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RESEARCH ARTICLE

Chemotherapy initiation with single-course methotrexate alone or combined with dactinomycin versus multi-course methotrexate for low-risk gestational trophoblastic neoplasia: a multi-centric randomized clinical trial

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Abstract **We aimed to evaluate the effectiveness and safety of single-course initial regimens in patients with low-risk gestational trophoblastic neoplasia (GTN). In this trial (NCT01823315), 276 patients were analyzed. Patients were allocated to three initiated regimens: single-course methotrexate (MTX), single-course MTX + dactinomycin (ACTD), and multi-course MTX (control arm). The primary endpoint was the complete remission (CR) rate by initial drug(s). The primary CR rate was 64.4% with multi-course MTX in the control arm. For the single-course MTX arm, the CR rate was 35.8% by one course; it increased to 59.3% after subsequent multi-course MTX, with non-inferiority to the control (difference –5.1%, 95% confidence interval (CI) –19.4% to 9.2%, $P = 0.014$). After further treatment with multi-course ACTD, the CR rate (93.3%) was similar to that of the control (95.2%, $P = 0.577$). For the single-course MTX + ACTD arm, the CR rate was 46.7% by one course, which increased to 89.1% after subsequent multi-course, with non-inferiority (difference 24.7%, 95% CI 12.8%–36.6%, $P < 0.001$) to the control. It was similar to the CR rate by MTX and further ACTD in the control arm (89.1% vs. 95.2%, $P = 0.135$). Four patients experienced recurrence, with no death, during the 2-year follow-up. We demonstrated that chemotherapy initiation with single-course MTX may be an alternative regimen for patients with low-risk GTN.**

Keywords gestational trophoblastic neoplasia (GTN); methotrexate (MTX); dactinomycin (ACTD)

Introduction

Low-risk gestational trophoblastic neoplasia (GTN) is a curable disease with a nearly 100% complete remission (CR) rate. The International Federation of Obstetrics and Gynecology (FIGO) and National Comprehensive Cancer Network (NCCN) all recommend multiple courses of single agents, methotrexate (MTX), or dactinomycin (ACTD), as primary chemotherapy for low-risk GTN [1–4]. Despite the use of different drugs and regimens, 4–7 courses are required to achieve CR [5–10].

In the early 1970s and 1980s, a regimen with single-course treatment initiation in low-risk GTN patients, with additional courses of chemotherapy given according to the fall in human chorionic gonadotropin (hCG) levels, was being used [11], and this practice was introduced in

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Novak's Gynecology (13th edition) in 2002 [12]. Moreover, several retrospective studies have reported that single-course treatment is adequate for some low-risk GTN patients. For instance, a retrospective study has reported that approximately 50% of the patients achieve CR after 1 course of MTX (100 mg/m^2 for 30 min and 200 mg/m^2 for 12 h) and that only 50% of the patients need additional multiple courses of MTX [13], but such a high dosage has not been recommended by the FIGO guidelines since 2002. In general, combined chemotherapy results in a more rapid and sustained efficacy than single-agent therapy, although it may lead to additional toxicity. A retrospective study has reported that 98% of the patients are cured after a mean of three courses of MTX and ACTD [14]. A single course or fewer courses of chemotherapy may improve some patients, but evidence from randomized control trial is lacking. In addition, the prognosis of the remaining patients who failed single-course treatment might be affected because they do not receive standard treatment with fixed intervals.

Considering that the overall survival in low-risk GTN is near 100%, the current strategy of therapy for those patients is to provide the treatment with minimum side effects while ensuring curative effects. Shorter duration of treatment gives patients the opportunity to be pregnant earlier. Therefore, we conducted the present multicenter, prospective, randomized controlled trial to assess the efficacy and safety of single-course MTX and single-course MTX with ACTD vs. multi-course MTX regimens for low-risk GTN patients.

Patients and methods

Patients and eligibility criteria

This work has been conducted in accordance with the *Declaration of Helsinki* (2000) of the World Medical Association. This study was approved by the Ethics Committee of Women's Hospital, Zhejiang University School of Medicine. Local institutional review board approval was separately obtained by each center before the study. Written informed consent was obtained before participation. The trial was registered at www.clinicaltrials.gov (No. NCT01823315, South-East-Middle China Gynecological Oncology Group (CSEM-GOG) trial 001). The participants were recruited from six hospitals across Chinese mainland from December 2012 to December 2015. All GTN patients met the FIGO criteria (2012). According to the diagnostic criteria of FIGO, PSTT and ETT were excluded in all the enrolled patients. The diagnosis of post-mole GTN was based on abnormal hCG levels and was possibly supported by radiologic and/or histological evidence. The criteria included at least four values of hCG plateau for 3 weeks or at least three values of hCG sequential increments for 2 weeks after curettage for hydatidiform mole. The diagnosis of GTN after non-molar pregnancy was based on abnormal vaginal bleeding or metastatic site with elevated hCG levels and by additional histological or radiologic evidence [15]. The eligible participants were enrolled in the study if their risk score was between 0 and 6; their age was no more than 60 years, and no previous chemotherapy had been administered. Pretreatment staging and scoring included chest X-ray, lung computed tomography scan, pelvic ultrasound, and serum hCG assay before the first day of chemotherapy. X-ray was used to calculate the number of lung lesions. All patients were randomly assigned to the three study arms (1:1:1, Fig. 1), using a concealed random allocation from computer-generated random numbers via the Women's Hospital, Zhejiang University School of Medicine, which was responsible for the multi-center trial, data review, protocol compliance, and adverse event review. Patient enrollment and evaluation were implemented by trial leaders in the six centers. The patients and investigators were aware of the assigned protocols from treatment initiation.

Procedures and response assessment

The patients were treated by the allocated initiated regimens: single-course MTX (0.4 mg/kg/day for 5 days, intramuscularly), single-course MTX plus ACTD (ACTD, 600 μg/m^2, days 1 and 2, intravenously; MTX, 100 mg/m^2 and 200 mg/m^2, day 1, intravenously, with tetrahydrofolic acid intramuscularly 24 h after MTX injection for detoxification), or multi-course MTX (0.4 mg/kg/day for 5 days, intramuscularly, biweekly) as a control, which was recommended as a first-line regimen by FIGO [15,16].

The response to treatment was assessed by weekly testing of the serum hCG levels. For both single-course arms, a decrease of at least 1 logarithmic (10-fold) of hCG levels within 18 days after the completion of primary chemotherapy was used as the cut-off for determining further treatment [16]. If a 10-fold decrease in hCG levels was achieved by a single course, then further treatment was withheld; otherwise, multi-course chemotherapy at regular intervals was referred. During the period of observation for those withheld of chemotherapy, if the hCG levels plateaued for at least 3 weeks or increased, then multi-course chemotherapy was also given. CR was defined as the achievement of a normal hCG level (< 5.3 IU/L), which was examined every week for 3 consecutive weeks and every month for 3 consecutive months. Abnormally elevated hCG levels within 3 months were defined as failure, and normal hCG levels within 3 months but then abnormally elevated were defined as relapse. Drug resistance was defined as follows: after two courses of treatment, the hCG levels did not fall logarithmically; the lesion size increased, or new lesions were detected by

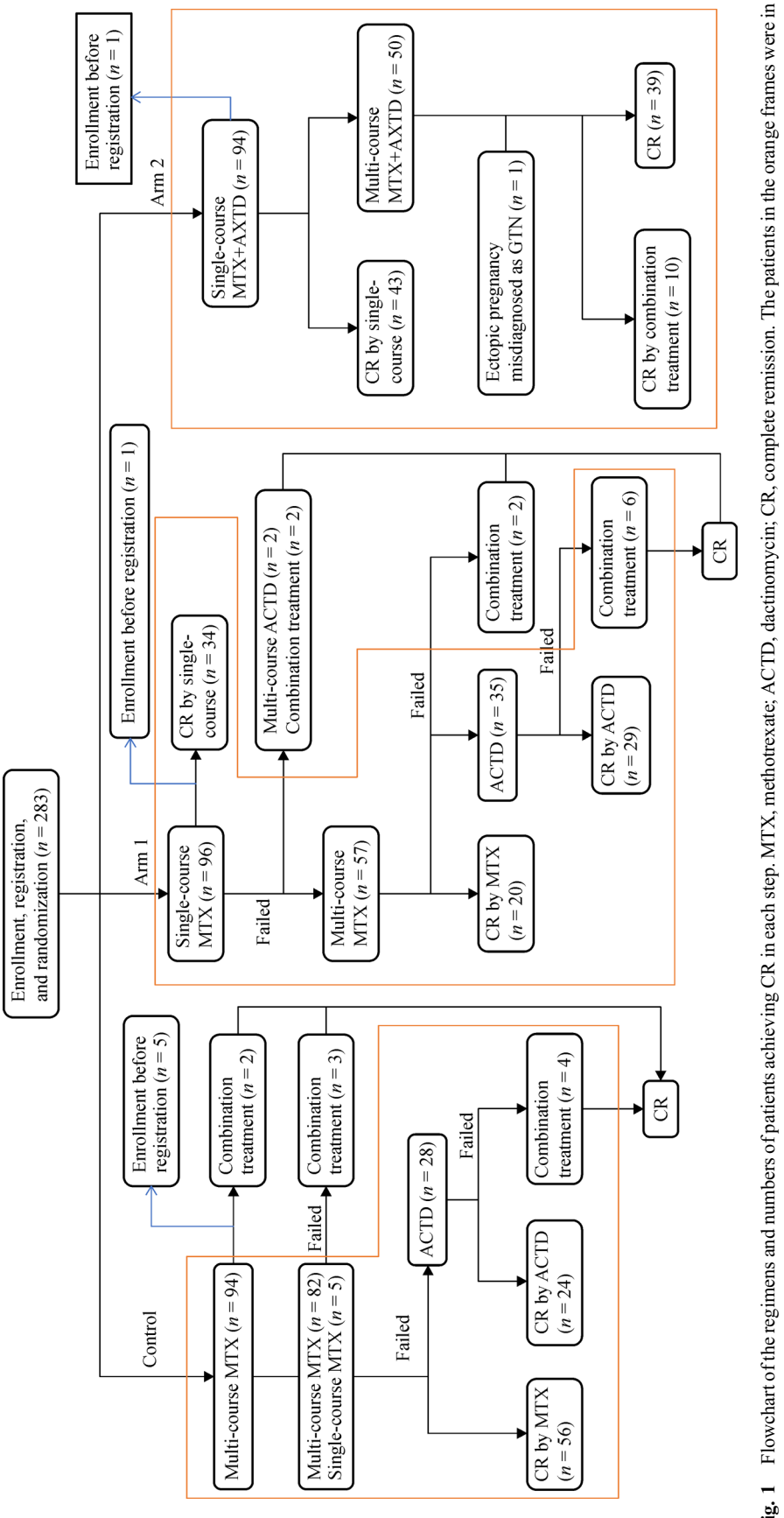

Fig. 1 Flowchart of the regimens and numbers of patients achieving CR in each step. MTX, methotrexate; ACTD, dactinomycin; CR, complete remission. The patients in the orange frames were in the per-protocol set.

imaging. Patients with resistance to MTX were treated with ACTD (10 μg/kg/day for 5 days biweekly, intravenously). Etoposide, MTX, ACTD, cyclophosphamide, vincristine (EMA-CO), or other combination chemotherapy regimens (etoposide, cisplatin/etoposide, MTX, and ACTD (EP-EMA) or EP, biweekly) were administered if MTX plus ACTD or second-line ACTD alone failed. Additional consolidation of chemotherapy using 1–3 courses was performed for those who achieved CR in multiple courses.

After treatment, all patients were requested to undergo regular hCG surveillance once a month for 3 consecutive months and once every 3 months for 2 years. The trial regimens and participant flowchart are shown in Fig. 1.

Study endpoints

The primary endpoint was the CR rate by the initial drug. First, we observed the CR rate by single-course of MTX or single-course of MTX + ACTD. Then, we compared the CR rate by MTX in the single-course MTX arm (arm 1) with that by MTX in the multi-course MTX arm (the control arm). Third, we compared the CR rate by MTX + ACTD in the single-course MTX + ACTD arm (arm 2) with that by MTX in the control arm.

The secondary endpoints were the CR rate by single-drug and salvage multi-drug chemotherapy, single-drug and multi-drug courses per case to achieve CR, recurrence rate, and drug toxicity according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v2.0.

Statistical analysis

The calculation of the sample size was based on the primary efficacy endpoint. Considering that the reported response rates of the conventional multi-course MTX regimen varied (48%–92%) [4], and the incidence of GTN was low, a sample size of 290 eligible patients (including a 20% over-enrollment rate to ensure sufficient eligible patients) equally allocated among treatment regimens was necessary to provide 80% power to detect non-inferiority at a non-inferiority margin of 0.2 in the proportion, with type I error set at 0.025 for a single-tailed test.

Continuous variables that were normally distributed were shown as mean ± standard deviation and were analyzed using *t*-test (two groups) or ANOVA (three groups). Non-normally distributed continuous variables were presented as medians (interquartile range (IQR)) and were compared using the Mann–Whitney U test (two groups) or Kruskal–Wallis test (three groups). Categorical variables were presented as *n* (%) and were analyzed using the chi-square test or Fisher Exact test, as appropriate. The unilateral Z-test was used for the non-inferiority test. A sequential test was used; arm 1 and the control group were compared first, and when the *P*-value was < 0.05, arm 2 and the control group were compared. Logistic regression was used for multivariable regression analysis. The statistical software used in this trial was SAS 9.4 (SAS Institute, Cary, NY, USA) and SPSS 22.0 (IBM, Armonk, NY, USA). All analyses were performed on a per-protocol (PP) basis.

Results

Patients and characteristics

A total of 283 participants were enrolled as the intent-to-treat (ITT) set by Women's Hospital, Zhejiang University School of Medicine; Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology; Qilu Hospital of Shandong University; West China Second University Hospital, Sichuan University; Shengjing Hospital of China Medical University; and Tianjin Central Hospital of Gynecology Obstetrics. Among which, seven patients (five in control arm, one in arm 1, and one in arm 2) were enrolled before the RCT was registered and published on April, 2013 on www.clinicaltrials.gov. They were all excluded from the PP set. Thus, 276 patients were included in the study, with 89 patients in the MTX multi-course (control) group, 95 cases in the MTX single-course group (arm 1), and 92 cases in the MTX + ACTD single-course group (arm 2). Data analyses were performed on a PP basis. The patients in the orange frames were in the PP set (Fig. 1).

The general clinical characteristics of ITT population are shown in Table 1.

Efficacy

In the control arm, 87 of 89 patients accepted multi-course MTX treatment, with two cases excluded for receiving multi-drug chemotherapy. Fifty-six patients (56/87, 64.4%, 95% CI 54.1%–74.6%) achieved CR by the regimen of 5-day MTX every 2 weeks. Of the remaining 31 patients, three were excluded from the PP set for receiving multi-drug regimen. Moreover, 28 patients accepted multi-course ACTD as salvage single-drug chemotherapy. Among which, 24 achieved CR. Thus, in the control arm, the CR rate by single drug was 95.2% (95% CI 90.6%–99.9%). Four cases (4.8%, 95% CI 0.1%–9.4%) who failed ACTD achieved CR by multi-drug chemotherapy (Table S1).

In the single-course MTX arm, 43 of 95 patients (45.3%) achieved a 1-log decrease in hCG levels within 18 days after 1 course of treatment; among the patients, 34 (34/95, 35.8%, 95% CI 26.0%–45.1%) achieved CR within 34 days (median, range: 14–62 days; Table S1). The remaining nine patients who did not achieve CR or presented an increased hCG level within a median of 34

Table 1 Baseline characteristics of the intention-to-treat population

Characteristics	Single-course MTX (n = 96)	Single-course MTX + ACTD (n = 93)	Multi-course MTX (n = 94)
Age (year, median (IQR))	30 (26–40)	30 (26–38)	30 (25–40)
Antecedent			
Mole	85 (89%)	81 (87%)	82 (87%)
Abortion	9 (9%)	11 (12%)	10 (11%)
Term delivery	2 (2%)	1 (1%)	2 (2%)
Interval from index pregnancy (month)			
<4	74 (77%)	76 (82%)	78 (83%)
⩾4	22 (23%)	17 (18%)	16 (17%)
Stage			
I	36 (38%)	28 (30%)	36 (38%)
II	4 (4%)	1 (1%)	5 (5%)
III	56 (58%)	64 (69%)	53 (56%)
WHO score			
0–2	56 (58%)	59 (63%)	55 (59%)
3–4	25 (26%)	22 (24%)	31 (33%)
5–6	15 (16%)	12 (13%)	8 (9%)
Pre-treatment hCG level (median (IQR))	1635.1 (302.1–1140.6)	3785 (1031–14 585.8)	2592.5 (463–12 726)
$<10^3$ IU/L	42 (43.8%)	22 (23.7%)	32 (34.0%)
$\geqslant 10^3$, $<10^4$ IU/L	29 (30.2%)	42 (45.2%)	36 (38.3%)
$\geqslant 10^4$ IU/L	25 (26.0%)	29 (31.2%)	26 (27.7%)
Largest tumor size (cm)			
<3	72 (75%)	78 (84%)	76 (81%)
⩾3	24 (25%)	15 (16%)	18 (19%)
Number of metastases identified			
0	66 (69%)	53 (57%)	65 (69%)
1–4	23 (24%)	27 (29%)	21 (22%)
⩾5	7 (7%)	13 (14%)	8 (9%)
Surgery during chemotherapy	8 (8%)	4 (4%)	3 (3%)

MTX, methotrexate; ACTD, dactinomycin; IQR, interquartile range; hCG, human chorionic gonadotropin.

days (range: 26–42 days) started multiple courses of MTX every 2 weeks. The other 48 patients, whose hCG levels failed to decrease by 1 log after 1 course, underwent a second course of MTX on the 24th day of chemotherapy initiation. Two patients who received a multi-drug regimen and two patients who received ACTD following one course of MTX were not included in the PP set.

Of the 57 patients in the single-course arm who underwent multiple courses of MTX, 20 achieved CR. Thus, 54 of 91 patients (59.3%, 95% CI 49.6%–70.0%) achieved CR with primary MTX chemotherapy (Table S1). By contrast, the control multi-course MTX arm had a CR rate of 64.4%, showing a –5.1% difference (95% CI –19.4% to 9.2%, P = 0.014; Fig. 2A). Two patients received combined chemotherapy after failing MTX treatment and were also excluded from the PP set at this stage. In the remaining patients who received ACTD, 28 achieved CR. Thus, the CR rate (83/89, 93.3%, 95% CI 88.1%–98.6%) by primary MTX and further multi-course ACTD was similar to that in the control arm (95.2%, P = 0.577). Furthermore, six patients (6.7%) in the single-course MTX arm underwent salvage multiple-drug chemotherapy and all achieved CR.

In the single-course MTX arm, the median course to achieve CR by initial MTX was 1 (IQR 1–3), which was significantly lower than that needed in the control group (median 3, IQR 2–4; P = 0.000). If the patients who developed drug resistance to MTX were considered, then no significant difference in median courses for CR by single drug could be found between arm 1 (median 5; IQR 4–6) and the control group (median 5; IQR 4–6; P = 0.568). Furthermore, no significant difference in courses

for CR among patients who needed multi-drug combination was found between arm 1 and the control group (P = 0.662; Table 2).

We further analyzed the effect of prognostic factors on failure to achieve CR following single-course MTX. Multivariable logistic regression analyses showed that high pretreatment hCG levels were significantly correlated to CR failure (Table 3). Patients with hCG levels ⩾ 10 000 IU/L had a fourfold increase in failure compared with those with hCG levels < 1000 IU/L.

In the single-course MTX + ACTD arm, 76.1% (70/92) of the cases achieved a 1-log decrease in hCG level within 18 days after the first course, among which, 43 (46.7%) of 92 patients naturally achieved CR within 34 days (median, range: 16–75 days). In total, 27 of 70 patients who failed to achieve CR after waiting for 33 days (median, range: 26–64 days) underwent multiple courses of MTX and ACTD chemotherapy. Finally, 49 of 92 patients underwent multi-course chemotherapy, 39 of whom achieved CR. A total of 82 of 92 patients (89.1%, Table S1) achieved CR by primary treatment of MTX plus ACTD, showing non-inferiority to the CR rate of primary MTX alone in the control arm (difference 24.7%; 95% CI 12.8%–36.6%; P < 0.001; Fig. 2B). Therefore, arms 1 and 2 were not inferior to the control group. However, the CR rate was similar to that by MTX and further ACTD in the control arm (89.1% vs. 95.2%, P = 0.135). In addition, all the patients treated by salvaged multiple drugs achieved CR.

The median course of treatment required for CR by MTX + ACTD in arm 2 was 1 course (IQR 1–3), which was significantly lower than that of the control group by initial MTX (P = 0.002). On the contrary, for patients who developed drug-resistance to MTX and ACTD, the median courses for CR in arm 2 was not significantly different from that of the control group (P = 0.513, Table 2).

Follow-up

Four patients experienced recurrence during the 2-year follow-up. One occurred in the 4th month and another in the 12th month after CR in the single-course MTX arm, 1 in the second month after CR in the MTX + ACTD arm, and 1 in the 8th month after CR in the multi-course MTX arm. All patients who experienced recurrence were treated by salvage chemotherapy. No patients died through the end of follow-up.

Safety

The common drug toxicities were myelosuppression, gastrointestinal disorders, hepatic damage, and alopecia. The occurrence of alopecia (grades III–IV) was significantly higher in the single-course MTX + ACTD arm (28%) compared with those in the single (13%, P = 0.008) and multi-course (12%, P = 0.001) MTX arms (Table 4).

Discussion

The results of the study showed that 35.8% of low-risk GTN patients achieved CR by single-course MTX. These patients avoided exposure to more courses of cytotoxic drugs, which might result in early childbearing. Previous studies reported a 44.8%–56.3% CR rate with a high dosage of MTX by single-course MTX [12,17,18]. However, such studies are retrospective and uncontrolled.

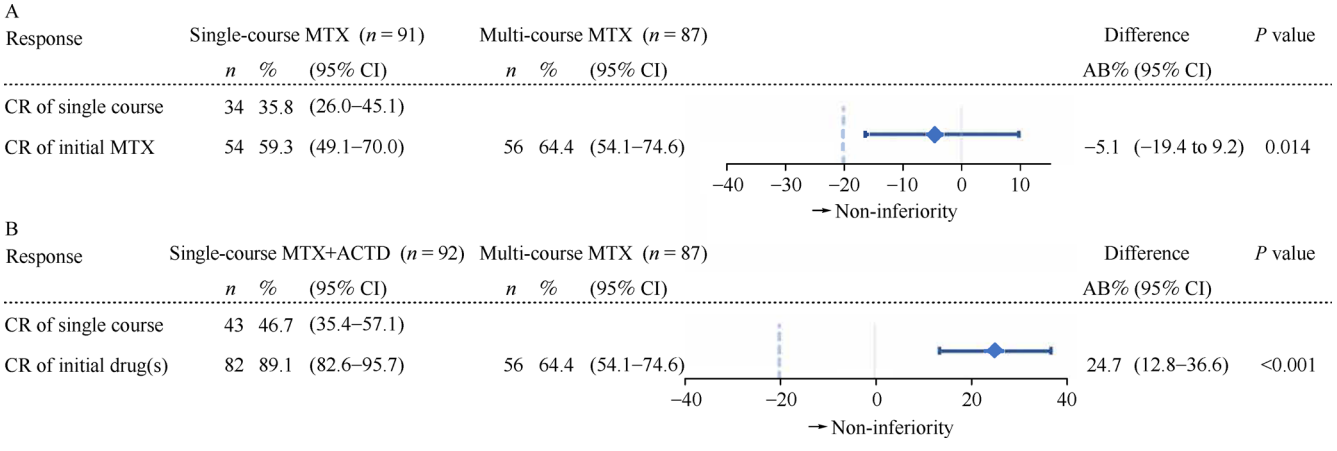

Fig. 2 Comparison of chemotherapy responses between single-course MTX versus multi-course MTX and single-course MTX + ACTD versus multi-course MTX arm. (A) Difference of CR rate between single-course and multi-course MTX arm. (B) Difference of CR rate between single-course MTX + ACTD and multi-course MTX arm. The dashed line denotes the non-inferiority margin of –20% points. Non-inferiority of CR would be declared if the lower boundary was not lower than this margin (i.e., to the right of this line). P value for non-inferiority is set at 0.025. MTX, methotrexate; ACTD, dactinomycin; CR, complete remission; AB, absolute.

Table 2 Comparison of courses for CR needed in different arms

	Control		Arm 1			Arm 2		
	Median	IQR	Median	IQR	*P*	Median	IQR	*P*
CR by initial drugs[a]	3	2–4	1	1–3	0.000	1	1–3	0.002
CR by single drugs[a]	5	4–6	5	4–6	0.568	NA	NA	
CR by salvage multi-drugs[a]	7	7–9[b]	8	7–11[b]	0.662	7	4–10[b]	0.513

[a]Per-protocol analysis. [b]Range, for the small number of patients. CR, complete remission; NA, not applicable.

Table 3 Multivariable analysis of factors related to the need for additional courses of MTX in single-course MTX arm

Factors	OR	95% CI	*P* value
Pre-treatment hCG level			0.027
$<10^3$ IU/L	Reference		
$\geqslant 10^3$, $<10^4$ IU/L	2.211	0.803–6.085	0.125
$\geqslant 10^4$ IU/L	3.979	1.236–12.809	0.021
Age	0.546	0.171–1.74	0.306
Largest tumor size, cm	1.756	0.506–6.092	0.375
Interval, month	0.962	0.313–2.963	0.947
Pregnancy before	1.982	0.498–7.882	0.332
Metastasis number			0.844
0	0.942	0.161–5.499	0.947
1–4	0.673	0.103–4.382	0.679
>4	Reference		

hCG, human chorionic gonadotropin; OR, odds ratio; CI, confidence interval.

Table 4 Main side effects of drugs in different allocated regimens

Main adverse effects	Single-course MTX					Single-course MTX + ACTD					Multi-course MTX				
	G1	G2	G3	G4	Total	G1	G2	G3	G4	Total	G1	G2	G3	G4	Total
Myelosuppression	19	8	18	6	51	31	13	16	4	64	29	16	19	9	73
Stomatitis	31	12	7	1	51	35	3	5	1	44	25	23	5	8	61
Hepatic/kidney	36	9	7	4	56	35	1	5	0	41	35	14	11	1	61
Alopecia	36	3	12	0	51	45	4	25	0	74	23	10	11	0	44

G1, G2, G3, and G4 represent drug toxicity grades 1, 2, 3, and 4, according to the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE) v2.0. MTX, methotrexate; ACTD, dactinomycin.

Based on previous reports, this prospective randomized study is the first to compare clinical outcomes of 5-day MTX with single-course followed by multi-course vs. standard bi-weekly 5-day MTX, which provides reliable evidence of single-course MTX for patients with low-risk GTN. Considering the second-course treatment delay, we should analyze the outcomes after subsequent multi-course chemotherapy in patients who failed single-course treatment. We found that the difference in the primary CR rate (–5.1%, 95% CI –19.4% to 9.2%) between the single-course and multi-course MTX arm was non-inferior. Furthermore, the CR rate by single drugs (initial MTX and subsequent ACTD) was similar to that in the control arm. In addition, compared with multi-course MTX, the single-course MTX regimen reduced median courses by MTX to achieve CR. Moreover, the treatment courses for CR by a single agent and salvage with multiple drugs in the single-course MTX arm were similar compared with those in the multi-course MTX arm. Furthermore, the outcomes of the 2-year follow-up showed that the rate of recurrence was not high in the single-course MTX arm. Thus, the results of the present study suggest that 35.8% of patients with low-risk GTN can avoid multiple courses of MTX, and those who fail to achieve CR with a single course do not present a decreased response to the subsequent single drugs and an increased courses of either single or multiple drugs, compared with patients receiving recommended multi-course MTX treatment every 2 weeks. Based on our result, we found that low serum hCG level before chemotherapy was associated with the success of the single-course MTX treatment. Our result was consistent with that of previous studies, showing that hCG level was a factor related to the CR rate of primary chemotherapy and drug resistance [19,20].

In general, the combination of active drugs yields higher response rates compared with single agents. Eiriksson [14] reported a primary CR rate of 98%; however, Renato [8] reported only a 79.1% CR rate by MTX and ACTD combination treatment. Thus, we designed a single-course MTX + ACTD arm to observe whether the combined regimen further increased the CR rate of single-course while decreasing the number of chemotherapy courses. We found that 46.7% of patients achieved CR by single-course MTX and ACTD. The CR rate of 89.1% for MTX and ACTD was non-inferior to that (64.4%) for primary multi-course MTX alone. However, 10.9% of patients received salvage chemotherapy, which was higher than the control group (4.8%), though no statistically significant difference was found. We found that the median courses required to achieve CR was significantly lower than that needed by multi-course MTX in the control group. However, if 46.7% of patients was excluded from analysis, then the median courses were not decreased (Table S2). Moreover, the median courses of treatment required after conversion to other salvage combined regimen were not reduced. Furthermore, the adverse effects were increased. Our results suggested that evidence to recommend the regimen of primary combination of MTX and ACTD in patients with low risk GTN is insufficient.

In this study, similar adverse events and drug toxicities were observed between the single- and multi-course MTX arms. In arm 2, if patients failed in the initial MTX + ACTD single-course treatment, then they continued to receive the same dose of multi-course drugs. The cumulative MTX was high in these patients, but calcium folinate was used to reduce the toxic side effects. Except for alopecia, the single-course MTX + ACTD arm presented similar toxicities, suggesting that combined MTX and ACTD chemotherapy is tolerable, although it is not significantly beneficial to patients.

Our trial has a limitation. The quality of life and costs of treatment were not assessed, which would support the conclusion of the study.

All patients with low-risk GTN ultimately achieved CR. The single-course MTX allowed 35.8% of patients to avoid multiple courses; moreover, the regimen of MTX with single-course initiation followed by multi-course did not reduce the response to subsequent chemotherapy. Our data suggested that chemotherapy with single-course MTX initiation was an alternative regimen. Furthermore, evidence to recommend the primary regimen of MTX combined with ACTD was insufficient.

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Compliance with ethics guidelines

Lili Chen , Ling Xi, Jie Jiang, Rutie Yin, Pengpeng Qu, Xiuqin Li, Xiaoyun Wan, Yaxia Chen, Dongxiao Hu, Yuyan Mao, Zimin Pan, Xiaodong Cheng, Xinyu Wang, Qingli Li, Danhui Weng, Xi Zhang, Hong Zhang, Quanhong Ping, Xiaomei Liu, Xing Xie, Beihua Kong, Ding Ma, and Weiguo Lu declare that they have no competing interests. The study was approved by the Ethics Committee of Women's Hospital, Zhejiang University School of Medicine (date of approval: June 24, 2012), and registered at ClinicalTrials.gov (No. NCT01823315).

Electronic Supplementary Material Supplementary material is available in the online version of this article at https://doi.org/10.1007/s11684-021-0855-4 and is accessible for authorized users.

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