



**Phase II single arm open label multicentre clinical trial to evaluate the efficacy and side effects of combination gefitinib and methotrexate to treat ectopic pregnancies (GEM II): study protocol**

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2013-002902
Article Type:	Protocol
Date Submitted by the Author:	18-Mar-2013
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<b>Primary Subject Heading</b>:	Obstetrics and gynaecology
Secondary Subject Heading:	Reproductive medicine, Evidence based practice
Keywords:	REPRODUCTIVE MEDICINE, GYNAECOLOGY, General endocrinology < DIABETES & ENDOCRINOLOGY

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3 1 **Phase II single arm open label multicentre clinical trial to evaluate the**  
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5 2 **efficacy and side effects of combination gefitinib and methotrexate to treat**  
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7 3 **ectopic pregnancies (GEM II): study protocol**  
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55 24 **Key words**  
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57 25 Ectopic pregnancy, methotrexate, gefitinib  
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3 **Abstract**  
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3 **Introduction:** Ectopic pregnancy (EP) is the most common life-threatening  
4 condition in gynaecology. Small EPs (pre-treatment serum human chorionic  
5 gonadotrophin [hCG] levels <1500IU/L) respond well to outpatient medical  
6 treatment with intramuscular methotrexate (MTX). Larger EPs take significant  
7 time to resolve with MTX and require multiple outpatient monitoring visits.

8 Gefitinib is an orally-active epidermal growth factor receptor (EGFR) antagonist.

9 In preclinical studies, we found that EP implantation sites express high levels of  
10 EGFR and that gefitinib augments MTX-induced regression of pregnancy-like  
11 tissue. We performed a Phase I toxicity study administering oral gefitinib and  
12 intramuscular MTX to 12 women with small stable EPs. The combination therapy  
13 did not cause significant toxicities and was well-tolerated. We noted that  
14 combination therapy resolved the EPs faster than MTX alone. We now describe  
15 the protocol of a larger single arm trial to estimate the efficacy and side effects of  
16 combination gefitinib and MTX to treat all stable ectopic pregnancies, including  
17 those of larger size currently considered unsuitable for medical therapy.

18 **Methods and analysis:** We propose undertaking a single-arm multicentre open  
19 label trial (in Edinburgh and Melbourne) and recruit 40 women with large stable  
20 ectopic pregnancies (pretreatment serum hCG >1500 IU/L). We will give single  
21 dose of intramuscular MTX (50 mg/m<sup>2</sup>) and oral gefitinib (250mg) daily for  
22 seven days. Our primary outcome is resolution of ectopic pregnancy within 25  
23 days to non-pregnant hCG levels <5 IU/L. Our secondary outcome is safety and  
24 tolerability as determined by clinical/biochemical assessment. Outcomes will be  
25 compared to historical controls given methotrexate only.

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3 1 **Ethics and dissemination:** Ethical approval has been obtained from Scotland A  
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5 2 Research Ethics Committee (MREC 11/AL/0350), Southern Health Human  
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7 3 Research Ethics Committee B (HREC 11180B) and the Mercy Health Human  
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9 4 Research Ethics Committee (R12/25). Data will be presented at international  
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11 5 conferences and published in peer-reviewed journals.  
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14 6 **Trial registration number:** ACTRN12611001056987.  
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3 **1 Article summary**  
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7 **3 Article focus**  
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10 4 Protocol of a study to determine:

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12 • Is combination therapy with methotrexate and gefitinib more effective at  
13 resolving ectopic pregnancies than methotrexate alone?  
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15 • Is combination therapy with methotrexate and gefitinib safe and well-  
16 tolerated?  
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23 **10 Key messages**  
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28 • Small stable ectopic pregnancies respond well to treatment with  
29 intramuscular methotrexate.  
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31 • Larger ectopic pregnancies require multiple hospital visits to resolve with  
32 methotrexate and often require surgery.  
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34 • Novel combination therapy of methotrexate and the oral EGFR antagonist,  
35 gefitinib, could reduce the number of hospital visits required to resolve  
36 larger ectopic pregnancies.  
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## 1 Strengths and limitations of this study

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- This is a Phase II exploratory efficacy trial, and will be the "first in man" to examine the efficacy of gefitinib and methotrexate to treat larger ectopic pregnancies.
  - This is a 'single arm' trial comparing outcome with historical controls. The data will be used to inform a future large multicentre randomised controlled trial comparing combination therapy to surgery for treatment of ectopic pregnancies.
  - The combination therapy described also has potential use in other pregnancy disorders where medical regression of placental tissue could be useful e.g. molar disease, retained products of conception after miscarriage, and regression of placenta accrete post-partum.

## 1 Introduction

2  
3 Ectopic pregnancy (EP) is the most common life-threatening condition in  
4 modern gynaecology in both the developed and developing world (Sivalingham  
5 et al. 2011; Wilkinson and Jurkovic, 2011). Small EPs (pre-treatment serum  
6 human chorionic gonadotrophin [hCG] levels <1500IU/L) respond well to  
7 outpatient medical treatment with an intramuscular injection of methotrexate  
8 (MTX). Indeed, it has been recently suggested that these small ectopic  
9 pregnancies could be managed safely, and equally efficiently, by expectant  
10 management without medical intervention (Mavrellos et al, 2013). In contrast, for  
11 larger EPs (~60% of total EPs) emergency laparoscopic surgical excision (with  
12 its inherent risks of damage to visceral organs) remains the most effective  
13 treatment (Mol et al. 2008). Larger EPs take a significant time to resolve with  
14 MTX and require multiple outpatient monitoring visits. There therefore exists a  
15 need for more effective medical treatments for larger EPs to reduce the need for  
16 emergency surgery and reduce the time to resolution associated with MTX  
17 management.

18  
19 Gefitinib is an orally active epidermal growth factor receptor (EGFR) antagonist  
20 licensed to treat non-small cell lung cancer (Herbst et al. 2004). In preclinical  
21 studies, we found that EP implantation sites express high levels of EGFR and that  
22 gefitinib augments MTX-induced regression of pregnancy-like tissue (Nilsson et  
23 al. 2010). To translate this into clinical care, we performed a Phase I single-arm  
24 open-label dose-escalation study administering a combination of 250mg oral  
25 gefitinib (one dose [n=3], three daily doses [n=3], seven daily doses [n=6]) and

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3 1 intramuscular MTX (50 mg/m<sup>2</sup>) to 12 women with EPs. The combination  
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5 2 therapy did not cause any significant toxicities and was well tolerated. We noted  
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7 3 that resolution (fall in serum hCG to <5IU/L) with combination therapy was  
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10 4 faster than the median time for EPs to resolve with MTX alone when compared  
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12 5 to contemporaneous controls (21 versus 32 days).  
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## 14 6

### 17 **Objectives**

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21 9 The objective of this trial is to evaluate the efficacy and side effects of  
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23 10 combination gefitinib and MTX to treat larger ectopic pregnancies (pretreatment  
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25 11 hCG >1500), including those currently considered unsuitable for medical  
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28 12 therapy.  
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3 **1 Methods and analysis**  
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7 **3 Study design**  
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10 4 Phase II single-arm multicentre open label trial (Edinburgh and two sites in  
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12 5 Melbourne).  
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16 **7 Subjects**  
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18 8 40 women with large stable ectopic pregnancies.  
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23 **10 Study settings**  
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25 11 We will recruit patients from gynaecology departments within NHS Lothian  
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27 12 (UK), and Southern Health and Mercy Health networks in Melbourne, Australia.  
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32 **14 Sample size**  
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34 15 We have calculated the sample size required to estimate the true treatment effect  
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36 16 with 90% confidence that the lower limit is at least 90%. We have assumed for  
37  
38 17 the sake of these calculations that the true treatment efficacy of  
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40 18 methotrexate/gefitinib in treating these larger ectopic pregnancies is 95%. We  
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42 19 have chosen in this scenario, a sample size of 36 to ensure the lower limit of the  
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44 20 90% CI will exceed 90% (assuming a treatment effect of 95%). Our sample size  
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46 21 of 40 will thus ensure we have sufficient precision in estimation of the true  
47  
48 22 treatment effect, allowing 10% loss to follow up. We believe that this is a  
49  
50 23 reasonable basis on which to conduct this initial efficacy study, of  
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52 24 methotrexate/gefitinib in the treatment of larger ectopic pregnancies that are  
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54 25 currently treated with surgery.  
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3 **1 Inclusion criteria**

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5 2 Women aged aged between 18-45; serum hCG of >1500 IU/L; ultrasound  
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7 3 diagnosis of an abnormal mass outside the uterine cavity; no clinical evidence of  
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10 4 intra-abdominal bleeding; no pallor; no guarding/rigidity on abdominal  
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12 5 examination; stable blood pressure and heart rate; normal haemoglobin on full  
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14 6 blood examination at day 1.  
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19 **8 Exclusion criteria**

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21 9 Women with a history of any significant pulmonary disease; abnormal  
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23 10 liver/renal/haematological indices; significant pre-existing dermatological  
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25 11 conditions; significant pre-existing gastrointestinal medical illnesses; Japanese  
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28 12 ethnicity.  
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32 **14 Participant enrolment**

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34 15 All gynaecology consultants within NHS Lothian (UK), Southern Health and  
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36 16 Mercy Health (both Australia) will be sent a letter informing them of the study  
37  
38 17 and requesting permission to approach their patients. The clinical research team  
39  
40 18 in NHS Lothian, Southern Health and Mercy Health will approach eligible women,  
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42 19 provide them with patient information sheets and offer them the opportunity to  
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44 20 discuss the trial, and obtain informed consent. Consent will only be taken once  
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46 21 the patient has had ample time to read the patient information sheet and had her  
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48 22 questions answered.  
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## 1 **Intervention**

2 Eligible women will be given a single dose intramuscular methotrexate (50  
3 mg/m<sup>2</sup>) injection with seven daily doses oral gefitinib (250 mg). The gefitinib  
4 will be started on the same date as the methotrexate injection is given.  
5

## 6 **Data collection**

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## 8 **Data storage**

9 A log with the patients' name and date of birth will be kept along with their  
10 unique study number in a separate file. All the data generated from the study will  
11 be stored in an anonymised form in a bespoke database, which will also be  
12 password protected. Only anonymised information will be stored on this, and  
13 participants will only be identifiable by their study number. All paperwork will  
14 be kept in a locked filing cabinet in a locked office. All data will be stored on  
15 university server (University of Edinburgh) on a password-protected computer  
16 with limited access to the research team, in accordance with the Data Protection  
17 Act (UK).

18

## 19 **Screening**

20 A member of the research team will carry out a screening visit to assess  
21 eligibility. All data will be recorded on a case record form and transferred to a  
22 secure database.

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## 24 **Participant log**

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3 1 The clinical research team will keep an electronic log of women who fulfil the  
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5 2 eligibility criteria, women who are invited to participate in the study, women  
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7 3 recruited and women who leave the trial early. Reasons for non-recruitment (eg,  
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10 4 non-eligibility, refusal to participate, administrative error) will also be recorded.  
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12 5 We will attempt to collect reasons for non-participation from women who  
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14 6 decline to take part after previously providing contact details. During the course  
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16 7 of the study, we will document reasons for withdrawal from the study and loss to  
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18 8 follow-up.  
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## 22 23 10 **Assessments**

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25 11 To monitor treatment response, we will follow protocols used clinically for single  
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27 12 dose methotrexate. Serum hCG levels will be measured on days 4, 7 and 11, then  
28  
29 13 weekly until hCG levels drop to non-pregnant levels (<5 IU/L). Participants will  
30  
31 14 be contacted at 3 and 6 months post treatment to document return of menstrual  
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33 15 cycles and any subsequent pregnancies. To monitor safety and tolerability,  
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35 16 women will be assessed clinically (history) and biochemically (haematological,  
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37 17 renal and liver function tests) on days 4, 7 and 11, then weekly until the ectopic  
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39 18 pregnancy has resolved (see below).  
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## 46 20 **Primary outcome**

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48 21 Our primary outcome is resolution of ectopic pregnancy within 25 days.  
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50 22 Resolution is defined by serum hCG levels (the current clinical marker to  
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52 23 monitor treatment response) falling to non-pregnant levels (<5 IU/L). We have  
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54 24 selected our primary outcome based on the data from our Phase I trial that  
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56 25 suggested that duration of resolution with combination therapy was faster than  
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1 the median time for EPs to resolve with MTX alone (21 versus 32 days). For  
2 practical purposes, we have chosen a primary outcome of resolution within 25  
3 days rather than 21 days because patients are not normally seen between 18 and  
4 25 days post treatment (our trial participants are followed up weekly from day  
5 11 to conform to standard monitoring protocols for treatment with MTX) (RCOG  
6 2004).

### 7 8 **Secondary outcome**

9 Safety and tolerability as determined by clinical and biochemical assessment.  
10 Both methotrexate and gefitinib have the potential to affect haematological, renal  
11 and liver function.

### 12 13 **Proposed analyses**

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15 Given this is a single arm efficacy trial, the majority of the data will be expressed  
16 as descriptive statistics.

### 17 18 **Ethics and dissemination**

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20 Ethical approval has been obtained from the Scotland A Research Ethics  
21 Committee (LREC 12/SS/0005) (UK), the Southern Health Human Research  
22 Ethics Committee B (SH HREC 11180B) and the Mercy Health Human Research  
23 Ethics Committee (R12/25) (both Australia). Data will be presented at  
24 international conferences and published in peer-reviewed journals. We will  
25 make the information obtained from the study available to the public through  
26 national bodies and charities (e.g. Ectopic Pregnancy Trust).

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3 **1 Adverse events**  
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5 2 Participants will collect information about adverse events in their treatment  
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7 3 diaries. However, they will be instructed to contact the clinical research team at  
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9 4 any time after consenting to join the trial if they have an event that requires  
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11 5 hospitalisation or an event that results in persistent or significant disability or  
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13 6 incapacity. Any serious adverse events that occur after joining the trial will be  
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15 7 reported in detail in the participant's medical notes, followed up until resolution  
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17 8 of the event and reported to the ACCORD Research Governance  
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19 9 (<http://www.accord.ed.ac.uk>) and QA Office based at the University of  
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21 10 Edinburgh, or the Southern Health/Mercy Health Human Research Ethics  
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23 11 Committees and Therapeutic Goods Administration of Australia's Office of  
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25 12 Scientific Evaluation immediately or within 24-72 hours.  
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## 1 Discussion

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3 If effective, we believe that this combination (gefitinib and methotrexate) could  
4 become standard of care for stable ectopic pregnancies. The combination also  
5 has potential use in other pregnancy disorders. There may be other important  
6 conditions where medical regression of pregnancy tissue could be useful, for  
7 example, women with complete molar pregnancies and persistent molar disease,  
8 women with retained products of conception after miscarriage, women with  
9 placenta accrete postpartum (to avoid hysterectomy), and therapeutic  
10 termination of pregnancy.

11  
12 However, we do not anticipate that this will be the final trial to determine  
13 whether further exploration of this area is worthwhile. We hope that the study  
14 will generate sufficient "signal" that gefitinib and methotrexate may be effective,  
15 to support a funding application for a larger trial with a comparative group. Such  
16 a trial could be designed as an "equivalence" trial in terms of treatment efficacy  
17 between surgery and the gefitinib/methotrexate comparison. It would aim to  
18 test the hypothesis that gefitinib/methotrexate was superior in a range of  
19 outcomes prioritised by consumer groups and clinicians. We anticipate that  
20 these outcomes could include: time in hospital, time to resumption of normal  
21 activities, SF-36 at intervals after treatment, and patient satisfaction scores.

22 Outcomes of a subsequent pregnancy are also important but would require long-  
23 term follow up studies. We anticipate that focus groups and surveys of patients  
24 and clinicians would be required to define the outcomes (other than efficacy) of  
25 these studies.

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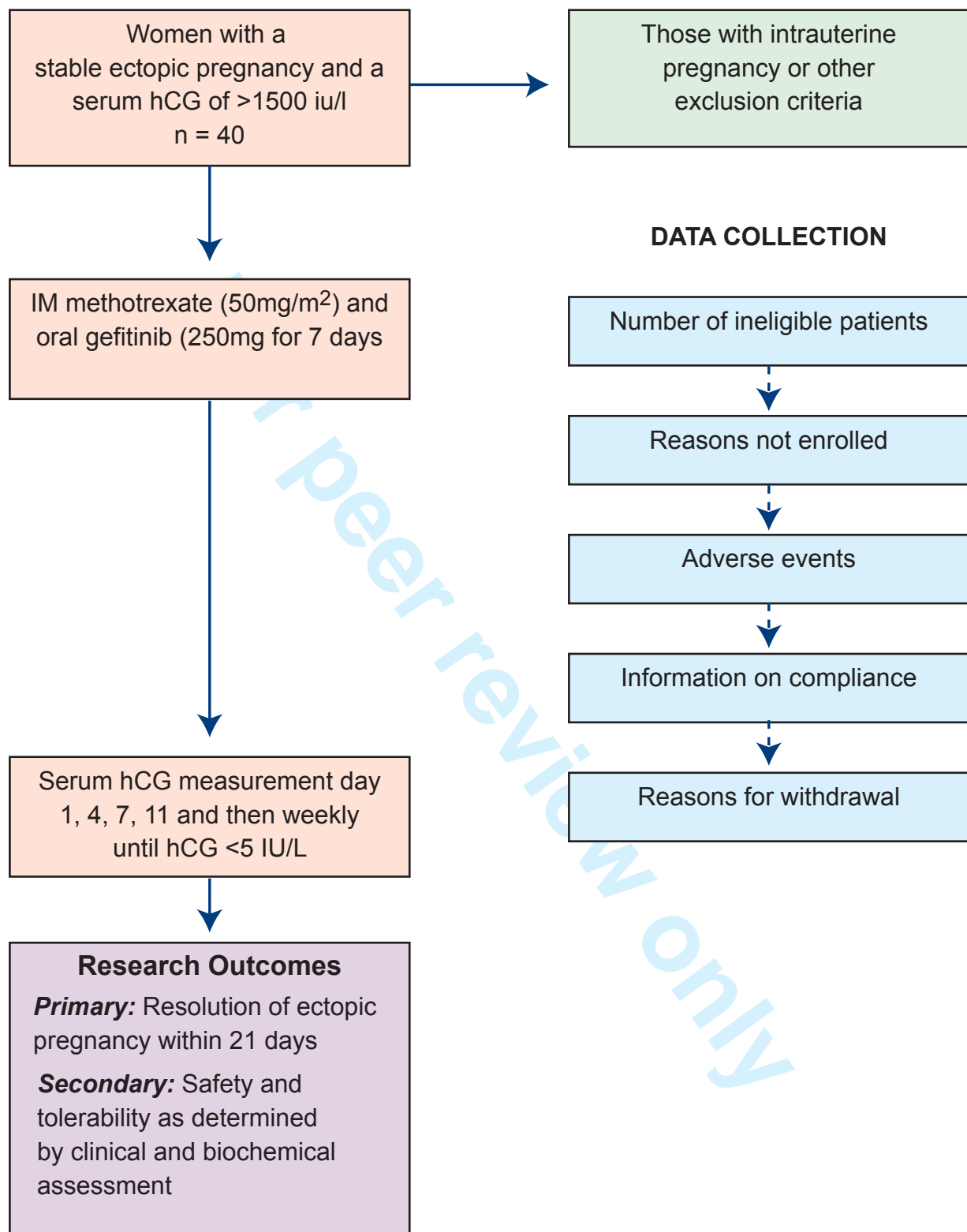
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3 **Figure 1.** Flow of participants through the study.

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OCT12-90



**Phase II single arm open label multicentre clinical trial to evaluate the efficacy and side effects of combination gefitinib and methotrexate to treat ectopic pregnancies (GEM II): study protocol**

Journal:	<i>BMJ Open</i>
Manuscript ID:	bmjopen-2013-002902.R1
Article Type:	Protocol
Date Submitted by the Author:	09-May-2013
Complete List of Authors:	Horne, Andrew Skubisz, Monika Doust, Ann Duncan, W Wallace, Euan Critchley, Hilary; University of Edinburgh, Obstetrics and Gynaecology Johns, Terry Norman, Jane Bhattacharya, Siladitya; Aberdeen University, Obstetrics and Gynaecology Mollison, Jill Rasmussen, Michael Tong, Stephen
<b>Primary Subject Heading</b>:	Obstetrics and gynaecology
Secondary Subject Heading:	Reproductive medicine, Evidence based practice, Pharmacology and therapeutics
Keywords:	REPRODUCTIVE MEDICINE, GYNAECOLOGY, General endocrinology < DIABETES & ENDOCRINOLOGY

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7 **3 tubal ectopic pregnancies (GEM II): study protocol**  
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55 **24 Key words**  
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57 Ectopic pregnancy, methotrexate, gefitinib  
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1  
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3 **Abstract**  
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7 **Introduction:** Tubal ectopic pregnancy (tEP) is the most common life-  
8  
9 threatening condition in gynaecology. TEPs with pre-treatment serum human  
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11 chorionic gonadotrophin [hCG] levels <1000IU/L respond well to outpatient  
12  
13 medical treatment with intramuscular methotrexate (MTX). TEPs with hCG  
14  
15 >1000IU/L take a significant time to resolve with MTX and require multiple  
16  
17 outpatient monitoring visits. Gefitinib is an orally active epidermal growth factor  
18  
19 receptor (EGFR) antagonist. In preclinical studies, we found that EP implantation  
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21 sites express high levels of EGFR and that gefitinib augments MTX-induced  
22  
23 regression of pregnancy-like tissue. We performed a Phase I toxicity study  
24  
25 administering oral gefitinib and intramuscular MTX to 12 women with tEPs. The  
26  
27 combination therapy did not cause significant toxicities and was well tolerated.  
28  
29 We noted that combination therapy resolved the tEPs faster than MTX alone. We  
30  
31 now describe the protocol of a larger single arm trial to estimate the efficacy and  
32  
33 side effects of combination gefitinib and MTX to treat stable tEPs with hCG 1,000-  
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35 10,000IU/L  
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41 **Methods and analysis:** We propose undertaking a single-arm multicentre open  
42  
43 label trial (in Edinburgh and Melbourne) and recruit 28 women with tEPs  
44  
45 (pretreatment serum hCG 1,000-10,000IU/L). We will give a single dose of  
46  
47 intramuscular MTX (50 mg/m<sup>2</sup>) and oral gefitinib (250mg) daily for seven days.  
48  
49 Our primary outcome is resolution of ectopic pregnancy to non-pregnant hCG  
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51 levels <15IU/L without requirement for surgery. Our secondary outcomes are  
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53 comparison of time to resolution against historical controls given MTX only, and  
54  
55 safety and tolerability as determined by clinical/biochemical assessment.  
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3 1 **Ethics and dissemination:** Ethical approval has been obtained from Scotland A  
4  
5 2 Research Ethics Committee (MREC 11/AL/0350), Southern Health Human  
6  
7 3 Research Ethics Committee B (HREC 11180B) and the Mercy Health Human  
8  
9 4 Research Ethics Committee (R12/25). Data will be presented at international  
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11 5 conferences and published in peer-reviewed journals.  
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14 6 **Trial registration number:** ACTRN12611001056987.  
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3 **Article summary**  
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8 **Article focus**  
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10 Protocol of a study to determine:

- 11  
12 • Is combination therapy with methotrexate and gefitinib effective at  
13 resolving tEPs?  
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15 • Is combination therapy with methotrexate and gefitinib safe and well  
16 tolerated?  
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23 **Key messages**  
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- 28 • tEPs with hCG levels <1,000IU/L respond well to treatment with  
29 intramuscular methotrexate.  
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31 • tEPs with hCG levels >1,000IU/L require multiple hospital visits to  
32 resolve with methotrexate and often require surgery.  
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34 • Novel combination therapy of methotrexate and the oral EGFR antagonist,  
35 gefitinib, could reduce the number of hospital visits required to resolve  
36 tEPs with hCG levels >1,000IU/L.  
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3 **1 Strengths and limitations of this study**  
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8 3 • This is a Phase II exploratory efficacy trial, and will be the "first in man" to  
9  
10 4 examine the efficacy of gefitinib and methotrexate to treat tEPs with hCG  
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12 5 levels >1,000IU/L  
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14 6 • This is a 'single arm' trial. The data will be used to inform a future large  
15  
16 7 multicentre randomised controlled trial comparing combination therapy  
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18 8 to conventional management of tEPs.  
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21 9 • The combination therapy described also has potential use in other  
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23 10 pregnancy disorders where medical regression of placental tissue could  
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25 11 be useful e.g. molar disease and regression of placenta accrete post-  
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## 1 Introduction

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Tubal ectopic pregnancy (tEP) is the most common life-threatening condition in modern gynaecology in both the developed and developing world (Sivalingham et al. 2011; Wilkinson and Jurkovic, 2011). TEPs with pre-treatment serum human chorionic gonadotrophin [hCG] levels <1,000IU/L respond well to outpatient medical treatment with an intramuscular injection of methotrexate (MTX). Indeed, it has been suggested that these tEPs could be managed safely, and equally efficiently by expectant management without medical intervention (RCOG, 2010; Mavrellos et al, 2013; van Mello et al, 2013). In contrast, single-dose MTX is only cost-effective in women with serum hCG concentrations <1,500 IU/l (Mol et al. 2008). In tEPs with higher hCG levels (>60% of total tEPs), emergency laparoscopic surgical excision (with its inherent risks of damage to visceral organs) remains the most effective treatment. TEPs with higher hCG levels take a significant time to resolve with MTX and require multiple outpatient monitoring visits. There therefore exists a need for more effective medical treatments for tEPs with higher hCG levels to reduce the need for emergency surgery and reduce the time to resolution associated with MTX management.

Gefitinib is an orally active epidermal growth factor receptor (EGFR) antagonist licensed to treat non-small cell lung cancer (Herbst et al. 2004). In preclinical studies, we found that EP implantation sites express high levels of EGFR and that gefitinib augments MTX-induced regression of pregnancy-like tissue (Nilsson et al. 2010). To translate this into clinical care, we performed a Phase I single-arm open-label dose-escalation study administering a combination of 250mg oral

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3 1 gefitinib (one dose [n=3], three daily doses [n=3], seven daily doses [n=6]) and  
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5 2 intramuscular MTX (50 mg/m<sup>2</sup>) to 12 women with tEPs. The combination  
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7 3 therapy did not cause any significant toxicities, and was well tolerated. We noted  
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10 4 that resolution (fall in serum hCG to <15IU/L) with combination therapy was  
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12 5 faster than the median time for tEPs to resolve with MTX alone when compared  
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14 6 to contemporaneous controls (21 versus 32 days).  
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## 19 **Objectives**

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23 10 The objective of this trial is to evaluate the efficacy and side effects of  
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25 11 combination gefitinib and MTX to treat tEPs (hCG 1,000-10,000IU/L).  
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3 **1 Methods and analysis**  
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3 **3 Study design**

4 Phase II single-arm multi-centre open label trial (Edinburgh and two sites in  
5 Melbourne).

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7 **7 Subjects**

8 28 women with tEPs with hCG levels 1,000-10,000IU/L.

9  
10 **10 Study settings**

11 We will recruit patients from gynaecology departments within NHS Lothian  
12 (UK), and Southern Health and Mercy Health networks in Melbourne, Australia.

13  
14 **14 Sample size**

15 We have calculated the sample size using A'Hern's formula for Phase II one-stage  
16 designs (A'Hern 2001). For treatment of tEPs with hCG levels 1,000-10,000IU/L  
17 by methotrexate/gefitinib to be considered effective, we would expect a success  
18 rate of at least 90%. However, a success rate of 70% or less would be considered  
19 unacceptable. With 80% power and a 5% level of significance, 28 patients are  
20 required to enable us to assess whether the proportion of patients with a  
21 successful outcome to treatment is  $\leq 70\%$  or  $\geq 90\%$ . If 24, or more, patients have  
22 a successful outcome, we can reject the hypothesis that the true efficacy of  
23 methotrexate/gefitinib is  $\leq 70\%$  and progress to a phase II trial.

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3 **1 Inclusion criteria**

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5 2 Women aged between 18-45 years; pre-treatment serum hCG of 1,000-  
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7 3 10,000IU/L (rising or static); ultrasound diagnosis of definite tEP (extrauterine  
8  
9 4 gestational sac with yolk sac and/or embryo, with or without cardiac activity) or  
10  
11 5 probable tEP (inhomogeneous adnexal mass or extrauterine sac-like structure)  
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13 6 (Barnhart et al, 2011) performed by a clinical team of trained, qualified and  
14  
15 7 experienced ultrasonographers; no clinical evidence of intra-abdominal  
16  
17 8 bleeding; no pallor; no guarding/rigidity on abdominal examination; stable blood  
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19 9 pressure and heart rate; haemoglobin on full blood examination at day 1  
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21 10 between 100-165 g/L) .  
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28 **12 Exclusion criteria**

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30 13 Women with a pregnancy of unknown location; evidence of a significant intra-  
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32 14 abdominal bleed on ultrasound defined by free fluid above the uterine fundus or  
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34 15 the surrounding ovary (Fauconnier et al, 2007); women with a history of any  
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36 16 significant pulmonary disease; abnormal liver/renal/haematological indices;  
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38 17 significant pre-existing dermatological conditions; significant pre-existing  
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40 18 gastrointestinal medical illnesses; Japanese ethnicity.  
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46 **20 Participant enrolment**

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48 21 All gynaecology consultants within NHS Lothian (UK), Southern Health and  
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50 22 Mercy Health (both Australia) will be sent a letter informing them of the study  
51  
52 23 and requesting permission to approach their patients. The clinical research team  
53  
54 24 in NHS Lothian, Southern Health and Mercy Health will approach eligible women,  
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56 25 provide them with patient information sheets and offer them the opportunity to  
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1 discuss the trial, and obtain informed consent. Consent will only be taken once  
2 the patient has had ample time to read the patient information sheet and had her  
3 questions answered.

#### 4 5 **Intervention**

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7 Eligible women will be given a single dose intramuscular methotrexate (50  
8 mg/m<sup>2</sup>) injection with seven daily doses oral gefitinib (250 mg). The gefitinib  
9 will be started on the same date as the methotrexate injection is given.

#### 10 11 **Data collection**

#### 12 13 **Data storage**

14 A log with the patients' name and date of birth will be kept along with their  
15 unique study number in a separate file. All the data generated from the study will  
16 be stored in an anonymised form in a bespoke database, which will also be  
17 password protected. Only anonymised information will be stored on this, and  
18 participants will only be identifiable by their study number. All paperwork will  
19 be kept in a locked filing cabinet in a locked office. All data will be stored on  
20 university server (University of Edinburgh) on a password-protected computer  
21 with limited access to the research team, in accordance with the Data Protection  
22 Act (UK).

#### 23 24 **Screening**

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3 1 A member of the research team will carry out a screening visit to assess  
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5 2 eligibility. All data will be recorded on a case record form and transferred to a  
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7 3 secure database.  
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#### 11 5 **Participant log**

12 6 The clinical research team will keep an electronic log of women who fulfil the  
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14 7 eligibility criteria, women who are invited to participate in the study, women  
15  
16 8 recruited and women who leave the trial early. Reasons for non-recruitment (eg,  
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18 9 non-eligibility, refusal to participate, administrative error) will also be recorded.  
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21 10 We will attempt to collect reasons for non-participation from women who  
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23 11 decline to take part after previously providing contact details. During the course  
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25 12 of the study, we will document reasons for withdrawal from the study and loss to  
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27 13 follow-up.  
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#### 35 15 **Assessments**

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37 16 To monitor treatment response, we will follow protocols used clinically for  
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39 17 medical management with methotrexate. Serum hCG levels will be measured on  
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41 18 days 4, 7 and 11, then weekly until hCG levels drop to non-pregnant levels (<15  
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43 19 IU/L). Medical management will be discontinued and patients will undergo  
44  
45 20 surgery based on their response to methotrexate and clinical picture (e.g. clinical  
46  
47 21 evidence of intra-abdominal bleeding) following standard clinical paradigms  
48  
49 22 documented by the assessing clinician. Participants will be contacted at 3 and 6  
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51 23 months post treatment to document return of menstrual cycles and any  
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53 24 subsequent pregnancies. To monitor safety and tolerability, women will be  
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55 25 assessed clinically (history) and biochemically (haematological, renal and liver  
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1 function tests) on days 4 and 7 (or if elevated, until return to normal  
2 physiological levels).

#### 3 4 **Primary outcome**

5 Our primary outcome is resolution of tEP without requirement for surgery.  
6 Resolution is defined by serum hCG levels (the current clinical marker to  
7 monitor treatment response) falling to non-pregnant levels (hCG <15IU/L). We  
8 have selected our primary outcome based on the data from our Phase I trial  
9 where two patients recruited with pretreatment hCG levels >1,000IU/L required  
10 surgery and previously published data (Menon et al, 2007). We are using a cut-  
11 off of <15IU/L, which corresponds to a negative urinary pregnancy test using the  
12 most sensitive assays.

#### 13 14 **Secondary outcome**

- 15 (i) Time to resolution (categorical variable) compared to historical  
16 controls of similar pre-treatment serum hCG levels (identified by an  
17 individual blinded to the study).  
18 (ii) Safety and tolerability as determined by clinical and biochemical  
19 assessment. Both methotrexate and gefitinib have the potential to  
20 affect haematological, renal and liver function.

#### 21 22 **Proposed analyses**

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24 Given this is a single arm efficacy trial, the majority of the data will be expressed  
25 as descriptive statistics.  
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3 **1 Ethics and dissemination**  
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6 3 Ethical approval has been obtained from the Scotland A Research Ethics  
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8 4 Committee (LREC 12/SS/0005) (UK), the Southern Health Human Research  
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10 5 Ethics Committee B (SH HREC 11180B) and the Mercy Health Human Research  
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12 6 Ethics Committee (R12/25) (both Australia). Data will be presented at  
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14 7 international conferences and published in peer-reviewed journals. We will  
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16 8 make the information obtained from the study available to the public through  
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18 9 national bodies and charities (e.g. Ectopic Pregnancy Trust).  
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24 **11 Adverse events**  
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27 12 Participants will collect information about adverse events in their treatment  
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29 13 diaries. However, they will be instructed to contact the clinical research team at  
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31 14 any time after consenting to join the trial if they have an event that requires  
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33 15 hospitalisation or an event that results in persistent or significant disability or  
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35 16 incapacity. Any serious adverse events that occur after joining the trial will be  
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37 17 reported in detail in the participant's medical notes, followed up until resolution  
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39 18 of the event and reported to the ACCORD Research Governance  
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41 19 (<http://www.accord.ed.ac.uk>) and QA Office based at the University of  
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43 20 Edinburgh, or the Southern Health/Mercy Health Human Research Ethics  
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45 21 Committees and Therapeutic Goods Administration of Australia's Office of  
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47 22 Scientific Evaluation immediately or within 24-72 hours.  
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## 1 Discussion

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3 If effective, we believe that this combination (gefitinib and methotrexate) could  
4 become standard of care for stable tEPs. The combination also has potential use  
5 in other pregnancy disorders. There may be other important conditions where  
6 medical regression of pregnancy tissue could be useful, for example, women with  
7 complete molar pregnancies and persistent molar disease, and women with  
8 placenta accrete postpartum (to avoid hysterectomy).

9  
10 Regarding the safety of gefitinib, data from post marketing surveillance  
11 representing over 92,000 patients exists, and has shown that EGFR inhibitors are  
12 well tolerated and largely free of serious side effects (FDA report: (Cohen et al,  
13 2004) . Of note, the data on tolerability is based on patients taking gefitinib daily  
14 on an ongoing, indefinite basis, after primary treatment of a cancer. Diarrhea and  
15 skin rash are the most common side effects (20-30%). The skin rash, described  
16 as acneiform, can be severe, but is generally self-limited. Skin rashes occur  
17 within a month of initiation of treatment, but rarely in the first week. Interstitial  
18 lung disease (ILD) is a very rare but serious side effect of gefitinib. It is a  
19 thickening of the lung parenchyma that can be fatal in a third of cases. Of the  
20 31,045 patients in the USA who took gefitinib (reported to the FDA), 84  
21 developed ILD (0.3%). We plan to administer seven 250mg gefitinib tablets, one  
22 daily for only seven days, in addition to methotrexate. This is an extremely short  
23 duration of treatment compared with gefitinib's current marketing indications  
24 and existing data usage. We would not expect this short course to have an  
25 adverse long-term effect on fertility but we will be assessing participants 3 and 6

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3 1 months post treatment to document return of menstrual cycles and any  
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5 2 subsequent pregnancies.  
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9 4 We do not anticipate that this will be the final trial to determine whether further  
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11 5 exploration of combination therapy with gefitinib and methotrexate is  
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13 6 worthwhile. We hope that the study will generate sufficient "signal" that gefitinib  
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15 7 and methotrexate may be effective and safe, to support a funding application for  
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17 8 a larger trial with a comparative group. Such a trial could be designed as an  
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19 9 "equivalence" trial in terms of treatment efficacy between conventional  
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21 10 management and the gefitinib/methotrexate comparison. It would aim to test  
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23 11 the hypothesis that gefitinib/methotrexate was superior in a range of outcomes  
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25 12 prioritised by consumer groups and clinicians. We anticipate that these  
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27 13 outcomes could include: time to resumption of normal activities, SF-36 at  
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29 14 intervals after treatment, and patient satisfaction scores. Outcomes of a  
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31 15 subsequent pregnancy are also important but would require long-term follow up  
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33 16 studies. We anticipate that focus groups and surveys of patients and clinicians  
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35 17 would be required to define the outcomes (other than efficacy) of these studies.  
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56 25 Kessel MA, Ankum WM, van der Veen F, Mol BW, Hajenius PJ. Methotrexate or  
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3 1 expectant management in women with an ectopic pregnancy or pregnancy of  
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5 2 unknown location and low serum hCG concentrations? A randomized  
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7 3 comparison. Hum Reprod. 2013 28(1):60-7.  
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3 **1 Funding**  
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5 2 This work is supported by an NHMRC Grant (#1008276) to ST, TJ and EW, and  
6  
7 3 an MRC Centenary Award (G0802808) to AH. The funders will have no role in the  
8  
9  
10 4 study design; collection, management, analysis and interpretation of data;  
11  
12 5 writing of the report; and the decision to submit the report for publication.  
13  
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16  
17 **7 Competing interests**  
18

19 8 AH is funded by an MRC Clinician Scientist Fellowship and MRC Centenary  
20  
21 9 Award (G0802808) and holds grants from the Chief Scientist's Office Scotland  
22  
23 10 (CZH/4/688) (HC co-investigator) and Wellbeing of Women. HC holds an MRC  
24  
25 11 DCS Grant (G003611), is a co-applicant of an MRC Centre Grant (G1002033) and  
26  
27 12 has research collaboration funding from Bayer Pharma AG. UN, TJ, and ST are  
28  
29 13 joint holders of patents that relate to the use of EGFR inhibition in treating  
30  
31 14 ectopic pregnancies.  
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37 **16 Authors' contributions**  
38

39 17 AH, ST: research, contribution of original material, editing and approval of final  
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41 18 manuscript; AD, MS: contribution of original material, editing and approval of  
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43 19 final manuscript; MS, MR, CD, EW, HC, TJ, SB, JM, JN: editing and approval of final  
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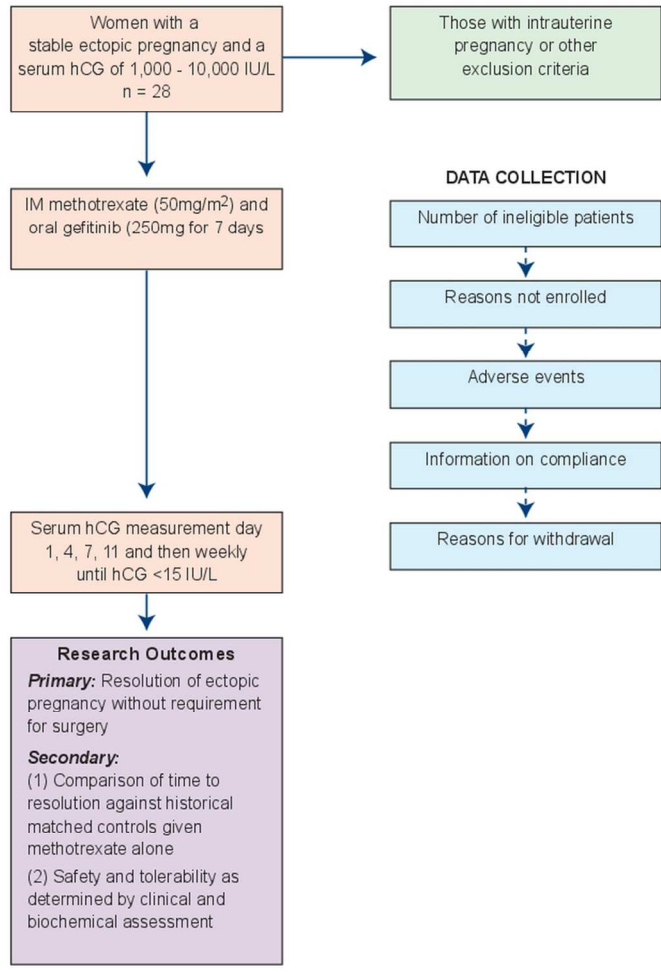
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3 **Figure 1.** Flow of participants through the study.

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6 | **Phase II single arm open label multi-centre clinical trial to evaluate the**  
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8 | **efficacy and side effects of combination gefitinib and methotrexate to treat**  
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10 | **tubal ectopic pregnancies (GEM II): study protocol**  
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14 Andrew W Horne<sup>1</sup>, Monika M Skubisz<sup>2</sup>, Ann Doust<sup>1</sup>, W Colin Duncan<sup>1</sup>, Euan  
15 Wallace<sup>3</sup>, Hilary OD Critchley<sup>1</sup>, Terrance G Johns<sup>3</sup>, Jane E Norman<sup>1</sup>, Siladitya  
16 Bhattacharya<sup>4</sup>, Jill Mollison<sup>4</sup>, Michael Rasmussen<sup>5</sup>, Stephen Tong<sup>2</sup>  
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47 | Tel: +44 131 242 6609      Email: andrew.horne@ed.ac.uk  
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52 | **Key words**

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54 | Ectopic pregnancy, methotrexate, gefitinib  
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6 **1 Abstract**  
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10 **3 Introduction:** ~~Ectopic-Tubal ectopic~~ pregnancy (tEP) is the most common life-  
11 threatening condition in gynaecology. ~~T~~Small EPs ~~(with~~ pre-treatment serum  
12 human chorionic gonadotrophin [hCG] levels ~~<10500IU/L)~~ respond well to  
13 outpatient medical treatment with intramuscular methotrexate (MTX). ~~T~~Larger  
14 EPs ~~with hCG >1000IU/L~~ take a significant time to resolve with MTX and require  
15 multiple outpatient monitoring visits. Gefitinib is an orally ~~-~~active epidermal  
16 growth factor receptor (EGFR) antagonist. In preclinical studies, we found that  
17 EP implantation sites express high levels of EGFR and that gefitinib augments  
18 MTX-induced regression of pregnancy-like tissue. We performed a Phase I  
19 toxicity study administering oral gefitinib and intramuscular MTX to 12 women  
20 with ~~small-stable~~ tEPs. The combination therapy did not cause significant  
21 toxicities and was well ~~-~~tolerated. We noted that combination therapy resolved  
22 the tEPs faster than MTX alone. We now describe the protocol of a larger single  
23 arm trial to estimate the efficacy and side effects of combination gefitinib and  
24 MTX to treat ~~all~~ stable ~~ectopic-pregnanciestEPs, including those with hCG 1,000-~~  
25 ~~10,000IU/L of larger size currently considered unsuitable for medical therapy.~~

26 **19 Methods and analysis:** We propose undertaking a single-arm multicentre open  
27 label trial (in Edinburgh and Melbourne) and recruit ~~28~~ women with ~~t~~large  
28 ~~stable-ectopic-pregnancies~~EPs (pretreatment serum hCG ~~>1,0500-10,000-~~IU/L).  
29 We will give a single dose of intramuscular MTX (50 mg/m<sup>2</sup>) and oral gefitinib  
30 (250mg) daily for seven days. Our primary outcome is resolution of ectopic  
31 pregnancy ~~to non-pregnant hCG levels <15IU/L within 25 dayswithout~~  
32 ~~requirement for surgery to non-pregnant hCG levels <5 IU/L.~~ Our secondary  
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6 | outcomes ~~is~~ are comparison of time to resolution against historical controls  
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8 | given MTX only, and safety and tolerability as determined by  
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10 | 3 clinical/biochemical assessment. ~~Outcomes will be compared to historical~~  
11  
12 | 4 ~~controls given methotrexate only.~~  
13

14 | 5 **Ethics and dissemination:** Ethical approval has been obtained from Scotland A  
15  
16 | 6 Research Ethics Committee (MREC 11/AL/0350), Southern Health Human  
17  
18 | 7 Research Ethics Committee B (HREC 11180B) and the Mercy Health Human  
19  
20 | 8 Research Ethics Committee (R12/25). Data will be presented at international  
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22 | 9 conferences and published in peer-reviewed journals.  
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24 | 10 **Trial registration number:** ACTRN12611001056987.  
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6 1 **Article summary**  
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10 3 **Article focus**  
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12 4 Protocol of a study to determine:

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14 5 | • Is combination therapy with methotrexate and gefitinib ~~more~~ effective at  
15 resolving ~~ectopic pregnancies~~ EPs than methotrexate alone?  
16  
17 6 | • Is combination therapy with methotrexate and gefitinib safe and well-  
18 7 | tolerated?  
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24 10 **Key messages**  
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- 26 11 |  
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28 12 | • Small stable ectopic pregnancies EPs with hCG levels <1,000IU/L respond  
29 well to treatment with intramuscular methotrexate.  
30  
31 13 | • Larger ectopic pregnancies EPs with hCG levels >1,000IU/L require  
32 multiple hospital visits to resolve with methotrexate and often require  
33 14 | surgery.  
34  
35 15 | • Novel combination therapy of methotrexate and the oral EGFR antagonist,  
36 16 | gefitinib, could reduce the number of hospital visits required to resolve  
37  
38 17 | EPs with hCG levels >1,000IU/L larger ectopic pregnancies.  
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6 1 Strengths and limitations of this study  
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- 10 3 • This is a Phase II exploratory efficacy trial, and will be the "first in man" to  
11  
12 4 examine the efficacy of gefitinib and methotrexate to treat TEPs with hCG  
13  
14 5 levels >1,000IU/L larger ectopic pregnancies.  
15  
16 6 • This is a 'single arm' trial ~~comparing outcome with historical controls.~~ The  
17  
18 7 data will be used to inform a future large multicentre randomised  
19  
20 8 controlled trial comparing combination therapy to ~~surgery for~~  
21  
22 9 ~~treatment~~ conventional management of ~~ectopic pregnancies~~ TEPs.  
23  
24 10 • The combination therapy described also has potential use in other  
25  
26 11 pregnancy disorders where medical regression of placental tissue could  
27  
28 12 be useful e.g. molar disease, ~~retained products of conception after~~  
29  
30 13 ~~miscarriage,~~ and regression of placenta accrete post-partum.  
31  
32 14

## 1 Introduction

3 ~~Ectopic Tubal ectopic~~ pregnancy (tEP) is the most common life-threatening  
4 condition in modern gynaecology in both the developed and developing world  
5 (Sivalingham et al. 2011; Wilkinson and Jurkovic, 2011). ~~Small TEPs (with pre-~~  
6 ~~treatment serum human chorionic gonadotrophin [hCG] levels <1,0500IU/L)~~  
7 respond well to outpatient medical treatment with an intramuscular injection of  
8 methotrexate (MTX). Indeed, it has been ~~recently~~ suggested that these ~~small~~  
9 ~~ectopic pregnancies~~tEPs could be managed safely, and equally efficiently, by  
10 expectant management without medical intervention (~~RCOG, 2010; Mavrelou et~~  
11 ~~al, 2013; van Mello et al, 2013~~). In contrast, ~~single-dose MTX is only cost-~~  
12 ~~effective in women with serum hCG concentrations <1,500 IU/l (Mol et al. 2008);~~  
13 ~~for larger~~In tEPs ~~with higher hCG levels (~>60% of total tEPs)~~, emergency  
14 laparoscopic surgical excision (with its inherent risks of damage to visceral  
15 organs) remains the most effective treatment. ~~(Mol et al. 2008)~~ ~~Larger~~EPs ~~with~~  
16 ~~higher hCG levels~~ take a significant time to resolve with MTX and require  
17 multiple outpatient monitoring visits. There therefore exists a need for more  
18 effective medical treatments for ~~larger tEPs with higher hCG levels~~ to reduce  
19 the need for emergency surgery and reduce the time to resolution associated  
20 with MTX management.

22 Gefitinib is an orally active epidermal growth factor receptor (EGFR) antagonist  
23 licensed to treat non-small cell lung cancer (Herbst et al. 2004). In preclinical  
24 studies, we found that EP implantation sites express high levels of EGFR and that  
25 gefitinib augments MTX-induced regression of pregnancy-like tissue (Nilsson et

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6 1 al. 2010). To translate this into clinical care, we performed a Phase I single-arm  
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8 2 open-label dose-escalation study administering a combination of 250mg oral  
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10 3 gefitinib (one dose [n=3], three daily doses [n=3], seven daily doses [n=6]) and  
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12 4 intramuscular MTX (50 mg/m<sup>2</sup>) to 12 women with tEPs. The combination  
13  
14 5 therapy did not cause any significant toxicities, and was well tolerated. We noted  
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16 6 that resolution (fall in serum hCG to <15IU/L) with combination therapy was  
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18 7 faster than the median time for tEPs to resolve with MTX alone when compared  
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20 8 to contemporaneous controls (21 versus 32 days).  
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## 10 Objectives

11  
12 The objective of this trial is to evaluate the efficacy and side effects of  
13  
14 13 combination gefitinib and MTX to treat ~~larger tEPs ectopic pregnancies~~  
15  
16 14 (~~pretreatment hCG >1,000-10,000IU/L1500~~), including those currently  
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18 15 ~~considered unsuitable for medical therapy~~.  
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6 **1 Methods and analysis**  
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10 **3 Study design**  
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12 4 Phase II single-arm multi-centre open label trial (Edinburgh and two sites in  
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14 5 Melbourne).

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18 **7 Subjects**  
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20 8 ~~40-28~~ women with ~~large stable ectopic pregnancies~~ EPs with hCG levels 1,000-  
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22 9 10,000IU/L.  
23

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26 **11 Study settings**  
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28 12 We will recruit patients from gynaecology departments within NHS Lothian  
29  
30 13 (UK), and Southern Health and Mercy Health networks in Melbourne, Australia.  
31

32 14

33  
34 **15 Sample size**  
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36 16 We have calculated the sample size using A'Hern's formula for Phase II one-  
37  
38 17 stage designs [(A'Hern RP (2001)1). Sample size tables for exact single stage  
39  
40 18 Phase II designs. Statistics in Medicine, 20, 859-866]. For treatment of tEPs with  
41  
42 19 hCG levels 1,000-10,000IU/L by methotrexate/gefitinib to be considered  
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44 20 effective, we would expect a success rate of at least 90%. However, a success  
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46 21 rate of 70% or less would be considered unacceptable. With 80% power and a  
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48 22 5% level of significance, 28 patients are required to enable us to assess whether  
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50 23 the proportion of patients with a successful outcome to treatment is  $\leq 70\%$  or  
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52 24  $\geq 90\%$ . -If 24, or more, patients have a successful outcome, we can reject the  
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1 [hypothesis that the true efficacy of methotrexate/gefitinib is  \$\leq 70\%\$  and progress](#)  
 2 [to a phase II trial.](#)

3 ~~required to estimate the true treatment effect with 90% confidence that the~~  
 4 ~~lower limit is at least 90%. We have assumed for the sake of these~~  
 5 ~~calculations that the true treatment efficacy of methotrexate/gefitinib in~~  
 6 ~~treating these tEPs with hCG levels 1,000-10,000IU/L larger ectopic~~  
 7 ~~pregnancies is 98.5%, based on previously published data (Menon et al,~~  
 8 ~~2007). We have chosen in this scenario, a sample size of 36 to ensure the~~  
 9 ~~lower limit of the 90% CI will exceed 90% (assuming a treatment effect of~~  
 10 ~~95.95%). Our sample size of 40 will thus ensure we have sufficient precision~~  
 11 ~~in estimation of the true treatment effect, allowing 10% loss to follow up. We~~  
 12 ~~believe that this is a reasonable basis on which to conduct this initial efficacy~~  
 13 ~~study, of methotrexate/gefitinib in the treatment of tEPs with hCG levels~~  
 14 ~~1,000-10,000IU/L larger ectopic pregnancies that are currently treated with~~  
 15 ~~surgery.~~

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## 17 Inclusion criteria

18 Women aged ~~aged~~ between 18-45 ~~years~~; ~~pre-treatment~~ serum hCG of ~~>1,000-~~  
 19 ~~10,000IU/L-1500 IU/L(rising or static)~~; ultrasound diagnosis of ~~definite tEP~~  
 20 ~~(extrauterine gestational sac with yolk sac and/or embryo, with or without~~  
 21 ~~cardiac activity) or probable tEP (inhomogeneous adnexal mass or extrauterine~~  
 22 ~~sac-like structure) (Barnhart et al, 2011) performed by a clinical team of trained,~~  
 23 ~~qualified and experienced ultrasonographersan abnormal mass outside the~~  
 24 ~~uterine cavity~~; no clinical evidence of intra-abdominal bleeding; no pallor; no  
 25 guarding/rigidity on abdominal examination; stable blood pressure and heart  
 26 rate; ~~normal~~ haemoglobin on full blood examination at day 1 ~~between 100-165~~  
 27 ~~g/L).~~

## 29 Exclusion criteria

30 ~~Women with a pregnancy of unknown location: evidence of a significant intra-~~  
 31 ~~abdominal bleed on ultrasound defined by free fluid above the uterine fundus or~~

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6 | [the surrounding ovary \(Fauconnier et al, 2007\); w](#)Women with a history of any  
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8 | significant pulmonary disease; abnormal liver/renal/haematological indices;  
9  
10 | significant pre-existing dermatological conditions; significant pre-existing  
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12 | gastrointestinal medical illnesses; Japanese ethnicity.  
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## 16 | **Participant enrolment**

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18 | All gynaecology consultants within NHS Lothian (UK), Southern Health and  
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20 | Mercy Health (both Australia) will be sent a letter informing them of the study  
21  
22 | and requesting permission to approach their patients. The clinical research team  
23  
24 | in NHS Lothian, Southern Health and Mercy Health will approach eligible women,  
25  
26 | provide them with patient information sheets and offer them the opportunity to  
27  
28 | discuss the trial, and obtain informed consent. Consent will only be taken once  
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30 | the patient has had ample time to read the patient information sheet and had her  
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## Intervention

Eligible women will be given a single dose intramuscular methotrexate (50 mg/m<sup>2</sup>) injection with seven daily doses oral gefitinib (250 mg). The gefitinib will be started on the same date as the methotrexate injection is given.

## Data collection

## Data storage

A log with the patients' name and date of birth will be kept along with their unique study number in a separate file. All the data generated from the study will be stored in an anonymised form in a bespoke database, which will also be password protected. Only anonymised information will be stored on this, and participants will only be identifiable by their study number. All paperwork will be kept in a locked filing cabinet in a locked office. All data will be stored on university server (University of Edinburgh) on a password-protected computer with limited access to the research team, in accordance with the Data Protection Act (UK).

## Screening

A member of the research team will carry out a screening visit to assess eligibility. All data will be recorded on a case record form and transferred to a secure database.

## Participant log

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6 1 The clinical research team will keep an electronic log of women who fulfil the  
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8 2 eligibility criteria, women who are invited to participate in the study, women  
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10 3 recruited and women who leave the trial early. Reasons for non-recruitment (eg,  
11  
12 4 non-eligibility, refusal to participate, administrative error) will also be recorded.  
13  
14 5 We will attempt to collect reasons for non-participation from women who  
15  
16 6 decline to take part after previously providing contact details. During the course  
17  
18 7 of the study, we will document reasons for withdrawal from the study and loss to  
19  
20 8 follow-up.  
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## 24 10 Assessments

26 11 To monitor treatment response, we will follow protocols used clinically for ~~single~~  
27  
28 12 ~~dose~~medical management with methotrexate. Serum hCG levels will be  
29  
30 13 measured on days 4, 7 and 11, then weekly until hCG levels drop to non-pregnant  
31  
32 14 levels (<15 IU/L). Medical management will be discontinued and patients will  
33  
34 15 undergo surgery based on their response to methotrexate and clinical picture  
35  
36 16 (e.g. clinical evidence of intra-abdominal bleeding) following standard clinical  
37  
38 17 paradigms documented by the assessing clinician. Participants will be contacted  
39  
40 18 at 3 and 6 months post treatment to document return of menstrual cycles and  
41  
42 19 any subsequent pregnancies. To monitor safety and tolerability, women will be  
43  
44 20 assessed clinically (history) and biochemically (haematological, renal and liver  
45  
46 21 function tests) on days 4, ~~and 7 and 11, then weekly until the ectopic pregnancy~~  
47  
48 22 ~~has resolved (see below)(or if elevated, until return to normal physiological~~  
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50 23 ~~levels).~~  
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## 56 Primary outcome

1 Our primary outcome is resolution of ~~ectopic pregnancy~~EP within 25  
 2 ~~days without requirement for surgery~~. Resolution is defined by serum hCG levels  
 3 (the current clinical marker to monitor treatment response) falling to non-  
 4 pregnant levels (hCG <15IU/L)-(~~<5 IU/L~~). We have selected our primary  
 5 outcome based on the data from our Phase I trial ~~that suggested that duration of~~  
 6 ~~resolution with combination therapy was faster than the median time for EPs to~~  
 7 ~~resolve with MTX alone (21 versus 32 days) where two patients recruited with~~  
 8 ~~pretreatment hCG levels >1,000IU/L required surgery and previously published~~  
 9 ~~data (Menon et al, 2007)~~. For practical purposes, we have chosen a primary  
 10 ~~outcome of resolution within 25 days rather than 21 days because patients are~~  
 11 ~~not normally seen between 18 and 25 days post treatment (our trial participants~~  
 12 ~~are followed up weekly from day 11 to conform to standard monitoring~~  
 13 ~~protocols for treatment with MTX) (RCOG 2004)~~-We are using a cut-off of  
 14 <15IU/L, which corresponds to a negative urinary pregnancy test using the most  
 15 sensitive assays.

## 17 Secondary outcome

- 18 (i) Time to resolution (categorical variable) compared to historical  
 19 controls of similar pre-treatment serum hCG levels (identified by an  
 20 individual blinded to the study).
- 21 (ii) Safety and tolerability as determined by clinical and biochemical  
 22 assessment. Both methotrexate and gefitinib have the potential to  
 23 affect haematological, renal and liver function.

## 25 Proposed analyses

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6 1 Given this is a single arm efficacy trial, the majority of the data will be expressed  
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8 2 as descriptive statistics.  
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11 4 **Ethics and dissemination**  
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14 6 Ethical approval has been obtained from the Scotland A Research Ethics  
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16 7 Committee (LREC 12/SS/0005) (UK), the Southern Health Human Research  
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18 8 Ethics Committee B (SH HREC 11180B) and the Mercy Health Human Research  
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20 9 Ethics Committee (R12/25) (both Australia). Data will be presented at  
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22 10 international conferences and published in peer-reviewed journals. We will  
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24 11 make the information obtained from the study available to the public through  
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26 12 national bodies and charities (e.g. Ectopic Pregnancy Trust).  
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30 14 **Adverse events**  
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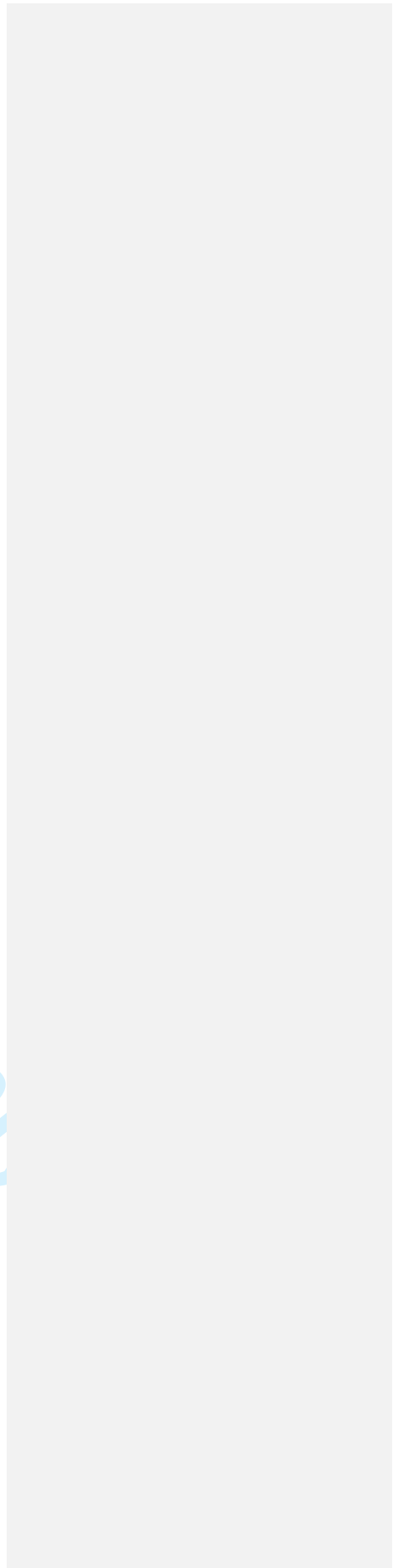
32 15 Participants will collect information about adverse events in their treatment  
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34 16 diaries. However, they will be instructed to contact the clinical research team at  
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36 17 any time after consenting to join the trial if they have an event that requires  
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38 18 hospitalisation or an event that results in persistent or significant disability or  
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40 19 incapacity. Any serious adverse events that occur after joining the trial will be  
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42 20 reported in detail in the participant's medical notes, followed up until resolution  
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44 21 of the event and reported to the ACCORD Research Governance  
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46 22 (<http://www.accord.ed.ac.uk>) and QA Office based at the University of  
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48 23 Edinburgh, or the Southern Health/Mercy Health Human Research Ethics  
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50 24 Committees and Therapeutic Goods Administration of Australia's Office of  
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52 25 Scientific Evaluation immediately or within 24-72 hours.  
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## Discussion

If effective, we believe that this combination (gefitinib and methotrexate) could become standard of care for stable ~~ectopic pregnancies~~EPs. The combination also has potential use in other pregnancy disorders. There may be other important conditions where medical regression of pregnancy tissue could be useful, for example, women with complete molar pregnancies and persistent molar disease, ~~and women with retained products of conception after miscarriage~~, women with placenta accrete postpartum (to avoid hysterectomy), ~~and therapeutic termination of pregnancy~~.

Regarding the safety of gefitinib, data from post marketing surveillance representing over 92,000 patients exists, and has shown that EGFR inhibitors are well tolerated and largely free of serious side effects (FDA report: (Cohen et al, 2004) . Of note, the data on tolerability is based on patients taking gefitinib daily on an ongoing, indefinite basis, after primary treatment of a cancer. Diarrhea and skin rash are the most common side effects (20-30%). The skin rash, described as acneiform, can be severe, but is generally self-limited. Skin rashes occur within a month of initiation of treatment, but rarely in the first week. Interstitial lung disease (ILD) is a very rare but serious side effect of gefitinib. It is a thickening of the lung parenchyma that can be fatal in a third of cases. Of the 31,045 patients in the USA who took gefitinib (reported to the FDA), 84 developed ILD (0.3%). We plan to administer seven 250mg gefitinib tablets, one daily for only seven days, in addition to methotrexate. This is an extremely short duration of treatment compared with gefitinib's current marketing indications

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6 1 [and existing data usage. We would not expect this short course to have an](#)  
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8 2 [adverse long-term effect on fertility but we will be assessing participants 3 and 6](#)  
9  
10 3 [months post treatment to document return of menstrual cycles and any](#)  
11  
12 4 [subsequent pregnancies.](#)  
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16 6 ~~However, w~~We do not anticipate that this will be the final trial to determine  
17  
18 7 whether further exploration of ~~this area~~[combination therapy with gefitinib and](#)  
19  
20 8 [methotrexate](#) is worthwhile. We hope that the study will generate sufficient  
21  
22 9 "signal" that gefitinib and methotrexate may be effective [and safe](#), to support a  
23  
24 10 funding application for a larger trial with a comparative group. Such a trial could  
25  
26 11 be designed as an "equivalence" trial in terms of treatment efficacy between  
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28 12 ~~surgery~~[conventional management](#) and the gefitinib/methotrexate comparison.  
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30 13 It would aim to test the hypothesis that gefitinib/methotrexate was superior in a  
31  
32 14 range of outcomes prioritised by consumer groups and clinicians. We anticipate  
33  
34 15 that these outcomes could include: ~~time in hospital~~, time to resumption of  
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36 16 normal activities, SF-36 at intervals after treatment, and patient satisfaction  
37  
38 17 scores. Outcomes of a subsequent pregnancy are also important but would  
39  
40 18 require long-term follow up studies. We anticipate that focus groups and surveys  
41  
42 19 of patients and clinicians would be required to define the outcomes (other than  
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44 20 efficacy) of these studies.  
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## 1 Funding

2 This work is supported by an NHMRC Grant (#1008276) to ST, TJ and EW, and  
3 an MRC Centenary Award (G0802808) to AH. The funders will have no role in the  
4 study design; collection, management, analysis and interpretation of data;  
5 writing of the report; and the decision to submit the report for publication.

## 7 Competing interests

8 AH is funded by an MRC Clinician Scientist Fellowship and MRC Centenary  
9 Award (G0802808) and holds grants from the Chief Scientist's Office Scotland  
10 (CZH/4/688) (HC co-investigator) and Wellbeing of Women. HC holds an MRC  
11 DCS Grant (G003611), is a co-applicant on an MRC Centre Grant (G1002033) and  
12 has research collaboration funding from Bayer Pharma AG. UN, TJ, and ST are  
13 joint holders of patents that relate to the use of EGFR inhibition in treating  
14 ectopic pregnancies. ~~AH and HC hold the University of Edinburgh Patent~~  
15 ~~'Identification of Ectopic Pregnancies' number 0712801.0.~~

## 17 Authors' contributions

18 AH, ST: research, contribution of original material, editing and approval of final  
19 manuscript; AD, MS: contribution of original material, editing and approval of  
20 final manuscript; MS, MR, CD, EW, HC, TJ, SB, JM, JN: editing and approval of final  
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1 **Figure legend**

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3 **Figure 1.** Flow of participants through the study.

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