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RESPONSE study group

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Recombinant human granulocyte–colony stimulating factor in women with unexplained recurrent pregnancy losses: a randomized clinical trial

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STUDY QUESTION: Does administration of recombinant human granulocyte colony stimulating factor (rhG-CSF) in the first trimester improve pregnancy outcomes, among women with a history of unexplained recurrent pregnancy loss?

SUMMARY ANSWER: rhG-CSF administered in the first trimester of pregnancy did not improve outcomes among women with a history of unexplained recurrent pregnancy loss.

WHAT IS KNOWN ALREADY: The only previous randomized controlled study of granulocyte colony stimulating factor in recurrent miscarriage in 68 women with unexplained primary recurrent miscarriage found a statistically significant reduction in miscarriage and improvement in live birth rates. A further four observational studies where G-CSF was used in a recurrent miscarriage population were identified in the literature, two of which confirmed statistically significant increase in clinical pregnancy and live birth rates.

STUDY DESIGN, SIZE, DURATION: A randomized, double-blind, placebo controlled clinical trial involving 150 women with a history of unexplained recurrent pregnancy loss was conducted at 21 sites with established recurrent miscarriage clinics in the United Kingdom between 23 June 2014 and 05 June 2016. The study was coordinated by University of Birmingham, UK.

PARTICIPANTS/MATERIALS, SETTING, METHODS: One hundred and fifty women with a history of unexplained recurrent pregnancy loss: 76 were randomized to rhG-CSF and 74 to placebo. Daily subcutaneous injections of recombinant human granulocyte – colony stimulating factor 130 µg or identical appearing placebo from as early as three to five weeks of gestation for a maximum of 9 weeks. The trial used central randomization with allocation concealment. The primary outcome was clinical pregnancy at 20 weeks of gestation, as demonstrated by an ultrasound scan. Secondary outcomes included miscarriages, livebirth, adverse events, stillbirth, neonatal birth weight, changes in clinical laboratory variables following study drug exposure, major congenital anomalies, preterm births and incidence of anti-drug antibody formation. Analysis was by intention to treat.

MAIN RESULTS AND THE ROLE OF CHANCE: A total of 340 participants were screened for eligibility of which 150 women were randomized. 76 women (median age, 32[*IQ*R, 29–34] years; mean BMI, 26.3[*SD*, 4.2]) and 74 women (median age, 31[*IQ*R, 26–33] years; mean BMI, 25.8[*SD*, 4.2]) were randomized to placebo. All women were followed-up to primary outcome, and beyond to live birth. The clinical pregnancy rate at 20 weeks, as well as the live birth rate, was 59.2% (45/76) in the rhG-CSF group, and 64.9% (48/74) in the placebo group, giving a relative risk of 0.9 (95% *CI*: 0.7–1.2; *P* = 0.48). There was no evidence of a significant difference between the groups for any of the secondary outcomes. Adverse events (AEs) occurred in 52 (68.4%) participants in rhG-CSF group and 43 (58.1%) participants in the

placebo group. Neonatal congenital anomalies were observed in 1/46 (2.1%) of babies in the rhG-CSF group versus 1/49 (2.0%) in the placebo group (RR of 0.9; 95% CI: 0.1–13.4; $P = 0.93$).

LIMITATIONS, REASONS FOR CAUTION: This trial was conducted in women diagnosed with unexplained recurrent pregnancy loss and therefore no screening tests (commercially available) were performed for immune dysfunction related pregnancy failure/s.

WIDER IMPLICATIONS OF THE FINDINGS: To our knowledge, this is the first multicentre study and largest randomized clinical trial to investigate the efficacy and safety of granulocyte human colony stimulating factor in women with recurrent miscarriages. Unlike the only available single center RCT, our trial showed no significant increase in clinical pregnancy or live births with the use of rhG-CSF in the first trimester of pregnancy.

STUDY FUNDING/COMPETING INTEREST(S): This study was sponsored and supported by Nora Therapeutics, Inc., 530 Lytton Avenue, 2nd Floor, Palo Alto, CA 94301, USA. Darryl Carter was the co-founder and VP of research, Nora Therapeutics, Inc. and held shares in the company. He holds a patent for the use of recombinant human granulocyte colony stimulating factor to reduce unexplained recurrent pregnancy loss. Mark Joing, Paul Kwon and Jeff Tong were or are employees of Nora Therapeutics, Inc. No other potential conflict of interest relevant to this article was reported.

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Key words: recombinant human granulocyte colony stimulating factor / cytokine / neutropenia / recurrent pregnancy loss / semi-allogenic fetus / immune mediated miscarriages / unexplained recurrent miscarriages

Introduction

Recurrent pregnancy losses (RPL) defined as the loss of three or more pregnancies, affects ~1–3% of couples attempting to have a child (Ford and Schust, 2009). Investigations do not provide a cause for recurrent pregnancy losses in approximately half of those investigated, and such couples are said to have unexplained recurrent pregnancy losses (Jeve and Davies, 2014). Whilst a range of treatments are currently offered to couples with unexplained recurrent pregnancy losses (uRPL), no effective treatment has yet been identified (ESHRE Recurrent Pregnancy Loss Guidelines, 2017).

Immune mediated mechanisms are thought to contribute to recurrent pregnancy losses. In particular, a failure of the maternal immune system to adapt to accommodate the semi-allogenic fetus may be important. Despite a lack of good evidence of their efficacy, a range of immunomodulatory treatments including, paternal lymphocyte infusion therapy (Mowbray et al., 1985, Cavalcante et al., 2014), corticosteroids, intravenous immunoglobulin therapy (Nyborg et al., 2014), intravenous intralipid infusion (Meng et al., 2016) and anti-TNF α monoclonal antibody therapy (Clark, 2009) to modulate the maternal immune response are offered to women with recurrent pregnancy losses.

Granulocyte colony stimulating factor (G-CSF) is a cytokine conventionally viewed as important in stimulating the proliferation and differentiation of neutrophils (Thomas et al., 2002). It is widely used in treatments associated with severe chronic neutropenia and chemotherapy induced neutropenia for mobilization of neutrophils (Carton et al., 2013). In addition, G-CSF is postulated to have immunomodulatory properties by inducing peripheral regulatory T-cells and myeloid derived suppressor cells, which have an important function curbing the immune response to infection, inflammation, and autoimmunity (Williams, 2012). Furthermore, studies in both humans and animals have showed that administration of G-CSF improves endometrial

thickness (Gleicher et al. 2011), ovarian follicular function and oocyte quality (Salmassi et al., 2004), which may enhance embryo implantation (Ledee et al., 2008).

A Phase I randomized double blind, placebo-controlled dose escalation (65 mcg, 130 mcg and 260 mcg) study of recombinant human (rh) G-CSF in 48 healthy female volunteers suggested changes in peripheral blood subsets including a temporary induction of toleragenic cell subsets and decreased percentages of pro-inflammatory and cytotoxic cell subsets, without evidence of global immune changes or suppression. These specific changes were observed only in the multidose groups and not in single dose or placebo groups (unpublished data; available on request through the corresponding author).

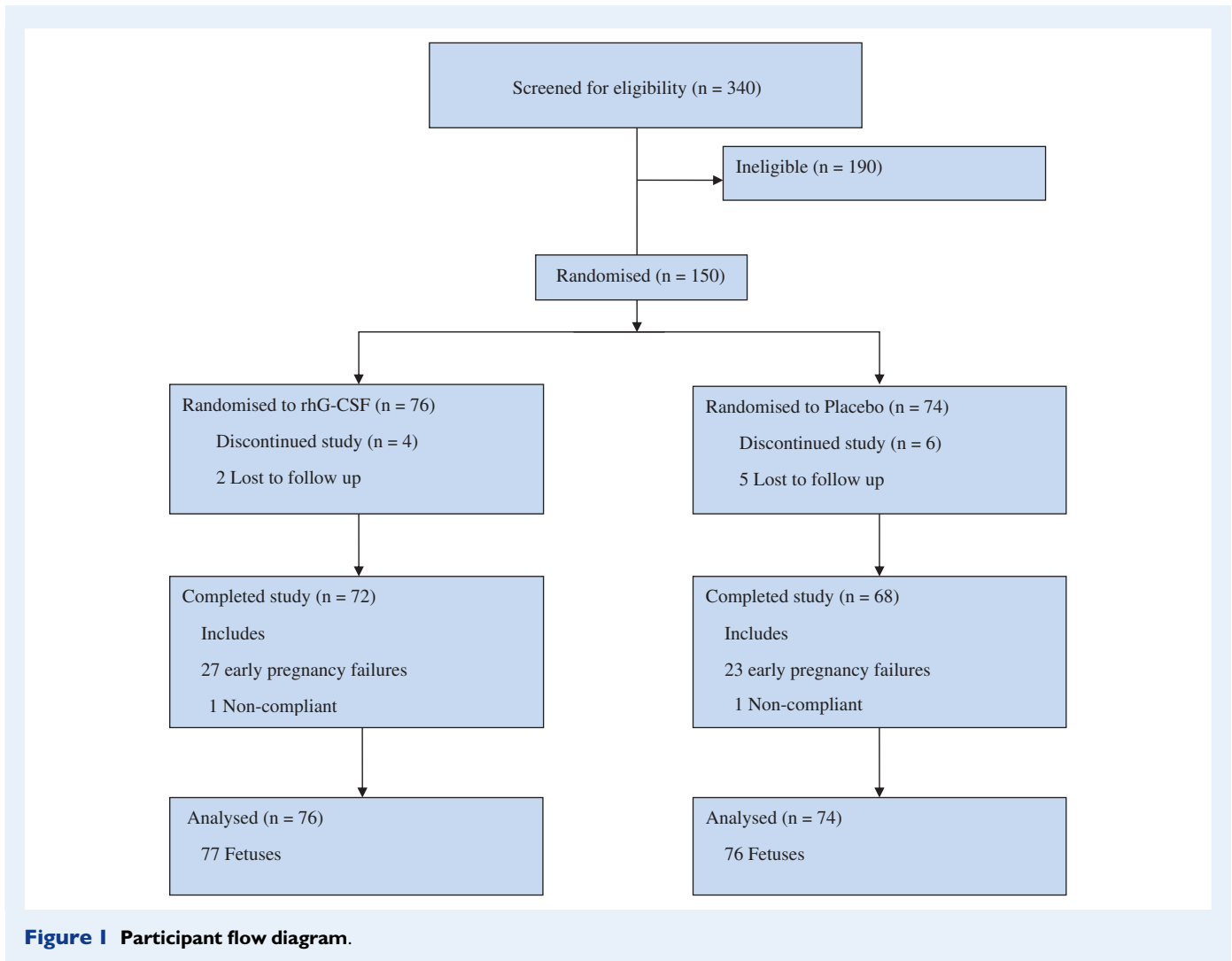
Furthermore, a single center, randomized controlled trial of 68 women diagnosed with recurrent pregnancy losses, suggested that rhG-CSF may be an effective treatment (Scarpellini and Sbracia, 2009). Although the clinical evidence is limited (ESHRE Recurrent Pregnancy Loss Guidelines, 2017), the use of G-CSF to treat recurrent miscarriage and implantation failure appears to be increasing. Therefore, there was an urgent need to determine the efficacy and safety of this treatment in a multicentre trial.

We conducted a multicentre, randomized, double-blind, placebo-controlled trial to evaluate the efficacy and safety of recombinant human granulocyte colony stimulating factor (rhG-CSF) in women with a history of unexplained recurrent pregnancy loss to provide a definitive answer on whether rhG-CSF administration improved pregnancy outcomes.

Materials and Methods

Study design

RESPONSE study was a multicentre, double blind; placebo controlled randomized clinical trial conducted to determine the effect of recombinant



(g) maternal adverse events and serious adverse events during the treatment period and within 4 weeks of the last dose of study drug, (h) changes in clinical laboratory variables following study drug exposure, (i) major congenital anomalies, (j) preterm births and (k) incidence of anti-drug antibody (ADA) formation.

For participants who maintained pregnancy through 20 weeks of gestation, phone visits were conducted every 8 weeks during pregnancy to assess pregnancy status/outcomes and prescription medication use. Within one month of delivery, additional information was obtained, including pregnancy outcome, gestational age at delivery, mode of delivery, birth weight and Apgar scores.

Sample size calculations

The target sample size was a total of 150 participants. Participants were randomized in a 1:1 ratio to the two treatment arms. This sample size had been selected to achieve >90% power to detect a difference in ongoing clinical pregnancy rates of 60% for the placebo group and 80% in the active treatment group. Efficacy analysis was based on an intent-to-treat principle. The difference in the primary efficacy outcome measure (ongoing clinical pregnancy rate at week 20 of gestation) between rhG-CSF and placebo was tested using a Cochran Mantel Haenszel (CMH) test.

Statistical analysis

The statistician who conducted the analysis was blinded to group allocation. Efficacy analysis was based on an intention to treat principle. The relative risks (RRs) with 95% CIs were calculated for the primary and secondary outcomes. Subgroup analysis was performed using the RR for the stratification factors. Missing data were imputed only for those with a clinical pregnancy and livebirth. This implied that summary measures were limited to participants who remained pregnant at that time point. The statistical analysis plan (SAP) is available in Supplementary material.

Results

Between 23 June 2014 and 05 June 2016, a total of 340 women were screened for inclusion criteria at 21 different recruiting centers in the United Kingdom. One hundred and ninety women did not meet the inclusion criteria. One hundred and fifty women who conceived naturally and remained willing to participate in the trial were randomized to receive either rhG-CSF (76 women) or placebo (74 women) (Fig. 1). Overall, 140/150 women (93.3%) completed the study and 10/150 women (6.7%) discontinued the study prematurely for reasons

Table 1 Baseline characteristics of the participants (intention to treat analysis)^a.

Characteristics	rhG-CSF (N = 76)	Placebo (N = 74)
Maternal age – yr ^b		
Median	32	31
Interquartile range	29–34	26–33
Maternal BMI	26.3 ± 4.2	25.8 ± 4.2
Maternal BMI >30 – no. (%)	17 (22.4)	13 (17.6)
Maternal race – no. (%) ^c		
White	71 (93.4)	63 (85.1)
Black	0	2 (2.7%)
Asian	4 (5.3)	5 (6.8)
Other, including mixed race	1 (1.3)	1 (5.4)
Maternal smoking – no (%)		
Nonsmoker	62 (81.6)	61 (82.4)
<10 cigarettes/day	10 (13.2)	7 (9.5)
10 to 19 cigarettes/day	3 (3.9)	6 (8.1)
≥20 cigarettes/day	1 (1.3)	0
Alcohol use – no. (%) ^d		
None	36 (47.4)	35 (47.3)
≤3 units/day	27 (35.5)	26 (35.1)
>3 to ≥20 units/day	13 (17.1)	13 (17.6)
>20 units/day	0	0
Parity		
Previous live birth – no. (%)	38 (50.0)	37 (50.0)
≥4 previous miscarriages – no. (%)	40 (52.6)	40 (54.1)
Previous pregnancy losses – no.		
Median	4.0	4.0
Interquartile range	3–5	3–5
Clinical risk factors – no. (%)		
Polycystic ovaries	2 (2.6)	6 (8.1)
Fibroids	5 (6.6)	3 (4.1)
Large-loop excision of the cervical transformation zone	2 (2.6)	8 (10.8)
Concurrent medications – no. (%)		
Metformin	1 (1.3)	2 (2.7)
Aspirin	13 (17.1)	10 (13.5)

^aPlus – minus values are means ± SD. The baseline data (age, body mass index [BMI]; the weight in kilograms divided by the square of the height in meters), maternal race, smoking status and parity) of the participants were similar in the two study groups.

^bListed is the maternal age at the time of randomization.

^cRace was self-reported.

^dOne unit is 10 g of pure alcohol.

including loss to follow up, withdrawal of consent, and other reasons (for e.g. use of any excluded therapy).

All participants were followed to primary outcome, and beyond to live birth. The baseline characteristics of the study population were similar across the study groups (Table 1). The median age at the time of recruitment was 30.6 years (IQR 29–34 years) and 37 participants (24.7%) had experienced more than three previous miscarriages. The mean BMI at the time of randomization was 26.06 kg/m². Ethnicity data was available for all 150 randomized women, and of these, 134 (89%) were white, 9 (6%) were Asian, 2 (1%) were black and 5 (3%)

were from other ethnic groups. Most of the women were non-smokers (123/150, 82%). Study records of concurrent medications showed that 3 (2%) participants were taking metformin at the time of participation, and 23 (15.3%) were taking low dose aspirin.

Treatment compliance

Subject compliance with study drug dosing was accessed via a site review of the returned syringes and the compliance record maintained by the participant. From these results, summaries of treatment

Table II Primary outcome and secondary outcomes of participants in this trial.

	rhG-CSF no./total	Placebo	Relative risk no. (%) (95% CI)	P value
Primary outcome				
Live birth after 20 weeks of gestation	45/76 (59.2)	48/74 (64.9)	0.9 (0.7, 1.2)	0.48
Secondary outcomes				
Pregnancy outcomes				
Clinical pregnancy at 6 weeks	67/76 (88.2)	69/74 (93.2)	0.9 (0.9, 1.0)	0.28
Ongoing pregnancy at 8 weeks	51/76 (67.1)	59/74 (79.7)	0.8 (0.7, 1.0)	0.09
Ongoing pregnancy at 12 weeks	45/76 (59.2)	51/74 (68.9)	0.9 (0.7, 1.1)	0.22
Live birth after 24 weeks of gestation	45/76 (59.2)	48/74 (64.9)	0.9 (0.7, 1.2)	0.48
Live birth after 34 weeks of gestation	45/76 (59.2)	42/74 (56.8)	1.0 (0.8, 1.4)	0.76
Ectopic pregnancy	1/76 (1.3)	0/74 (0.0)	NA	NA
Miscarriage ^a	28/76 (36.8)	25/74 (33.8)	1.1 (0.7, 1.7)	0.70
Stillbirth	0/76 (0.0)	0/76 (0.0)	NA	NA
Preterm birth (before 37 weeks 0 days of gestation)	5/45 (11.1)	8/48 (16.7)	0.7 (0.3, 2.0)	0.54
Infant birth weight (g)				
Median	3420.0	3300.0	NA	NA
Range	3005–3920	2690–3610	NA	NA
Neonatal outcomes ^b				
Infants discharged alive from hospital	46/46 (100.0)	49/49 (100.0)	NA	NA
Any congenital anomaly	1/46 (2.2)	1/49 (2.0)	0.9 (0.1, 13.4)	0.93
Adverse events ^c				
	n/N (%)	n/N(%)		
Maternal adverse events	52/76 (68.4)	43/74 (58.1)	1.2 (0.9, 1.5)	0.20
Serious adverse events	4/76 (5.2)	2/74 (2.7)	1.9 (0.3, 10.3)	0.43
Incidence of anti-drug antibody formation	0/76 (0.0)	NA	NA	NA

^aMiscarriage was defined as spontaneous loss of a pregnancy less than 24 weeks of gestation; the median gestational age at miscarriage was 6.0 weeks (interquartile range, 6–7) in the rhG-CSF and 6.5 weeks (interquartile range, 6–9) in the placebo group. There were three pregnancies of unknown location in the rhG-CSF group.

^bThe end point is listed per neonate.

^cPlease see Supplementary Table S1 for details.

compliance and exposure were produced. In the overall population, the mean (SD) compliance rate was 98.7% (3.99).

Outcomes of the participants

The clinical pregnancy rate at 20 weeks of gestation was 59% (45/76) in the rhG-CSF group, compared with 65% (48/74) in the placebo group, giving a RR of 0.9 (95% CI: 0.7–1.2; $P = 0.48$) (Table II). There were no pregnancy losses in the time period from primary outcome to live birth; therefore the live birth rate was 59.2% (45/76) in the rhG-CSF group, and 64.9% (48/74) in the placebo group, giving a relative risk of 0.9 (95% CI: 0.7–1.2; $P = 0.48$). During the study, clinical pregnancies were confirmed by ultrasound scan at 6 weeks of gestation in 136 (90.7%) of the 150 randomized participants [67/76, 88.2% in the rhG-CSF group vs 69/74, 93.2% in the placebo group, RR of 0.9 (95% CI: 0.9–1.0; $P = 0.28$)]. Ongoing pregnancies were confirmed at ~12 weeks in 96 (64.0%) of the women [45/76, 59.2% in the rhG-CSF group versus 51/74, 68.9% in the placebo group, RR of 0.9 (95% CI: 0.7–1.1; $P = 0.224$)].

Miscarriage rates were not significantly different between the study groups (rhG-CSF 28/76, 36.8% versus placebo 25/74, 33.8%, RR of 1.1 (95% CI: 0.7–1.7, $P = 0.70$)). Amongst the 28 pregnancies that

ended in miscarriage for participants receiving rhG-CSF, the median gestation was 6 weeks (IQR 6–7 weeks). Amongst the 25 pregnancies that ended in miscarriage for participants receiving placebo, the median gestation was 6.5 weeks (IQR 6–9 weeks). The distributions of gestational age at live birth delivery for the rhG-CSF and placebo groups are given in Fig. 2. All infants were discharged home alive from the hospital.

Adverse events (AEs) occurred in 52 (68.4%) participants in rhG-CSF group and 43 (58.1%) participants in the placebo group (Supplementary Table S1). Neonatal congenital anomalies were observed in 1/46 (2.1%) of babies in the rhG-CSF group versus 1/49 (2.0%) in the placebo group (RR of 0.9; 95% CI: 0.1–13.4; $P = 0.93$).

Findings of subgroup analyses are given in Supplementary Table SII; no significant subgroup effects were identified. Exploratory analyses for a number of key obstetric outcomes did not find any significant differences between the rhG-CSF and placebo arms.

Discussion

This randomized clinical trial investigating the effect of recombinant human granulocyte colony stimulating factor in the first trimester of pregnancy in women, diagnosed with unexplained recurrent pregnancy

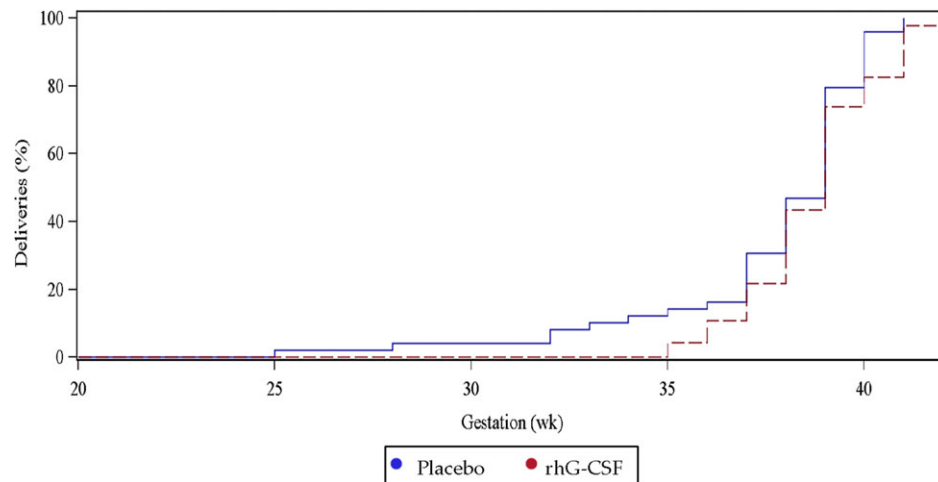


Figure 2 Distribution of gestational age according to study group assignment. Only pregnancies which continued beyond 24 weeks are shown.

loss, found no improvement in clinical pregnancies at 20 weeks, or live births, compared to placebo.

Comparison with previous studies

The findings from this large multicentre, randomized, placebo-controlled trial do not support the findings of the only previous randomized control study evaluating recombinant G-CSF in recurrent pregnancy loss (Scarpellini and Sbracia, 2009). In this previous, smaller, single center study by Scarpellini and Sbracia, 68 women with a previous history of unexplained recurrent miscarriage were randomized to G-CSF or placebo. Women in the intervention group were started on a dose of 1 mcg/kg/day starting on sixth day after ovulation. The live birth rates in the rhG-CSF group was 82.8% versus 48.5% in the placebo group ($P = 0.0061$, OR = 5.1; 95% confidence interval 1.5–18.4), suggesting a statistically significant improvement in outcomes.

There are also two retrospective cohort studies in women with recurrent miscarriage which suggested improved outcomes with administration of G-CSF (Santjohanser *et al.*, 2013, Würfel, 2013). Observational data from two separate population registry was also identified. Boxer *et al.*, identified 224 pregnancy events in women diagnosed with chronic neutropenia and identified a decrease in abortion rates with no adverse side effects (Boxer *et al.*, 2010). However, Zeidler *et al.*, used data from severe chronic neutropenia international registry (SCNIR) and observed no improvement in pregnancy outcomes after administration of G-CSF (Zeidler *et al.*, 2014). All the above studies used a varying dose and duration of G-CSF and were of poor quality (ESHRE Recurrent Pregnancy Loss Guidelines, 2017).

rhG-CSF is also widely used in assisted conception treatment. A previous review of G-CSF in reproductive medicine studies also suggested therapeutic benefit based on body weight dependent target dose or use of G-CSF as an intrauterine infusion (Cavalcante *et al.*, 2015). This review included 1 RCT, 5 cohort studies and 1 case report in a varied range of patients. The included studies were of poor quality and the researchers called for larger well-designed studies. A more recent randomized open label clinical trial of G-CSF (using a single

dose of 300 µg as an intrauterine infusion on day of oocyte recovery) in 100 infertile women undergoing *in-vitro* fertilization treatment did not show a benefit of G-CSF in improving pregnancy outcomes (Eftekhar *et al.*, 2016).

Strength and limitations of this study

The strengths of our study include the multicentre study design involving 21 hospitals spread across the United Kingdom. After standard screening tests, as practiced in the United Kingdom, 150 women with unexplained recurrent pregnancy losses from different ethnic background were randomized. Thus, our study represents the largest placebo-controlled randomized control study of rhG-CSF in women with unexplained recurrent pregnancy losses. Participant compliance rate was high and all participants were followed up until completion of study endpoints, as appropriate. Unlike other studies where a varying dose of G-CSF was utilized, we initiated optimal dosage of rhG-CSF treatment as soon as the pregnancy was confirmed, which started as early as 7 days after ovulation. A Phase I, randomized, double-blind, placebo-controlled, dose-escalation study was conducted prior to this study for dosage determination. This study consisted of six single- and multiple-dose cohorts with eight participants in each dose cohort, randomized in a 3:1 ratio to receive either rhG-CSF or placebo. Transient neutrophilia and increases in white blood cell (WBC) counts were observed following both single and multiple doses. Changes in peripheral blood cell subsets were observed consistent with supporting a state of maternal–fetal immune tolerance. These changes include the temporary induction of toleragenic cell subsets including an increase in toleragenic myeloid derived suppressor cells (MDSC) and a decrease in cytotoxic natural killer (NK) cells, without evidence of global immune changes or suppression. These changes were observed only in the multidose rhG-CSF treatment groups, and not in any placebo group. The weakness of our study was that women were not screened prior to inclusion to demonstrate immune dysfunction as the reason for their pregnancy losses. This is because there is no accepted test(s) for immune dysfunction in reproductive immunology.

There was no increased risk of congenital anomalies among offspring of women treated with rhG-CSF, although the study was not powered for such rare outcomes.

Conclusion

Among women with a history of unexplained recurrent pregnancy loss, administration of rhG-CSF in the first trimester of pregnancy, compared with placebo, did not improve the clinical pregnancy rates at 20 weeks or live birth rates.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Acknowledgements

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Authors' roles

The first, second and last author vouch for the accuracy of the data and analyses and for the fidelity of the study to the protocol. Conception of the study: Darryl Carter, Mark Joing, Paul Kwon and Jeffrey Tong. Data interpretation and writing of the report: Abey Eapen, Mark Joing, David Lissauer and Darryl Carter. Members of trial steering committee: Abey Eapen, Mark Joing and, Paul Kwon. Biomarker study: Ebtehaj Maneta, Carmela De, Francis Mussai and David Lissauer.

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Conflict of interest

Darryl Carter was the co-founder and VP of research, Nora Therapeutics, Inc. and held shares in the company. He holds a patent for the use of recombinant human granulocyte colony stimulating factor to reduce unexplained recurrent pregnancy loss. Mark Joing, Paul Kwon and Jeff Tong were or are employees of Nora Therapeutics, Inc. No other potential conflict of interest relevant to this article was reported.

Appendix

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