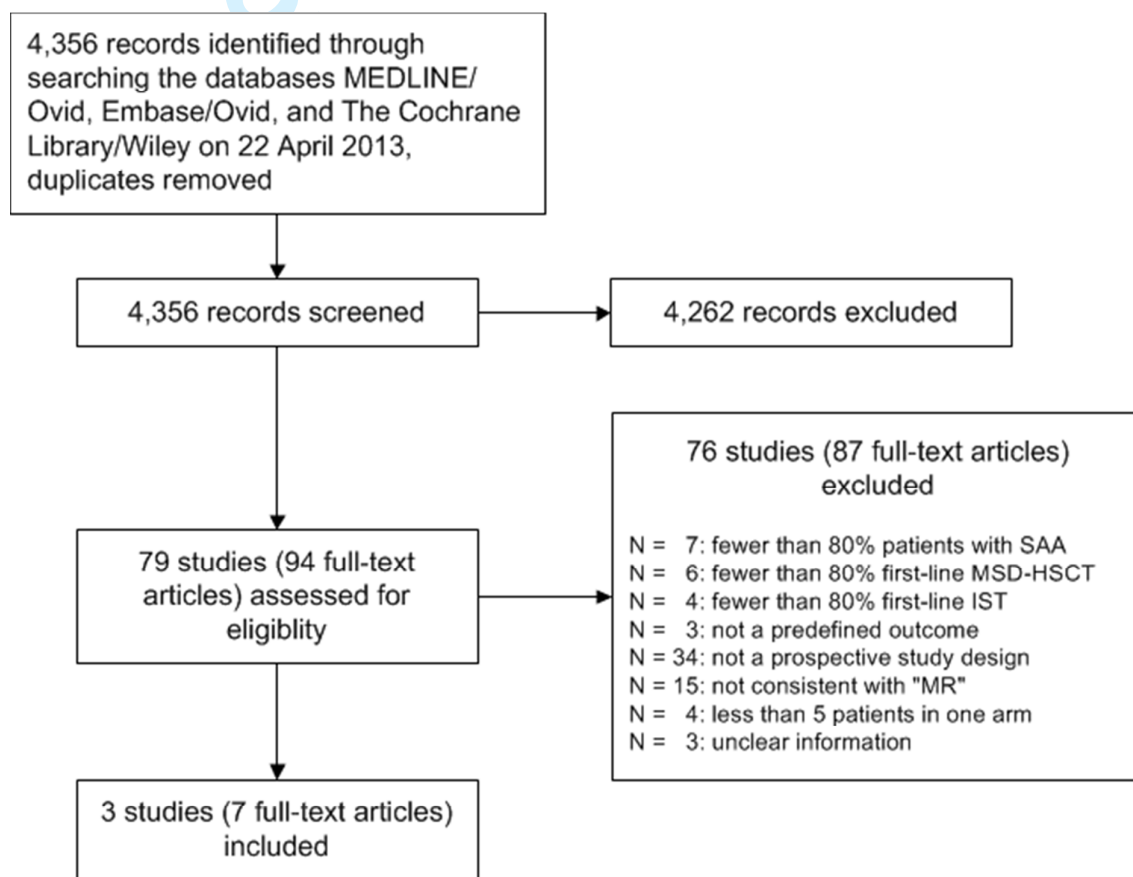
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Subject: Transplantation for SAA

## 321 Figure legends

322 Figure 1. Study flow

323 Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic  
 324 hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors;  
 325 "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia



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Subject: Transplantation for SAA

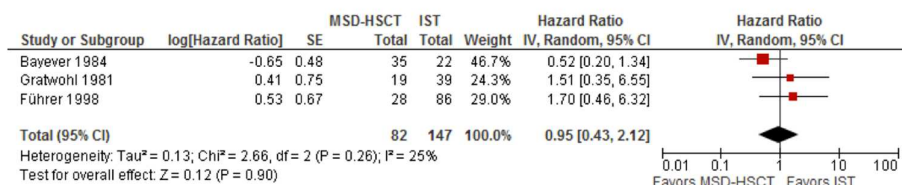
328 Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model.

329 Standard error calculated from data presented in the Kaplan-Meier graph of the article.

330 Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem

331 cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST:

332 first-line immunosuppressive therapy; IV: inverse variance; SE: standard error



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Subject: Transplantation for SAA

### Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median follow up	Setting, center, country	Patients, no. <sup>1</sup>	Median age, years (range) <sup>1</sup>	Fraction of males, % <sup>1</sup>	Median interval, days <sup>1,2</sup>	Stem cell source	IST components	ATG source
Bayever 1984	1977 to 1982	N.R.	Single, United States	35 vs. 22	17 (2 to 24) vs. 15 (1 to 23)	67 vs. 68	60 vs. 58	bone marrow	ATG	horse
Führer 1998	1993 to 1997	N.R.	Multi, Germany, Austria	28 vs. 86	10.1 (2.3 to 15.8) vs. 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	N.R.	Single, Switzerland	19 vs. 13	18 (4 to 29) vs. 23 (7 to 37)	53 vs. 54	105 vs. 180	bone marrow	ATG Ciclosporin	N.R.

<sup>1</sup>MSD-HSCT vs. IST

<sup>2</sup>Median time interval between diagnosis and begin of treatment

Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

Subject: Transplantation for SAA

Table 2. Risk of bias of included studies

Study ID	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias	Comparable baseline characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	High	Low	Unclear	High <sup>1</sup>	Low	Low	High
Führer 1998	High	High	High <sup>2</sup>	High <sup>3</sup>	Low	Low	High
Gratwohl 1981	High	High	Unclear	Unclear	Low	Low	High

<sup>1</sup>Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".

<sup>2</sup>Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.

<sup>3</sup>Führer 1998: Financial support was provided by 2 pharmaceutical companies.

Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Subject: Transplantation for SAA

Table 3. Overall survival

Study ID	MSD-HSCT		IST		FU <sup>1</sup> Year	P value
	N	OS (95% CI)	N	OS (95% CI)		
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69% <sup>2</sup> (N.R.)	5	0.56 <sup>3</sup>

<sup>1</sup>Time point of Kaplan-Meier estimate.

<sup>2</sup>Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line HSCT from the then again available MSD that was offered after the patients showed no response to IST.

<sup>3</sup>The P value was not reported and we calculated the P value using Fisher's exact test.

Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival

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Subject: Transplantation for SAA

Table 4. Secondary outcomes

Study ID	TRM after MSD-HSCT <sup>1,2</sup>	Graft failure after MSD-HSCT <sup>1</sup>	GVHD after MSD-HSCT <sup>1</sup>	No response to IST <sup>1</sup>	Relapse at 5 years after IST <sup>1</sup>
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

<sup>1</sup>In parenthesis: number of affected of number of evaluable patients

<sup>2</sup>Treatment-related mortality was not reported for IST.

Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

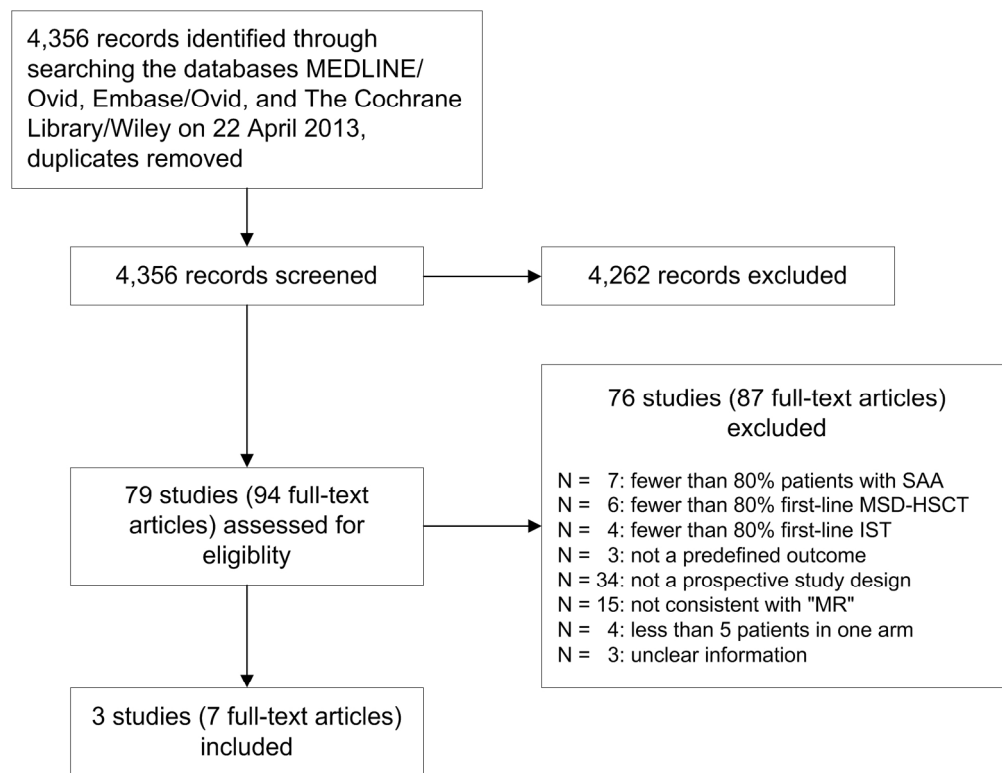


Figure 1. Study flow

Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia

170x130mm (300 x 300 DPI)



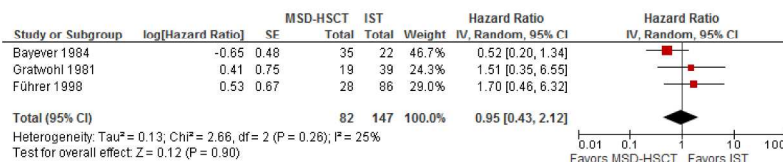


Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model. Standard error calculated from data presented in the Kaplan-Meier graph of the article. Ab-breviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error  
 254x190mm (300 x 300 DPI)



# PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
<b>TITLE</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
<b>ABSTRACT</b>			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
<b>INTRODUCTION</b>			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
<b>METHODS</b>			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	in the Cochrane review
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6 to 7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7



# PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$ ) for each meta-analysis.	7
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Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
<b>RESULTS</b>			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	in the Cochrane review
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8, table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8 to 9, table 3 to 4, figure 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	figure 1
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	table 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	none
<b>DISCUSSION</b>			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
<b>FUNDING</b>			



# PRISMA 2009 Checklist

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Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12
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*From:* Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: [www.prisma-statement.org](http://www.prisma-statement.org).

Page 2 of 2

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# BMJ Open

## Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2014-005039.R1
Article Type:	Research
Date Submitted by the Author:	09-Jun-2014
Complete List of Authors:	Peinemann, Frank; University of Cologne, Children's Hospital Labeit, Alexander; University of Illinois College of Medicine, Center for Outcomes Research
<b>Primary Subject Heading</b>:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Paediatrics, Pharmacology and therapeutics
Keywords:	CHEMOTHERAPY, Anaemia < HAEMATOLOGY, Bone marrow transplantation < HAEMATOLOGY, Paediatric oncology < ONCOLOGY, STATISTICS & RESEARCH METHODS, TRANSPLANT MEDICINE

SCHOLARONE™  
Manuscripts

1 *Subject: bmjopen-2014-005039-R1: SAA-MSD*

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4 1 Stem cell transplantation of matched sibling donors compared with immunosuppressive ther-  
5 2 apy for acquired severe aplastic anemia – a Cochrane Systematic Review\*

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8 3 Frank Peinemann,<sup>1†</sup> Alexander Labeit,<sup>2</sup>

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10 4 <sup>1</sup>Children's Hospital, University of Cologne, Cologne, Germany

11 5 <sup>2</sup>Center for Outcomes Research, University of Illinois College of Medicine at Peoria, Illinois,  
12 6 USA

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21 11 \*This article is based on a Cochrane Systematic Review published in the Cochrane Database  
22 12 of Systematic Reviews 2013, Issue 7. Art. No.: CD006407. DOI:

23 13 10.1002/14651858.CD006407.pub2. (see [www.thecochranelibrary.com](http://www.thecochranelibrary.com) for information).

24 14 Cochrane Systematic Reviews are regularly updated as new evidence emerges and in response  
25 15 to feedback, and the CDSR should be consulted for the most recent version of the review.

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29 17 †Corresponding author: Frank Peinemann, M.D., M.Sc., Children's Hospital, University of  
30 18 Cologne, Kerpener Str. 62, 50937 Cologne, Germany; e-mail: [pubmedprjournal@gmail.com](mailto:pubmedprjournal@gmail.com).  
31 19 Phone: +49-176-31130745.

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39 21 **Keywords**

40 22 severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosup-  
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Subject: *bmjopen-2014-005039-R1: SAA-MSD*

## 29 **Abstract**

30 Objectives: Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of  
31 this Cochrane review was to evaluate the effectiveness and adverse events of first-line  
32 allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors compared  
33 to first-line immunosuppressive therapy.

34 Setting: Specialised stem cell transplantations units in primary care hospitals

35 Participants: We included 302 participants with newly diagnosed acquired severe aplastic  
36 anemia. The age ranged from early childhood to young adulthood. We excluded studies on  
37 participants with secondary aplastic anemia.

38 Interventions: We included allogeneic haematopoietic stem cell transplantation as the test  
39 intervention harvested from any source of matched sibling donor and serving as a first-line  
40 therapy. We included immunosuppressive therapy as comparator with either antithymocyte/-  
41 antilymphocyte globulin or ciclosporin or a combination of the two.

42 Primary and secondary outcome measures planned and finally measured: The primary  
43 outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft  
44 failure, graft-versus-host disease, no response to immunosuppressive therapy, relapse after  
45 initial successful treatment, secondary clonal disease or malignancies, health-related quality  
46 of life, and performance scores.

47 Results: We identified three prospective non-randomized controlled trials with a study design  
48 that was consistent with the principle of 'Mendelian randomization' in allocating patients to  
49 treatment groups. All studies had a high risk of bias due to the study design and were  
50 conducted more than 15 years. The pooled hazard ratio for overall mortality for the

1 *Subject: bmjopen-2014-005039-R1: SAA-MSD*

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3 51 transplanted group versus the not transplanted group was 0.95 (95% confidence interval 0.43  
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5 52 to 2.12, P = 0.90).

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8 53 Conclusions: There are insufficient and biased data that do not allow any firm conclusions to  
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10 54 be made about the comparative effectiveness of first-line allogeneic hematopoietic stem cell  
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12 55 transplantation of HLA-matched sibling donors and first-line immunosuppressive therapy of  
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14 56 patients with acquired severe aplastic anemia.

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21 58 **Strengths and limitations of this study**

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24 59 • We conducted a comprehensive literature search and strictly adhered to the projected  
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26 60 methodology.
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29 61 • We restricted the study design to randomized controlled trials and prospective non-  
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31 62 randomized controlled trials and the studies had to be compatible with 'Mendelian  
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33 63 Randomization' to avoid excess risk of bias.
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36 64 • The included data are too scarce and too biased to allow any conclusion on the com-  
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38 65 parative effectiveness of MSD-HSCT and IST.
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40 66 • The included data were collected 15 to more than 30 years ago. Thus, the results may  
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42 67 not be applicable to current modern standard care.
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Subject: *bmjopen-2014-005039-R1: SAA-MSD*

## 69 Introduction

70 Acquired severe aplastic anemia (SAA) is a rare [1] and potentially fatal disease which is  
71 characterized by hypocellular bone marrow and pancytopenia; it mainly affects young adults.  
72 The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2].  
73 The underlying pathophysiology is thought to be an aberrant immune response involving the  
74 T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are  
75 severe infections, bleeding, and exhaustion and patients may experience paleness, weakness,  
76 fatigue, and shortness of breath.

77 According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of  
78 the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic  
79 stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen  
80 (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly  
81 diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the  
82 conditioning regimen may lead to severe non-hematological organ toxicities.

83 According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of  
84 the British Committee for Standards in Haematology [4], first-line immunosuppressive  
85 therapy (IST) is a combination of antithymocyte globulin (ATG) and ciclosporin. First-line  
86 IST is indicated for patients where no MSD is available, which can be expected for 70% of  
87 patients with SAA [3]. Ciclosporin is an immunosuppressant drug that is not lymphocytotoxic  
88 but has specific inhibitory effects on T-lymphocyte function [5]. In the past, antilymphocyte  
89 globulin (ALG) was reported interchangeably in the literature alongside ATG, therefore, ALG  
90 is reported in the present study on equal terms. ATG as well as ALG are polyclonal antibodies  
91 that recognize a variety of human lymphocyte cell surface antigens, reduce the number of  
92 lymphocytes and induce an immunosuppressive effect. They originate in animals immunized

1 *Subject: bmjopen-2014-005039-R1: SAA-MSD*

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3 93 with either normal human thymocytes, collected at pediatric cardiac surgery or thoracic duct  
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5 94 lymphocytes, collected during therapeutic cannulation [5]. Concerning the hematologic  
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7 95 response and the survival of patients after a first treatment for severe aplastic anemia, it may  
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9 96 be crucial in what type of animal ATG originates, as a randomized study showed that rabbit  
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11 97 ATG was inferior in this respect to horse ATG [6]. The currently recommended combination  
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13 98 of ciclosporin with ATG in the treatment of severe aplastic anemia is based on their separate  
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15 99 and potentially complementary modes of action[5]. Some patients do not respond well to IST  
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17 100 or show no response at all. Frequent transfusions increase the risk of adverse events such as  
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19 101 iron overload and early death. If a diagnosis of SAA is established at an early patient age,  
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21 102 then it is crucial to know which treatment promises more benefit and less harm in the long  
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23 103 run. We aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT  
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25 104 compared to IST in patients with SAA.  
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## 105 **Methods**

106 This article is based on a Cochrane Systematic Review published in The Cochrane Library  
107 [7]. Publication of this work is in agreement with the policy of The Cochrane Collaboration  
108 [8]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA  
109 statement, adhered to its principles and conformed to its checklist [9].

### 110 **Study inclusion criteria**

111 We included randomized controlled trials (RCTs) and prospective non-randomized controlled  
112 trials as long as the study design was consistent with the principle of 'Mendelian  
113 randomization' in allocating patients to treatment groups. We required a minimum of 80% of  
114 relevant patients per group and we set a minimum sample size of five participants per group.  
115 We set no limits on language, year of publication, or year of treatment. We included  
116 participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age  
117 limits for participants. We excluded studies on participants with secondary aplastic anemia.  
118 We included HSCT as the test intervention harvested from any source of MSD and serving as  
119 a first-line therapy [10]. That means, no other HSCT or IST has been offered to the patients  
120 before. We included IST as comparator with ciclosporin combined with ATG as the current  
121 mode of IST [10]. To accommodate also former modes of IST, we also included ciclosporin  
122 combined with ALG, cyclosporine alone, ATG alone, and ALG. Other agents such as  
123 corticosteroids and androgens were not considered. The primary outcome was overall  
124 mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-  
125 host disease, no response to IST, relapse after initial successful treatment, secondary clonal  
126 disease or malignancies, health-related quality of life, and performance scores.

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3 **128 Principle of 'Mendelian randomization'**

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6 129 There are ethical concerns around randomization of patients with severe aplastic anemia to  
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8 130 transplantation versus non-transplantation. In general, MSD-HSCT is a life-threatening  
9  
10 131 treatment that can lead to early severe adverse events including death. Gray 1991 [11] and  
11  
12 132 Wheatley 2004 [12] described the potential of 'Mendelian randomization' to minimize bias  
13  
14 133 when comparing MSD-HSCT with an alternative therapy. The base concept has been ascribed  
15  
16 134 to Katan 1986 [13]. 'Mendelian randomization' means the view that nature itself has already  
17  
18 135 'randomized' the paternal and maternal part of a gene given that donor and recipient are  
19  
20 136 siblings. Thus, 'Mendelian randomization' by definition accepts only siblings as transplant  
21  
22 137 donors and these sibling donors are required to have 'identical' or matched features of specific  
23  
24 138 transplant-relevant HLA sites when compared with the transplant recipient. Therefore,  
25  
26 139 patients with an HLA-matched sibling will be allocated to the MSD-HSCT group. On the  
27  
28 140 other hand, patients with siblings that are not HLA compatible will be allocated to the  
29  
30 141 immunosuppressive therapy group. The term 'Mendelian randomization' refers to the fact that  
31  
32 142 the genetic distribution of paternal and maternal alleles follows a random process and is  
33  
34 143 determined before birth. This concept takes advantage of an instrumental variable for  
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36 144 allocating the patients to treatment groups and, at the same time, this variable is neither  
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38 145 associated with the treatment nor associated with the outcome.

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44 **146 Search strategy and selection of studies**

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47 147 We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ov-  
48  
49 148 id), and Cochrane Library CENTRAL (Wiley) including articles published from inception to  
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51 149 22 April 2013. The corresponding search strategies are depicted in the original Cochrane Re-  
52  
53 150 view [7]. We retrieved all titles and abstracts by electronic searching and downloaded them to  
54  
55 151 the reference management database EndNote Version X3 [14]. Two authors assessed the eli-

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2  
3 152 gibility of retrieved papers independently. We considered studies written in languages other  
4  
5 153 than English. We judged studies to be prospective if an explicit statement was reported or  
6  
7 154 there were clues suggesting a prospective design (e.g. prior approval of treatment, informed  
8  
9 155 consent). We judged studies to be retrospective if an explicit statement was reported or it was  
10  
11 156 implied by description that data were reviewed from an existing source. We regarded each of  
12  
13 157 the following items as an indication of a retrospective design: registry reports and reviewing  
14  
15 158 of medical records. We judged studies as consistent with the principle of 'Mendelian randomi-  
16  
17 159 zation' if all transplant donors were clearly siblings and if the allocation of patients to treat-  
18  
19 160 ment groups was not based on age. We regarded studies as not consistent with the principle of  
20  
21 161 'Mendelian randomization' if age was not balanced between groups, indicating that age played  
22  
23 162 a role in the group assignment. Example for imbalance: distribution of age categories was  
24  
25 163 statistically not comparable (P value less than 0.05).  
26  
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#### 30 164 **Assessment of risk of bias in included studies**

31  
32  
33 165 Two review authors independently assessed the risk of bias in the included studies using six  
34  
35 166 criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk  
36  
37 167 of bias [15]: blinding of outcome assessment, complete outcome data such as missing data,  
38  
39 168 selective reporting such as not reporting pre-specified outcomes, and other sources of bias  
40  
41 169 such as bias related to the specific study design and competing interest. We extended the  
42  
43 170 Cochrane tool for assessing risk of bias with two additional criteria that are specific to the  
44  
45 171 inclusion criteria for the present review and critical for confidence in results: comparable  
46  
47 172 baseline characteristics and concurrent control. We applied The Cochrane Collaboration's  
48  
49 173 criteria for judging risk of bias [16].  
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#### 54 174 **Data synthesis**

55  
56  
57 175 One review author entered the data into Review Manager [17]. Another review author  
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59  
60

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2  
3 176 checked the entered data. We synthesized data on mortality (MSD-HSCT versus IST) by  
4  
5 177 using the hazard ratio (HR) for time-to-event data as the primary effect measure with a  
6  
7 178 random-effects model. If the hazard ratio was not directly given in the publication, we  
8  
9 179 estimated hazard ratios according to methods proposed by [18] and [19].  
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## 180 **Results**

### 181 **Search results**

182 We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure**  
183 **1**). Bayever 1984 [20] and Gratwohl 1981 [21] reported their results in a single original arti-  
184 cle, respectively. Führer 1998 reported five publications including one original article [22], a  
185 follow up article [23], one protocol [24], and two abstracts [25 26]. We did not identify any  
186 RCTs.

### 187 **Characteristics of included articles**

188 The main study, patients and interventions characteristics are shown in **Table 1**. The patients  
189 were treated and observed between 1976 and 1997. Thus, the reported data were collected  
190 more than 15 years ago. Median follow up was not reported. Median age, fraction of males,  
191 and median days of time interval between diagnosis and begin of treatment were roughly  
192 comparable between the treatment groups within each study. The age ranged from early  
193 childhood to young adulthood. In the study by Führer 1998, all patients were less than 17  
194 years old by definition of the inclusion criteria [22]. Bone marrow was used as source for all  
195 transplants. All three included studies had a high risk of bias due to the study design (**Table**  
196 **2**). We judged a low risk of bias for blinding the assessment of overall mortality. Blinding or  
197 lack of blinding is not expected to make a difference concerning overall mortality. The au-  
198 thors of all included studies did not report that 'Mendelian randomization' was planned and  
199 the authors did not report the size of the involved families. The authors did not report the  
200 numbers of siblings and the results of the individual genetic analyses.

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3 201 **Effects of intervention**

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6 202 The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence inter-  
7  
8 203 val of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data  
9  
10 204 from all three included studies, overall mortality was not statistically significantly different  
11  
12 205 between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT  
13  
14 206 group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary out-  
15  
16 207 comes including treatment-related mortality after MSD-HSCT, graft failure after MSD-  
17  
18 208 HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse  
19  
20 209 after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies,  
21  
22 210 Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and  
23  
24 211 Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST.  
25  
26 212 Health-related quality of life questionnaires were not used in any of the included studies. Ba-  
27  
28 213 yever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and  
29  
30 214 less than half of the patients in the IST group had a Karnofsky Performance Status of higher  
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32 215 than 70%.



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## 216 **Discussion**

### 217 **Interpretation of main results**

218 We identified three prospective, non-randomized controlled trials [20-22] including 302 par-  
219 ticipants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found  
220 insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST.  
221 Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in  
222 the IST group and in a meta-analysis, overall mortality was not statistically significantly dif-  
223 ferent between the treatment groups. Treatment-related mortality in the MSD-HSCT group  
224 was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the  
225 death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplanted  
226 patients. More than half of patients in one study did not respond to IST. Relapse affected  
227 up to one in eight patients after IST in one study. Secondary clonal disease or malignancies  
228 were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone  
229 marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance  
230 of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of stud-  
231 ies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at  
232 three to five years [27]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT  
233 to patients older than 40 years of age who are without significant co-morbidities [28]. The  
234 results of the studies included in the present systematic review appear to roughly match the  
235 recent estimates reported by others.

### 236 **Recent therapeutic improvement**

237 Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for  
238 IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

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2  
3 239 ment of overall survival in the group of matched related donor transplants but not in the IST  
4  
5 240 group [29]. Several factors may have contributed to recent improvements in HSCT, such as  
6  
7 241 detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Con-  
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9 242 ference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used  
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11 243 as the source of stem cells and that the upper age limit should be 50 years and that the combi-  
12  
13 244 nation of antithymocyte globulin and ciclosporin remains the gold standard for immunosup-  
14  
15 245 pressive therapy [30]. Scheinberg 2012 provided an overview and update of various treatment  
16  
17 246 options for severe aplastic anemia including immunosuppressive therapy and transplantation  
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21 247 [31].  
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### 23 24 248 **Strengths and limitations**

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27 249 One of the strengths of this review is the broadness of the search strategy such that study re-  
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29 250 trieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled  
30  
31 251 trials and prospective non-randomized controlled trials that were compatible with 'Mendelian  
32  
33 252 Randomization' to avoid excess risk of bias. Nevertheless, the included data are too scarce and  
34  
35 253 too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST.  
36  
37 254 The rates of adverse events, such as treatment-related mortality, graft failure, no response to  
38  
39 255 IST, and GVHD, are unusually high, which may be explained by the age of the studies (start-  
40  
41 256 ing in 1976). We did not separate horse ATG from rabbit ATG, although the type of animal as  
42  
43 257 the origin of ATG was reported as a serious effect modifier [6]. All data were collected about  
44  
45 258 15 up to more than 30 years ago. Thus, the results may not be applicable to current modern  
46  
47 259 standard care. Use of 'Mendelian randomization' is no guarantee that bias is minimized. This  
48  
49 260 may be because tissue typing data may not be accurate. Patients may have only one sibling  
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51 261 either in the donor or in the no donor group. Large families have a greater chance of finding a  
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53 262 donor. Therefore, designing a non-randomized controlled trial by applying 'Mendelian ran-  
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2  
3 263 domization' requires careful thought to effectively reduce bias and control for potential con-  
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5 264 founders. There is a time lag in patients with siblings because tissue typing and readiness for  
6  
7 265 assignment to treatment group may possibly take several months [12]. On the other hand, pa-  
8  
9 266 tients with no siblings can be assigned immediately and are at earlier risk for adverse events.  
10  
11 267 Nitsch 2006 described the limits to causal inference based on 'Mendelian randomization' [32].  
12  
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## 14 268 **Conclusions**

15  
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18 269 There are insufficient and biased data that do not allow any firm conclusions to be made about  
19  
20 270 the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the  
21  
22 271 early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with  
23  
24 272 IST should also be made aware that the disease may recur after initial successful treatment,  
25  
26 273 and that life-threatening late clonal and malignant disease after IST may occur in a higher  
27  
28 274 percentage compared to HSCT.  
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## 280 **Ethics statement**

281 An ethics statement was not required for this work.

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284 in study design, data collection and analysis, decision to publish, or preparation of the manu-  
285 script.

## 286 **Conflict of Interest Statement**

287 No authors have any competing interests.

## 288 **Data Sharing Statement**

289 No additional data available.

## 290 **Contributorship Statement**

291 FP: design, search strategy, study selection, data extraction, data analysis, writing the  
292 manuscript

293 AL: methodological perspective, reviewing the manuscript

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4 380 **Figure legends**

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8 381 Figure 1. Study flow

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11 382 Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic  
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13 383 hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors;  
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15 384 "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia  
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22 386 Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model.

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24 388 Standard error calculated from data presented in the Kaplan-Meier graph of the article.

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26 389 Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem

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28 390 cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST:

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30 391 first-line immunosuppressive therapy; IV: inverse variance; SE: standard error  
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## Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median follow up	Setting, center, country	Patients, no. <sup>1</sup>	Median age, years (range) <sup>1</sup>	Fraction of males, % <sup>1</sup>	Median interval, days <sup>1,2</sup>	Stem cell source	IST components	ATG source
Bayever 1984	1977 to 1982	N.R.	Single, United States	35 vs. 22	17 (2 to 24) vs. 15 (1 to 23)	67 vs. 68	60 vs. 58	bone marrow	ATG	horse
Führer 1998	1993 to 1997	N.R.	Multi, Germany, Austria	28 vs. 86	10.1 (2.3 to 15.8) vs. 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	N.R.	Single, Switzerland	19 vs. 13	18 (4 to 29) vs. 23 (7 to 37)	53 vs. 54	105 vs. 180	bone marrow	ATG Ciclosporin	N.R.

<sup>1</sup>MSD-HSCT vs. IST

<sup>2</sup>Median time interval between diagnosis and begin of treatment

Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Table 2. Risk of bias of included studies

Study ID	Blinding of assessment of overall mortality	Incomplete outcome data	Selective reporting	Other bias	Comparable baseline characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	Low	Low	Unclear	High <sup>1</sup>	Low	Low	High
Führer 1998	Low	High	High <sup>2</sup>	High <sup>3</sup>	Low	Low	High
Gratwohl 1981	Low	High	Unclear	Unclear	Low	Low	High

<sup>1</sup>Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".  
<sup>2</sup>Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.  
<sup>3</sup>Führer 1998: Financial support was provided by 2 pharmaceutical companies.  
Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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3 *Subject: bmjopen-2014-005039-R1: SAA-MSD*

4  
5 Table 3. Overall survival

Study ID	MSD-HSCT		IST		FU <sup>1</sup>	P value
	N	OS (95% CI)	N	OS (95% CI)	Year	
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69% <sup>2</sup> (N.R.)	5	0.56 <sup>3</sup>

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12 <sup>1</sup>Time point of Kaplan-Meier estimate.

13 <sup>2</sup>Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line HSCT from the then again available MSD that was offered after the patients showed no response to IST.

14 <sup>3</sup>The P value was not reported and we calculated the P value using Fisher's exact test.

15 Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival

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Table 4. Secondary outcomes

Study ID	TRM after MSD-HSCT <sup>1,2</sup>	Graft failure after MSD-HSCT <sup>1</sup>	GVHD after MSD-HSCT <sup>1</sup>	No response to IST <sup>1</sup>	Relapse at 5 years after IST <sup>1</sup>
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

<sup>1</sup>In parenthesis: number of affected of number of evaluable patients

<sup>2</sup>Treatment-related mortality was not reported for IST.

Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

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| Subject: *bmjopen-2014-005039-RI: SAA-MSD*

1 Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review\*

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9 10.1002/14651858.CD006407.pub2. (see [www.thecochranelibrary.com](http://www.thecochranelibrary.com) for information).

10 Cochrane Systematic Reviews are regularly updated as new evidence emerges and in response to feedback, and the CDSR should be consulted for the most recent version of the review.

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13 Phone: +49-176-31130745.

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## 22 Keywords

23 severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosup-  
24 pressive therapy, systematic review

## 27 Strengths and limitations of this study

- 28 • We conducted a comprehensive literature search and strictly adhered to the projected  
29 methodology.
- 30 • We restricted the study design to randomized controlled trials and prospective non-  
31 randomized controlled trials and the studies had to be compatible with 'Mendelian  
32 Randomization' to avoid excess risk of bias.
- 33 • The included data are too scarce and too biased to allow any conclusion on the com-  
34 parative effectiveness of MSD-HSCT and IST.
- 35 • The included data were collected 15 to more than 30 years ago. Thus, the results may  
36 not be applicable to current modern standard care.

| Subject: *bmjopen-2014-005039-RI: SAA-MSD*

## 40 Abstract

### 41 Background

42 Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of this  
43 Cochrane review was to evaluate the effectiveness and adverse events of first-line allogeneic  
44 hematopoietic stem cell transplantation of HLA-matched sibling donors compared to first-line  
45 immunosuppressive therapy.

### 46 Procedure

47 We searched the electronic databases MEDLINE (Ovid), EMBASE (Ovid), and The  
48 Cochrane Library CENTRAL (Wiley) for published articles from 1946 to 22 April 2013. We  
49 included randomized controlled trials and prospective non-randomized controlled trials as  
50 long as the study design was consistent with the principle of 'Mendelian randomization' in  
51 allocating patients to treatment groups.

### 52 Results

53 We identified three prospective non-randomized controlled trials with 302 participants. We  
54 did not identify a randomized controlled trial. All studies had a high risk of bias due to the  
55 study design and were conducted more than 15 years ago and may not be applicable to the  
56 standard of care of today. The pooled hazard ratio for overall mortality for the transplanted  
57 group versus the not transplanted group was 0.95 (95% confidence interval 0.43 to 2.12, P =  
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61 Conclusions

62 There are insufficient and biased data that do not allow any firm conclusions to be made about  
63 the comparative effectiveness of first-line allogeneic hematopoietic stem cell transplantation  
64 of HLA-matched sibling donors and first-line immunosuppressive therapy of patients with  
65 acquired severe aplastic anemia.

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## 67 Introduction

68 Acquired severe aplastic anemia (SAA) is a rare [1] and potentially fatal disease which is  
69 characterized by hypocellular bone marrow and pancytopenia; it mainly affects young adults.  
70 The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2].  
71 The underlying pathophysiology is thought to be an aberrant immune response involving the  
72 T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are  
73 severe infections, bleeding, and exhaustion and patients may experience paleness, weakness,  
74 fatigue, and shortness of breath.

75 According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of  
76 the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic  
77 stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen  
78 (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly  
79 diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the  
80 conditioning regimen may lead to severe non-hematological organ toxicities.

81 According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of  
82 the British Committee for Standards in Haematology [4], first-line immunosuppressive  
83 therapy (IST) is a combination of antithymocyte globulin (ATG) and ciclosporin. First-line  
84 ~~IST ciclosporin and/or antithymocyte or antilymphocyte globulin denoted as first line~~  
85 ~~immunosuppressive therapy (IST)~~ is indicated for patients where no MSD is available, which  
86 can be expected for 70% of patients with SAA [3].

87 ~~Ciclosporin is an immunosuppressant drug that is not lymphocytotoxic but has specific~~  
88 ~~inhibitory effects on T-lymphocyte function [5]. In the past, antilymphocyte globulin (ALG)~~  
89 ~~was reported interchangeably in the literature alongside ATG, therefore, ALG is reported in~~

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90 the present study on equal terms. ATG as well as ALG are polyclonal antibodies that  
91 recognize a variety of human lymphocyte cell surface antigens, reduce the number of  
92 lymphocytes and induce an immunosuppressive effect. They originate in animals immunized  
93 with either normal human thymocytes, collected at pediatric cardiac surgery or thoracic duct  
94 lymphocytes, collected during therapeutic cannulation [5]. Concerning the hematologic  
95 response and the survival of patients after a first treatment for severe aplastic anemia, it may  
96 be crucial in what type of animal ATG originates, as a randomized study showed that rabbit  
97 ATG was inferior in this respect to horse ATG [6]. The currently recommended combination  
98 of ciclosporin with ATG in the treatment of severe aplastic anemia is based on their separate  
99 and potentially complementary modes of action[5]. Some patients do not respond well to IST  
100 or show no response at all. Frequent transfusions increase the risk of adverse events such as  
101 iron overload and early death. If a diagnosis of SAA is established at an early patient age,  
102 then it is crucial to know which treatment promises more benefit and less harm in the long  
103 run. We aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT  
104 compared to IST in patients with SAA.

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## 7 105 **Methods**

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10 106 This article is based on a Cochrane Systematic Review published in The Cochrane Library  
11 107 [7]. Publication of this work is in agreement with the policy of The Cochrane Collaboration  
12 108 [8]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA  
13 109 statement, adhered to its principles and conformed to its checklist [9].  
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### 16 110 **Study inclusion criteria**

17  
18  
19  
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21 111 We included randomized controlled trials (RCTs) and prospective non-randomized controlled  
22 112 trials as long as the study design was consistent with the principle of 'Mendelian  
23 113 randomization' in allocating patients to treatment groups. We required a minimum of 80% of  
24 114 relevant patients per group and we set a minimum sample size of five participants per group.

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27 115 We set no limits on language, year of publication, or year of treatment. We included  
28 116 participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age  
29 117 limits for participants. We excluded studies on participants with secondary aplastic anemia.

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32 118 We included HSCT as the test intervention harvested from any source of MSD and serving as  
33 119 a first-line therapy [10]. That means, no other HSCT or IST has been offered to the patients  
34 120 before. We included IST as comparator with ~~either ciclosporin combined with ATG as the~~

35 121 ~~current mode of IST~~ [10]. ~~To accommodate also former modes of IST, we also included~~  
36 122 ~~ciclosporin combined with ALG, cyclosporine alone, ATG alone, and ALG. Other agents~~  
37 123 ~~such as corticosteroids and androgens were not considered. antithymocyte/antilymphocyte~~  
38 124 ~~globulin or ciclosporin or a combination of the two~~—The primary outcome was overall

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47 125 mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-  
48 126 host disease, no response to IST, relapse after initial successful treatment, secondary clonal  
49 127 disease or malignancies, health-related quality of life, and performance scores.  
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## Principle of 'Mendelian randomization'

There are ethical concerns around randomization of patients with severe aplastic anemia to transplantation versus non-transplantation. In general, MSD-HSCT is a life-threatening treatment that can lead to early severe adverse events including death. Gray 1991 [11] and Wheatley 2004 [12] described the potential of 'Mendelian randomization' to minimize bias when comparing MSD-HSCT with an alternative therapy. The base concept has been ascribed to Katan 1986 [13]. 'Mendelian randomization' means the view that nature itself has already 'randomized' the paternal and maternal part of a gene given that donor and recipient are siblings. Thus, 'Mendelian randomization' by definition accepts only siblings as transplant donors and these sibling donors are required to have 'identical' or matched features of specific transplant-relevant HLA sites when compared with the transplant recipient. Therefore, patients with an HLA-matched sibling will be allocated to the MSD-HSCT group. On the other hand, patients with siblings that are not HLA compatible will be allocated to the immunosuppressive therapy group. The term 'Mendelian randomization' refers to the fact that the genetic distribution of paternal and maternal alleles follows a random process and is determined before birth. This concept takes advantage of an instrumental variable for allocating the patients to treatment groups and, at the same time, this variable is neither associated with the treatment nor associated with the outcome.

### **Search strategy and selection of studies**

We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ovid), and Cochrane Library CENTRAL (Wiley) including articles published from inception to 22 April 2013. The corresponding search strategies are depicted in the original Cochrane Review [7]. We retrieved all titles and abstracts by electronic searching and downloaded them to

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7 152 the reference management database EndNote Version X3 [14]. Two authors assessed the eli-  
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9 153 gibility of retrieved papers independently. We considered studies written in languages other  
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11 154 than English. We judged studies to be prospective if an explicit statement was reported or  
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13 155 there were clues suggesting a prospective design (e.g. prior approval of treatment, informed  
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15 156 consent). We judged studies to be retrospective if an explicit statement was reported or it was  
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17 157 implied by description that data were reviewed from an existing source. We regarded each of  
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19 158 the following items as an indication of a retrospective design: registry reports and reviewing  
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21 159 of medical records. ~~Gray 1991 and Wheatley 2004 described the potential of 'Mendelian ran-~~  
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23 160 ~~domization' to minimize bias when comparing MSD-HSCT with an alternative therapy [11~~  
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25 161 12]. We judged studies as consistent with the principle of 'Mendelian randomization' if all  
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27 162 transplant donors were clearly siblings and if the allocation of patients to treatment groups  
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29 163 was not based on age. We regarded studies as not consistent with the principle of 'Mendelian  
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31 164 randomization' if age was not balanced between groups, indicating that age played a role in  
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33 165 the group assignment. Example for imbalance: distribution of age categories was statistically  
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35 166 not comparable (P value less than 0.05).

### 36 167 **Assessment of risk of bias in included studies**

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39 168 Two review authors independently assessed the risk of bias in the included studies using six  
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41 169 criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk  
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43 170 of bias [15]: blinding of outcome assessment, complete outcome data such as missing data,  
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45 171 selective reporting such as not reporting pre-specified outcomes, and other sources of bias  
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47 172 such as bias related to the specific study design and competing interest. We extended the  
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49 173 Cochrane tool for assessing risk of bias with two additional criteria that are specific to the  
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51 174 inclusion criteria for the present review and critical for confidence in results: comparable  
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7 175 baseline characteristics and concurrent control. We applied The Cochrane Collaboration's  
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9 176 criteria for judging risk of bias [16].

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11 177 **Data synthesis**

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14 178 One review author entered the data into Review Manager [17]. Another review author  
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16 179 checked the entered data. We synthesized data on mortality (MSD-HSCT versus IST) by  
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18 180 using the hazard ratio (HR) for time-to-event data as the primary effect measure with a  
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20 181 random-effects model. If the hazard ratio was not directly given in the publication, we  
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22 182 estimated hazard ratios according to methods proposed by [18] and [19].  
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## 7 183 **Results**

### 10 184 **Search results**

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12 185 We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure**  
13 186 **1**). Bayever 1984 [20] and Gratwohl 1981 [21] reported their results in a single original arti-  
14 187 cle, respectively. Führer 1998 reported five publications including one original article [22], a  
15 188 follow up article [23], one protocol [24], and two abstracts [25 26]. We did not identify any  
16 189 RCTs.

### 23 190 **Characteristics of included articles**

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26 191 The main study, patients and interventions characteristics are shown in **Table 1**. The patients  
27 192 were treated and observed between 1976 and 1997. Thus, the reported data were collected  
28 193 more than 15 years ago. Median follow up was not reported. Median age, fraction of males,  
29 194 and median days of time interval between diagnosis and begin of treatment were roughly  
30 195 comparable between the treatment groups within each study. The age ranged from early  
31 196 childhood to young adulthood. In the study by Führer 1998, all patients were less than 17  
32 197 years old by definition of the inclusion criteria [22]. Bone marrow was used as source for all  
33 198 transplants. All three included studies had a high risk of bias due to the study design (**Table**  
34 199 **2**). We judged a low risk of bias for blinding the assessment of overall mortality. Blinding or  
35 200 lack of blinding is not expected to make a difference concerning overall mortality. The au-  
36 201 thors of all included studies did not report that 'Mendelian randomization' was planned and  
37 202 the authors did not report the size of the involved families. The authors did not report the  
38 203 numbers of siblings and the results of the individual genetic analyses.  
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7 204 **Effects of intervention**

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9 205 The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence inter-  
10 206 val of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data  
11 207 from all three included studies, overall mortality was not statistically significantly different  
12 208 between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT  
13 209 group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary out-  
14 210 comes including treatment-related mortality after MSD-HSCT, graft failure after MSD-  
15 211 HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse  
16 212 after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies,  
17 213 Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and  
18 214 Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST.  
19 215 Health-related quality of life questionnaires were not used in any of the included studies. Ba-  
20 216 yever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and  
21 217 less than half of the patients in the IST group had a Karnofsky Performance Status of higher  
22 218 than 70%.



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## 219 Discussion

### 220 Interpretation of main results

221 We identified three prospective, non-randomized controlled trials [20-22] including 302 par-  
222 ticipants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found  
223 insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST.  
224 Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in  
225 the IST group and in a meta-analysis, overall mortality was not statistically significantly dif-  
226 ferent between the treatment groups. Treatment-related mortality in the MSD-HSCT group  
227 was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the  
228 death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplant-  
229 ed patients. More than half of patients in one study did not respond to IST. Relapse affected  
230 up to one in eight patients after IST in one study. Secondary clonal disease or malignancies  
231 were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone  
232 marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance  
233 of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of stud-  
234 ies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at  
235 three to five years [27]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT  
236 to patients older than 40 years of age who are without significant co-morbidities [28]. The  
237 results of the studies included in the present systematic review appear to roughly match the  
238 recent estimates reported by others.

### 239 Recent therapeutic improvement

240 Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for  
241 IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

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242 ment of overall survival in the group of matched related donor transplants but not in the IST  
243 group [29]. Several factors may have contributed to recent improvements in HSCT, such as  
244 detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Con-  
245 ference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used  
246 as the source of stem cells and that the upper age limit should be 50 years and that the combi-  
247 nation of antithymocyte globulin and ciclosporin remains the gold standard for immunosup-  
248 pressive therapy [30]. Scheinberg 2012 provided an overview and update of various treatment  
249 options for severe aplastic anemia including immunosuppressive therapy and transplantation  
250 [31].

#### 251 **Strengths and limitations**

252 One of the strengths of this review is the broadness of the search strategy such that study  
253 retrieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled  
254 trials and prospective non-randomized controlled trials that were compatible with 'Mendelian  
255 Randomization' to avoid excess risk of bias. Nevertheless, the included data are too scarce and  
256 too biased to allow any conclusion on the comparative effectiveness of MSD-HSCT and IST.  
257 The rates of adverse events, such as treatment-related mortality, graft failure, no response to  
258 IST, and GVHD, are unusually high, which may be explained by the age of the studies (start-  
259 ing in 1976). We did not separate horse ATG from rabbit ATG, although the type of animal as  
260 the origin of ATG was reported as a serious effect modifier [6]. All data were collected about  
261 15 up to more than 30 years ago. Thus, the results may not be applicable to current modern  
262 standard care. Use of 'Mendelian randomization' is no guarantee that bias is minimized. This  
263 may be because tissue typing data may not be accurate. Patients may have only one sibling  
264 either in the donor or in the no donor group. Large families have a greater chance of finding a  
265 donor. Therefore, designing a non-randomized controlled trial by applying 'Mendelian ran-

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7 266 | domization' requires careful thought to effectively reduce bias and control for potential con-  
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9 267 | founders. There is a time lag in patients with siblings because tissue typing and readiness for  
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11 268 | assignment to treatment group may possibly take several months [12]. On the other hand, pa-  
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13 269 | tients with no siblings can be assigned immediately and are at earlier risk for adverse events.  
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15 270 | ~~and~~ Nitsch 2006 described the limits to causal inference based on 'Mendelian randomization'  
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17 271 | [32].  
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22 273 | **Conclusions**

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24 274 | There are insufficient and biased data that do not allow any firm conclusions to be made about  
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26 275 | the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the  
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28 276 | early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with  
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30 277 | IST should also be made aware that the disease may recur after initial successful treatment,  
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32 278 | and that life-threatening late clonal and malignant disease after IST may occur in a higher  
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34 279 | percentage compared to HSCT.  
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283 Group, Cologne, Germany, especially Nicole Skoetz for advice on the review. We thank the  
284 University of Cologne, Germany, for provision of fulltexts.

## 285 **Ethics statement**

286 An ethics statement was not required for this work.

## 287 **Financial Disclosure**

288 Provision of fulltexts by the University of Cologne, Germany. No funding bodies had any role  
289 in study design, data collection and analysis, decision to publish, or preparation of the manu-  
290 script.

## 291 **Conflict of Interest Statement**

292 No authors have any competing interests.

293

| Subject: *bmjopen-2014-005039-RI: SAA-MSD*

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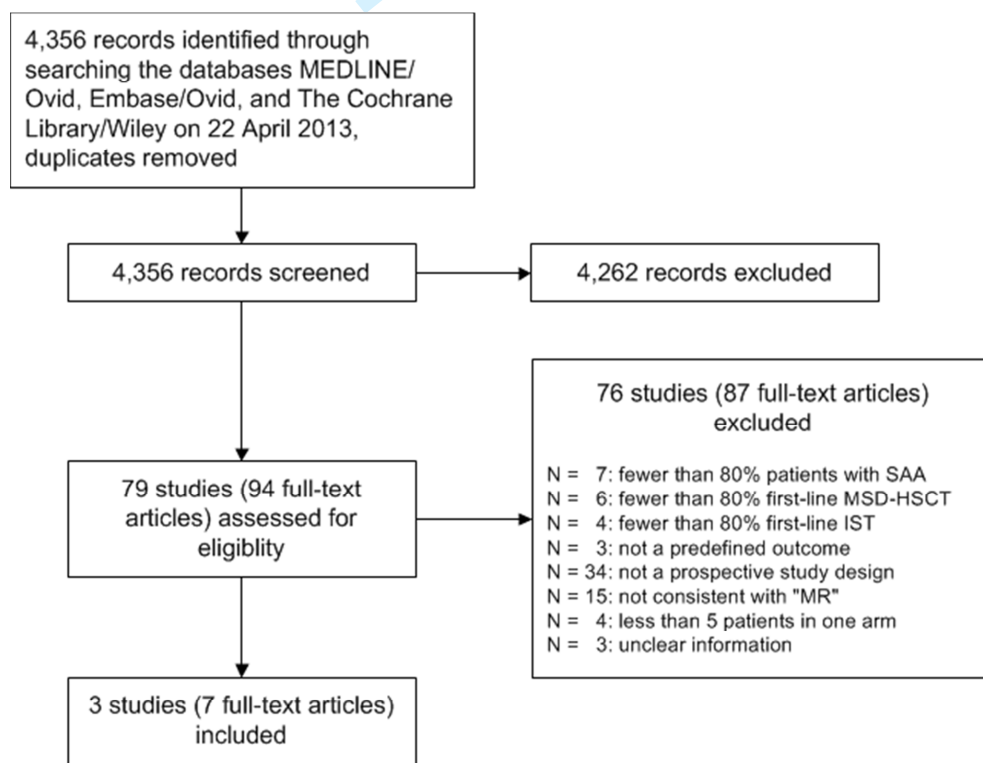
## 380 Figure legends

381 Figure 1. Study flow

382 Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic

383 hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors;

384 "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia



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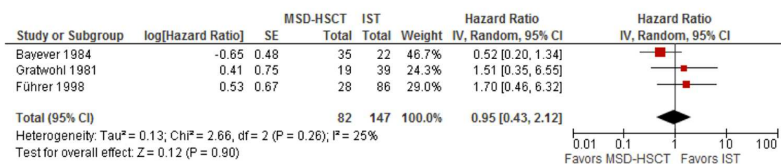
387 Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model.

388 Standard error calculated from data presented in the Kaplan-Meier graph of the article.

389 Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem

390 cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST:

391 first-line immunosuppressive therapy; IV: inverse variance; SE: standard error



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| Subject: *bmjopen-2014-005039-RI*: SAA-MSD

Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median follow up	Setting, center, country	Patients, no. <sup>1</sup>	Median age, years (range) <sup>1</sup>	Fraction of males, % <sup>1</sup>	Median interval, days <sup>1,2</sup>	Stem cell source	IST components	ATG source
Bayever 1984	1977 to 1982	N.R.	Single, United States	35 vs. 22	17 (2 to 24) <del>vs.</del> 15 (1 to 23)	67 vs. 68	60 vs. 58	bone marrow	ATG	horse
Führer 1998	1993 to 1997	N.R.	Multi, Germany, Austria	28 vs. 86	10.1 (2.3 to 15.8) <del>vs.</del> 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	N.R.	Single, Switzerland	19 vs. 13	18 (4 to 29) <del>vs.</del> 23 (7 to 37)	53 vs. 54	105 vs. 180	bone marrow	ATG Ciclosporin	N.R.

<sup>1</sup>MSD-HSCT vs. IST

<sup>2</sup>Median time interval between diagnosis and begin of treatment

Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Subject: *bmjopen-2014-005039-RI*: SAA-MSD

Table 2. Risk of bias of included studies

Study ID	Blinding of <del>assessment of overall mortality outcome assessment</del> <u>as-sessment of over-all mortality outcome assessment</u>	Incomplete outcome data	Selective reporting	Other bias	Comparable baseline characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	<del>High</del> Low	Low	Unclear	High <sup>1</sup>	Low	Low	High
Führer 1998	<del>High</del> Low	High	High <sup>2</sup>	High <sup>3</sup>	Low	Low	High
Gratwohl 1981	<del>High</del> Low	High	Unclear	Unclear	Low	Low	High

<sup>1</sup>Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".

<sup>2</sup>Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.

<sup>3</sup>Führer 1998: Financial support was provided by 2 pharmaceutical companies.

Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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Table 3. Overall survival

Study ID	MSD-HSCT		IST		FU <sup>1</sup> Year	P value
	N	OS (95% CI)	N	OS (95% CI)		
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69% <sup>2</sup> (N.R.)	5	0.56 <sup>3</sup>

<sup>1</sup>Time point of Kaplan-Meier estimate.

<sup>2</sup>Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line HSCT from the then again available MSD that was offered after the patients showed no response to IST.

<sup>3</sup>The P value was not reported and we calculated the P value using Fisher's exact test.

Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival

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| Subject: *bmjopen-2014-005039-RI*: SAA-MSD

Table 4. Secondary outcomes

Study ID	TRM after MSD-HSCT <sup>1,2</sup>	Graft failure after MSD-HSCT <sup>1</sup>	GVHD after MSD-HSCT <sup>1</sup>	No response to IST <sup>1</sup>	Relapse at 5 years after IST <sup>1</sup>
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

<sup>1</sup>In parenthesis: number of affected of number of evaluable patients

<sup>2</sup>Treatment-related mortality was not reported for IST.

Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

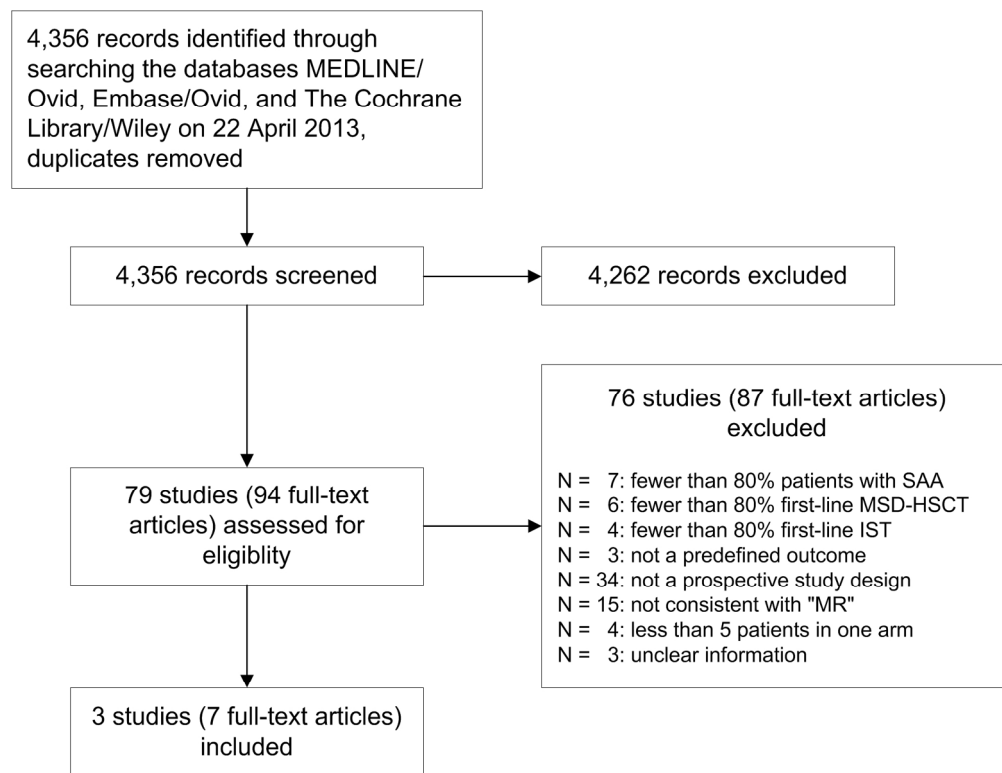


Figure 1. Study flow

Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia

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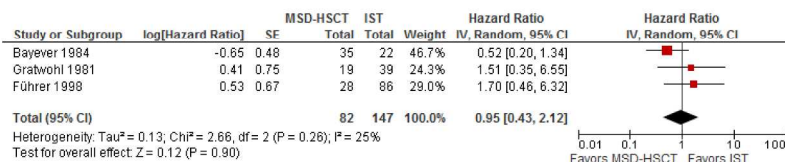


Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model. Standard error calculated from data presented in the Kaplan-Meier graph of the article. Ab-breviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error  
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# PRISMA 2009 Checklist

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Section/topic	#	Checklist item	Reported on page #
<b>TITLE</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
<b>ABSTRACT</b>			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
<b>INTRODUCTION</b>			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
<b>METHODS</b>			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	in the Cochrane review
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6 to 7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7





# PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$ ) for each meta-analysis.	7
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Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
<b>RESULTS</b>			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	in the Cochrane review
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8, table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8 to 9, table 3 to 4, figure 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	figure 1
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	table 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	none
<b>DISCUSSION</b>			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
<b>FUNDING</b>			



# PRISMA 2009 Checklist

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Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12
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*From:* Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: [www.prisma-statement.org](http://www.prisma-statement.org).

Page 2 of 2

For peer review only

# BMJ Open

## Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2014-005039.R2
Article Type:	Research
Date Submitted by the Author:	13-Jun-2014
Complete List of Authors:	Peinemann, Frank; University of Cologne, Children's Hospital Labeit, Alexander; University of Illinois College of Medicine, Center for Outcomes Research
<b>Primary Subject Heading</b>:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Paediatrics, Pharmacology and therapeutics
Keywords:	CHEMOTHERAPY, Anaemia < HAEMATOLOGY, Bone marrow transplantation < HAEMATOLOGY, Paediatric oncology < ONCOLOGY, STATISTICS & RESEARCH METHODS, TRANSPLANT MEDICINE

SCHOLARONE™  
Manuscripts

Subject: *bmjopen-2014-005039-R2: SAA-MSD*

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4 1 **Stem cell transplantation of matched sibling donors compared with immunosuppressive**  
5 2 **therapy for acquired severe aplastic anemia – a Cochrane Systematic Review\***

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8 3 Frank Peinemann,<sup>1†</sup> Alexander Labeit,<sup>2</sup>

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21 11 \*This article is based on a Cochrane Systematic Review published in the Cochrane Database  
22 12 of Systematic Reviews 2013, Issue 7. Art. No.: CD006407. DOI:

23 13 10.1002/14651858.CD006407.pub2. (see [www.thecochranelibrary.com](http://www.thecochranelibrary.com) for information).

24 14 Cochrane Systematic Reviews are regularly updated as new evidence emerges and in response  
25 15 to feedback, and the CDSR should be consulted for the most recent version of the review.

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28 16  
29 17 †Corresponding author: Frank Peinemann, M.D., M.Sc., Children's Hospital, University of  
30 18 Cologne, Kerpener Str. 62, 50937 Cologne, Germany; e-mail: [pubmedprjournal@gmail.com](mailto:pubmedprjournal@gmail.com).  
31 19 Phone: +49-176-31130745.

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37 20  
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39 21 **Keywords**

40 22 severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosup-  
41 23 pressive therapy, systematic review

Subject: *bmjopen-2014-005039-R2: SAA-MSD*

## 33 Abstract

34 Objectives: Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of  
35 this Cochrane review was to evaluate the effectiveness and adverse events of first-line  
36 allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors compared  
37 to first-line immunosuppressive therapy.

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39 Setting: Specialised stem cell transplantations units in primary care hospitals

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41 Participants: We included 302 participants with newly diagnosed acquired severe aplastic  
42 anemia. The age ranged from early childhood to young adulthood. We excluded studies on  
43 participants with secondary aplastic anemia.

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45 Interventions: We included allogeneic haematopoietic stem cell transplantation as the test  
46 intervention harvested from any source of matched sibling donor and serving as a first-line  
47 therapy. We included immunosuppressive therapy as comparator with either antithymocyte/-  
48 antilymphocyte globulin or ciclosporin or a combination of the two.

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50 Primary and secondary outcome measures planned and finally measured: The primary  
51 outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft  
52 failure, graft-versus-host disease, no response to immunosuppressive therapy, relapse after  
53 initial successful treatment, secondary clonal disease or malignancies, health-related quality  
54 of life, and performance scores.

55  
56 Results: We identified three prospective non-randomized controlled trials with a study design  
57 that was consistent with the principle of 'Mendelian randomization' in allocating patients to  
58 treatment groups. All studies had a high risk of bias due to the study design and were  
59 conducted more than 15 years. The pooled hazard ratio for overall mortality for the donor  
60 group versus the no donor group was 0.95 (95% confidence interval 0.43 to 2.12, P = 0.90).

61  
62 Conclusions: There are insufficient and biased data that do not allow any firm conclusions to  
63 be made about the comparative effectiveness of first-line allogeneic hematopoietic stem cell

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3 64 transplantation of HLA-matched sibling donors and first-line immunosuppressive therapy of  
4 65 patients with acquired severe aplastic anemia.

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10 68 **Strengths and limitations of this study**

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13 69 • We conducted a comprehensive literature search and strictly adhered to the projected  
14 70 methodology.

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16  
17 71 • We restricted the study design to randomized controlled trials and prospective non-  
18 72 randomized controlled trials and the studies had to be compatible with 'Mendelian  
19 73 Randomization' to avoid excess risk of bias.

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21  
22 74 • The included data are too scarce and too biased to allow any conclusion on the com-  
23 75 parative effectiveness of MSD-HSCT and IST.

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25  
26 76 • The included data were collected 15 to more than 30 years ago. Thus, the results may  
27 77 not be applicable to current modern standard care.

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## 81 Introduction

82 Acquired severe aplastic anemia (SAA) is a rare [1] and potentially fatal disease which is  
83 characterized by hypocellular bone marrow and pancytopenia; it mainly affects young adults.  
84 The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2].  
85 The underlying pathophysiology is thought to be an aberrant immune response involving the  
86 T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are  
87 severe infections, bleeding, and exhaustion and patients may experience paleness, weakness,  
88 fatigue, and shortness of breath.

89 According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of  
90 the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic  
91 stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen  
92 (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly  
93 diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the  
94 conditioning regimen may lead to severe non-hematological organ toxicities.

95 According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of  
96 the British Committee for Standards in Haematology [4], first-line immunosuppressive  
97 therapy (IST) is a combination of antithymocyte globulin (ATG) and ciclosporin. First-line  
98 IST is indicated for patients where no MSD is available, which can be expected for 70% of  
99 patients with SAA [3]. Ciclosporin is an immunosuppressant drug that is not lymphocytotoxic  
100 but has specific inhibitory effects on T-lymphocyte function [5]. In the past, antilymphocyte  
101 globulin (ALG) was reported interchangeably in the literature alongside ATG, therefore, ALG  
102 is reported in the present study on equal terms. ATG as well as ALG are polyclonal antibodies  
103 that recognize a variety of human lymphocyte cell surface antigens, reduce the number of

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3 104 lymphocytes and induce an immunosuppressive effect. They originate in animals immunized  
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6 105 with either normal human thymocytes, collected at pediatric cardiac surgery or thoracic duct  
7  
8 106 lymphocytes, collected during therapeutic cannulation [5]. Concerning the hematologic  
9  
10 107 response and the survival of patients after a first treatment for severe aplastic anemia, it may  
11  
12 108 be crucial in what type of animal ATG originates, as a randomized study showed that rabbit  
13  
14 109 ATG was inferior in this respect to horse ATG [6]. The currently recommended combination  
15  
16 110 of ciclosporin with ATG in the treatment of severe aplastic anemia is based on their separate  
17  
18 111 and potentially complementary modes of action[5]. Some patients do not respond well to IST  
19  
20 112 or show no response at all. Frequent transfusions increase the risk of adverse events such as  
21  
22 113 iron overload and early death. If a diagnosis of SAA is established at an early patient age,  
23  
24 114 then it is crucial to know which treatment promises more benefit and less harm in the long  
25  
26 115 run. We aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT  
27  
28 116 compared to IST in patients with SAA.  
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## 117 **Methods**

118 This article is based on a Cochrane Systematic Review published in The Cochrane Library  
119 [7]. Publication of this work is in agreement with the policy of The Cochrane Collaboration  
120 [8]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA  
121 statement, adhered to its principles and conformed to its checklist [9].

### 122 **Study inclusion criteria**

123 We included randomized controlled trials (RCTs) and prospective non-randomized controlled  
124 trials as long as the study design was consistent with the principle of 'Mendelian  
125 randomization' in allocating patients to treatment groups. We required a minimum of 80% of  
126 relevant patients per group and we set a minimum sample size of five participants per group.  
127 We set no limits on language, year of publication, or year of treatment. We included  
128 participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age  
129 limits for participants. We excluded studies on participants with secondary aplastic anemia.  
130 We included HSCT as the test intervention harvested from any source of MSD and serving as  
131 a first-line therapy [10]. That means, no other HSCT or IST has been offered to the patients  
132 before. We included IST as comparator with ciclosporin combined with ATG as the current  
133 mode of IST [10]. To accommodate also former modes of IST, we also included ciclosporin  
134 combined with ALG, ciclosporine alone, ATG alone, and ALG. Other agents such as  
135 corticosteroids and androgens were not considered. The primary outcome was overall  
136 mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-  
137 host disease, no response to IST, relapse after initial successful treatment, secondary clonal  
138 disease or malignancies, health-related quality of life, and performance scores.

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3 140 **Principle of 'Mendelian randomization'**  
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6 141 There are ethical concerns around randomization of patients with severe aplastic anemia to  
7  
8 142 transplantation versus non-transplantation. In general, MSD-HSCT is a life-threatening  
9  
10 143 treatment that can lead to early severe adverse events including death. Gray 1991 [11] and  
11  
12 144 Wheatley 2004 [12] described the potential of 'Mendelian randomization' to minimize bias  
13  
14 145 when comparing MSD-HSCT with an alternative therapy. The base concept has been ascribed  
15  
16 146 to Katan 1986 [13]. 'Mendelian randomization' means the view that nature itself has already  
17  
18 147 'randomized' the paternal and maternal part of a gene given that donor and recipient are  
19  
20 148 siblings. Thus, 'Mendelian randomization' by definition accepts only siblings as transplant  
21  
22 149 donors and these sibling donors are required to have 'identical' or matched features of specific  
23  
24 150 transplant-relevant HLA sites when compared with the transplant recipient. Therefore,  
25  
26 151 patients with an HLA-matched sibling will be allocated to the MSD-HSCT group. On the  
27  
28 152 other hand, patients with siblings that are not HLA compatible will be allocated to the  
29  
30 153 immunosuppressive therapy group. The term 'Mendelian randomization' refers to the fact that  
31  
32 154 the genetic distribution of paternal and maternal alleles follows a random process and is  
33  
34 155 determined before birth. This concept takes advantage of an instrumental variable for  
35  
36 156 allocating the patients to treatment groups and, at the same time, this variable is neither  
37  
38 157 associated with the treatment nor associated with the outcome.  
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45 158 **Search strategy and selection of studies**  
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48 159 We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ov-  
49  
50 160 id), and Cochrane Library CENTRAL (Wiley) including articles published from inception to  
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52 161 22 April 2013. The corresponding search strategies are depicted in the original Cochrane Re-  
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54 162 view [7]. We retrieved all titles and abstracts by electronic searching and downloaded them to  
55  
56 163 the reference management database EndNote Version X3 [14]. Two authors assessed the eli-  
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2  
3 164 gibility of retrieved papers independently. We considered studies written in languages other  
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5 165 than English. We judged studies to be prospective if an explicit statement was reported or  
6  
7 166 there were clues suggesting a prospective design (e.g. prior approval of treatment, informed  
8  
9 167 consent). We judged studies to be retrospective if an explicit statement was reported or it was  
10  
11 168 implied by description that data were reviewed from an existing source. We regarded each of  
12  
13 169 the following items as an indication of a retrospective design: registry reports and reviewing  
14  
15 170 of medical records. We judged studies as consistent with the principle of 'Mendelian randomi-  
16  
17 171 zation' if all transplant donors were clearly siblings and if the allocation of patients to treat-  
18  
19 172 ment groups was not based on age. We regarded studies as not consistent with the principle of  
20  
21 173 'Mendelian randomization' if age was not balanced between groups, indicating that age played  
22  
23 174 a role in the group assignment. Example for imbalance: distribution of age categories was  
24  
25 175 statistically not comparable (P value less than 0.05).  
26  
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### 30 **176 Assessment of risk of bias in included studies**

31  
32  
33 177 Two review authors independently assessed the risk of bias in the included studies using six  
34  
35 178 criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk  
36  
37 179 of bias [15]: blinding of outcome assessment, complete outcome data such as missing data,  
38  
39 180 selective reporting such as not reporting pre-specified outcomes, and other sources of bias  
40  
41 181 such as bias related to the specific study design and competing interest. We extended the  
42  
43 182 Cochrane tool for assessing risk of bias with two additional criteria that are specific to the  
44  
45 183 inclusion criteria for the present review and critical for confidence in results: comparable  
46  
47 184 baseline characteristics and concurrent control. We applied The Cochrane Collaboration's  
48  
49 185 criteria for judging risk of bias [16].  
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### 54 **186 Data synthesis**

55  
56  
57 187 One review author entered the data into Review Manager [17]. Another review author  
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2  
3 188 checked the entered data. We synthesized data on mortality for the donor group (MSD-HSCT)  
4  
5 189 versus the no donor group (IST) by using the hazard ratio (HR) for time-to-event data as the  
6  
7 190 primary effect measure with a random-effects model. If the hazard ratio was not directly given  
8  
9 191 in the publication, we estimated hazard ratios according to methods proposed by [18] and  
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11 192 [19].  
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## 193 **Results**

### 194 **Search results**

195 We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure**  
196 **1**). Bayever 1984 [20] and Gratwohl 1981 [21] reported their results in a single original arti-  
197 cle, respectively. Führer 1998 reported five publications including one original article [22], a  
198 follow up article [23], one protocol [24], and two abstracts [25 26]. We did not identify any  
199 RCTs.

### 200 **Characteristics of included articles**

201 The main study, patients and interventions characteristics are shown in **Table 1**. The patients  
202 were treated and observed between 1976 and 1997. Thus, the reported data were collected  
203 more than 15 years ago. Median follow up was not reported. Median age, fraction of males,  
204 and median days of time interval between diagnosis and begin of treatment were roughly  
205 comparable between the treatment groups within each study. The age ranged from early  
206 childhood to young adulthood. In the study by Führer 1998, all patients were less than 17  
207 years old by definition of the inclusion criteria [22]. Bone marrow was used as source for all  
208 transplants. All three included studies had a high risk of bias due to the study design (**Table**  
209 **2**). We judged a low risk of bias for blinding the assessment of overall mortality. Blinding or  
210 lack of blinding is not expected to make a difference concerning overall mortality. The au-  
211 thors of all included studies did not report that 'Mendelian randomization' was planned and  
212 the authors did not report the size of the involved families. The authors did not report the  
213 numbers of siblings and the results of the individual genetic analyses.

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3 214 **Effects of intervention**

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6 215 The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence inter-  
7  
8 216 val of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data  
9  
10 217 from all three included studies, overall mortality was not statistically significantly different  
11  
12 218 between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT  
13  
14 219 group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary out-  
15  
16 220 comes including treatment-related mortality after MSD-HSCT, graft failure after MSD-  
17  
18 221 HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse  
19  
20 222 after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies,  
21  
22 223 Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and  
23  
24 224 Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST.  
25  
26 225 Health-related quality of life questionnaires were not used in any of the included studies. Ba-  
27  
28 226 yever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and  
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30 227 less than half of the patients in the IST group had a Karnofsky Performance Status of higher  
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32 228 than 70%.

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## 229 **Discussion**

### 230 **Interpretation of main results**

231 We identified three prospective, non-randomized controlled trials [20-22] including 302 par-  
232 ticipants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found  
233 insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST.  
234 Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in  
235 the IST group and in a meta-analysis, overall mortality was not statistically significantly dif-  
236 ferent between the treatment groups. Treatment-related mortality in the MSD-HSCT group  
237 was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the  
238 death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplanted  
239 patients. More than half of patients in one study did not respond to IST. Relapse affected  
240 up to one in eight patients after IST in one study. Secondary clonal disease or malignancies  
241 were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone  
242 marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance  
243 of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of stud-  
244 ies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at  
245 three to five years [27]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT  
246 to patients older than 40 years of age who are without significant co-morbidities [28]. The  
247 results of the studies included in the present systematic review appear to roughly match the  
248 recent estimates reported by others.

### 249 **Recent therapeutic improvement**

250 Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for  
251 IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

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2  
3 252 ment of overall survival in the group of matched related donor transplants but not in the IST  
4  
5 253 group [29]. Several factors may have contributed to recent improvements in HSCT, such as  
6  
7 254 detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Con-  
8  
9 255 ference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used  
10  
11 256 as the source of stem cells and that the upper age limit should be 50 years and that the combi-  
12  
13 257 nation of antithymocyte globulin and ciclosporin remains the gold standard for immunosup-  
14  
15 258 pressive therapy [30]. Scheinberg 2012 provided an overview and update of various treatment  
16  
17 259 options for severe aplastic anemia including immunosuppressive therapy and transplantation  
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20  
21 260 [31].  
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23

### 24 261 **Strengths and limitations**

25  
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27 262 One of the strengths of this review is the broadness of the search strategy such that study re-  
28  
29 263 trieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled  
30  
31 264 trials and prospective non-randomized controlled trials that were compatible with 'Mendelian  
32  
33 265 Randomization' to avoid excess risk of bias. We assumed a possible 'Mendelian Randomiza-  
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35 266 tion' in the three included studies, though, the authors did not mention this approach and did  
36  
37 267 not report what proportion of patients with a matched sibling donor actually received the  
38  
39 268 transplant. Thus, crucial information is lacking to judge the compliance of patients and the  
40  
41 269 significance of the assumed concept of natural allocation. Nevertheless, the included data are  
42  
43 270 too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-  
44  
45 271 HSCT and IST. The rates of adverse events, such as treatment-related mortality, graft failure,  
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47 272 no response to IST, and GVHD, are unusually high, which may be explained by the age of the  
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49 273 studies (starting in 1976). We did not separate horse ATG from rabbit ATG, although the type  
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51 274 of animal as the origin of ATG was reported as a serious effect modifier [6]. All data were  
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53 275 collected about 15 up to more than 30 years ago. Thus, the results may not be applicable to  
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2  
3 276 current modern standard care. Use of 'Mendelian randomization' is no guarantee that bias is  
4  
5 277 minimized. This may be because tissue typing data may not be accurate. Patients may have  
6  
7 278 only one sibling either in the donor or in the no donor group. Large families have a greater  
8  
9 279 chance of finding a donor. Therefore, designing a non-randomized controlled trial by applying  
10  
11 280 'Mendelian randomization' requires careful thought to effectively reduce bias and control for  
12  
13 281 potential confounders. There is a time lag in patients with siblings because tissue typing and  
14  
15 282 readiness for assignment to treatment group may possibly take several months [12]. On the  
16  
17 283 other hand, patients with no siblings can be assigned immediately and are at earlier risk for  
18  
19 284 adverse events. Nitsch 2006 described the limits to causal inference based on 'Mendelian ran-  
20  
21 285 domization' [32].  
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## 26 **Conclusions**

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28  
29 287 There are insufficient and biased data that do not allow any firm conclusions to be made about  
30  
31 288 the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the  
32  
33 289 early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with  
34  
35 290 IST should also be made aware that the disease may recur after initial successful treatment,  
36  
37 291 and that life-threatening late clonal and malignant disease after IST may occur in a higher  
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39 292 percentage compared to HSCT.  
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9  
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11  
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13  
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15  
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18  
19 300 in study design, data collection and analysis, decision to publish, or preparation of the manu-  
20  
21 301 script.  
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28 303 **Contributorship Statement**

29  
30  
31  
32 304 FP: design, search strategy, study selection, data extraction, data analysis, writing the  
33 305 manuscript  
34 306  
35 307 AL: methodological perspective, reviewing the manuscript  
36

37 308 **Conflict of Interest Statement**

38  
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40  
41 309 No authors have any competing interests.  
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45 310 **Data Sharing Statement**

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48 311 No additional data available  
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52 313 **Ethics statement**

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55 314 An ethics statement was not required for this work.  
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## 404 **Figure legends**

405 Figure 1. Study flow

406 Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic  
407 hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors;  
408 "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia

409 Figure 2. Mortality in the donor group (MSD-HSCT) vs. the no donor group (IST); effect:  
410 hazard ratio; random-effects model.

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412 Standard error calculated from data presented in the Kaplan-Meier graph of the article.

413 Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem  
414 cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST:  
415 first-line immunosuppressive therapy; IV: inverse variance; SE: standard error

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## Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median FU	Setting, center, country	Patients, no. <sup>1</sup>	Median age, years (range) <sup>1</sup>	Fraction of males, % <sup>1</sup>	Median interval, days <sup>1,2</sup>	Stem cell source	IST components	ATG source
Bayever 1984	1977 to 1982	NR	Single, United States	35 vs. 22	17 (2 to 24) vs. 15 (1 to 23)	67 vs. 68	60 vs. 58	Bone marrow	ATG	horse
Führer 1998	1993 to 1997	NR	Multi, Germany, Austria	28 vs. 86	10.1 (2.3 to 15.8) vs. 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	Bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	NR	Single, Switzerland	19 vs. 13	18 (4 to 29) vs. 23 (7 to 37)	53 vs. 54	105 vs. 180	Bone marrow	ATG Ciclosporin	N.R.

<sup>1</sup>Donor group (MSD-HSCT) versus No donor group (IST)

<sup>2</sup>Median time interval between diagnosis and begin of treatment

Abbreviations. ATG: anti-thymocyte globulin; FU: follow-up; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; NR: not reported; no.: number

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Table 2. Risk of bias of included studies

Study ID	Blinding of assessment of overall mortality	Incomplete outcome data	Selective reporting	Other bias	Comparable baseline characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	Low	Low	Unclear	High <sup>1</sup>	Low	Low	High
Führer 1998	Low	High	High <sup>2</sup>	High <sup>3</sup>	Low	Low	High
Gratwohl 1981	Low	High	Unclear	Unclear	Low	Low	High

<sup>1</sup>Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".  
<sup>2</sup>Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.  
<sup>3</sup>Führer 1998: Financial support was provided by 2 pharmaceutical companies.  
Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number



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Table 3. Overall survival

Study ID	Donor group (MSD-HSCT)		No donor group (IST)		FU <sup>1</sup>	P value
	N	OS (95% CI)	N	OS (95% CI)	Year	
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69% <sup>2</sup> (N.R.)	5	0.56 <sup>3</sup>

<sup>1</sup>Time point of Kaplan-Meier estimate.

<sup>2</sup>Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line HSCT from the then again available MSD that was offered after the patients showed no response to IST.

<sup>3</sup>The P value was not reported and we calculated the P value using Fisher's exact test.

Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival

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Table 4. Secondary outcomes

Study ID	TRM after MSD-HSCT <sup>1,2</sup>	Graft failure after MSD-HSCT <sup>1</sup>	GVHD after MSD-HSCT <sup>1</sup>	No response to IST <sup>1</sup>	Relapse at 5 years after IST <sup>1</sup>
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

<sup>1</sup>In parenthesis: number of affected of number of evaluable patients

<sup>2</sup>Treatment-related mortality was not reported for IST.

Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

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| Subject: *bmjopen-2014-005039-~~R1~~R2*: SAA-MSD

1 **Stem cell transplantation of matched sibling donors compared with immunosuppressive**  
2 **therapy for acquired severe aplastic anemia – a Cochrane Systematic Review\***

3 Frank Peinemann,<sup>1†</sup> Alexander Labeit,<sup>2</sup>

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5 <sup>2</sup>Center for Outcomes Research, University of Illinois College of Medicine at Peoria, Illinois,  
6 USA

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8 FP: [pubmedprjournal@gmail.com](mailto:pubmedprjournal@gmail.com)

9 AL: [alabeit.publications@gmail.com](mailto:alabeit.publications@gmail.com)

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11 \*This article is based on a Cochrane Systematic Review published in the Cochrane Database  
12 of Systematic Reviews 2013, Issue 7. Art. No.: CD006407. DOI:  
13 10.1002/14651858.CD006407.pub2. (see [www.thecochranelibrary.com](http://www.thecochranelibrary.com) for information).

14 Cochrane Systematic Reviews are regularly updated as new evidence emerges and in response  
15 to feedback, and the CDSR should be consulted for the most recent version of the review.

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17 †Corresponding author: Frank Peinemann, M.D., M.Sc., Children's Hospital, University of  
18 Cologne, Kerpener Str. 62, 50937 Cologne, Germany; e-mail: [pubmedprjournal@gmail.com](mailto:pubmedprjournal@gmail.com).  
19 Phone: +49-176-31130745.

| Subject: *bmjopen-2014-005039-~~R1~~R2*: SAA-MSD

22 **Keywords**

23 severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosup-  
24 pressive therapy, systematic review

27 **Strengths and limitations of this study**

- 28 • We conducted a comprehensive literature search and strictly adhered to the projected  
29 methodology.
- 30 • We restricted the study design to randomized controlled trials and prospective non-  
31 randomized controlled trials and the studies had to be compatible with 'Mendelian  
32 Randomization' to avoid excess risk of bias.
- 33 • The included data are too scarce and too biased to allow any conclusion on the com-  
34 parative effectiveness of MSD-HSCT and IST.
- 35 • The included data were collected 15 to more than 30 years ago. Thus, the results may  
36 not be applicable to current modern standard care.

1 | *Subject: bmjopen-2014-005039-~~R1R2~~: SAA-MSD*

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4 | **Abstract**

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7 | 41 Background

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11 | 42 Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of this  
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13 | 43 Cochrane review was to evaluate the effectiveness and adverse events of first-line allogeneic  
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15 | 44 hematopoietic stem cell transplantation of HLA-matched sibling donors compared to first-line  
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17 | 45 immunosuppressive therapy.

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20 | 46 Procedure

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23 | 47 We searched the electronic databases MEDLINE (Ovid), EMBASE (Ovid), and The  
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25 | 48 Cochrane Library CENTRAL (Wiley) for published articles from 1946 to 22 April 2013. We  
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27 | 49 included randomized controlled trials and prospective non-randomized controlled trials as  
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29 | 50 long as the study design was consistent with the principle of 'Mendelian randomization' in  
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31 | 51 allocating patients to treatment groups.

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34 | 52 Results

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37 | 53 We identified three prospective non-randomized controlled trials with 302 participants. We  
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39 | 54 did not identify a randomized controlled trial. All studies had a high risk of bias due to the  
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41 | 55 study design and were conducted more than 15 years ago and may not be applicable to the  
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43 | 56 standard of care of today. The pooled hazard ratio for overall mortality for the ~~transplanted~~  
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45 | 57 donor group versus the ~~not-transplanted~~no donor group was 0.95 (95% confidence interval  
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47 | 58 0.43 to 2.12, P = 0.90).

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3 61 Conclusions

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8 63 the comparative effectiveness of first-line allogeneic hematopoietic stem cell transplantation  
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## 67 Introduction

68 Acquired severe aplastic anemia (SAA) is a rare [1] and potentially fatal disease which is  
69 characterized by hypocellular bone marrow and pancytopenia; it mainly affects young adults.

70 The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2].

71 The underlying pathophysiology is thought to be an aberrant immune response involving the

72 T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are

73 severe infections, bleeding, and exhaustion and patients may experience paleness, weakness,

74 fatigue, and shortness of breath.

75 According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of

76 the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic

77 stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen

78 (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly

79 diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the

80 conditioning regimen may lead to severe non-hematological organ toxicities.

81 According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of

82 the British Committee for Standards in Haematology [4], first-line immunosuppressive

83 therapy (IST) is a combination of antithymocyte globulin (ATG) and ciclosporin. First-line

84 IST is indicated for patients where no MSD is available, which can be expected for 70% of

85 patients with SAA [3]. Ciclosporin is an immunosuppressant drug that is not lymphocytotoxic

86 but has specific inhibitory effects on T-lymphocyte function [5]. In the past, antilymphocyte

87 globulin (ALG) was reported interchangeably in the literature alongside ATG, therefore, ALG

88 is reported in the present study on equal terms. ATG as well as ALG are polyclonal antibodies

89 that recognize a variety of human lymphocyte cell surface antigens, reduce the number of

90 lymphocytes and induce an immunosuppressive effect. They originate in animals immunized

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3 91 with either normal human thymocytes, collected at pediatric cardiac surgery or thoracic duct  
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5 92 lymphocytes, collected during therapeutic cannulation [5]. Concerning the hematologic  
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7 93 response and the survival of patients after a first treatment for severe aplastic anemia, it may  
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9 94 be crucial in what type of animal ATG originates, as a randomized study showed that rabbit  
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11 95 ATG was inferior in this respect to horse ATG [6]. The currently recommended combination  
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13 96 of ciclosporin with ATG in the treatment of severe aplastic anemia is based on their separate  
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15 97 and potentially complementary modes of action[5]. Some patients do not respond well to IST  
16  
17 98 or show no response at all. Frequent transfusions increase the risk of adverse events such as  
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19 99 iron overload and early death. If a diagnosis of SAA is established at an early patient age,  
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21 100 then it is crucial to know which treatment promises more benefit and less harm in the long  
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23 101 run. We aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT  
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25 102 compared to IST in patients with SAA.  
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## 103 **Methods**

104 This article is based on a Cochrane Systematic Review published in The Cochrane Library  
105 [7]. Publication of this work is in agreement with the policy of The Cochrane Collaboration  
106 [8]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA  
107 statement, adhered to its principles and conformed to its checklist [9].

### 108 **Study inclusion criteria**

109 We included randomized controlled trials (RCTs) and prospective non-randomized controlled  
110 trials as long as the study design was consistent with the principle of 'Mendelian  
111 randomization' in allocating patients to treatment groups. We required a minimum of 80% of  
112 relevant patients per group and we set a minimum sample size of five participants per group.  
113 We set no limits on language, year of publication, or year of treatment. We included  
114 participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age  
115 limits for participants. We excluded studies on participants with secondary aplastic anemia.  
116 We included HSCT as the test intervention harvested from any source of MSD and serving as  
117 a first-line therapy [10]. That means, no other HSCT or IST has been offered to the patients  
118 before. We included IST as comparator with ciclosporin combined with ATG as the current  
119 mode of IST [10]. To accommodate also former modes of IST, we also included ciclosporin  
120 combined with ALG, ciclosporine alone, ATG alone, and ALG. Other agents such as  
121 corticosteroids and androgens were not considered. The primary outcome was overall  
122 mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-  
123 host disease, no response to IST, relapse after initial successful treatment, secondary clonal  
124 disease or malignancies, health-related quality of life, and performance scores.

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## 126 **Principle of 'Mendelian randomization'**

127 There are ethical concerns around randomization of patients with severe aplastic anemia to  
128 transplantation versus non-transplantation. In general, MSD-HSCT is a life-threatening  
129 treatment that can lead to early severe adverse events including death. Gray 1991 [11] and  
130 Wheatley 2004 [12] described the potential of 'Mendelian randomization' to minimize bias  
131 when comparing MSD-HSCT with an alternative therapy. The base concept has been ascribed  
132 to Katan 1986 [13]. 'Mendelian randomization' means the view that nature itself has already  
133 'randomized' the paternal and maternal part of a gene given that donor and recipient are  
134 siblings. Thus, 'Mendelian randomization' by definition accepts only siblings as transplant  
135 donors and these sibling donors are required to have 'identical' or matched features of specific  
136 transplant-relevant HLA sites when compared with the transplant recipient. Therefore,  
137 patients with an HLA-matched sibling will be allocated to the MSD-HSCT group. On the  
138 other hand, patients with siblings that are not HLA compatible will be allocated to the  
139 immunosuppressive therapy group. The term 'Mendelian randomization' refers to the fact that  
140 the genetic distribution of paternal and maternal alleles follows a random process and is  
141 determined before birth. This concept takes advantage of an instrumental variable for  
142 allocating the patients to treatment groups and, at the same time, this variable is neither  
143 associated with the treatment nor associated with the outcome.

## 144 **Search strategy and selection of studies**

145 We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ov-  
146 id), and Cochrane Library CENTRAL (Wiley) including articles published from inception to  
147 22 April 2013. The corresponding search strategies are depicted in the original Cochrane Re-  
148 view [7]. We retrieved all titles and abstracts by electronic searching and downloaded them to  
149 the reference management database EndNote Version X3 [14]. Two authors assessed the eli-

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2  
3 150 gibility of retrieved papers independently. We considered studies written in languages other  
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5 151 than English. We judged studies to be prospective if an explicit statement was reported or  
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7 152 there were clues suggesting a prospective design (e.g. prior approval of treatment, informed  
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9 153 consent). We judged studies to be retrospective if an explicit statement was reported or it was  
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11 154 implied by description that data were reviewed from an existing source. We regarded each of  
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13 155 the following items as an indication of a retrospective design: registry reports and reviewing  
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15 156 of medical records. We judged studies as consistent with the principle of 'Mendelian randomi-  
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17 157 zation' if all transplant donors were clearly siblings and if the allocation of patients to treat-  
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19 158 ment groups was not based on age. We regarded studies as not consistent with the principle of  
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21 159 'Mendelian randomization' if age was not balanced between groups, indicating that age played  
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23 160 a role in the group assignment. Example for imbalance: distribution of age categories was  
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25 161 statistically not comparable (P value less than 0.05).  
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### 30 162 **Assessment of risk of bias in included studies**

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33 163 Two review authors independently assessed the risk of bias in the included studies using six  
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35 164 criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk  
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37 165 of bias [15]: blinding of outcome assessment, complete outcome data such as missing data,  
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39 166 selective reporting such as not reporting pre-specified outcomes, and other sources of bias  
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41 167 such as bias related to the specific study design and competing interest. We extended the  
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43 168 Cochrane tool for assessing risk of bias with two additional criteria that are specific to the  
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45 169 inclusion criteria for the present review and critical for confidence in results: comparable  
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47 170 baseline characteristics and concurrent control. We applied The Cochrane Collaboration's  
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49 171 criteria for judging risk of bias [16].  
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### 54 172 **Data synthesis**

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57 173 One review author entered the data into Review Manager [17]. Another review author  
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3 | 174 | checked the entered data. We synthesized data on mortality for the donor group (MSD-HSCT)  
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5 | 175 | versus the no donor group (IST) by using the hazard ratio (HR) for time-to-event data as the  
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7 | 176 | primary effect measure with a random-effects model. If the hazard ratio was not directly given  
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9 | 177 | in the publication, we estimated hazard ratios according to methods proposed by [18] and  
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## 179 Results

### 180 Search results

181 We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure**  
182 **1**). Bayever 1984 [20] and Gratwohl 1981 [21] reported their results in a single original arti-  
183 cle, respectively. Führer 1998 reported five publications including one original article [22], a  
184 follow up article [23], one protocol [24], and two abstracts [25 26]. We did not identify any  
185 RCTs.

### 186 Characteristics of included articles

187 The main study, patients and interventions characteristics are shown in **Table 1**. The patients  
188 were treated and observed between 1976 and 1997. Thus, the reported data were collected  
189 more than 15 years ago. Median follow up was not reported. Median age, fraction of males,  
190 and median days of time interval between diagnosis and begin of treatment were roughly  
191 comparable between the treatment groups within each study. The age ranged from early  
192 childhood to young adulthood. In the study by Führer 1998, all patients were less than 17  
193 years old by definition of the inclusion criteria [22]. Bone marrow was used as source for all  
194 transplants. All three included studies had a high risk of bias due to the study design (**Table**  
195 **2**). We judged a low risk of bias for blinding the assessment of overall mortality. Blinding or  
196 lack of blinding is not expected to make a difference concerning overall mortality. The au-  
197 thors of all included studies did not report that 'Mendelian randomization' was planned and  
198 the authors did not report the size of the involved families. The authors did not report the  
199 numbers of siblings and the results of the individual genetic analyses.

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200 **Effects of intervention**

201 The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence inter-  
202 val of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data  
203 from all three included studies, overall mortality was not statistically significantly different  
204 between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT  
205 group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary out-  
206 comes including treatment-related mortality after MSD-HSCT, graft failure after MSD-  
207 HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse  
208 after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies,  
209 Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and  
210 Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST.  
211 Health-related quality of life questionnaires were not used in any of the included studies. Ba-  
212 yever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and  
213 less than half of the patients in the IST group had a Karnofsky Performance Status of higher  
214 than 70%.

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## 215 **Discussion**

### 216 **Interpretation of main results**

217 We identified three prospective, non-randomized controlled trials [20-22] including 302 par-  
218 ticipants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found  
219 insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST.  
220 Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in  
221 the IST group and in a meta-analysis, overall mortality was not statistically significantly dif-  
222 ferent between the treatment groups. Treatment-related mortality in the MSD-HSCT group  
223 was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the  
224 death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplanted  
225 patients. More than half of patients in one study did not respond to IST. Relapse affected  
226 up to one in eight patients after IST in one study. Secondary clonal disease or malignancies  
227 were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone  
228 marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance  
229 of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of stud-  
230 ies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at  
231 three to five years [27]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT  
232 to patients older than 40 years of age who are without significant co-morbidities [28]. The  
233 results of the studies included in the present systematic review appear to roughly match the  
234 recent estimates reported by others.

### 235 **Recent therapeutic improvement**

236 Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for  
237 IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

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2  
3 238 ment of overall survival in the group of matched related donor transplants but not in the IST  
4  
5 239 group [29]. Several factors may have contributed to recent improvements in HSCT, such as  
6  
7 240 detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Con-  
8  
9 241 ference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used  
10  
11 242 as the source of stem cells and that the upper age limit should be 50 years and that the combi-  
12  
13 243 nation of antithymocyte globulin and ciclosporin remains the gold standard for immunosup-  
14  
15 244 pressive therapy [30]. Scheinberg 2012 provided an overview and update of various treatment  
16  
17 245 options for severe aplastic anemia including immunosuppressive therapy and transplantation  
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19 246 [31].  
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### 23 247 **Strengths and limitations**

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27 248 One of the strengths of this review is the broadness of the search strategy such that study re-  
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29 249 trieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled  
30  
31 250 trials and prospective non-randomized controlled trials that were compatible with 'Mendelian  
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33 251 Randomization' to avoid excess risk of bias. We assumed a possible 'Mendelian Randomiza-  
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35 252 tion' in the three included studies, though, the authors did not mention this approach and did  
36  
37 253 not report what proportion of patients with a matched sibling donor actually received the  
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39 254 transplant. Thus, crucial information is lacking to judge the compliance of patients and the  
40  
41 255 significance of the assumed concept of natural allocation. Nevertheless, the included data are  
42  
43 256 too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-  
44  
45 257 HSCT and IST. The rates of adverse events, such as treatment-related mortality, graft failure,  
46  
47 258 no response to IST, and GVHD, are unusually high, which may be explained by the age of the  
48  
49 259 studies (starting in 1976). We did not separate horse ATG from rabbit ATG, although the type  
50  
51 260 of animal as the origin of ATG was reported as a serious effect modifier [6]. All data were  
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53 261 collected about 15 up to more than 30 years ago. Thus, the results may not be applicable to  
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3 262 current modern standard care. Use of 'Mendelian randomization' is no guarantee that bias is  
4  
5 263 minimized. This may be because tissue typing data may not be accurate. Patients may have  
6  
7 264 only one sibling either in the donor or in the no donor group. Large families have a greater  
8  
9 265 chance of finding a donor. Therefore, designing a non-randomized controlled trial by applying  
10  
11 266 'Mendelian randomization' requires careful thought to effectively reduce bias and control for  
12  
13 267 potential confounders. There is a time lag in patients with siblings because tissue typing and  
14  
15 268 readiness for assignment to treatment group may possibly take several months [12]. On the  
16  
17 269 other hand, patients with no siblings can be assigned immediately and are at earlier risk for  
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19 270 adverse events. Nitsch 2006 described the limits to causal inference based on 'Mendelian ran-  
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21 271 domization' [32].  
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## 26 272 **Conclusions**

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29 273 There are insufficient and biased data that do not allow any firm conclusions to be made about  
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31 274 the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the  
32  
33 275 early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with  
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35 276 IST should also be made aware that the disease may recur after initial successful treatment,  
36  
37 277 and that life-threatening late clonal and malignant disease after IST may occur in a higher  
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39 278 percentage compared to HSCT.  
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282 Group, Cologne, Germany, especially Nicole Skoetz for advice on the review. We thank the  
283 University of Cologne, Germany, for provision of fulltexts.

## 284 **Ethics statement**

285 An ethics statement was not required for this work.

## 286 **Financial Disclosure**

287 Provision of fulltexts by the University of Cologne, Germany. No funding bodies had any role  
288 in study design, data collection and analysis, decision to publish, or preparation of the manu-  
289 script.

## 290 **Conflict of Interest Statement**

291 No authors have any competing interests.

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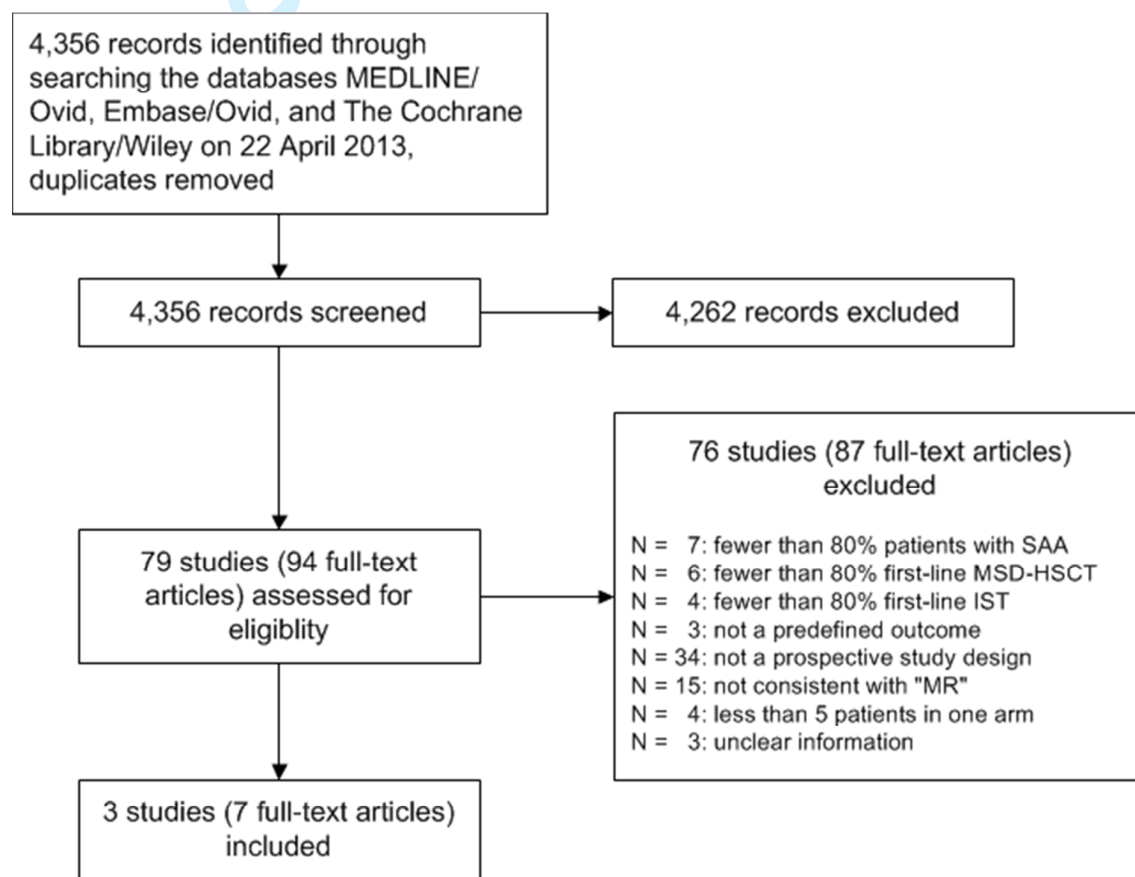
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## 379 Figure legends

380 Figure 1. Study flow

381 Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic  
 382 hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors;  
 383 "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia



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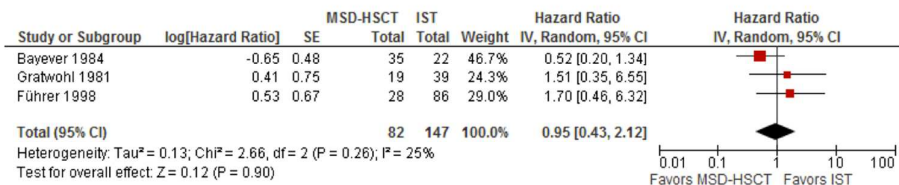
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386 Figure 2. Mortality in the donor group (MSD-HSCT) vs. the no donor group (IST); effect:  
 387 hazard ratio; random-effects model.

388 Standard error calculated from data presented in the Kaplan-Meier graph of the article.

389 Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem  
 390 cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST:  
 391 first-line immunosuppressive therapy; IV: inverse variance; SE: standard error



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### Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median FU	Setting, center, country	Patients, no. <sup>1</sup>	Median age, years (range) <sup>1</sup>	Fraction of males, % <sup>1</sup>	Median interval, days <sup>1,2</sup>	Stem cell source	IST components	ATG source
Bayever 1984	1977 to 1982	NR	Single, United States	35 vs. 22	17 (2 to 24) vs. 15 (1 to 23)	67 vs. 68	60 vs. 58	Bone marrow	ATG	horse
Führer 1998	1993 to 1997	NR	Multi, Germany, Austria	28 vs. 86	10.1 (2.3 to 15.8) vs. 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	Bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	NR	Single, Switzerland	19 vs. 13	18 (4 to 29) vs. 23 (7 to 37)	53 vs. 54	105 vs. 180	Bone marrow	ATG Ciclosporin	N.R.

<sup>1</sup> Donor group (MSD-HSCT) versus No donor group (IST)

<sup>2</sup>Median time interval between diagnosis and begin of treatment

Abbreviations. ATG: anti-thymocyte globulin; FU: follow-up; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; NR: not reported; no.: number



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3 | Subject: *bmjopen-2014-005039-~~R1R2~~*: SAA-MSD  
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5 Table 2. Risk of bias of included studies  
6

Study ID	Blinding of assessment of overall mortality	Incomplete outcome data	Selective reporting	Other bias	Comparable baseline characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	Low	Low	Unclear	High <sup>1</sup>	Low	Low	High
Führer 1998	Low	High	High <sup>2</sup>	High <sup>3</sup>	Low	Low	High
Gratwohl 1981	Low	High	Unclear	Unclear	Low	Low	High

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13 <sup>1</sup>Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".

14 <sup>2</sup>Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.

15  
16 <sup>3</sup>Führer 1998: Financial support was provided by 2 pharmaceutical companies.

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18 Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number  
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Table 3. Overall survival

Study ID	<u>Donor group (MSD-HSCT)</u>		<u>No donor group (IST)</u>		FU <sup>1</sup>	P value
	N	OS (95% CI)	N	OS (95% CI)	Year	
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69% <sup>2</sup> (N.R.)	5	0.56 <sup>3</sup>

<sup>1</sup>Time point of Kaplan-Meier estimate.

<sup>2</sup>Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line HSCT from the then again available MSD that was offered after the patients showed no response to IST.

<sup>3</sup>The P value was not reported and we calculated the P value using Fisher's exact test.

Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival

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| Subject: *bmjopen-2014-005039-~~R1R2~~*: SAA-MSD

Table 4. Secondary outcomes

Study ID	TRM after MSD-HSCT <sup>1,2</sup>	Graft failure after MSD-HSCT <sup>1</sup>	GVHD after MSD-HSCT <sup>1</sup>	No response to IST <sup>1</sup>	Relapse at 5 years after IST <sup>1</sup>
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

<sup>1</sup>In parenthesis: number of affected of number of evaluable patients

<sup>2</sup>Treatment-related mortality was not reported for IST.

Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

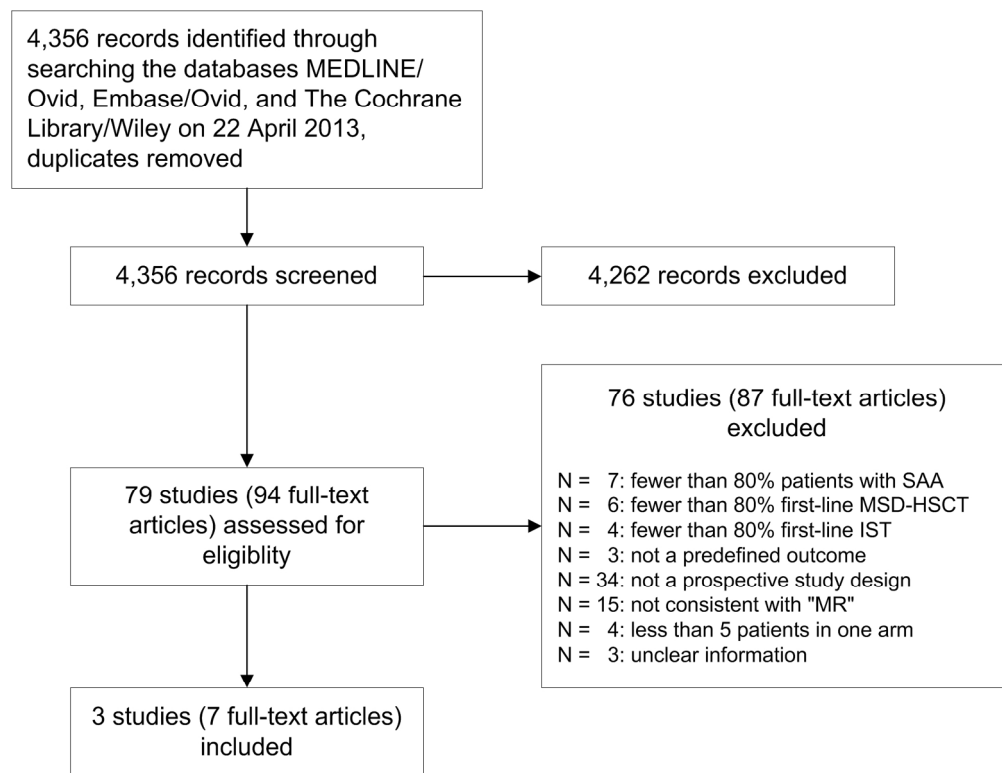


Figure 1. Study flow

Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia

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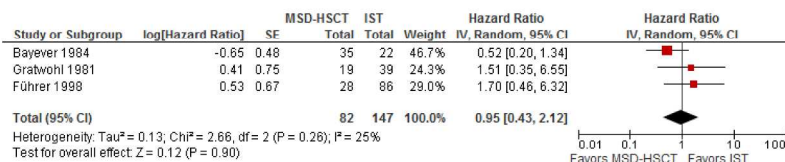


Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model. Standard error calculated from data presented in the Kaplan-Meier graph of the article. Ab-breviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error  
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# PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
<b>TITLE</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
<b>ABSTRACT</b>			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
<b>INTRODUCTION</b>			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
<b>METHODS</b>			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	in the Cochrane review
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6 to 7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7



# PRISMA 2009 Checklist

Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$ ) for each meta-analysis.	7
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Page 1 of 2

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
<b>RESULTS</b>			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	in the Cochrane review
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8, table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8 to 9, table 3 to 4, figure 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	figure 1
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	table 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	none
<b>DISCUSSION</b>			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
<b>FUNDING</b>			



# PRISMA 2009 Checklist

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Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12
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From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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Page 2 of 2

For peer review only



# BMJ Open

## Stem cell transplantation of matched sibling donors compared with immunosuppressive therapy for acquired severe aplastic anemia – a Cochrane Systematic Review

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2014-005039.R3
Article Type:	Research
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Complete List of Authors:	Peinemann, Frank; University of Cologne, Children's Hospital Labeit, Alexander; University of Illinois College of Medicine, Center for Outcomes Research
<b>Primary Subject Heading</b>:	Haematology (incl blood transfusion)
Secondary Subject Heading:	Paediatrics, Pharmacology and therapeutics
Keywords:	CHEMOTHERAPY, Anaemia < HAEMATOLOGY, Bone marrow transplantation < HAEMATOLOGY, Paediatric oncology < ONCOLOGY, STATISTICS & RESEARCH METHODS, TRANSPLANT MEDICINE

SCHOLARONE™  
Manuscripts

Subject: *bmjopen-2014-005039-R3: SAA-MSD*

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4 **1 Stem cell transplantation of matched sibling donors compared with immunosuppressive**  
5 **2 therapy for acquired severe aplastic anemia – a Cochrane Systematic Review\***

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8 Frank Peinemann,<sup>1†</sup> Alexander Labeit,<sup>2</sup>

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22 \*This article is based on a Cochrane Systematic Review published in the Cochrane Database  
23 of Systematic Reviews 2013, Issue 7. Art. No.: CD006407. DOI:

24  
25 10.1002/14651858.CD006407.pub2. (see [www.thecochranelibrary.com](http://www.thecochranelibrary.com) for information).

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Cochrane Systematic Reviews are regularly updated as new evidence emerges and in response  
15 to feedback, and the CDSR should be consulted for the most recent version of the review.

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1 *Subject: bmjopen-2014-005039-R3: SAA-MSD*

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3 **22 Keywords**

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6 23 severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosup-  
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8 24 pressive therapy, systematic review

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Subject: *bmjopen-2014-005039-R3: SAA-MSD*

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4 29 **Abstract**  
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8 30 Objectives: Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of  
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10 31 this Cochrane review was to evaluate the effectiveness and adverse events of first-line  
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12 32 allogeneic hematopoietic stem cell transplantation of HLA-matched sibling donors compared  
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14 33 to first-line immunosuppressive therapy.  
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17 34 Setting: Specialised stem cell transplantations units in primary care hospitals  
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20 35 Participants: We included 302 participants with newly diagnosed acquired severe aplastic  
21  
22 36 anemia. The age ranged from early childhood to young adulthood. We excluded studies on  
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24 37 participants with secondary aplastic anemia.  
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28 38 Interventions: We included allogeneic haematopoietic stem cell transplantation as the test  
29  
30 39 intervention harvested from any source of matched sibling donor and serving as a first-line  
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32 40 therapy. We included immunosuppressive therapy as comparator with either antithymocyte/-  
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34 41 antilymphocyte globulin or ciclosporin or a combination of the two.  
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38 42 Primary and secondary outcome measures planned and finally measured: The primary  
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40 43 outcome was overall mortality. Secondary outcomes were treatment-related mortality, graft  
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42 44 failure, graft-versus-host disease, no response to immunosuppressive therapy, relapse after  
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44 45 initial successful treatment, secondary clonal disease or malignancies, health-related quality  
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46 46 of life, and performance scores.  
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50 47 Results: We identified three prospective non-randomized controlled trials with a study design  
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52 48 that was consistent with the principle of 'Mendelian randomization' in allocating patients to  
53  
54 49 treatment groups. All studies had a high risk of bias due to the study design and were  
55  
56 50 conducted more than 15 years. The pooled hazard ratio for overall mortality for the donor  
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1 *Subject: bmjopen-2014-005039-R3: SAA-MSD*

2  
3 51 group versus the no donor group was 0.95 (95% confidence interval 0.43 to 2.12, P = 0.90).

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6 52 Conclusions: There are insufficient and biased data that do not allow any firm conclusions to  
7  
8 53 be made about the comparative effectiveness of first-line allogeneic hematopoietic stem cell  
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10 54 transplantation of HLA-matched sibling donors and first-line immunosuppressive therapy of  
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12 55 patients with acquired severe aplastic anemia.  
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17 57 **Strengths and limitations of this study**

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21 58 • We conducted a comprehensive literature search and strictly adhered to the projected  
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23 59 methodology.  
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25 60 • We restricted the study design to randomized controlled trials and prospective non-  
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27 61 randomized controlled trials and the studies had to be compatible with 'Mendelian  
28  
29 62 Randomization' to avoid excess risk of bias.  
30  
31 63 • The included data are too scarce and too biased to allow any conclusion on the com-  
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33 64 parative effectiveness of MSD-HSCT and IST.  
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35 65 • The included data were collected 15 to more than 30 years ago. Thus, the results may  
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37 66 not be applicable to current modern standard care.  
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Subject: *bmjopen-2014-005039-R3: SAA-MSD*

## 68 Introduction

69 Acquired severe aplastic anemia (SAA) is a rare [1] and potentially fatal disease which is  
70 characterized by hypocellular bone marrow and pancytopenia; it mainly affects young adults.

71 The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2].

72 The underlying pathophysiology is thought to be an aberrant immune response involving the

73 T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are  
74 severe infections, bleeding, and exhaustion and patients may experience paleness, weakness,  
75 fatigue, and shortness of breath.

76 According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of  
77 the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic  
78 stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen  
79 (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly  
80 diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the  
81 conditioning regimen may lead to severe non-hematological organ toxicities.

82 According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of  
83 the British Committee for Standards in Haematology [4], first-line immunosuppressive  
84 therapy (IST) is a combination of antithymocyte globulin (ATG) and ciclosporin. First-line  
85 IST is indicated for patients where no MSD is available, which can be expected for 70% of  
86 patients with SAA [3]. Ciclosporin is an immunosuppressant drug that is not lymphocytotoxic  
87 but has specific inhibitory effects on T-lymphocyte function [5]. In the past, antilymphocyte  
88 globulin (ALG) was reported interchangeably in the literature alongside ATG, therefore, ALG  
89 is reported in the present study on equal terms. ATG as well as ALG are polyclonal antibodies  
90 that recognize a variety of human lymphocyte cell surface antigens, reduce the number of  
91 lymphocytes and induce an immunosuppressive effect. They originate in animals immunized

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2  
3 92 with either normal human thymocytes, collected at pediatric cardiac surgery or thoracic duct  
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5 93 lymphocytes, collected during therapeutic cannulation [5]. Concerning the hematologic  
6  
7 94 response and the survival of patients after a first treatment for severe aplastic anemia, it may  
8  
9 95 be crucial in what type of animal ATG originates, as a randomized study showed that rabbit  
10  
11 96 ATG was inferior in this respect to horse ATG [6]. The currently recommended combination  
12  
13 97 of ciclosporin with ATG in the treatment of severe aplastic anemia is based on their separate  
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15 98 and potentially complementary modes of action[5]. Some patients do not respond well to IST  
16  
17 99 or show no response at all. Frequent transfusions increase the risk of adverse events such as  
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20 100 iron overload and early death. If a diagnosis of SAA is established at an early patient age,  
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22 101 then it is crucial to know which treatment promises more benefit and less harm in the long  
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24 102 run. We aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT  
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27 103 compared to IST in patients with SAA.  
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3 104 **Methods**

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7 105 This article is based on a Cochrane Systematic Review published in The Cochrane Library  
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9 106 [7]. Publication of this work is in agreement with the policy of The Cochrane Collaboration  
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11 107 [8]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA  
12  
13 108 statement, adhered to its principles and conformed to its checklist [9].  
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17 109 **Study inclusion criteria**

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20 110 We included randomized controlled trials (RCTs) and prospective non-randomized controlled  
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22 111 trials as long as the study design was consistent with the principle of 'Mendelian  
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24 112 randomization' in allocating patients to treatment groups. We required a minimum of 80% of  
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26 113 relevant patients per group and we set a minimum sample size of five participants per group.  
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28 114 We set no limits on language, year of publication, or year of treatment. We included  
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30 115 participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age  
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32 116 limits for participants. We excluded studies on participants with secondary aplastic anemia.  
33  
34 117 We included HSCT as the test intervention harvested from any source of MSD and serving as  
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36 118 a first-line therapy [10]. That means, no other HSCT or IST has been offered to the patients  
37  
38 119 before. We included IST as comparator with ciclosporin combined with ATG as the current  
39  
40 120 mode of IST [10]. To accommodate also former modes of IST, we also included ciclosporin  
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42 121 combined with ALG, cyclosporine alone, ATG alone, and ALG. Other agents such as  
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44 122 corticosteroids and androgens were not considered. The primary outcome was overall  
45  
46 123 mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-  
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48 124 host disease, no response to IST, relapse after initial successful treatment, secondary clonal  
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50 125 disease or malignancies, health-related quality of life, and performance scores.  
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1 *Subject: bmjopen-2014-005039-R3: SAA-MSD*

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3 **126 Principle of 'Mendelian randomization'**

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6 127 There are ethical concerns around randomization of patients with severe aplastic anemia to  
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8 128 transplantation versus non-transplantation because the risk of early death is expected to be  
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10 129 higher in the transplantation group than in the non-transplantation group. The reason is the  
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12 130 potentially life-threatening graft-versus-host disease occurring only in the transplanted  
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14 131 patients. Gray 1991 [11] and Wheatley 2004 [12] described the potential of 'Mendelian  
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16 132 randomization' to minimize bias when comparing MSD-HSCT with an alternative therapy.  
17  
18 133 The base concept has been ascribed to Katan 1986 [13]. 'Mendelian randomization' means the  
19  
20 134 view that nature itself has already 'randomized' the paternal and maternal part of a gene given  
21  
22 135 that donor and recipient are siblings. Thus, 'Mendelian randomization' by definition accepts  
23  
24 136 only siblings as transplant donors and these sibling donors are required to have 'identical' or  
25  
26 137 matched features of specific transplant-relevant HLA sites when compared with the transplant  
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28 138 recipient. Therefore, patients with an HLA-matched sibling will be allocated to the MSD-  
29  
30 139 HSCT group. On the other hand, patients with siblings that are not HLA compatible will be  
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32 140 allocated to the immunosuppressive therapy group. The term 'Mendelian randomization' refers  
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34 141 to the fact that the genetic distribution of paternal and maternal alleles follows a random  
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36 142 process and is determined before birth. This concept takes advantage of an instrumental  
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38 143 variable for allocating the patients to treatment groups and, at the same time, this variable is  
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40 144 neither associated with the treatment nor associated with the outcome.  
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47 **145 Search strategy and selection of studies**

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50 146 We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ov-  
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52 147 id), and Cochrane Library CENTRAL (Wiley) including articles published from inception to  
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54 148 22 April 2013. The corresponding search strategies are depicted in the original Cochrane Re-  
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56 149 view [7]. We retrieved all titles and abstracts by electronic searching and downloaded them to  
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3 150 the reference management database EndNote Version X3 [14]. Two authors assessed the eli-  
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5 151 gibility of retrieved papers independently. We considered studies written in languages other  
6  
7 152 than English. We judged studies to be prospective if an explicit statement was reported or  
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9 153 there were clues suggesting a prospective design (e.g. prior approval of treatment, informed  
10  
11 154 consent). We judged studies to be retrospective if an explicit statement was reported or it was  
12  
13 155 implied by description that data were reviewed from an existing source. We regarded each of  
14  
15 156 the following items as an indication of a retrospective design: registry reports and reviewing  
16  
17 157 of medical records. We judged studies as consistent with the principle of 'Mendelian randomi-  
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19 158 zation' if all transplant donors were clearly siblings and if the allocation of patients to treat-  
20  
21 159 ment groups was not based on age. We regarded studies as not consistent with the principle of  
22  
23 160 'Mendelian randomization' if age was not balanced between groups, indicating that age played  
24  
25 161 a role in the group assignment. Example for imbalance: distribution of age categories was  
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27 162 statistically not comparable (P value less than 0.05).  
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### 32 **Assessment of risk of bias in included studies**

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36 164 Two review authors independently assessed the risk of bias in the included studies using six  
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38 165 criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk  
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40 166 of bias [15]: blinding of outcome assessment, complete outcome data such as missing data,  
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42 167 selective reporting such as not reporting pre-specified outcomes, and other sources of bias  
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44 168 such as bias related to the specific study design and competing interest. We extended the  
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46 169 Cochrane tool for assessing risk of bias with two additional criteria that are specific to the  
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48 170 inclusion criteria for the present review and critical for confidence in results: comparable  
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50 171 baseline characteristics and concurrent control. We applied The Cochrane Collaboration's  
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52 172 criteria for judging risk of bias [16].  
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1 *Subject: bmjopen-2014-005039-R3: SAA-MSD*

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3 **173 Data synthesis**

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6 174 One review author entered the data into Review Manager [17]. Another review author  
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8 175 checked the entered data. We synthesized data on mortality for the donor group (MSD-HSCT)  
9  
10 176 versus the no donor group (IST) by using the hazard ratio (HR) for time-to-event data as the  
11  
12 177 primary effect measure with a random-effects model. If the hazard ratio was not directly given  
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14 178 in the publication, we estimated hazard ratios according to methods proposed by [18] and  
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16 179 [19].  
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1 *Subject: bmjopen-2014-005039-R3: SAA-MSD*

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3 180 **Results**

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7 181 **Search results**

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10 182 We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure**  
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12 183 **1**). Bayever 1984 [20] and Gratwohl 1981 [21] reported their results in a single original arti-  
13  
14 184 cle, respectively. Führer 1998 reported five publications including one original article [22], a  
15  
16 185 follow up article [23], one protocol [24], and two abstracts [25 26]. We did not identify any  
17  
18 186 RCTs.

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22 187 **Characteristics of included articles**

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24  
25 188 The main study, patients and interventions characteristics are shown in **Table 1**. The patients  
26  
27 189 were treated and observed between 1976 and 1997. Thus, the reported data were collected  
28  
29 190 more than 15 years ago. Median follow up was not reported. Median age, fraction of males,  
30  
31 191 and median days of time interval between diagnosis and begin of treatment were roughly  
32  
33 192 comparable between the treatment groups within each study. The age ranged from early  
34  
35 193 childhood to young adulthood. In the study by Führer 1998, all patients were less than 17  
36  
37 194 years old by definition of the inclusion criteria [22]. Bone marrow was used as source for all  
38  
39 195 transplants. All three included studies had a high risk of bias due to the study design (**Table**  
40  
41 196 **2**). We judged a low risk of bias for blinding the assessment of overall mortality. Blinding or  
42  
43 197 lack of blinding is not expected to make a difference concerning overall mortality. The au-  
44  
45 198 thors of all included studies did not report that 'Mendelian randomization' was planned and  
46  
47 199 the authors did not report the size of the involved families. The authors did not report the  
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49 200 numbers of siblings and the results of the individual genetic analyses.  
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Subject: *bmjopen-2014-005039-R3: SAA-MSD*

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3 201 **Effects of intervention**  
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6 202 The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence inter-  
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8 203 val of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data  
9  
10 204 from all three included studies, overall mortality was not statistically significantly different  
11  
12 205 between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT  
13  
14 206 group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary out-  
15  
16 207 comes including treatment-related mortality after MSD-HSCT, graft failure after MSD-  
17  
18 208 HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse  
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20 209 after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies,  
21  
22 210 Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and  
23  
24 211 Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST.  
25  
26 212 Health-related quality of life questionnaires were not used in any of the included studies. Ba-  
27  
28 213 yever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and  
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30 214 less than half of the patients in the IST group had a Karnofsky Performance Status of higher  
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32 215 than 70%.  
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## 216 Discussion

### 217 Interpretation of main results

218 We identified three prospective, non-randomized controlled trials [20-22] including 302 par-  
219 ticipants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found  
220 insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST.  
221 Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in  
222 the IST group and in a meta-analysis, overall mortality was not statistically significantly dif-  
223 ferent between the treatment groups. Treatment-related mortality in the MSD-HSCT group  
224 was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the  
225 death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplanted  
226 patients. More than half of patients in one study did not respond to IST. Relapse affected  
227 up to one in eight patients after IST in one study. Secondary clonal disease or malignancies  
228 were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone  
229 marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance  
230 of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of stud-  
231 ies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at  
232 three to five years [27]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT  
233 to patients older than 40 years of age who are without significant co-morbidities [28]. The  
234 results of the studies included in the present systematic review appear to roughly match the  
235 recent estimates reported by others.

### 236 Recent therapeutic improvement

237 Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for  
238 IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

1 *Subject: bmjopen-2014-005039-R3: SAA-MSD*

2  
3 239 ment of overall survival in the group of matched related donor transplants but not in the IST  
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5 240 group [29]. Several factors may have contributed to recent improvements in HSCT, such as  
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7 241 detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Con-  
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9 242 ference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used  
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11 243 as the source of stem cells and that the upper age limit should be 50 years and that the combi-  
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13 244 nation of antithymocyte globulin and ciclosporin remains the gold standard for immunosup-  
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15 245 pressive therapy [30]. Scheinberg 2012 provided an overview and update of various treatment  
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17 246 options for severe aplastic anemia including immunosuppressive therapy and transplantation  
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21 247 [31].  
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### 23 24 248 **Strengths and limitations**

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27 249 One of the strengths of this review is the broadness of the search strategy such that study re-  
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29 250 trieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled  
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31 251 trials and prospective non-randomized controlled trials that were compatible with 'Mendelian  
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33 252 Randomization' to avoid excess risk of bias. We assumed a possible 'Mendelian Randomiza-  
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35 253 tion' in the three included studies, though, the authors did not mention this approach and did  
36  
37 254 not report what proportion of patients with a matched sibling donor actually received the  
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39 255 transplant. Thus, crucial information is lacking to judge the compliance of patients and the  
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41 256 significance of the assumed concept of natural allocation. Nevertheless, the included data are  
42  
43 257 too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-  
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45 258 HSCT and IST. The rates of adverse events, such as treatment-related mortality, graft failure,  
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47 259 no response to IST, and GVHD, are unusually high, which may be explained by the age of the  
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49 260 studies (starting in 1976). We did not separate horse ATG from rabbit ATG, although the type  
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51 261 of animal as the origin of ATG was reported as a serious effect modifier [6]. All data were  
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53 262 collected about 15 up to more than 30 years ago. Thus, the results may not be applicable to  
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3 263 current modern standard care. Use of 'Mendelian randomization' is no guarantee that bias is  
4  
5 264 minimized. This may be because tissue typing data may not be accurate. Patients may have  
6  
7 265 only one sibling either in the donor or in the no donor group. Large families have a greater  
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9 266 chance of finding a donor. Therefore, designing a non-randomized controlled trial by applying  
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11 267 'Mendelian randomization' requires careful thought to effectively reduce bias and control for  
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13 268 potential confounders. There is a time lag in patients with siblings because tissue typing and  
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15 269 readiness for assignment to treatment group may possibly take several months [12]. On the  
16  
17 270 other hand, patients with no siblings can be assigned immediately and are at earlier risk for  
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19 271 adverse events. Nitsch 2006 described the limits to causal inference based on 'Mendelian ran-  
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21 272 domization' [32].  
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## 26 **Conclusions**

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29 274 There are insufficient and biased data that do not allow any firm conclusions to be made about  
30  
31 275 the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the  
32  
33 276 early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with  
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35 277 IST should also be made aware that the disease may recur after initial successful treatment,  
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37 278 and that life-threatening late clonal and malignant disease after IST may occur in a higher  
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39 279 percentage compared to HSCT.  
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8  
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10  
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14 **285 Ethics statement**

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18 286 An ethics statement was not required for this work.

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21 **287 Financial Disclosure**

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26  
27 289 in study design, data collection and analysis, decision to publish, or preparation of the manu-  
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29 290 script.

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33 **291 Conflict of Interest Statement**

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37 292 No authors have any competing interests.

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40 **293 Contributorship Statement**

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44 294 FP: design, search strategy, study selection, data extraction, data analysis, writing the  
45 295 manuscript  
46 296 AL: methodological perspective, reviewing the manuscript  
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51 **298 Data Sharing Statement**

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55 299 No additional data available.

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## Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median FU	Setting, center, country	Patients, no. <sup>1</sup>	Median age, years (range) <sup>1</sup>	Fraction of males, % <sup>1</sup>	Median interval, days <sup>1,2</sup>	Stem cell source	IST components	ATG source
Bayever 1984	1977 to 1982	NR	Single, United States	35 vs. 22	17 (2 to 24) vs. 15 (1 to 23)	67 vs. 68	60 vs. 58	Bone marrow	ATG	horse
Führer 1998	1993 to 1997	NR	Multi, Germany, Austria	28 vs. 86	10.1 (2.3 to 15.8) vs. 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	Bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	NR	Single, Switzerland	19 vs. 13	18 (4 to 29) vs. 23 (7 to 37)	53 vs. 54	105 vs. 180	Bone marrow	ATG Ciclosporin	N.R.

<sup>1</sup>Donor group (MSD-HSCT) versus No donor group (IST)

<sup>2</sup>Median time interval between diagnosis and begin of treatment

Abbreviations. ATG: anti-thymocyte globulin; FU: follow-up; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; NR: not reported; no.: number

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Table 2. Risk of bias of included studies

Study ID	Blinding of assessment of overall mortality	Incomplete outcome data	Selective reporting	Other bias	Comparable baseline characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	Low	Low	Unclear	High <sup>1</sup>	Low	Low	High
Führer 1998	Low	High	High <sup>2</sup>	High <sup>3</sup>	Low	Low	High
Gratwohl 1981	Low	High	Unclear	Unclear	Low	Low	High

<sup>1</sup>Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".

<sup>2</sup>Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.

<sup>3</sup>Führer 1998: Financial support was provided by 2 pharmaceutical companies.

Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number

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5 Table 3. Overall survival  
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Study ID	Donor group (MSD-HSCT)		No donor group (IST)		FU <sup>1</sup>	P value
	N	OS (95% CI)	N	OS (95% CI)	Year	
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69% <sup>2</sup> (N.R.)	5	0.56 <sup>3</sup>

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12 <sup>1</sup>Time point of Kaplan-Meier estimate.

13 <sup>2</sup>Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line  
14 HSCT from the then again available MSD that was offered after the patients showed no response to IST.

15 <sup>3</sup>The P value was not reported and we calculated the P value using Fisher's exact test.

16 Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT:  
17 first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival  
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Table 4. Secondary outcomes

Study ID	TRM after MSD-HSCT <sup>1,2</sup>	Graft failure after MSD-HSCT <sup>1</sup>	GVHD after MSD-HSCT <sup>1</sup>	No response to IST <sup>1</sup>	Relapse at 5 years after IST <sup>1</sup>
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

<sup>1</sup>In parenthesis: number of affected of number of evaluable patients

<sup>2</sup>Treatment-related mortality was not reported for IST.

Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

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## 6 **Figure legends** 7

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10 Figure 1. Study flow

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13 Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone  
14 marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia  
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21 Figure 2. Mortality in the donor group (MSD-HSCT) vs. the no donor group (IST); effect: hazard ratio; random-effects model.

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23 Standard error calculated from data presented in the Kaplan-Meier graph of the article. Abbreviations: CI: confidence interval; MSD-HSCT:  
24 first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line  
25 immunosuppressive therapy; IV: inverse variance; SE: standard error  
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| Subject: *bmjopen-2014-005039-R2R3*: SAA-MSD

1 **Stem cell transplantation of matched sibling donors compared with immunosuppressive**  
2 **therapy for acquired severe aplastic anemia – a Cochrane Systematic Review\***

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11 \*This article is based on a Cochrane Systematic Review published in the Cochrane Database  
12 of Systematic Reviews 2013, Issue 7. Art. No.: CD006407. DOI:

13 10.1002/14651858.CD006407.pub2. (see [www.thecochranelibrary.com](http://www.thecochranelibrary.com) for information).

14 Cochrane Systematic Reviews are regularly updated as new evidence emerges and in response  
15 to feedback, and the CDSR should be consulted for the most recent version of the review.

16  
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19 Phone: +49-176-31130745.

| Subject: *bmjopen-2014-005039-R2R3*: SAA-MSD

22 **Keywords**

23 severe aplastic anemia, stem cell transplantation, bone marrow transplantation, immunosup-  
24 pressive therapy, systematic review

27 **Strengths and limitations of this study**

- 28 • We conducted a comprehensive literature search and strictly adhered to the projected  
29 methodology.
- 30 • We restricted the study design to randomized controlled trials and prospective non-  
31 randomized controlled trials and the studies had to be compatible with 'Mendelian  
32 Randomization' to avoid excess risk of bias.
- 33 • The included data are too scarce and too biased to allow any conclusion on the com-  
34 parative effectiveness of MSD-HSCT and IST.
- 35 • The included data were collected 15 to more than 30 years ago. Thus, the results may  
36 not be applicable to current modern standard care.

| Subject: *bmjopen-2014-005039-R2R3*: SAA-MSD

## 40 Abstract

### 41 Background

42 Acquired severe aplastic anemia is a rare and potentially fatal disease. The aim of this  
43 Cochrane review was to evaluate the effectiveness and adverse events of first-line allogeneic  
44 hematopoietic stem cell transplantation of HLA-matched sibling donors compared to first-line  
45 immunosuppressive therapy.

### 46 Procedure

47 We searched the electronic databases MEDLINE (Ovid), EMBASE (Ovid), and The  
48 Cochrane Library CENTRAL (Wiley) for published articles from 1946 to 22 April 2013. We  
49 included randomized controlled trials and prospective non-randomized controlled trials as  
50 long as the study design was consistent with the principle of 'Mendelian randomization' in  
51 allocating patients to treatment groups.

### 52 Results

53 We identified three prospective non-randomized controlled trials with 302 participants. We  
54 did not identify a randomized controlled trial. All studies had a high risk of bias due to the  
55 study design and were conducted more than 15 years ago and may not be applicable to the  
56 standard of care of today. The pooled hazard ratio for overall mortality for the donor group  
57 versus the no donor group was 0.95 (95% confidence interval 0.43 to 2.12, P = 0.90).

### 60 Conclusions

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3 61 There are insufficient and biased data that do not allow any firm conclusions to be made about  
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5 62 the comparative effectiveness of first-line allogeneic hematopoietic stem cell transplantation  
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7 63 of HLA-matched sibling donors and first-line immunosuppressive therapy of patients with  
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9 64 acquired severe aplastic anemia.  
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For peer review only

| *Subject: bmjopen-2014-005039-R2R3: SAA-MSD*

## 66 Introduction

67 Acquired severe aplastic anemia (SAA) is a rare [1] and potentially fatal disease which is  
68 characterized by hypocellular bone marrow and pancytopenia; it mainly affects young adults.

69 The estimated incidence rate of SAA ranges from 0.7 to 4.1 per million people per year [2].

70 The underlying pathophysiology is thought to be an aberrant immune response involving the

71 T-cell mediated destruction of hematopoietic stem cells [3]. Major signs and symptoms are  
72 severe infections, bleeding, and exhaustion and patients may experience paleness, weakness,  
73 fatigue, and shortness of breath.

74 According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of

75 the British Committee for Standards in Haematology [4], first-line allogeneic hematopoietic

76 stem cell transplantation (HSCT) from the bone marrow of an human leukocyte antigen

77 (HLA)-matched sibling donor (MSD) is regarded as the initial treatment of choice for newly

78 diagnosed patients with severe aplastic anemia. Graft failure may lead to early death and the

79 conditioning regimen may lead to severe non-hematological organ toxicities.

80 According to the 2009 Guidelines for the diagnosis and management of aplastic anaemia of

81 the British Committee for Standards in Haematology [4], first-line immunosuppressive

82 therapy (IST) is a combination of antithymocyte globulin (ATG) and ciclosporin. First-line

83 IST is indicated for patients where no MSD is available, which can be expected for 70% of

84 patients with SAA [3]. Ciclosporin is an immunosuppressant drug that is not lymphocytotoxic

85 but has specific inhibitory effects on T-lymphocyte function [5]. In the past, antilymphocyte

86 globulin (ALG) was reported interchangeably in the literature alongside ATG, therefore, ALG

87 is reported in the present study on equal terms. ATG as well as ALG are polyclonal antibodies

88 that recognize a variety of human lymphocyte cell surface antigens, reduce the number of

89 lymphocytes and induce an immunosuppressive effect. They originate in animals immunized

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3 90 with either normal human thymocytes, collected at pediatric cardiac surgery or thoracic duct  
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5 91 lymphocytes, collected during therapeutic cannulation [5]. Concerning the hematologic  
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7 92 response and the survival of patients after a first treatment for severe aplastic anemia, it may  
8  
9 93 be crucial in what type of animal ATG originates, as a randomized study showed that rabbit  
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11 94 ATG was inferior in this respect to horse ATG [6]. The currently recommended combination  
12  
13 95 of ciclosporin with ATG in the treatment of severe aplastic anemia is based on their separate  
14  
15 96 and potentially complementary modes of action[5]. Some patients do not respond well to IST  
16  
17 97 or show no response at all. Frequent transfusions increase the risk of adverse events such as  
18  
19 98 iron overload and early death. If a diagnosis of SAA is established at an early patient age,  
20  
21 99 then it is crucial to know which treatment promises more benefit and less harm in the long  
22  
23 100 run. We aimed to evaluate the effectiveness and severe adverse events of MSD-HSCT  
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25 101 compared to IST in patients with SAA.  
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## 102 **Methods**

103 This article is based on a Cochrane Systematic Review published in The Cochrane Library  
104 [7]. Publication of this work is in agreement with the policy of The Cochrane Collaboration  
105 [8]. While preparing this systematic review and meta-analysis, we endorsed the PRISMA  
106 statement, adhered to its principles and conformed to its checklist [9].

### 107 **Study inclusion criteria**

108 We included randomized controlled trials (RCTs) and prospective non-randomized controlled  
109 trials as long as the study design was consistent with the principle of 'Mendelian  
110 randomization' in allocating patients to treatment groups. We required a minimum of 80% of  
111 relevant patients per group and we set a minimum sample size of five participants per group.  
112 We set no limits on language, year of publication, or year of treatment. We included  
113 participants with newly diagnosed acquired severe aplastic anemia [4]. We did not set any age  
114 limits for participants. We excluded studies on participants with secondary aplastic anemia.  
115 We included HSCT as the test intervention harvested from any source of MSD and serving as  
116 a first-line therapy [10]. That means, no other HSCT or IST has been offered to the patients  
117 before. We included IST as comparator with ciclosporin combined with ATG as the current  
118 mode of IST [10]. To accommodate also former modes of IST, we also included ciclosporin  
119 combined with ALG, ciclosporine alone, ATG alone, and ALG. Other agents such as  
120 corticosteroids and androgens were not considered. The primary outcome was overall  
121 mortality. Secondary outcomes were treatment-related mortality, graft failure, graft-versus-  
122 host disease, no response to IST, relapse after initial successful treatment, secondary clonal  
123 disease or malignancies, health-related quality of life, and performance scores.

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3 | 125 **Principle of 'Mendelian randomization'**

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6 | 126 There are ethical concerns around randomization of patients with severe aplastic anemia to  
7  
8 | 127 transplantation versus non-transplantation because the risk of early death is expected to be  
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10 | 128 higher in the transplantation group than in the non-transplantation group. The reason is the  
11  
12 | 129 potentially life-threatening graft-versus-host disease occurring only in the transplanted  
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14 | 130 patients. In general, MSD-HSCT is a life-threatening treatment that can lead to early severe  
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16 | 131 adverse events including death. Gray 1991 [11] and Wheatley 2004 [12] described the  
17  
18 | 132 potential of 'Mendelian randomization' to minimize bias when comparing MSD-HSCT with  
19  
20 | 133 an alternative therapy. The base concept has been ascribed to Katan 1986 [13]. 'Mendelian  
21  
22 | 134 randomization' means the view that nature itself has already 'randomized' the paternal and  
23  
24 | 135 maternal part of a gene given that donor and recipient are siblings. Thus, 'Mendelian  
25  
26 | 136 randomization' by definition accepts only siblings as transplant donors and these sibling  
27  
28 | 137 donors are required to have 'identical' or matched features of specific transplant-relevant HLA  
29  
30 | 138 sites when compared with the transplant recipient. Therefore, patients with an HLA-matched  
31  
32 | 139 sibling will be allocated to the MSD-HSCT group. On the other hand, patients with siblings  
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34 | 140 that are not HLA compatible will be allocated to the immunosuppressive therapy group. The  
35  
36 | 141 term 'Mendelian randomization' refers to the fact that the genetic distribution of paternal and  
37  
38 | 142 maternal alleles follows a random process and is determined before birth. This concept takes  
39  
40 | 143 advantage of an instrumental variable for allocating the patients to treatment groups and, at  
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42 | 144 the same time, this variable is neither associated with the treatment nor associated with the  
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44 | 145 outcome.

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50 | 146 **Search strategy and selection of studies**

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54 | 147 We conducted an electronic literature database search in MEDLINE (Ovid), EMBASE (Ov-  
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56 | 148 id), and Cochrane Library CENTRAL (Wiley) including articles published from inception to

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3 149 22 April 2013. The corresponding search strategies are depicted in the original Cochrane Re-  
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5 150 view [7]. We retrieved all titles and abstracts by electronic searching and downloaded them to  
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7 151 the reference management database EndNote Version X3 [14]. Two authors assessed the eli-  
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9 152 gibility of retrieved papers independently. We considered studies written in languages other  
10  
11 153 than English. We judged studies to be prospective if an explicit statement was reported or  
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13 154 there were clues suggesting a prospective design (e.g. prior approval of treatment, informed  
14  
15 155 consent). We judged studies to be retrospective if an explicit statement was reported or it was  
16  
17 156 implied by description that data were reviewed from an existing source. We regarded each of  
18  
19 157 the following items as an indication of a retrospective design: registry reports and reviewing  
20  
21 158 of medical records. We judged studies as consistent with the principle of 'Mendelian randomi-  
22  
23 159 zation' if all transplant donors were clearly siblings and if the allocation of patients to treat-  
24  
25 160 ment groups was not based on age. We regarded studies as not consistent with the principle of  
26  
27 161 'Mendelian randomization' if age was not balanced between groups, indicating that age played  
28  
29 162 a role in the group assignment. Example for imbalance: distribution of age categories was  
30  
31 163 statistically not comparable (P value less than 0.05).  
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#### 37 164 **Assessment of risk of bias in included studies**

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39  
40 165 Two review authors independently assessed the risk of bias in the included studies using six  
41  
42 166 criteria. We have used four criteria from The Cochrane Collaboration's tool for assessing risk  
43  
44 167 of bias [15]: blinding of outcome assessment, complete outcome data such as missing data,  
45  
46 168 selective reporting such as not reporting pre-specified outcomes, and other sources of bias  
47  
48 169 such as bias related to the specific study design and competing interest. We extended the  
49  
50 170 Cochrane tool for assessing risk of bias with two additional criteria that are specific to the  
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52 171 inclusion criteria for the present review and critical for confidence in results: comparable  
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3 172 baseline characteristics and concurrent control. We applied The Cochrane Collaboration's  
4  
5 173 criteria for judging risk of bias [16].  
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8 174 **Data synthesis**  
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10  
11 175 One review author entered the data into Review Manager [17]. Another review author  
12  
13 176 checked the entered data. We synthesized data on mortality for the donor group (MSD-HSCT)  
14  
15 177 versus the no donor group (IST) by using the hazard ratio (HR) for time-to-event data as the  
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17 178 primary effect measure with a random-effects model. If the hazard ratio was not directly given  
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19 179 in the publication, we estimated hazard ratios according to methods proposed by [18] and  
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21 180 [19].  
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## 181 Results

### 182 Search results

183 We identified three non-randomized, prospective, parallel, controlled clinical trials (**Figure**  
184 **1**). Bayever 1984 [20] and Gratwohl 1981 [21] reported their results in a single original arti-  
185 cle, respectively. Führer 1998 reported five publications including one original article [22], a  
186 follow up article [23], one protocol [24], and two abstracts [25 26]. We did not identify any  
187 RCTs.

### 188 Characteristics of included articles

189 The main study, patients and interventions characteristics are shown in **Table 1**. The patients  
190 were treated and observed between 1976 and 1997. Thus, the reported data were collected  
191 more than 15 years ago. Median follow up was not reported. Median age, fraction of males,  
192 and median days of time interval between diagnosis and begin of treatment were roughly  
193 comparable between the treatment groups within each study. The age ranged from early  
194 childhood to young adulthood. In the study by Führer 1998, all patients were less than 17  
195 years old by definition of the inclusion criteria [22]. Bone marrow was used as source for all  
196 transplants. All three included studies had a high risk of bias due to the study design (**Table**  
197 **2**). We judged a low risk of bias for blinding the assessment of overall mortality. Blinding or  
198 lack of blinding is not expected to make a difference concerning overall mortality. The au-  
199 thors of all included studies did not report that 'Mendelian randomization' was planned and  
200 the authors did not report the size of the involved families. The authors did not report the  
201 numbers of siblings and the results of the individual genetic analyses.

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3 202 **Effects of intervention**

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6 203 The pooled hazard ratio estimate for overall mortality was 0.95 with a 95% confidence inter-  
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8 204 val of 0.43 to 2.12 (P value = 0.90) (**Figure 2**). According to the meta-analysis based on data  
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10 205 from all three included studies, overall mortality was not statistically significantly different  
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12 206 between MSD-HSCT and IST. Overall survival ranged from 47% to 84% in the MSD-HSCT  
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14 207 group and from 45% to 87% in the IST group (**Table 3**). The results for the secondary out-  
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16 208 comes including treatment-related mortality after MSD-HSCT, graft failure after MSD-  
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18 209 HSCT, graft-versus-host-disease (GVHD) after MSD-HSCT, no response to IST, and relapse  
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20 210 after IST are shown in **Table 4**. With respect to secondary clonal disease or malignancies,  
21  
22 211 Bayever 1984 reported one patient who developed T-cell lymphoma after MSD-HSCT and  
23  
24 212 Führer 1998 reported 4 patients who developed acute myelogenous leukemia after IST.  
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26 213 Health-related quality of life questionnaires were not used in any of the included studies. Ba-  
27  
28 214 yever 1984 reported that almost all evaluable patients in the MSD-HSCT group (92%) and  
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30 215 less than half of the patients in the IST group had a Karnofsky Performance Status of higher  
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32 216 than 70%.

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## 217 Discussion

### 218 Interpretation of main results

219 We identified three prospective, non-randomized controlled trials [20-22] including 302 par-  
220 ticipants; 121 received MSD-HSCT and 181 received IST. Based on these trials we found  
221 insufficient evidence to clarify whether MSD-HSCT leads to better overall survival than IST.  
222 Overall survival ranged from 47% to 84% in the MSD-HSCT group and from 45% to 87% in  
223 the IST group and in a meta-analysis, overall mortality was not statistically significantly dif-  
224 ferent between the treatment groups. Treatment-related mortality in the MSD-HSCT group  
225 was considerable ranging from 20% to 42%. The graft failure rate was variable and caused the  
226 death of 3% to 16% of transplanted patients. GVHD affected a quarter to a half of transplanted  
227 patients. More than half of patients in one study did not respond to IST. Relapse affected  
228 up to one in eight patients after IST in one study. Secondary clonal disease or malignancies  
229 were detected rarely in both treatment groups. Marsh 2009 estimated that allogeneic bone  
230 marrow transplantation from an HLA-identical sibling donor provides a 75% to 90% chance  
231 of long-term cure in patients younger than 40 years of age [4]. Similarly, in a review of stud-  
232 ies with younger patients, Guinan 2009 reported an overall survival between 75% and 95% at  
233 three to five years [27]. Sangiolo 2010 concluded that their data favors extending MSD-HSCT  
234 to patients older than 40 years of age who are without significant co-morbidities [28]. The  
235 results of the studies included in the present systematic review appear to roughly match the  
236 recent estimates reported by others.

### 237 Recent therapeutic improvement

238 Bacigalupo 2008 reported that the outcome has improved since 1996 for HSCT but not for  
239 IST. Peinemann 2011 identified three studies that reported a statistically significant improve-

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2  
3 240 ment of overall survival in the group of matched related donor transplants but not in the IST  
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5 241 group [29]. Several factors may have contributed to recent improvements in HSCT, such as  
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7 242 detailed HLA-matching and less irradiation-based conditioning. The Third Consensus Con-  
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9 243 ference on the treatment of aplastic anemia agreed in 2010 that bone marrow should be used  
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11 244 as the source of stem cells and that the upper age limit should be 50 years and that the combi-  
12  
13 245 nation of antithymocyte globulin and ciclosporin remains the gold standard for immunosup-  
14  
15 246 pressive therapy [30]. Scheinberg 2012 provided an overview and update of various treatment  
16  
17 247 options for severe aplastic anemia including immunosuppressive therapy and transplantation  
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19 248 [31].  
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### 23 249 **Strengths and limitations**

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27 250 One of the strengths of this review is the broadness of the search strategy such that study re-  
28  
29 251 trieval bias is very unlikely. We restricted the inclusion to studies to randomized controlled  
30  
31 252 trials and prospective non-randomized controlled trials that were compatible with 'Mendelian  
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33 253 Randomization' to avoid excess risk of bias. We assumed a possible 'Mendelian Randomiza-  
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35 254 tion' in the three included studies, though, the authors did not mention this approach and did  
36  
37 255 not report what proportion of patients with a matched sibling donor actually received the  
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39 256 transplant. Thus, crucial information is lacking to judge the compliance of patients and the  
40  
41 257 significance of the assumed concept of natural allocation. Nevertheless, the included data are  
42  
43 258 too scarce and too biased to allow any conclusion on the comparative effectiveness of MSD-  
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45 259 HSCT and IST. The rates of adverse events, such as treatment-related mortality, graft failure,  
46  
47 260 no response to IST, and GVHD, are unusually high, which may be explained by the age of the  
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49 261 studies (starting in 1976). We did not separate horse ATG from rabbit ATG, although the type  
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51 262 of animal as the origin of ATG was reported as a serious effect modifier [6]. All data were  
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53 263 collected about 15 up to more than 30 years ago. Thus, the results may not be applicable to  
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3 264 current modern standard care. Use of 'Mendelian randomization' is no guarantee that bias is  
4  
5 265 minimized. This may be because tissue typing data may not be accurate. Patients may have  
6  
7 266 only one sibling either in the donor or in the no donor group. Large families have a greater  
8  
9 267 chance of finding a donor. Therefore, designing a non-randomized controlled trial by applying  
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11 268 'Mendelian randomization' requires careful thought to effectively reduce bias and control for  
12  
13 269 potential confounders. There is a time lag in patients with siblings because tissue typing and  
14  
15 270 readiness for assignment to treatment group may possibly take several months [12]. On the  
16  
17 271 other hand, patients with no siblings can be assigned immediately and are at earlier risk for  
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19 272 adverse events. Nitsch 2006 described the limits to causal inference based on 'Mendelian ran-  
20  
21 273 domization' [32].  
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## 26 274 **Conclusions**

27  
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29 275 There are insufficient and biased data that do not allow any firm conclusions to be made about  
30  
31 276 the comparative effectiveness of MSD-HSCT and IST. Patients should be made aware of the  
32  
33 277 early treatment-related mortality and the burden of GVHD after HSCT. Patients treated with  
34  
35 278 IST should also be made aware that the disease may recur after initial successful treatment,  
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37 279 and that life-threatening late clonal and malignant disease after IST may occur in a higher  
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39 280 percentage compared to HSCT.  
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## 286 **Ethics statement**

287 An ethics statement was not required for this work.

## 288 **Financial Disclosure**

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290 in study design, data collection and analysis, decision to publish, or preparation of the manu-  
291 script.

## 292 **Conflict of Interest Statement**

293 No authors have any competing interests.

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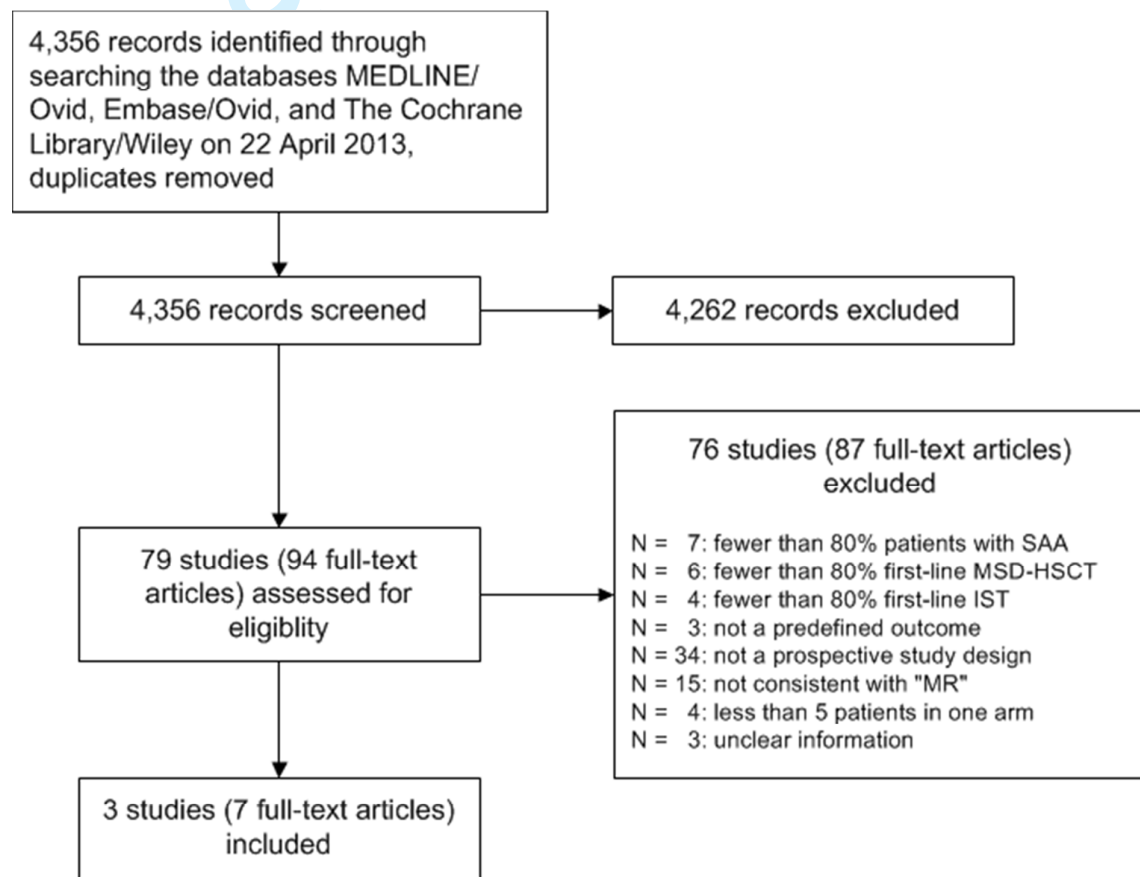
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4 368 transplantation from HLA-identical siblings for severe aplastic anemia in patients over  
5 369 40 years of age. *Biol Blood Marrow Transplant* 2010;**16**(10):1411-18  
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| Subject: *bmjopen-2014-005039-R2R3*: SAA-MSD

## 381 Figure legends

382 Figure 1. Study flow

383 Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic  
 384 hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors;  
 385 "MR": "Mendelian Randomization"; SAA: acquired severe aplastic anemia



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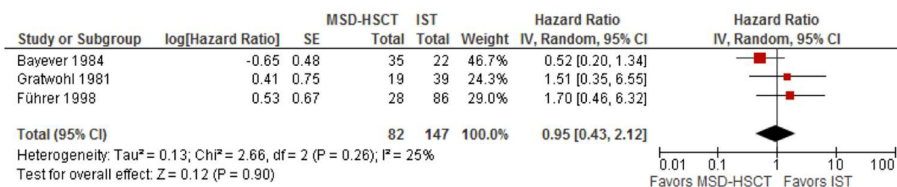
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| Subject: *bmjopen-2014-005039-R2R3*: SAA-MSD

388 Figure 2. Mortality in the donor group (MSD-HSCT) vs. the no donor group (IST); effect:  
389 hazard ratio; random-effects model.

390 Standard error calculated from data presented in the Kaplan-Meier graph of the article.

391 Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem  
392 cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST:  
393 first-line immunosuppressive therapy; IV: inverse variance; SE: standard error



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## Tables

Table 1. Characteristics of included studies

Study ID	Duration of study	Median FU	Setting, center, country	Patients, no. <sup>1</sup>	Median age, years (range) <sup>1</sup>	Fraction of males, % <sup>1</sup>	Median interval, days <sup>1,2</sup>	Stem cell source	IST components	ATG source
Bayever 1984	1977 to 1982	NR	Single, United States	35 vs. 22	17 (2 to 24) vs. 15 (1 to 23)	67 vs. 68	60 vs. 58	Bone marrow	ATG	horse
Führer 1998	1993 to 1997	NR	Multi, Germany, Austria	28 vs. 86	10.1 (2.3 to 15.8) vs. 9.1 (0.9 to 15.2)	43 vs. 62	49 vs. 23	Bone marrow	ATG Ciclosporin	horse
Gratwohl 1981	1976 to 1980	NR	Single, Switzerland	19 vs. 13	18 (4 to 29) vs. 23 (7 to 37)	53 vs. 54	105 vs. 180	Bone marrow	ATG Ciclosporin	N.R.

<sup>1</sup>Donor group (MSD-HSCT) versus No donor group (IST)

<sup>2</sup>Median time interval between diagnosis and begin of treatment

Abbreviations. ATG: anti-thymocyte globulin; FU: follow-up; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; NR: not reported; no.: number

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Table 2. Risk of bias of included studies

Study ID	Blinding of assessment of overall mortality	Incomplete outcome data	Selective reporting	Other bias	Comparable baseline characteristics	Concurrent control	Overall judgement of bias
Bayever 1984	Low	Low	Unclear	High <sup>1</sup>	Low	Low	High
Führer 1998	Low	High	High <sup>2</sup>	High <sup>3</sup>	Low	Low	High
Gratwohl 1981	Low	High	Unclear	Unclear	Low	Low	High

<sup>1</sup>Bayever 1984: The authors reported the study results at an early time point before all planned data had been gathered: "We present this interim report(...)".  
<sup>2</sup>Führer 1998: In the 2005 update, overall survival, secondary clonal disease or malignancies, and also relapse were not reported separately for the 2 distinct treatment groups. Rather, the results were presented for 2 subgroups according to disease severity. This was different from the earlier report of the same study published in 1998 covering the study period from 1993 to 1997. See Fig. 1 and Tab. 1 of the article.  
<sup>3</sup>Führer 1998: Financial support was provided by 2 pharmaceutical companies.  
 Abbreviations. ATG: anti-thymocyte globulin; IST: immunosuppressive therapy; MSD-HSCT: HLA-matched sibling donor hematopoietic stem cell transplantation; N.R.: not reported; no.: number



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3 | Subject: *bmjopen-2014-005039-R2R3*: SAA-MSD  
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5 Table 3. Overall survival  
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Study ID	Donor group (MSD-HSCT)		No donor group (IST)		FU <sup>1</sup>	P value
	N	OS (95% CI)	N	OS (95% CI)	Year	
Bayever 1984	35	72% (64 to 80)	22	45% (29 to 61)	2	0.18
Führer 1998	28	84% (N.R.)	86	87% (N.R.)	4	0.43
Gratwohl 1981	19	47% (N.R.)	13	69% <sup>2</sup> (N.R.)	5	0.56 <sup>3</sup>

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13 <sup>1</sup>Time point of Kaplan-Meier estimate.

14 <sup>2</sup>Gratwohl 1981: Two of 13 patients were eligible for MSD-HSCT but donors were not available in the first place; the two patients died after they received a second-line HSCT from the then again available MSD that was offered after the patients showed no response to IST.

15 <sup>3</sup>The P value was not reported and we calculated the P value using Fisher's exact test.

16 Abbreviations: CI: confidence interval; FU: follow up; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT:  
17 first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N: number of analyzed patients; N.R.: not reported; OS: overall survival  
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Table 4. Secondary outcomes

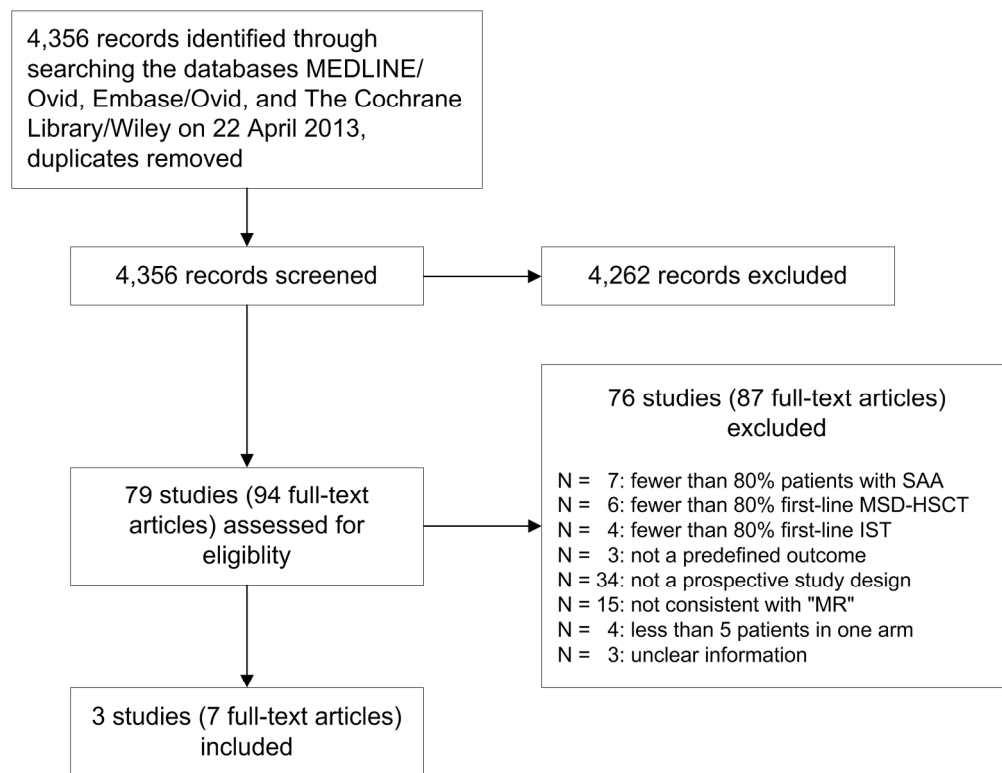
Study ID	TRM after MSD-HSCT <sup>1,2</sup>	Graft failure after MSD-HSCT <sup>1</sup>	GVHD after MSD-HSCT <sup>1</sup>	No response to IST <sup>1</sup>	Relapse at 5 years after IST <sup>1</sup>
Bayever 1984	20% (7 of 35)	3% (1 of 35)	51% (17 of 33)	64% (14 of 22)	12.5% (1 of 8)
Führer 1998	N.R.	N.R.	N.R.	N.R.	N.R.
Gratwohl 1981	42% (8 of 19)	16% (3 of 19)	26% (5 of 19)	15% (2 of 13)	N.R.

<sup>1</sup>In parenthesis: number of affected of number of evaluable patients

<sup>2</sup>Treatment-related mortality was not reported for IST.

Abbreviations. GVHD: graft-versus-host disease; IST: immunosuppressive therapy including ciclosporin and/or antithymocyte or antilymphocyte globulin; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation from HLA-matched sibling donor; N.R.: not reported; TRM: treatment-related mortality

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33 Figure 1. Study flow

34 Abbreviations. IST: first-line immunosuppressive therapy; MSD-HSCT: first-line allogeneic hematopoietic  
 35 stem cell transplantation of bone marrow of HLA-matched sibling donors; "MR": "Mendelian Randomization";  
 36 SAA: acquired severe aplastic anemia  
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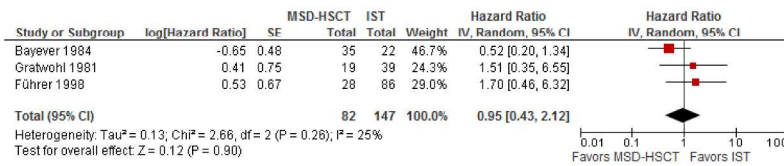


Figure 2. Mortality (MSD-HSCT vs. IST); effect: hazard ratio; random-effects model. Standard error calculated from data presented in the Kaplan-Meier graph of the article. Abbreviations: CI: confidence interval; MSD-HSCT: first-line allogeneic hematopoietic stem cell transplantation of bone marrow of HLA-matched sibling donors; log: logarithm; IST: first-line immunosuppressive therapy; IV: inverse variance; SE: standard error  
 254x190mm (300 x 300 DPI)



# PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
<b>TITLE</b>			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
<b>ABSTRACT</b>			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	3
<b>INTRODUCTION</b>			
Rationale	3	Describe the rationale for the review in the context of what is already known.	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	5
<b>METHODS</b>			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	5
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	6
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	in the Cochrane review
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	6
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6 to 7
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	7



# PRISMA 2009 Checklist

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Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $I^2$ ) for each meta-analysis.	7
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Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	7
<b>RESULTS</b>			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	in the Cochrane review
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	8, table 1
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	8 to 9, table 3 to 4, figure 1
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	figure 1
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	table 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	none
<b>DISCUSSION</b>			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	10
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	12
<b>FUNDING</b>			



## PRISMA 2009 Checklist

Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	12
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From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

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