

Combination of gefitinib and methotrexate to treat tubal ectopic pregnancy (GEM3): a multicentre, randomised, double-blind, placebo-controlled trial

Andrew W Horne, Stephen Tong, Catherine A Moakes, Lee J Middleton, W Colin Duncan, Ben W Mol, Lucy H R Whitaker, Davor Jurkovic, Arri Coomarasamy, Natalie Nunes, Tom Holland, Fiona Clarke, Ann M Doust, Jane P Daniels, for the GEM3 collaborative



Summary

Background Tubal ectopic pregnancies can cause substantial morbidity or even death. Current treatment is with methotrexate or surgery. Methotrexate treatment fails in approximately 30% of women who subsequently require rescue surgery. Gefitinib, an epidermal growth factor receptor inhibitor, might improve the effects of methotrexate. We assessed the efficacy of oral gefitinib with methotrexate, versus methotrexate alone, to treat tubal ectopic pregnancy.

Methods We performed a multicentre, randomised, double-blind, placebo-controlled trial across 50 UK hospitals. Participants diagnosed with tubal ectopic pregnancy were administered a single dose of intramuscular methotrexate (50 mg/m²) and randomised (1:1 ratio) to 7 days of additional oral gefitinib (250 mg daily) or placebo. The primary outcome, analysed by intention to treat, was surgical intervention to resolve the ectopic pregnancy. Secondary outcomes included time to resolution of ectopic pregnancy and serious adverse events. This trial is registered at the ISRCTN registry, ISRCTN 67795930.

Findings Between Nov 2, 2016, and Oct 6, 2021, 328 participants were allocated to methotrexate and gefitinib (n=165) or methotrexate and placebo (n=163). Three participants in the placebo group withdrew. Surgical intervention occurred in 50 (30%) of 165 participants in the gefitinib group and in 47 (29%) of 160 participants in the placebo group (adjusted risk ratio 1.15, 95% CI 0.85 to 1.58; adjusted risk difference -0.01, 95% CI -0.10 to 0.09; p=0.37). Without surgical intervention, median time to resolution was 28.0 days in the gefitinib group and 28.0 days in the placebo group (subdistribution hazard ratio 1.03, 95% CI 0.75 to 1.40). Serious adverse events occurred in five (3%) of 165 participants in the gefitinib group and in six (4%) of 162 participants in the placebo group. Diarrhoea and rash were more common in the gefitinib group.

Interpretation In women with a tubal ectopic pregnancy, adding oral gefitinib to parenteral methotrexate does not offer clinical benefit over methotrexate and increases minor adverse reactions.

Funding National Institute of Health Research.

Copyright © 2023 The Author(s). Published by Elsevier Ltd. This is an Open Access article under the CC BY 4.0 license.

Introduction

Ectopic pregnancies are a significant contributor to maternal morbidity and mortality in both high-income and low-income countries.¹ More than 90% of ectopic pregnancies occur in a fallopian tube. Without medical intervention, the ectopic pregnancy can continue to grow, cause the tube to rupture, and lead to internal abdominal bleeding, which might be life threatening. In most cases, laparoscopic surgery is carried out to remove the ectopic pregnancy (usually along with the affected fallopian tube), ideally before it ruptures. Surgery carries inherent risks of damage to visceral organs.

First proposed in 1991,² medical management with a single intramuscular injection of methotrexate is a recognised treatment for women with tubal ectopic pregnancies without signs of tubal rupture.³ Methotrexate is a chemotherapeutic that targets trophoblast DNA synthesis, traditionally described as a dihydrofolate

reductase inhibitor.⁴ Adverse reactions such as stomatitis and nausea are usually mild and self-limiting. More severe adverse reactions are rare but include hepatotoxicity, myelosuppression, and nephrotoxicity.

The evidence on the resolution rate of methotrexate treatment for ectopic pregnancy is scarce; however, a retrospective study suggests it is around 70% effective.⁵ Treatment failure carries a risk of requiring a second dose of methotrexate and the subsequent risk of emergency laparoscopic surgery. In addition, ectopic pregnancies with higher human chorionic gonadotrophin (hCG) concentrations (>1000 IU/L) at the start of treatment with methotrexate take a substantial length of time to resolve and require multiple outpatient monitoring visits. More effective medical treatments for tubal ectopic pregnancy are needed to reduce the requirement for additional methotrexate, to reduce the need for emergency surgery,

Published Online
February 1, 2023
[https://doi.org/10.1016/S0140-6736\(22\)02478-3](https://doi.org/10.1016/S0140-6736(22)02478-3)
See Online/Comment
[https://doi.org/10.1016/S0140-6736\(23\)00181-2](https://doi.org/10.1016/S0140-6736(23)00181-2)

MRC Centre for Reproductive Health, University of Edinburgh, Edinburgh, UK (Prof A W Horne PhD, A M Doust DipN, Prof W C Duncan MD, L H R Whitaker MD); Department of Obstetrics and Gynaecology, University of Melbourne, Melbourne, VIC, Australia (Prof S Tong PhD, Prof B W Mol PhD); Birmingham Clinical Trials Unit (C A Moakes MSc, L J Middleton MSc) and Tommy's National Centre for Miscarriage Research (Prof A Coomarasamy MD), University of Birmingham, Birmingham, UK; Aberdeen Centre for Women's Health Research, Institute of Applied Health Sciences, School of Medicine, Medical Sciences and Nutrition, University of Aberdeen, Aberdeen, UK (Prof B W Mol); Institute for Women's Health, University College Hospital, London, UK (Prof D Jurkovic, PhD); Department of Obstetrics and Gynaecology, West Middlesex University Hospital, Chelsea and Westminster Hospital Foundation Trust, London, UK (N Nunes MD); Department of Obstetrics and Gynaecology, Guy's and St Thomas' NHS Foundation Trust, London, UK (T Holland MD); Department of Obstetrics and Gynaecology, East Lancashire Hospitals NHS Trust, Burnley, UK (F Clarke MA); Nottingham Clinical Trials Unit, University of Nottingham, Nottingham, UK (Prof J P Daniels PhD);

Correspondence to:
 Prof Andrew W Horne, MRC
 Centre for Reproductive Health,
 Queen's Medical Research
 Institute, Edinburgh
 EH16 4TJ, UK
andrew.horne@ed.ac.uk

Research in context

Evidence before this study

We searched PubMed for articles published from database inception to March 28, 2022. We used the search terms “ectopic pregnancy” AND “treatments” AND “gefitinib”. There is already extensive literature evaluating the use of methotrexate to treat tubal ectopic pregnancies and reviewing each of these trials was beyond the remit of our study (given both trial groups received the same dose of this drug). Instead, for methotrexate treatment we examined meta-analyses, reviews, and international clinical guidelines on the medical management of ectopic pregnancies. We also searched for general reviews on the topic of ectopic pregnancy treatment (search terms “ectopic pregnancy” AND “review”).

At the time of the design of the trial, it had been shown in preclinical studies that tubal implantation sites express high concentrations of epidermal growth factor receptor (EGFR) and that gefitinib (an EGFR antagonist) augments methotrexate-induced regression of pregnancy-like tissue. There was also clinical evidence from uncontrolled phase 1 and 2 trials that raised the possibility that combination of methotrexate and gefitinib could be a more effective medical treatment than methotrexate alone to treat stable ectopic pregnancies.

Added value of this study

This is the first randomised placebo-controlled clinical trial to evaluate the treatment of tubal ectopic pregnancy with a combination of methotrexate and gefitinib. The robustness of the study design, including blinding to treatment allocation of both participants and investigators, ensured internal validity and enabled the findings to be interpreted with confidence. Groups were balanced with respect to serum human chorionic

gonadotrophin (hCG) concentrations, BMI, and ectopic pregnancy size: variables that are prognostic for the likelihood of success with methotrexate treatment. The range of serum hCG concentrations (1003–4946 IU/L) reflects the diversity of the participants studied and the generalisability of the results.

Implications of all the available evidence

In light of our clinical trial results, we can confidently conclude that women with a tubal ectopic pregnancy should not be offered the combination of gefitinib and methotrexate because it is no more effective than treatment with methotrexate alone. The combination treatment might also cause additional symptoms, such as a transient rash or diarrhoea. Our trial results also provide high-quality evidence that women with ectopic pregnancies (with an hCG of 1000–5000 IU/L before treatment) who are treated with intramuscular methotrexate take a median of 28 days for the ectopic pregnancy to resolve (when medical treatment is successful), require a second dose of methotrexate in 14% of cases (95% CI 9–20%), require surgery in 29% (95% CI 22–36%) of cases, return to normal menstruation after a median of 24 days (IQR 24–38 days) from resolution, and have a high level of satisfaction with their treatment. This information will be useful for counselling and for inclusion in early pregnancy guidelines. In our opinion, no further research is required to evaluate the role of gefitinib in the management of women with tubal ectopic pregnancies. Questions that remain unaddressed relate to the use of combination treatment for other extrauterine and uterine ectopic pregnancies, such as caesarean scar pregnancies, or in the management of choriocarcinoma.

and to reduce the time to resolution associated with methotrexate management.

Preclinical⁶ and small clinical studies^{7–10} suggest that adding oral gefitinib to intramuscular methotrexate could improve its efficacy. Gefitinib is an epidermal growth factor receptor (EGFR) inhibitor¹¹ used in the treatment of non-small-cell lung cancer with adverse effects similar to methotrexate, except that it has a high incidence of acneiform rash. Gefitinib could plausibly disrupt the ectopic implantation site, because placental tissue exhibits very high expression of EGFR and the developing placenta seems crucially dependent on this pathway for survival. So far, the clinical evidence is limited to uncontrolled phase 1 and 2 trials. A phase 1, single-arm, open-label, dose-escalation trial (GEM1) found that 12 women with tubal ectopic pregnancy who were administered intramuscular methotrexate and oral gefitinib had a faster resolution of ectopic pregnancy (fall in serum hCG to ≤ 15 IU/L) compared with a historic cohort treated with methotrexate alone (21 days vs 32 days).⁷ A subsequent phase 2, single-arm, open-label trial (GEM2) showed that combination of methotrexate

and gefitinib met an a priori analysis of being at least 70% effective in resolving tubal ectopic pregnancies in 28 women with a serum hCG of 1000–10 000 IU/L before treatment.⁹

In the Gefitinib for Ectopic pregnancy Management (GEM3) study, we evaluated the efficacy and safety of combining methotrexate and gefitinib to treat tubal ectopic pregnancies, compared with methotrexate alone.

Methods

Study design

We conducted a multicentre, randomised, double-blind, placebo-controlled trial in 50 UK hospitals. Ethics approval for the trial was obtained from the Scotland A Research Ethics Committee (REC 16/SS/0014) and clinical trial authorisation from the Medicines and Healthcare products Regulatory Authority. A trial steering committee provided independent oversight of the trial. Confidential inspection of all available data alongside anonymised reports of serious adverse events experienced by participants was reviewed by a data monitoring committee; no reason to recommend halting or modifying the trial was identified.

The trial protocol has been published¹² and is available in the appendix (pp 8–28).

Participants

Eligible participants were women with a tubal ectopic pregnancy who were deemed suitable for medical management with methotrexate. Inclusion criteria were: aged 18–50 years; serum hCG concentrations of 1000–5000 IU/L before treatment (within 1 calendar day of randomisation); clinically stable; haemoglobin between 100 g/L and 165 g/L (no more than 3 calendar days before randomisation); and either a definite diagnosis of tubal ectopic pregnancy (extrauterine gestational sac with yolk sac or embryo, or both, without cardiac activity on ultrasound scan) or a clinical judgment of probable tubal ectopic pregnancy (extrauterine sac-like structure or inhomogeneous adnexal swelling on ultrasound scan, with a background of suboptimal hCG concentrations on at least 2 different days). Participants were excluded if they had a pregnancy of unknown location; evidence of an intrauterine pregnancy;³ an ectopic pregnancy on ultrasound greater than 3.5 cm (mean dimensions); evidence of substantial intra-abdominal bleed on ultrasound scan (defined by echogenic free fluid above the uterine fundus or surrounding ovary, within 1 calendar day of treatment); clinically significant abnormal liver, renal, or haematological indices noted before randomisation (according to local thresholds, where investigations were done no more than 3 calendar days before randomisation); significant pulmonary, dermatological, or gastrointestinal disease, or if they were of Japanese ethnicity (because they could incur an increased risk of interstitial lung disease with gefitinib administration).¹³ All participants provided written informed consent. Most of the exclusion criteria were related to known contraindications to methotrexate treatment.

Randomisation and masking

Participants were randomly assigned (1:1) to receive either methotrexate and gefitinib or methotrexate and matched placebo through a secure online central randomisation system provided by the Birmingham Clinical Trials Unit, with the use of minimisation to balance trial-group assignments according to baseline hCG concentrations (1000 to <1500 IU/L, ≥1500 to <2500 IU/L, or ≥2500 IU/L), BMI (<25 kg/m² or ≥25 kg/m²), sonographic ectopic size (<2 cm or ≥2 cm) and by hospital centre. The appearance, route, and administration of the assigned intervention were identical in both groups. Participants, clinicians, and research staff were unaware of the trial group assignments throughout the trial. Participants were unblinded if a serious adverse event requiring knowledge of the study drug occurred.

Study interventions

Participants were given a single-dose injection of intramuscular methotrexate (50 mg/m²) and an oral dose

of the assigned study drug once a day for 7 days, taken from the time of randomisation, or up to the point of resolution (if this occurred by day 7 after randomisation). The daily dose of gefitinib was 250 mg, which is the standard dosage used in oncology, and its use for 7 days was supported by the dose-escalation study.⁶ The study drugs were supplied by AstraZeneca (Macclesfield, UK), who manufactured the gefitinib and placebo capsules, and dispensed them into numbered containers.

Serum hCG monitoring and monitoring visits after treatment followed each site's local clinical care protocol for treatment with methotrexate (usually day 4 and day 7 after treatment, then weekly thereafter until hCG concentrations fell to <30 IU/L). Relevant information was extracted by case note review by the local clinical research team.

Outcomes

Our primary outcome was surgical intervention for treatment of the index ectopic pregnancy (salpingectomy or salpingostomy by laparoscopy or laparotomy). The criteria for surgical intervention were up to the attending clinician's discretion and could include worsening clinical symptoms, increasing serum hCG concentrations following administration of the study drugs, clinical, ultrasound, or laboratory signs of intra-abdominal bleeding, or participant's request for surgical intervention.

Our secondary outcomes included the need for additional methotrexate doses; number of days until resolution of tubal ectopic pregnancy (resolution was defined by serum hCG concentrations falling to pre-pregnancy nadir of ≤15 IU/L); number of treatment-associated hospital visits until resolution or emergency rescue surgery, and adverse events. Time to return to menses from resolution and acceptability of treatment (by participant-reported Likert scores) were assessed 3 months from resolution via a telephone interview. Each participant was assessed clinically (at each contact as per local policies) and biochemically (haematological, renal, and liver function tests between days 14 and 21 after treatment and these were repeated if deemed clinically significant).

Adherence to treatment was assessed by both participant's self-reported account of total number of tablets taken and clinician-reported data on whether the methotrexate injection was given. We predefined adherence as participants who received their initial methotrexate injection and at least 75% of their allocated treatment (gefitinib or placebo) before resolution (up to a maximum of seven daily doses if resolution had not occurred by day 7 after randomisation).

Sample size

The sample size was based on data taken from the GEM2 phase 2 study,⁹ published cohort data⁸ and an unpublished audit of women undergoing usual care in 2012 at

See Online for appendix

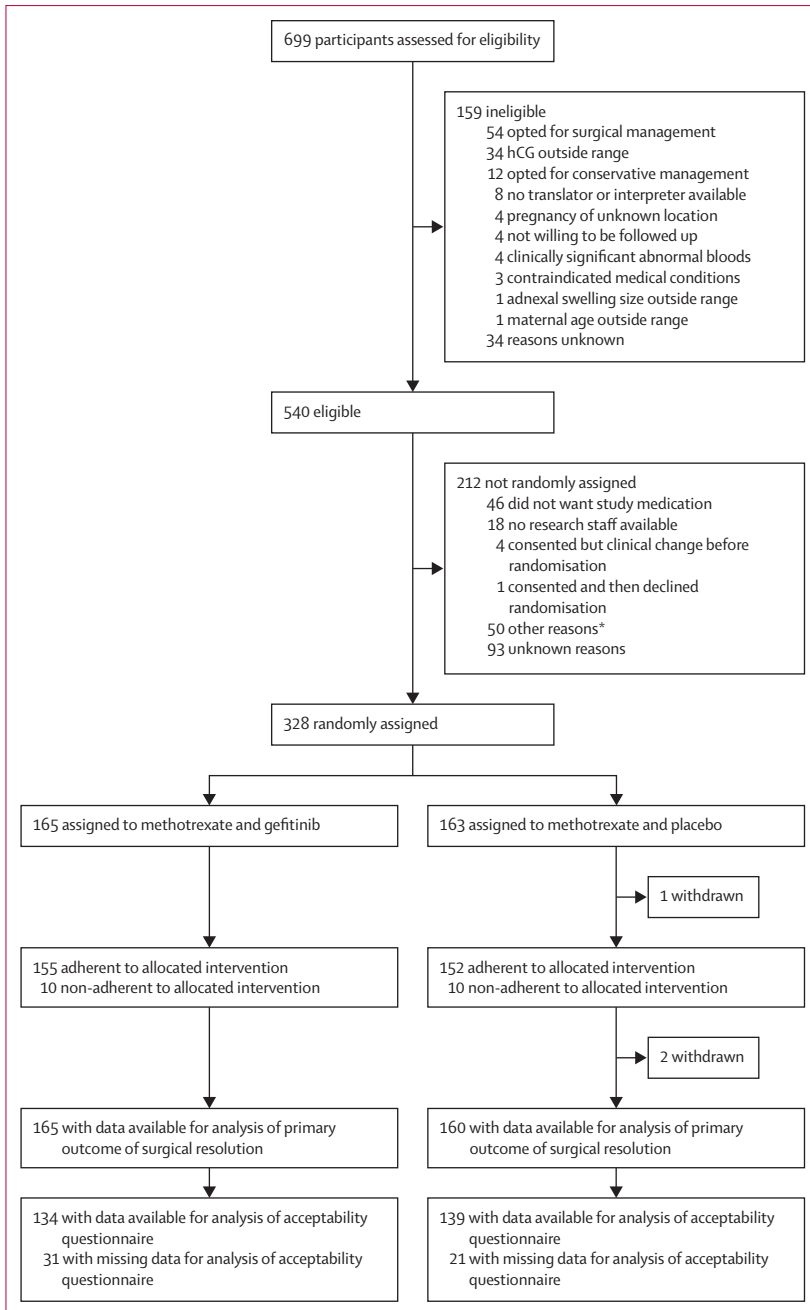


Figure 1: Trial profile

hCG=human chorionic gonadotropin. *Concerns regarding side-effects, general anxiety or worry, family did not want the woman to participate, unable to swallow tablets, and woman did not want primary care physician notified.

two participating sites: Edinburgh, UK, and Melbourne, VIC, Australia. These data suggested 30% of women would require surgical intervention in the methotrexate only group, with a halving of this proportion to 15% plausible in the gefitinib and methotrexate group (a 50% relative reduction). A sample size of 322 participants was required to provide 90% power with an α error rate of 5% to detect this size of difference. We planned to include

328 participants in the trial to account for up to 2% attrition.

Statistical analysis

A comprehensive statistical analysis plan (appendix pp 8–28) was drawn up before any analysis. In brief, categorical data were summarised with frequencies and percentages. Continuous variables were summarised with means and standard deviations unless there was evidence of skew, where medians and IQRs were presented. In the first instance, participants were analysed in the treatment group to which they were randomly allocated (intention to treat), irrespective of adherence with the treatment protocol. All estimates of differences between groups were presented with 95%, two-sided CIs, adjusted for the minimisation variables (where possible).

The primary outcome was analysed using a mixed effects log-binomial model to generate an adjusted risk ratio (RR) and an adjusted risk difference (using an identity link function), including centre as a random effect. Statistical significance of the treatment group parameter was determined (p value generated) through examination of the associated χ^2 statistic (obtained from the log-binomial model which produced the RR).

Binary secondary outcomes were analysed as per the primary outcome. Time to hCG resolution was considered in a competing risk framework to account for participants who had surgical intervention for their ectopic pregnancy.¹⁴ A cumulative incidence function was used to estimate the probability of occurrence (hCG resolution) over time. A Fine and Gray model was then used to estimate a subdistribution adjusted hazard ratio (HR) directly from the cumulative incidence function. In addition, a further Cox proportional hazard model was fitted and applied to the cause-specific (non-surgical resolution) hazard function and used to generate an adjusted HR.¹⁵ Return to menses was analysed using a Cox regression model. Number of hospital visits associated with treatment was analysed using a Poisson regression model, including centre as a random effect to generate an adjusted incidence ratio. Acceptability of treatment was analysed using an ordinal logistic regression model, including centre as a random effect to generate an adjusted odds ratio.

Sensitivity and supportive analyses of the primary outcome included a per-protocol analysis, an analysis which excluded any participants found to violate the inclusion or exclusion criteria after randomisation and an analysis to investigate the small amount of missing primary outcome data by means of a tipping point approach, which explored the possibility that missing responses were missing not at random. A sensitivity analysis for time to hCG resolution was also conducted where the threshold for resolution was considered as 30 IU/L. At the inception of the trial, a number of UK early pregnancy units had moved to urinary testing to

	Methotrexate and gefitinib (n=165)	Methotrexate and placebo (n=162)*
hCG concentration (IU/L)†		
<1500	44 (27%)	40 (25%)
≥1500 to <2500	64 (39%)	65 (40%)
≥2500	57 (34%)	57 (35%)
Median (IQR)	1972 (1457–2820)	2023 (1523–2809)
BMI (kg/m²)†		
<25	79 (48%)	78 (48%)
≥25	86 (52%)	84 (52%)
Mean (SD)	26.7 (5.9)	26.9 (6.3)
Ectopic size (cm)†		
<2	121 (73%)	124 (77%)
≥2	44 (27%)	38 (23%)
Participant's age, years		
Mean (SD)	31.7 (5.6)	31.7 (5.3)
Vaginal bleeding		
No vaginal blood loss	57 (34%)	77 (48%)
Light bleeding	89 (54%)	73 (45%)
Moderate bleeding	17 (10%)	10 (6%)
Heavy bleeding	1 (1%)	2 (1%)
Clots or flooding	1 (1%)	0
Ethnicity		
White	122 (74%)	126 (78%)
Asian	19 (12%)	19 (12%)
Chinese	1 (1%)	3 (2%)
Black	12 (7%)	9 (6%)
Mixed	10 (6%)	2 (1%)
Other‡	0	2 (1%)
Missing or unknown	1	1
Smoking status		
Current smoker	38 (24%)	36 (23%)
Ex-smoker	27 (17%)	32 (21%)
Never smoked	95 (59%)	86 (56%)
Missing or unknown	5	8
Previous chlamydia infection		
Yes	25 (17%)	21 (15%)
No	119 (83%)	120 (85%)
Missing or unknown	21	21
Number of presumed patent tubes		
0	0	1 (1%)
1	24 (15%)	24 (15%)
2	140 (85%)	137 (85%)
Missing	1	0
Nulliparous		
Yes	91 (55%)	85 (52%)
No	73 (45%)	77 (48%)
Missing or unknown	1	0

(Table 1 continues in next column)

	Methotrexate and gefitinib (n=165)	Methotrexate and placebo (n=162)*
(Continued from previous column)		
Number of previous presumed ectopic pregnancies or pregnancies of unknown location		
0	138 (84%)	133 (82%)
1	22 (13%)	21 (13%)
2	3 (2%)	7 (4%)
≥3	1 (1%)	1 (1%)
Missing	1	0
Current IVF pregnancy		
Yes	5 (3%)	2 (1%)
No	159 (97%)	159 (99%)
Missing or unknown	1	1
Ultrasound scan findings		
Extrauterine inhomogeneous swelling	82 (50%)	75 (46%)
Extrauterine sac-like structure	47 (28%)	48 (30%)
Extrauterine gestation sac with yolk sac	31 (19%)	33 (20%)
Extrauterine gestation sac with yolk sac or embryo, or both	5 (3%)	6 (4%)
Data are n (%), median (IQR), or mean (SD). hCG=human chorionic gonadotrophin. IVF=in-vitro fertilisation. *Excluding the participant who did not provide consent. †Minimisation variable. ‡Defined as size of adnexal mass seen on ultrasound. ‡Mixed-White-Asian (n=1) and Latina (n=1).		
Table 1: Demographic and baseline characteristics of the participants in the intention-to-treat population		

Preplanned subgroup analyses (limited to the primary outcome measure only) were completed for the following: baseline serum hCG concentrations (1000 to <1500 IU/L, ≥1500 to <2500 IU/L, and ≥2500 IU/L), BMI (<25 kg/m² or ≥25 kg/m²),¹⁶ and ectopic size on ultrasound (<2 cm or ≥2 cm). The effects of these subgroups were examined by adding the subgroup by treatment group interaction parameters to the regression model. We present p values from the tests for statistical heterogeneity alongside the effect estimate and estimates of uncertainty within each subgroup. In addition to this, ratios were provided to quantify the difference between the treatment effects estimated within each subgroup.

Interim analyses of effectiveness and safety endpoints were performed on behalf of the data monitoring committee on an approximately annual basis during the period of recruitment. These analyses were done with the use of the Haybittle–Peto principle¹⁷ and hence no adjustment was made in the final p values to determine significance.

All analyses were performed in SAS (version 9.4) or Stata (version 17.0). This trial is registered at the ISRCTN registry, ISRCTN 67795930.

Role of the funding source

The National Institute of Health Research (who funded the study), AstraZeneca (who supplied the gefitinib and

determine whether an ectopic pregnancy had resolved after medical treatment. Urinary pregnancy tests for hCG had a sensitivity of approximately 30 IU/L, hence, our inclusion of a sensitivity analysis at this threshold.

	Methotrexate and gefitinib (n=165)	Methotrexate and placebo (n=162)*	Odds ratio (95% CI)	Odds ratio (95% CI)
Primary outcome				
Surgical intervention	50/165 (30%)	47/160 (29%)	1.15† (0.85 to 1.58); p=0.37	-0.01‡ (-0.10 to 0.09)
Secondary outcomes				
Additional methotrexate	20/165 (12%)	23/162 (14%)	0.86† (0.57 to 1.28)	0.01‡ (-0.07 to 0.09)
Time to hCG resolution, days§	28.0 (23.5 to 36.0, 108)	28.0 (21.0 to 36.5, 108)	0.96¶ (0.69 to 1.33)	1.03 (0.75 to 1.40)
Time to hCG resolution, days**	27.5 (20.0 to 35.0, 112)	27.0 (20.0 to 35.0, 111)	1.14¶ (0.83 to 1.58)	1.08 (0.79 to 1.46)
Number of hospital visits	5.0 (4.0 to 7.0, 163)	5.0 (3.0 to 6.0, 162)	1.01†† (0.92 to 1.12)	..
Satisfaction with effects of treatment				
Very satisfied	59/134 (44%)	72/138 (52%)	0.89‡‡ (0.71 to 1.12)	..
Mostly satisfied	44/134 (33%)	33/138 (24%)	NA	..
Neither satisfied nor dissatisfied	24/134 (18%)	23/138 (17%)	NA	..
Mostly dissatisfied	3/134 (2%)	4/138 (3%)	NA	..
Very dissatisfied	4/134 (3%)	6/138 (4%)	NA	..
Acceptability of study treatment				
Very acceptable	78/134 (58%)	87/138 (63%)	0.79§§ (0.49 to 1.28)	..
Mostly acceptable	32/134 (24%)	32/138 (23%)	NA	..
Neither acceptable nor unacceptable	19/134 (14%)	13/138 (9%)	NA	..
Mostly unacceptable	3/134 (2%)	4/138 (3%)	NA	..
Very unacceptable	2/134 (1%)	2/138 (1%)	NA	..
Likely to recommend study treatment				
Very likely to recommend	78/134 (58%)	93/139 (67%)	0.61§§ (0.37 to 1.01)	..
Fairly likely to recommend	32/134 (24%)	33/139 (24%)	NA	..
Neither likely to recommend or recommend against	15/134 (11%)	4/139 (3%)	NA	..
Fairly likely to recommend against	4/134 (3%)	6/139 (4%)	NA	..
Very likely to recommend against	5/134 (4%)	3/139 (2%)	NA	..
Time to return to menses, days¶¶	24.0 (24.0 to 38.0, 132)	24.0 (24.0 to 38.0, 134)	1.08 (0.83 to 1.40)	..

Data are n/N (%), median (IQR, N), or odds ratio (95% CI). hCG=human chorionic gonadotrophin. NA=not available. Minimisation parameters include hCG concentration, BMI, ectopic size, and centre. *Excluding the participant who did not provide consent. †Risk ratio, adjusted for the minimisation parameters. Values <1 favour methotrexate and gefitinib. ‡Risk difference, adjusted for the minimisation parameters with the exclusion of centre parameter (removed from the model due to convergence issues). Values <0 favour methotrexate and gefitinib. §In participants whose pregnancy is resolved (where a participant was followed up to a hCG ≤15 IU/L and did not receive surgical intervention). ¶Cause-specific hazard ratio, adjusted for the minimisation parameters; obtained from a Cox proportional hazard model, censoring participants who received surgical intervention at the point of surgery. Values >1 favour methotrexate and gefitinib. ||Subdistribution hazard ratio, adjusted for the minimisation parameters; obtained from a Fine and Gray model, where surgical intervention is considered a competing event which prevents hCG resolution occurring. Values >1 favour methotrexate and gefitinib. **Sensitivity analysis: in participants whose pregnancy is resolved (where a participant was followed up to a hCG ≤30 IU/L and did not receive surgical intervention). ††Incidence rate ratio, adjusted for the minimisation parameters. Values <1 favour methotrexate and gefitinib. ‡‡Odds ratio, adjusted for the minimisation parameters with the exclusion of centre parameter (removed from the model due to convergence issues). Values >1 indicate participants allocated to methotrexate and gefitinib have higher odds for satisfaction with treatment than participants allocated to methotrexate and placebo. §§Odds ratio, adjusted for the minimisation parameters. Values >1 indicate participants allocated to methotrexate and gefitinib have higher odds for acceptability of treatment or likeliness to recommend treatment than participants allocated to methotrexate and placebo. ¶¶Post resolution. |||Hazard ratio, adjusted for the minimisation parameters. Values >1 favour methotrexate and gefitinib.

Table 2: Primary and secondary outcomes

placebo free of charge) and Sharp Clinical Services (who distributed the drug and placebo) had no role in study design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the results for publication.

Results

Screening of participants commenced on Nov 2, 2016, and the last participant was randomly allocated on Oct 6, 2021 (figure 1). Recruitment was paused during the COVID-19 pandemic from March 20 to June 2, 2020, but otherwise COVID-19 had little effect on the trial process and data collection. Out of 328 participants

providing informed consent, 165 were randomly allocated to gefitinib and 163 to placebo. Three participants in the placebo group withdrew or were withdrawn. Two of the participants withdrew because it was discovered after commencing treatment that their pregnancies were in fact intrauterine, and the third participant was withdrawn immediately after randomisation because consent had not been sought. Data for the primary outcome analysis were available for 165 participants in the gefitinib group and 160 participants in the placebo group.

At enrolment, baseline characteristics were balanced between groups (table 1). The mean age was 31.7 years

(SD 5.5 years), mean BMI was 26.8 kg/m² (SD 6.1 kg/m²), the median hCG concentrations before treatment were 1994.0 (IQR 1487.0–2819.0), and 25% had a starting ectopic size of 2 cm or greater measured on ultrasound. Adherence to study drug was high in both treatment groups (94%).

There was no evidence of a difference in the primary outcome. The surgical intervention rate in the gefitinib group was 30% (50 of 165) versus 29% (47 of 160) in the placebo group (adjusted RR 1.15, 95% CI 0.85 to 1.58; adjusted risk difference -0.01, 95% CI -0.10 to 0.09; *p*=0.37; table 2). Sensitivity and supportive analyses had minimal impact on effect estimates (appendix pp 5–6).

There were no differences in the secondary outcomes (table 2). The median time to resolution of the ectopic pregnancy (defined as serum hCG declining to ≤15 IU/L) for those in whom medical management successfully resolved the ectopic pregnancy was 28.0 (IQR 23.5–36.0, *n*=108) days in the gefitinib group and 28.0 (IQR 21.0–36.5, *n*=108) days in the placebo group (cause-specific HR 0.96, 95% CI 0.69–1.33; subdistribution HR 1.03, 95% CI 0.75–1.40; figure 2). A second dose of methotrexate was administered to 20 (12%) of 165 participants in the gefitinib group and 23 (14%) of 162 participants in the placebo group (adjusted RR 0.86, 95% CI 0.57–1.28). The number of hospital visits were similar in both groups.

Five (3%) of 165 participants in the gefitinib group and six (4%) of 162 in the placebo group (*p*=0.74; table 3) experienced a serious adverse side-effect. One participant in the gefitinib group experienced an unexpected serious adverse reaction, possibly to methotrexate, but was discharged from hospital after 24 h observation. The proportions of participants who reported diarrhoea or a rash were higher in the gefitinib group than the placebo group (table 3). The median number of days before a return to menses after resolution was the same in both groups: 24.0 (IQR 24.0–38.0, *n*=132) days in the gefitinib group versus 24.0 (IQR 24.0–38.0, *n*=134) days in the placebo group (adjusted HR 1.08, 95% CI 0.83–1.40). Both treatments had high rates of satisfaction (percentage of participants who were very satisfied or mostly satisfied was 77% in the gefitinib group and 76% in the placebo group; table 2).

Discussion

This multicentre, randomised, double-blind, placebo-controlled trial showed that adding oral gefitinib to standard medical treatment with methotrexate in women with a tubal ectopic pregnancy did not reduce the rate of surgical interventions. The confidence intervals around our comparative estimate exclude our target difference of 50% relative reduction (15% absolute reduction) and also exclude a smaller relative reduction of 15% or more and, when considered with the higher incidence of reported adverse symptoms, we can conclude that gefitinib is not clinically effective.

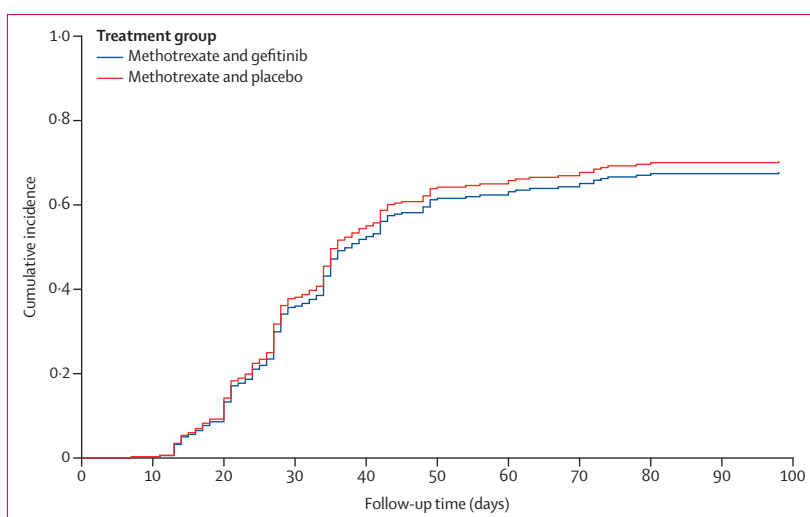


Figure 2: Cumulative incidence function plot for time to human chorionic gonadotropin of 15 IU/L or less (by treatment group)

	Methotrexate and gefitinib (n=165)	Methotrexate and placebo (n=162)*
Reported symptoms		
Abdominal pain	135/162 (83%)	133/160 (83%)
Dizziness	72/160 (45%)	61/160 (38%)
Nausea	104/161 (65%)	97/160 (61%)
Diarrhoea	75/160 (47%)	39/161 (24%)
Rash	97/159 (61%)	36/160 (23%)
Mouth ulcers	42/160 (26%)	24/160 (15%)
Fatigue	127/161 (79%)	115/161 (71%)
Vomiting	32/160 (20%)	33/160 (21%)
Adverse events		
Number of participants who experienced an adverse event	37/165 (22%)	38/162 (23%)
Number of adverse events	52	63
Serious adverse events		
Total number of participants experiencing a serious adverse event	5/165 (3%)	6/162 (4%)
Number of serious adverse events reported	5	6

Data are n or n/N (%). *Excluding the participant who did not provide consent.

Table 3: Summary of reported symptoms and adverse events

Collectively, preclinical studies⁶ and three previous single-arm human trials^{7–9} suggested that combination gefitinib and methotrexate could have been a new medical treatment to resolve most ectopic pregnancies in clinically stable women presenting with hCG concentrations of 5000 IU/L or less. Although there are issues of bias associated with these non-randomised trials, the use of complementary combination chemotherapeutic treatments is a very plausible therapeutic strategy and we proceeded to a phase 3 trial. However, the robustness of our design, including our

large sample size, blinding to treatment allocation of both participants and investigators, near complete capture of the primary outcome, and high adherence ensures internal validity and enables our findings to be interpreted with confidence. Our design ensured groups were balanced with respect to serum hCG concentrations, BMI, and ectopic pregnancy size, all potentially prognostic for the success of methotrexate treatment.

Limitations of the trial include the fact that we only tested one dose regimen. It is possible gefitinib might be effective if a different protocol were used, such as a longer period of administration. Also, we did not do pharmacokinetic studies to ensure good drug coverage, although the incidence of rash suggests there was likely to have been good systemic absorption. However, if an absence of local tissue penetration at the ectopic site was the reason for our negative findings, it could mean that gefitinib might still prove useful for other placental-related disorders where drug penetration can be better (as a result of more collateral vessel blood flow), such as non-tubal ectopic pregnancy or choriocarcinoma. Furthermore, side-effects caused by gefitinib, such as an acneiform rash, might have caused some unblinding. Nonetheless, we believe that it is unlikely that this meaningfully impacted on the primary outcome given rash also occurred among those in the methotrexate-only arm (23% incidence), and the incidence of other minor side-effects was similar in both groups. Even if a few participants correctly guessed their treatment allocation, this is unlikely to have significantly affected the clinical decision to proceed with surgery, or not.

Two cases with slow-rising hCG concentrations associated with a heterogeneous adnexal mass were subsequently diagnosed as a failing intrauterine pregnancy rather than a tubal ectopic pregnancy. This highlights the limitations of the current non-invasive diagnostic tools for ectopic pregnancy. In a detailed retrospective audit of 537 patients treated with methotrexate, focusing on current practice, in a single US centre, 16 (3%) presumed ectopic pregnancies were subsequently found to be intrauterine.¹⁸

Although our trial does not provide any supporting evidence for a new medical treatment approach for women with tubal ectopic pregnancies, it does provide useful information for counselling patients who receive a single-dose regimen of methotrexate (the most common treatment approach in current clinical practice) and for inclusion in early pregnancy guidelines. Our results provide the first high-quality evidence that women with ectopic pregnancies (with an hCG of 1000–5000 IU/L before treatment) who are treated with intramuscular methotrexate take a median of 28 days to resolve (when medical treatment is successful), require a second dose of methotrexate in 14% of cases, require rescue surgery in 29% of cases, return to normal menstruation after a median of

24 days from resolution, and have a high level of satisfaction with their treatment.

In conclusion, our results show that the addition of gefitinib to standard medical management with methotrexate to treat tubal ectopic pregnancy is not clinically effective because it does not reduce subsequent surgical intervention and is associated with higher rates of reported symptoms than placebo.

Contributors

AWH, ST, CAM, LJM, AMD, WCD, BWM, LHRW and JPD contributed to the design, delivery, and interpretation of the trial. CAM and LJM directly accessed and verified the underlying data, and performed the statistical analysis. AMD was responsible for the day-to-day management of the trial. AWH, ST, CAM, LJM, WCD, BWM, LHRW, AC, DJ, NN, TH, FC, and JPD drafted the report and all authors provided input into the editing for publication. All authors in the writing team shared final responsibility for the decision to submit for publication, and have seen and approved the final report.

Declaration of interests

AWH has received funding from Roche Diagnostics, and honoraria for consultancy for Ferring, Roche, Nordic Pharma, AbbVie, Benevolent AI, and GSK. ST is named on patents relating to use of epidermal growth factor receptor inhibitors to treat ectopic pregnancy and is on an advisory board for the Menzies Medical Research Institute. WCD has received honoraria from Merck and Guerbet and research funding from Galvani Biosciences. BWM reports consultancy for ObsEva and Merck and travel support from Merck. LHRW has received grant funding from National Institute of Health Research (NIHR) Health Technology Assessment (HTA) and Roche Diagnostics. TH has received honoraria from Olympus for teaching. All other authors declare no competing interests.

Data sharing

Requests for data should be directed to the corresponding author. Participant-level data will be made available within 6 months of publication. Requests will be assessed for scientific rigour before being granted. Data will be anonymised and securely transferred. A data-sharing agreement might be required.

Acknowledgments

AWH has received funding from NIHR HTA and UK Research and Innovation. WCD is immediate past chair of the Society for Reproduction and Fertility. BWM is supported by an the National Health and Medical Research Council Investigator grant (GNT1176437). AC is immediate past member of the NIHR Efficacy and Mechanism Evaluation Programme Funding Committee. JPD is a member of the NIHR Clinical Trials Unit Standing Advisory Committee.

References

- Say L, Chou D, Gemmill A, et al. Global causes of maternal death: a WHO systematic analysis. *Lancet Glob Health* 2014; 2: e323–33.
- Stovall TG, Ling FW, Gray LA. Single-dose methotrexate for treatment of ectopic pregnancy. *Obstet Gynecol* 1991; 77: 754–7.
- National Institute for Health and Care Excellence. Ectopic pregnancy and miscarriage: diagnosis and initial management. 2021. <https://www.nice.org.uk/guidance/ng126> (accessed Jan 6, 2023).
- Bertino JR, Donohue DR, Gabrio BW, et al. Increased level of dihydrofolic reductase in leucocytes of patients treated with amethopterin. *Nature* 1962; 193: 140–2.
- Sendy F, AlShehri E, AlAjmi A, Bamanie E, Appani S, Shams T. Failure rate of single dose methotrexate in management of ectopic pregnancy. *Obstet Gynecol Int* 2015; 2015: 902426.
- Nilsson UW, Johns TG, Wilmann T, et al. Effects of gefitinib, an epidermal growth factor receptor inhibitor, on human placental cell growth. *Obstet Gynecol* 2013; 122: 737–44.
- Skubisz MM, Horne AW, Johns TG, et al. Combination gefitinib and methotrexate compared with methotrexate alone to treat ectopic pregnancy. *Obstet Gynecol* 2013; 122: 745–51.
- Horne AW, Skubisz MM, Tong S, et al. Combination gefitinib and methotrexate treatment for non-tubal ectopic pregnancies: a case series. *Human Reprod* 2014; 29: 1375–9.

- 9 Skubisz MM, Tong S, Doust A, et al. Gefitinib and methotrexate to treat ectopic pregnancies with a pre-treatment serum hCG 1000–10,000IU/L: phase II open label, single arm multi-centre trial. *EBioMedicine* 2018; **33**: 276–81.
- 10 Italiano S, Tong S, Readman E, Tassone M, Hastie R, Pritchard N. Combination methotrexate and gefitinib: a potential medical treatment for inoperable nontubal ectopic pregnancy. *J Obstet Gynaecol Res* 2020; **46**: 531–5.
- 11 Dhillon S. Gefitinib: a review of its use in adults with advanced non-small cell lung cancer. *Target Oncology* 2015; **10**: 154–70.
- 12 May J, Duncan C, Mol B, et al. A multi-centre, double-blind, placebo-controlled, randomised trial of combination methotrexate and gefitinib versus methotrexate alone to treat tubal ectopic pregnancies (GEM3): trial protocol. *Trials* 2018; **19**: 643.
- 13 Jiang H. Overview of gefitinib in non-small cell lung cancer: an Asian perspective. *Japan J Clin Oncol* 2009; **39**: 137–50.
- 14 Austin PC, Fine JP. Accounting for competing risks in randomized controlled trials: a review and recommendations for improvement. *Stat Med* 2017; **36**: 1203–9.
- 15 Latouche A, Allignol A, Beyersmann J, Labopin M, Fine JP. A competing risks analysis should report results on all cause-specific hazards and cumulative incidence functions. *J Clin Epidemiol* 2013; **66**: 648–53.
- 16 Levin G, Meyer R, Dior U, et al. Outcome of methotrexate treatment for ectopic pregnancies among obese women. *J Gynecol Obstet Human Reprod* 2020; published online May 12. <https://doi.org/10.1016/j.jogoh.2020.101790>.
- 17 Peto R, Pike MC, Armitage P, et al. Design and analysis of randomized clinical trials requiring prolonged observation of each patient. I. Introduction and design. *Br J Cancer* 1976; **34**: 585–612.
- 18 Fridman D, Hawkins E, Dar P, et al. Methotrexate administration to patients with presumed ectopic pregnancy leads to methotrexate exposure of intrauterine pregnancies. *J Ultrasound Medicine* 2019; **38**: 675–84.