The multiple-center study reported recently by Hoeijmakers et al.[1] retrospectively analyzed 45 cases with the low-risk gestational trophoblastic neoplasia (GTN), who treated but failed with the first-line chemotherapy, methotrexate (MTX), and then switched to the second-line regimen, dactinomycin (Act-D)(BJOG 2020; https://doi.org/10.1111/1471-0528.16198). This research identified the high human chorionic gonadotropin (hCG) levels before Act-D (hCG_{Act-D} > 40 ng/ml) as a risk factor for the subsequent Act-D failure. Hoeijmakers et al. indicated that the second-line Act-D chemotherapy was an effective option for patients with hCG_{Act-D}<20 ng/mL, while EMA-CO (etoposide, MTX, Act-D/cyclophosphamide, and vincristine) should be considered for patients with hCG_{Act-D} > 40 ng/ml. These findings are interesting and support possible independent associations of hCG_{Act-D} with second-line Act-D failure risk.

By pooling data from the most recent studies and guidelines, Act-D is one of the most recommended salvage choices due to its high effectiveness and low toxicity[2-6]. However, several situations should be considered to use multi-agent salvage therapy rather than Act-D. These situations include a significant elevation of hCG level during initial single chemotherapy[5, 6], and a high serum hCG value at the time of first-line treatment failure[7, 8]. Notably, it is still unclear at the numerical definition of "significant" elevation and the precise cut-off value of hCG for the choice of multi-agent salvage therapy. The cut-off value of hCG varied from 100 to 1,000 IU/L in the different reports[4, 7-9]. Thus Hoeijmakers et al. did try to answer this question[1].

Almost at the same time, we reported our 16-year experience of treating low-risk GTN with Act-D salvage therapy after MTX treatment in the Journal of Gynecologic Oncology[10]. This report showed consistent results with Hoeijmakers et al.[1], which was the hCG_{Act-D} was significantly higher in the cases of Act-D resistance compard with the responders (median 605 vs. 103 IU/L, P=0.009). However, the range of hCG_{Act-D} values in Act-D responders was wider than that in Act-D-resistant cases (5.76–16,664 IU/L vs. 11.43–6,732 IU/L). Considering the individual setting, we difficult to determine a general cut-off value. Another consideration was 82.02% of cases with therapeutic effect and 97.80% of cases with tolerable toxicity. Therefore, we recommended Act-D salvage therapy for all patients with low-risk GTN who fail primary MTX chemotherapy.

To validate the above single-center results, we extracted independent samples from our hospital

and a multi-center research (NCT01823315) as the testing set. This multi-center research was a randomized controlled trial, enrolling patients with low-risk GTN to explore the effective regimen. Besides our center, the other four locations were involved, including Qilu Hospital of Shangdong University, Shengjing Hospital of China Medical University, Tianjin Central Hospital of Gynecology Obstetrics, and West China Second University Hospital, Sichuan University. According to the inclusion criteria of our previous paper[10], the new 46 eligible GTN patients (median age 33.5 years) were extracted in the testing set. **Table.1** summarizes the clinical characteristics of patients, which showed no significantly different characteristics between the training (data from our previous paper [10]) and the testing data sets (all P>0.05). Consistent with the results of the training set, the testing set showed the group of Act-D resistance cases had a significantly higher hCG_{Act-D} value compared with the group of the responders (median 2,638 vs. 35.95 IU/L, P=0.002). For the individual, the range of hCG_{Act-D} also showed an overlap between the Act-D-resistant cases and responders (19.20–147,321 IU/L vs. 5.8–29,172 IU/L, **Fig.1**). Therefore, the results of training set were validated by the independent-sample testing set.

To validate the study by Hoeijmakers et al., we analyzed 135 subjects (median age 29 years) from six centers in China to keep the same methodology of multicenter research. The response rate to the second-line Act-D therapy was 78.52% (106/135). The remaining 21.48% of cases (29/135) required the third-line therapy (multiple-agent EMA-CO) and achieved the final complete-remission. The hCG_{Act-D} still stood as a significant risk-factor in the multivariate analysis. It was higher in the Act-D-resistant cases than those in the Act-D responders (median 740.80 vs. 77.35 IU/L, P<0.001). The receiver operating characteristic (ROC) curve analysis showed hCG_{Act-D} with an area under the ROC curve (AUC) of 0.74 (95% confidence interval (CI) 0.64–0.84), indicating its discriminatory potential **(Fig.2)**. The optimal hCG_{Act-D} cut-off value for Act-D resistance was 140 IU/L (approximation from 139.75 IU/L), with 79.30% sensitivity and 58.50% specificity. Patients with $hCG_{Act-D}\geq$140 IU/L had a 5.4-fold higher risk of Act-D resistance than that in cases with hCG_{Act-D} <140 IU/L (risk ratio 5.40; 95%CI 2.03–14.36; P<0.001). Therefore, the conclusion that hCG_{Act-D} was a risk factor for the Act-D failure was consistent in both Chinese and European multi-center data. [1] However, on the classification analysis, the risk of Act-D failure did not increase with the increasing hCG_{Act-D} levels of 100 IU/L, 300 IU/L, 500IU/L, and 1,000IU/L (**Table.2**). The scatter diagram of hCG_{Act-D} for each patient showed an

apparent overlap between the responders and Act-D-resistant cases (5.76–29,172 IU/L vs. 11.43–147,321 IU/L, **Fig.3**). Therefore, the predictive power of hCG_{Act-D} to identify individuals with Act-D resistance was considered to be limited.

In conclusion, we introduced an independent set of multi-center cases to verified our previous single-center results but also to repeat the results of Hoeijmakers et al.'s study. Agreeing with Hoeijmakers et al.'s work, our results showed the hCG_{Act-D} predictive value for the second-line Act-D failure in low-risk GTN patients with failed primary MTX. However, we still insisted that this predictive power was limited for individuals as to the overlap of hCG_{Act-D} values for Act-D responders and resistance cases, although the overlap pattern is hard to validate in the dataset of Hoeijmakers et al. as these individual hCG_{Act-D} levels were not published. Based on the current data, we still recommended 5-day Act-D salvage therapy for all patients who fail primary MTX chemotherapy regardless of the hCG_{Act-D}.

Disclosure of interests

None declared. A completed disclosure of interest form is available to view online as supporting information.

Contribution to authorship

Q.J.L. and W.X.D. have been involved in the study design. W.X.D., S.T., and C.L.L collected data for this study. W.X.D. and Q.J.L. analyzed the data, L.W.G., F.W.D., and X.X interpreted the data. All authors were equally involved in writing, reviewing and editing the manuscript.

Details of ethical approval

This study was approved by the Ethics Committee of Women's Hospital, Zhejiang University School of Medicine (09/27/2018, No.20180106).

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Table 2. The response rates of Act-D salvage therapy divided in different serum $hCG_{Act\text{-}D}$ levels (n=135)

hCG levels (IU/L)	Response (n)	Resistance (n)	Response rate (%)
<100	57	6	90.48%
100–300	15	4	78.95%
300–500	9	2	81.82%
500–1000	9	5	64.29%
≥1000	16	12	57.14%

Act-D, actinomycinD; $hCG_{Act\text{-}D}$, serum hCG level before Act-D salvage therapy.