

# Sensitivity Assessment of Chemotherapeutic Agents for Ovarian Tumors Based on Test in Vitro Culture: A Blind Study

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## Research

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## Abstract

**Background:** Ovarian cancer is common malignant tumors but the survival rate has not been improved well for a long time. One of the important reasons is that the current clinical guidelines for treatment still do not solve the problem of patient heterogeneity and drug resistance, which are exactly the key factors affecting the efficacy of chemotherapy. In addition, there are limited research data that can help accurately identify patients' preferred drugs and combination regimen, which fails to meet the needs of individualized treatment.

**Methods:** The objective was used the Hydrogel Embedded Histoculture Drug Sensitivity Test (HDST), to verified its accuracy in predicting the response to chemotherapy in ovarian tumors and evaluate the sensitivity of chemotherapy drugs. Tissue samples of 82 ovarian tumor patients were tested for HDST drug sensitivity and follow-up who were included in randomized study. The consistency of drug sensitivity results and clinical outcomes were analyzed through preliminary study to determine the feasibility of this technology, and then a comprehensive blind study was conducted to evaluate Drug sensitivity.

**Result:** HDST in determining the efficacy of chemotherapy drugs were consistent with the actual clinical efficacy of patients ( $Kappa=0.535$ ), and the sensitivity, specificity, AUC were 82.14%, 100.00%, 0.911. Single-drug resistance rates of TXL, DOC, CDDP, CBP, Lobaplatin, and VP-16 were 21.95%, 74.39%, 62.20% , 52.44%, 39.02%, 60.98%, respectively. Highest Secondary drug resistance rate of CBP was 44.44%, followed by CDDP and VP-16 (22.22%). TXL's IR was significantly higher than DOC wherever the location, and Loplatin's was higher than CDDP ( $P<0.05$ ). TXL's IR was higher than VP-16 in primary foci, but VP-16's was not only higher than DOC, but also CDDP, and similar to the CBP and Lobaplatin in abdominal metastases( $P<0.05$ ).

**Conclusion:** HDST can effectively predict the response to existing chemotherapy regimens as new tool to screen individualized treatment for patients. Paclitaxel combined with Lobaplatin regimen may have more advantages in chemotherapy and Etoposide should not be ignored in ovarian tumors.

## Background

Ovarian cancer is one of the most common malignant tumors in women. Because of its lack of typical symptoms and effective screening methods, it is difficult to achieve early diagnosis and has the characteristics of easy implantation and distant metastasis [1]. There are 239,000 new cases and 152,000 deaths in the world every year [2], but incidence still increasing year by year [3]. Although a large number of new drug research and development, molecular mechanisms of etiology and genetic basic research have been carried out, the survival rate of the disease has not improved well [4]. 70.0% of ovarian cancer is not diagnosed until it is advanced [5]. Besides this way, one of the important reasons is that the current clinical guidelines for treatment still do not solve the problem of patient heterogeneity and drug resistance, which are exactly the key factors affecting the efficacy of chemotherapy.

Currently, the mainstream treatment for ovarian cancer patients is tumor cell reduction combined with paclitaxel-platinum chemotherapy regimen [6]. High-grade serous carcinoma (HGSC) is the most common tissue type (75%) [7], and first-line treatment guidelines are primarily based on HGSC ovarian cancer, which usually only ensures that the drug is universally effective to the patient, ignoring individual differences. However studies have found that different histological types of ovarian cancer have different biological characteristics and genetic abnormalities [8]. Different pathological subtypes have different signaling pathways and can show different sensitivity and drug resistance to systematic treatment [9], especially ovarian clear cell carcinoma (OCCC) and low-grade serous ovarian cancer (LGSOC) is less responsive to standard treatment and more resistant to chemotherapy [10]. Recent studies have further confirmed that there is considerable heterogeneity in the anatomical location of diseases in the same individual at the genomic and immune levels [9]. Vaughan also showed that individual heterogeneity in predicting factors of chemotherapy sensitivity response and mechanism of drug resistance in ovarian cancer tissue led to differences in chemotherapy efficacy among patients [11]. There is no doubt that the current clinical method of completely using guidelines to guide patients in chemotherapy has some defects [12], which cannot solve the problems of individual heterogeneity and drug resistance of patients.

Drug resistance is a critical unmet clinical need in the treatment of ovarian cancer. The incidence of primary drug resistance of ovarian cancer is about 15.0%~25.0% [13]. Most ovarian cancer recurs after treatment and eventually develops into acquired drug resistance [14], which is also the main cause of death of most ovarian tumors [15]. Most patients do not get the expected benefits from the universal and systematic treatment, but suffer serious side effects [16]. For example, the treatment of drug-resistant patients is generally based on the standard regimen with the addition of other new drugs such as liposomal doxorubicin and topotecan [14], but several trials have shown that the combination of chemotherapy drugs not only fails to improve the efficacy, but may even increase the toxicity of the drug [17]. It can be considered that improving the survival rate of ovarian cancer depends on individualized precision chemotherapy in response to the development of drug resistance [18]. The priority of drug selection for drug-resistant patients is a key factor in the efficacy of cancer patients [19]. Moreover, the prediction of the efficacy of second-line chemotherapy in patients with recurrent ovarian cancer can only be made based on the platinum-free drug interval after first-line chemotherapy [20]. However, there is limited research data that can clearly identify the combination regimen and the order of drug use of patients' preferred treatment [21].

Up to now, the relative efficacy of many single agents and the role of combination therapy in ovarian tumors have not been clarified [22]. Predicting the response of existing or new regimens have greater potential for individualized treatment and survival rate improvement of ovarian tumor patients [9]. The techniques used to assess the sensitivity and drug resistance of tumors to treatment may be an accurate indicator to judge the efficacy of diseases [23]. In vitro tissue culture test is a reliable and cost-effective technique for evaluating the sensitivity of ovarian tumors to chemotherapy and the effect of new therapies [12]. The value of Hydrogel embedded histoculture drug sensitivity test (HDST) as a new 3D tissue culture technology, has been preliminarily clarified in a small sample study of 11 cases in our team in the screening effect of chemotherapy regimens for ovarian cancer [24]. Therefore, this study will further verify the predictive value of HDST in chemotherapy regimens for ovarian tumor patients through an expanded sample size blind study. In addition, a prospective double-blind study was conducted to evaluate the effectiveness and sensitivity of drugs for ovarian tumors in the regimens.

## Methods

### Study design

According to the inclusion and exclusion criteria, 82 patients with ovarian tumor who were admitted to the gynecological oncology department of Sun Yat-sen Memorial Hospital, Sun Yat-sen University, from October 2019 to December 2020 were recruited in this study, and tumor tissue samples were collected during biopsy or surgery. Among them, 32 patients with neoadjuvant chemotherapy (NACT) were included in the preliminary study, and the predictive accuracy and feasibility of this technique in ovarian tumors were again verified, laying foundation for prospective analysis. All patients were included in the subsequent prospective double-blind study to evaluate their drug sensitivity (Fig. 1). The experimental operation of HDST and clinical follow-up were performed by two independent observers respectively. The results of the two observers were blind and not visible. All patients received NCCN standardized chemotherapy and clinician's choice of chemotherapy regimen was not interfered. After unblinding, the drugs efficacy and prediction accuracy of HDST were evaluated. The study was conducted with the approval of the ethics committee, in accordance with the principles of the Helsinki Declaration and all local laws, and with the informed consent and signature of the patients.

### Inclusion and exclusion criteria.

Inclusion criteria:  Histological diagnosis was consistent with ovarian tumor;  Tumor tissue was removed intraoperatively, or tumor tissue samples were obtained after biopsy for HDST detection. Exclusion criteria:  histopathological non-ovarian tumor;  In addition to chemotherapy, the patients received other types of adjuvant therapy (radiotherapy or endocrine therapy);  Pregnant or lactating women.

### Clinical follow-up

Sociodemographic data and clinical data of patients were used to collected, including age, pathological type, clinical stage, chemotherapy regimen, imaging examination (Ultrasound, CT, MRI), tumor markers. In the process of biopsy or surgery, the clinician collects tumor tissue samples from the patients, and sends them to the team laboratory staff according to the HDST trial operation plan to independently complete HDST testing and report. The patient is admitted to the hospital for chemotherapy approximately every 3 weeks. During the treatment in the hospital, the patient's chemotherapy regimen, CA125, HE4 levels, recurrence, death and other information were collected through inpatient or outpatient follow-up. When the patient is discharged from the hospital or the treatment ends, the follow-up staff will continue to follow-up by telephone until the patient dies or is lost to follow-up.

### Outcome variables and evaluation.

Included sensitivity to response to chemotherapy regimens in HDST reports, tumor marker, such as CA125 and HE4, and also Fagotti score (PIV), CT scores before and after NACT. Response Evaluation Criteria in Solid Tumors version 1.1 (RECIST 1.1) were used by clinicians to evaluate the outcome of treatment, which was classified as progressive disease (PD) or ineffective, poor response or stable disease (SD), partial remission or partial response (PR), complete response (CR) or effective [25].

### HDST

The detailed experimental methods are as follows (Fig. 2).  Sample collection: Fresh ovarian tumor tissue excised by surgery was collected within 15 minutes in vitro, transported to the cell laboratory within 24 hours under low temperature (2 ~ 8).  In vitro culture of tumor tissue: the tumor tissue was cleaned, evaluated and modified into tissue blocks weighing about 10mg(2×2×2 mm). One tissue fragment was placed in each well of 96-well cell culture plate, then added 80μl hydrogel solution to submerge the tissue fragment, and incubate for 1 h in a cell incubator with 5% carbon dioxide at 37 °C. Add proper amount of tissue medium (100μL/well) after solidification of hydrogel for culture.  Medication: After cultured for 24 h, Aspirate and discard the original tissue culture medium, add fresh tissue medium containing the test drug or control solvent. Change the tissue culture medium containing the test drug each 24 hours. Details of the drug, manufacturer and quality control can be found in the supplementary materials.  Drug susceptibility testing: After culturing for 3–10 days, the drug-containing medium was aspirated and washed with PBS. Then add MTT solution (American Sigma company) to incubate for 6 hours to develop color, and use a microplate reader to detect the absorbance at 490nm.  Result analysis: The inhibitory effect of the drug on the tumor tissue was calculated according to the formula: Inhibition rate of cancer cell survival(IR)%=(Ac-As)/Ac×100%, Ac is the optical density (OD) value of the control group, and As is the OD value of the experimental group. The criterion for drug sensitivity testing defines that IR ≥ 30.0% is an effective drug, and IR < 30.0% is considered invalid. The experiment was repeated 3 times and the average value was taken.

Statistical analysis The measurement data is described by the mean ± standard deviation (), non-normal distribution is described by the median and quartile [ $M(P_{25} \sim P_{75})$ ], and the number of count data is n(%) to describe. *Kappa* coefficient and ROC curve were used to determine the feasibility of the technique. Paired t test, Chi Square test, Willcoxon Rank Sum test, Willcoxon Sign-Rank test and Kruskal-Wallis test were used to analyze drug IR. If there are no special remarks, P values equal to or less than 0.05 were considered significant. *SPSS 25.0* was used for statistical analysis. *GraphPad Prism 8* were used to draw figures.

## Result

### General conditions of patients.

82 patients and 116 samples were included in the prospective, double-blind cohort study, which including 62 samples of primary nidus and 54 samples of metastases. The success rate of HDST culture was 100.00%. There had described the drug sensitivity distribution according to their age, pathological type, and pathological stage (Table 1). According to the results of HDST, IR of same drug was not related to the age ( $P>0.05$ ). Other pathological types such as non-serous or early stage (stage I) ovarian tumors have higher IR. The IR distribution of Paclitaxel (TXL), Docetaxel (DOC) and Etoposide (VP-16) was not affected by the pathological types and stages ( $P>0.05$ ), and the IR of PTX, Docetaxel were both higher in primary lesions than in metastatic lesions ( $P<0.05$ ). The IR of VP-16, Cisplatin(CDDP), Carboplatin (CBP), and Lobaplatin are not affected by changes in tumor nidus sites, and the IR of Lobaplatin is higher than that of CDDP or CBP regardless of the nidus sites, age, pathological types, and staging. Moreover, the IR of Lobaplatin was not related to the pathological types of patients.

### Consistency analysis of clinical efficacy of HDST.

In the preliminary study, 32 patients with ovarian tumors who completed NACT were evaluated for the IR and clinical outcomes of HDST. The results of HDST in determining the efficacy of chemotherapy drugs were consistent with the actual clinical efficacy of patients (Kappa=0.535,  $P=0.004$ , Table 2). AUC of ROC curve was 0.911(95%CI, 0.808-1.000, Figure 3). It is indicated that the judgment result of IR based on HDST is closely related to the clinical response of patients ( $P=0.009$ ). As a new drug susceptibility screening method, HDST has a sensitivity of 82.14%, a specificity of 100.00% and a correct index of 0.82 in predicting the clinical efficacy of patients with ovarian tumors, which has a high authenticity.

### Drug sensitivity analysis.

After a median time of 16.62 months of follow-up of 82 patients with ovarian tumors, 11 patients did not require chemotherapy, and 1 patient had missed the record of markers who was completed initial treatment in other hospitals. Of the 70 patients, CA125 returned to normal in 53 patients (75.71%) and HE4 levels decreased to normal in 59 patients (84.29%). The level of CA125 or HE4 decreased to normal in 6 patients and then increased again, while the level of marker in 1 patient increased with the increase of chemotherapy course. After unblinding, the results of the study showed that the initial chemotherapy regimen of the above 7 patients did not match the HDST recommendations (supplementary materials). The IR of 32 patients with NACT was stratified ( $IR<30%$ ,  $30\% \leq IR<60%$ ,  $IR \geq 60%$ ), and there was no statistical difference in tumor marker levels in the 3 groups before chemotherapy (Figure 4C, 4E). After NACT and 3 times of chemotherapy, the levels of tumor markers in all patients returned to normal. Among them, the decline rate of patients with  $IR \geq 60%$  level during NACT was higher than that of the other two groups, which generally showed that the decline rate of CA125 and HE4 increased with the increase of IR. The decrease curves of CA125 and HE4 crossed several times during chemotherapy, and the curves of HE4 increased in those patients with  $IR<30%$  after the first chemotherapy (Figure 4D, 4F). Among the 70 patients who receiving postoperative adjuvant chemotherapy, the overall decrease trend of tumor markers was more obvious in the  $IR \geq 60%$  group, and the advantage of continuous decrease could be maintained even after the decrease to the normal range (Figure 4G, 4H).

10.98% patients had a maximum IR lower than 30 for all chemotherapeutic agents. The single-drug resistance rates of TXL, DOC, CDDP, CBP, Lobaplatin, and VP-16 were 21.95%, 74.39%, 62.20%, 52.44%, 39.02%, 60.98%, respectively, and the rate of platinum-resistant was 29.27%. Among the 9 patients whose samples were collected before and after interval debulking surgery (IDS), the primary drug resistance rate of TXL (21.95%) was the lowest, followed by Loplatin (39.02%) and DOC (74.39%). Highest secondary drug resistance was CBP (44.44%), followed by CDDP and VP-16 (22.22%). The secondary drug resistance rate of TXL, DOC and Lobaplatin was the same as 11.11%.

### Relationship between IR of chemotherapeutic agents and nidus.

Based on the HDST, not only different chemotherapy drugs were used to test the same site of tissue, but also the same chemotherapy drugs were used to test the same patient's primary and metastatic tissues. The sensitivity level of CDDP, CBP and Lobaplatin was not related to site, and the IR level of Lobaplatin was the most stable in different sites. The sensitivity of TXL, DOC and VP-16 in different sites varied to different degrees, and both TXL and DOC were higher in primary sites than in metastatic sites ( $P<0.05$ , Figure 5). There was no statistically significant difference in the IR of VP-16 between the primary and metastatic nidus, and abdominal metastasis was higher than that for pelvic metastasis. Further self-matching test showed that TXL and DOC in the same patient had higher IR primary sites than metastatic sites, which was consistent with the results of non-self-matching. In addition, after excluding individual differences, the IR of CDDP and CBP in primary foci was higher than that in metastatic foci ( $P<0.05$ ), while the IR changes of Lobaplatin and VP-16 in different lesions in the same patient were not significant (Figure 5I, 5K). By comparing the drug sensitivity of TXL and DOC, the sensitivity of TXL was significantly higher than DOC, and this difference was not affected by location (Figure 6A-D). There were significant differences in the sensitivity between CDDP and Loplatin in primary metastatic lesions (Figure 6E-H), which was mainly manifested as that Loplatin was higher than CDDP, while there was no significant difference in sensitivity between Loplatin and CBP in different sites. IR of paclitaxel was higher than VP-16 in primary foci;  $TXL>VP-16>DOC$  in metastases. In abdominal metastases, the IR of VP-16 was not only higher than DOC, but also higher than CDDP. No matter in the primary or metastatic focus, the IR of VP-16 was similar to that of CBP and Lobaplatin, without statistical significance (Figure 6I-L).

### Heterogeneity of ovarian tumors.

In present study, 17 patients (20.73%) had higher drug IR than the primary tumor in their metastatic tissues, and 9 patients (10.98%) had higher drug IR in IDS than biopsy. Among them, TXL, DOC, VP-16, CDDP, CBP, Lobaplatin had higher IR rates of metastatic lesions than the primary lesions were 4.88%, 6.10%, 10.98%, 8.54%, 12.20%, 12.20%, respectively. And the corresponding rates of IR in IDS lower than biopsy drugs were 6.10%, 4.88%, 4.88%, 2.44%, 3.66%, and 6.10%.

## Discussion

Solving the problem of drug resistance remains the key to the treatment of ovarian tumors, and the biggest challenge is to determine the most effective drug therapy for each patient. This study demonstrated that the chemotherapy sensitivity of HDST resembled the chemotherapy sensitivity of the corresponding patient in the clinic, which was similar to our previous study [24]. Our data showed that the sensitivity, specificity, AUC of the HDST model for predicting drug responses were 82.14%, 100.00%, 0.911, respectively, which indicates that HDST can serve as a prediction model to guide the individualized selection of chemotherapy regimens for ovarian patients. After unblinding, researchers evaluated the prediction accuracy of HDST response to chemotherapy regimen based on HDSTreport and clinical follow-up results. The results confirmed heterogeneity, revealing the different responses of ovarian tumors to chemotherapy regimens. It was not only shows the difference in the sensitivity of the lesions in different parts of the same individual; but also shows the difference in the sensitivity of the same tumor to different drugs, which is consistent with previous studies [9, 11].

Experimental technologies can predict responses to chemotherapy and new therapies which will allow for more effective and personalized patient management and, importantly, free patients from the side effects of ineffective chemotherapy drugs. The feasibility of in vitro tissue culture trials to predict responses to existing or new therapies has great potential to improve the survival rate of patients with ovarian tumors, given the time cost [12]. There is no doubt that cell line tests, while enabling high-throughput screening, are not representative of patient-derived tumor tissue. Although the PDX model can simulate the biological characteristics of the tumor, it takes 2–4 months at least [26], and has disadvantages of low implantation efficiency and high cost [27]. 3D tissue block hydrogel culture method preserves the heterogeneity and microenvironment of tumors, and can be used as a method for evaluating tumor drug sensitivity, and the Histoculture drug response assay (HDRA) method [28–30] has gradually used to evaluate clinical drug sensitivity.

The tissue culture method of HDST is similar to HDRA [28], but the operation process and hydrogel formula are optimized and upgraded. In contrast to the solid gelatin used in HDRA, the HDST test used a hydrogel solution derived from collagen polysaccharide to encase the tumor tissue, and a semi-solid gel with elastic pores constituted the three-dimensional structure of the tumor microtissue culture model. In this structure, the chance of tumor tissue exposure to the solution is more uniform, the absorption of tissue nutrients is better than the HDRA method with only one side exposed to the solution, and the survival rate of tissue mass is higher. The histological characteristics of solid tumors can be reproduced in culture, maintaining tissue activity and tumor microenvironment [31], and overcoming the abnormal cell-cell interactions and inappropriate cell polarity shown in two-dimensional tissue culture [32]. HDST only takes 3–10 days to provide test results for screening tumor chemotherapeutic drugs. Due to its simple operation, quick and convenient, economical and practical, and high feasibility, the best solution can be quickly and effectively selected based on the experimental results under the premise of complying with the guidelines for clinical application.

CA125 and HE4 all returned to normal in patients with ovarian tumor NACT after standard chemotherapy (Fig. 4). In the early stage, it can still be clearly seen that the higher the IR of the drug based on the HDST test, rate of decline of patients with IR  $\geq$  60% level was significantly higher than that of the other two groups, and the marker level dropped to the normal range during postoperative adjuvant chemotherapy and still maintained an advantage. This means that in the early stage of treatment, if patients can use drugs with higher sensitivity, the advantages of long-term chemotherapy may be more obvious. Due to the heterogeneity, even tumors of the same histological type and degree of differentiation, or even tumors of the same individual, have significantly different sensitivity spectra to drugs [9, 11, 33]. It is generally believed that the tolerance of tumor to drugs increases with the increase of the number of times of administration and the prolongation of treatment time, and the sensitivity of primary lesions is higher than that of metastatic lesions. However, through analysis of real data in present study, it was found that there were still 20.73% patients whose tissue samples showed higher IR of chemotherapy drugs in the metastatic lesion than in the primary lesion, while 10.98% patients whose IDS samples showed higher IR than biopsy. These results objectively confirmed the existence of individual heterogeneity in ovarian tumors, which leads to differences in the chemotherapy response of different patients to the same regimen. Heterogeneity of tumor and drug resistance are the key factors affecting the efficacy of tumor, while drug resistance are the important factors restricting the clinical efficacy of patients.

Based on HDST technology, our study found that the primary drug resistance rate of ovarian tumor patients was 10.98%, which was lower than the reported rate of 15.0%-25.0% [13]. The primary drug resistance rates of DOC, CDDP and VP-16 were ranking the top 3 among the 6 conventional chemotherapy drugs. The objective total effective rate of DOC was 23% in Paclitaxel-resistant patients [34], 25%-30% in platinum-resistant advanced ovarian cancer [35–36], 33% in platinum-sensitive patients [35], and the drug resistance rate was as high as 67–77%, which was consistent with the results of this study. VP-16 in O'Dwyer PJ and Muggia FM studies also had a single drug effective rate of only 21% in ovarian tumors [37], which was higher than the primary single drug resistance rate in this study (79% vs 60.98%). A study of 104 patients with advanced epithelial cancer showed 54% and 58% resistance to CDDP and CBP, respectively [29]. In another study of 79 patients with ovarian tumors, the drug resistance rates of CDDP and CBP were 49.2% and 34.7%, respectively [30]. In this study, the drug resistance rate of CDDP (62.20%) was higher than that of Jung PS [29] and Lee SW [30], while the drug resistance rate of CBP (52.44%) was between the two results. In addition, our study found that the single-drug resistance rates of TXL (21.95%) and Loplaplatin (39.02%) were the lowest. Single-drug resistance rates of TXL were lower than 46% reported by Jung PS, while Loplaplatin was slightly higher than 32.2% reported by Lee SW. In the IR comparison of our study, the IR of TXL was significantly higher than DOC, and Lobaplatin was higher than CDDP, and this difference was not affected by the site. If only considering the action of various drugs on IR of ovarian tumor tissue, Lobaplatin is similar to CBP; TXL was superior to VP-16, which was superior to DOC and CDDP.

TXL combined with CBP regimen has been accepted as the first-line standard treatment for primary epithelial ovarian cancer [38]. As progresses, even if there are other alternatives, most of them add chemotherapy to the Platinum-Paclitaxel class and/or change the administration of chemotherapy drugs [39]. Note that the treatment itself can induce drug resistance [40]. Whether the change of sensitivity difference is caused by the drug resistance induced after drug treatment or the development of new drug resistance mechanism in the treatment or caused by the improper selection of drugs in the early stage is still a puzzling fact [41]. The choice of medication is usually affected by the side effects and convenience of the medication.

Kelland LR determined the cytotoxicity of chemotherapy drugs to human ovarian cancer cell lines and found that the cytotoxicity of DOC was stronger than that of TXL, especially in the cell lines that developed resistance to CDDP or CBP; the cytotoxicity of DOC was 3.9 times that of TXL [42]. What's more, DOC and TXL have a cross resistance spectrum [43]. In this study, the IR of DOC was significantly lower than that of TXL (Fig. 5), and with higher drug resistance. Based on the clinical efficacy and experimental results, the role of TXL in ovarian tumor chemotherapy was more recognized. In TXL combined with platinum-based chemotherapy regimens, CBP is limited due to its shortcomings such as severe secondary resistance to myelosuppression and high cross resistance with CDDP [9]. From the perspective of pharmacokinetics, Lobaplatin basically maintains its structural integrity during metabolism in vivo, as the third generation of platinum anticancer drugs, and its stable and powerful structure maintains its anti-tumor activity, and its performance is more prominent than that of CDDP and CBP in some models [44]. Compared with CDDP, Lobaplatin has no obvious hepatorenal toxicity, neurotoxicity, ototoxicity and digestive tract reactions. The incidence and severity of side effect are similar to those of CDDP [39]. The sensitivity of CDDP and CBP in different pathological types was significantly different, while Lobaplatin was not affected by pathological types, which was consistent with the results of Galluzzi L[45]. It showed that Lobaplatin IR was significantly higher than that of CDDP, and both primary and secondary drug resistance rates were the lowest. From the perspective of optimal drugs and comprehensive side effects, Lobaplatin is more suitable for patients with platinum-sensitive ovarian tumors. In the 32 patients with NACT in this study, compared with CDDP and CBP, the IR of Loplatin remained relatively stable during the course of treatment (Table 1, Fig. 3–4), which is more worthy of clinical promotion, such as maintenance chemotherapy after IDS. In conclusion, TXL combined with Lobaplatin can be used as the preferred drug combination after NACT based on the TXL combined with platinum regimen in the standard of treatment for ovarian cancer patients, which needs to be further verified in a randomized controlled clinical trial.

In exploring the role of VP-16 in ovarian tumors, this study also found that the IR was better than that of DOC and CDDP. Oral VP-16 has been used in many clinical trials for the treatment of recurrent ovarian cancer and is one of the options for patients with recurrent ovarian cancer [25, 46]. In a phase 2 trial using long-term oral VP-16 as the second-line treatment for platinum-resistant and platinum-sensitive ovarian cancer, the objective response rate of 41 patients with platinum-resistant ovarian cancer was 26.8%, and the response rate in platinum-sensitive patients was 34.1% [46]. Long-term oral VP-16 is effective in paclitaxel-resistant ovarian cancer, which objectively proves the efficacy of oral etoposide monotherapy in ovarian cancer. In recent years, a Phase 2 clinical trial of apatinib combined with oral VP-16 also showed that nearly 55% of patients with platinum-resistant or platinum-refractory ovarian cancer achieved objective outcomes [25]. This further proves the role of VP-16 in ovarian tumor chemotherapy. More importantly, from the perspective of ease of administration, VP-16 can be administered orally without hospitalization or infusion pump, which means that the treatment regimen may improve patient compliance and economic benefits, making it a cost-effective oral therapy for outpatients.

HDST also has some limitations. As an in vitro chemotherapeutic drug screening method, the tumor microenvironment of the HDST test does not mimic the overall human immunotherapeutic response. For tumors with strong heterogeneity, the response of some tumor tissues to drugs may not well reflect the intervention effect of drugs on the whole body. This view is consistent with Kandice Tanner [47] in the 3D in vitro culture model to evaluate the efficacy of patients' medications. Furthermore, the quality of samples can also affect the results of subsequent tests. With the advancement of chemotherapy, the predictive effect of drugs is better as soon as possible and multiple sampling and inspection. Although this study was a double-blind study without intervention. Further prospective clinical trials are needed to clarify the survival advantage of HDST in the selection of chemotherapy regimens for cervical cancer patients.

## Conclusion

The high mortality rate of ovarian cancer urgently requires new treatment strategies. Clinical oncology research on drug-sensitive screening has also been developing, with the goal of achieving personalized treatment to improve survival. At present, human tissues and animal models are the most commonly used methods in biomedical research. The choice of drug screening experiments also depends on the time, cost and accuracy of simulating the complex microenvironment of cancer. Regardless of the experimental model, it should have the characteristics of high prediction, simple method, technically feasible, and reasonable economy, so that it can be flexibly applied in the clinical environment, especially in developing countries where medical resources are relatively scarce. The patient-derived tumor tissue collected during surgery or biopsy has characteristics such as heredity, microenvironment, and phenotypic characteristics, which are suitable for clinical drug screening and translational research. Given the time cost or others aspects, The vitro tissue culture trials has great potential for individualized treatment in predicting responses to exist or new therapies. As a time-consuming, cost-effective, and mature technology, HDST requires less skills for test operators. And based on its outstanding predictive value, under the constraints of external conditions such as animal ethics, HDST can be used to test the efficacy of a larger number of drug combinations on tumor tissue samples, which is especially suitable for widespread clinical use. The main limitation is that most in vitro experiments are often difficult to simulate the overall immune treatment response of the human body and are affected by the quality of tissue samples. This will be the direction that needs to be optimized in the future. As surgical and biopsy sampling techniques mature, sample quality may improve. In short, as a new tool for screening individualized treatments, HDST provides a supplement to precision medicine methods, and provides more opportunities for patients to screen out drugs in vitro and make decisions about faster and better chemotherapy combinations. In addition, with the help of HDST, this study analyzed the sensitivity test results and drug resistance of TXL, DOC, CDDP, CBP, Lobaplatin, VP-16, and other traditional ovarian cancer drugs. It is believed that TXL combined with Lobaplatin regimen may have more advantages in chemotherapy and Etoposide should not be ignored in ovarian tumors, more importantly.

## Abbreviations

HDST: Hydrogel Embedded Histoculture Drug Sensitivity Test; HGSC: High-grade serous carcinoma; OCCC: ovarian clear cell carcinoma; LGSOC: low-grade serous ovarian cancer; NACT: neoadjuvant chemotherapy; RECIST 1.1: Response Evaluation Criteria in Solid Tumors version 1.1; PD: progressive disease; SD: stable disease; PR: partial response; CR: complete response; IDS: in interval debulking surgery; IR: Inhibition rate of cancer cell survival(%)=(Ac-

As)/Ac×100%; OD: optical density; Ac: the OD value of the control group; As: the OD value of the experimental group. TXL: Paclitaxel; DOC: Docetaxel; CDDP: Cisplatin; CBP: Carboplatin; VP-16: Etoposide.

## Declarations

### Ethics approval and consent to participate

The study received ethical approval and patient signatures.

### Consent for publication

Not applicable.

### Availability of data and material

The datasets supporting the conclusions of this article are included within the article (and its additional files).

### Competing interests

The study without any potential competing interests.

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### Authors' contributions

QXR and YW performed the literature search and Conceptualization. QXR, YW and YQH

analyzed selected papers. YW and XH realized Figures and Tables. QXR and YW were dedicated to Writing and Original Draft Preparation. TRS, YS and XH were dedicated to methodology and investigation. QC, MMG, HZ, HWL, XHX, YQH, QSC supervised the whole process. All authors read and approved the final manuscript.

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## Tables

Table 1. General patient conditions and drug sensitivity analysis table  $\chi^2M(P_{25}-P_{75})/n(\%)$

Variables	n(%)	TXL(%)	P	DOC(%)	P	CDDP(%)	P	CBP(%)	P	Lobaplatin(%)	P	VP-16(%)	P
<b>Age (year)</b>													
<60	51(62.20)	43.00 (24.50-71.00)	0.714	18.00 (6.00-30.50)	0.482	20.00 (5.00-34.00)	0.272	24.00 (8.00-42.00)	0.950	32.00 (11.50-42.00)	0.475	29.00 (17.00-37.00)	0.201
≥60	31(37.80)	47.00 (29.00-75.00)		14.00 (7.00-26.00)		16.00 (5.00-21.00)		23.00 (13.00-42.00)		32.00 (20.00-43.00)		25.00 (7.00-43.00)	
<b>Pathology</b>													
Serous	56(68.29)	43.00 (23.00-66.50)	0.076	15.00 (5.50-29.00)	0.218	13.00 (4.00-29.50)	0.001	20.00 (8.00-41.50)	0.030	30.00 (10.00-40.00)	0.111	29.00 (8.00-37.00)	0.080
others	26(31.71)	60.00 (34.00-81.00)		20.00 (12.00-29.00)		30.00 (18.00-37.00)		31.00 (22.00-47.00)		32.00 (26.00-52.00)		29.00 (23.00-48.00)	
<b>Stage</b>													
I	9(10.84)	54.00 (36.00-78.50)	0.236	28.00 (19.00-36.00)	0.239	30.00 (23.50-46.00)	0.026	31.00 (18.50-52.00)	0.033	32.00 (31.00-43.50)	0.017	35.00 (20.00-36.50)	0.693
II	5(6.02)	42.00 (35.00-60.00)		14.00 (6.00-28.50)		21.00 (17.50-23.50)		31.00 (17.50-35.00)		32.00 (26.00-46.50)		18.00 (7.50-48.00)	
III	54(65.06)	41.00 (19.00-70.00)		13.00 (6.00-28.00)		12.00 (4.00-34.00)		19.00 (6.00-39.00)		29.00 (8.00-38.00)		29.00 (11.00-37.00)	
IV	15(18.07)	64.00 (25.00-82.00)		18.00 (10.00-21.00)		20.00 (10.00-35.00)		31.00 (17.00-65.00)		37.00 (25.00-67.00)		29.00 (8.00-47.00)	
<b>Nidus</b>													
primary	62(53.45)	52.50 (32.75-80.25)	0.034	20.00 (12.00-34.00)	0.012	18.50 (6.50-30.25)	0.137	24.00 (11.75-42.25)	0.361	32.00 (19.00-43.25)	0.688	29.00 (16.75-47.20)	0.072
metastatic	54(46.55)	39.00 (19.25-63.75)		11.50 (5.25-23.25)		13.00 (3.75-34.25)		24.00 (8.00-42.25)		29.50 (9.75-41.50)		28.00 (8.00-37.00)	

Willcoxon and Kruskal-Wallis tests were used for others. Cisplatin: CDDP; Carboplatin: CBP; Docetaxel: DOC ; neoadjuvant chemotherapy: NACT; Paclitaxel: TXL; Etoposide: VP-16.  $\alpha=0.05$ .

Table 2. Consistency between drug sensitivity results of HDST and clinical efficacy (n=32)

HDST	Clinical efficacy			Kappa	P
	CR	PD or PR	Total		
Valid	23	0	23	11.683	0.535
Invalid	5	4	9		0.004
Total	28	4	32		

Valid signified IR $\geq$ 30% and invalid are IR<30%. 1 case was PD, 3 cases were PR.

## Figures

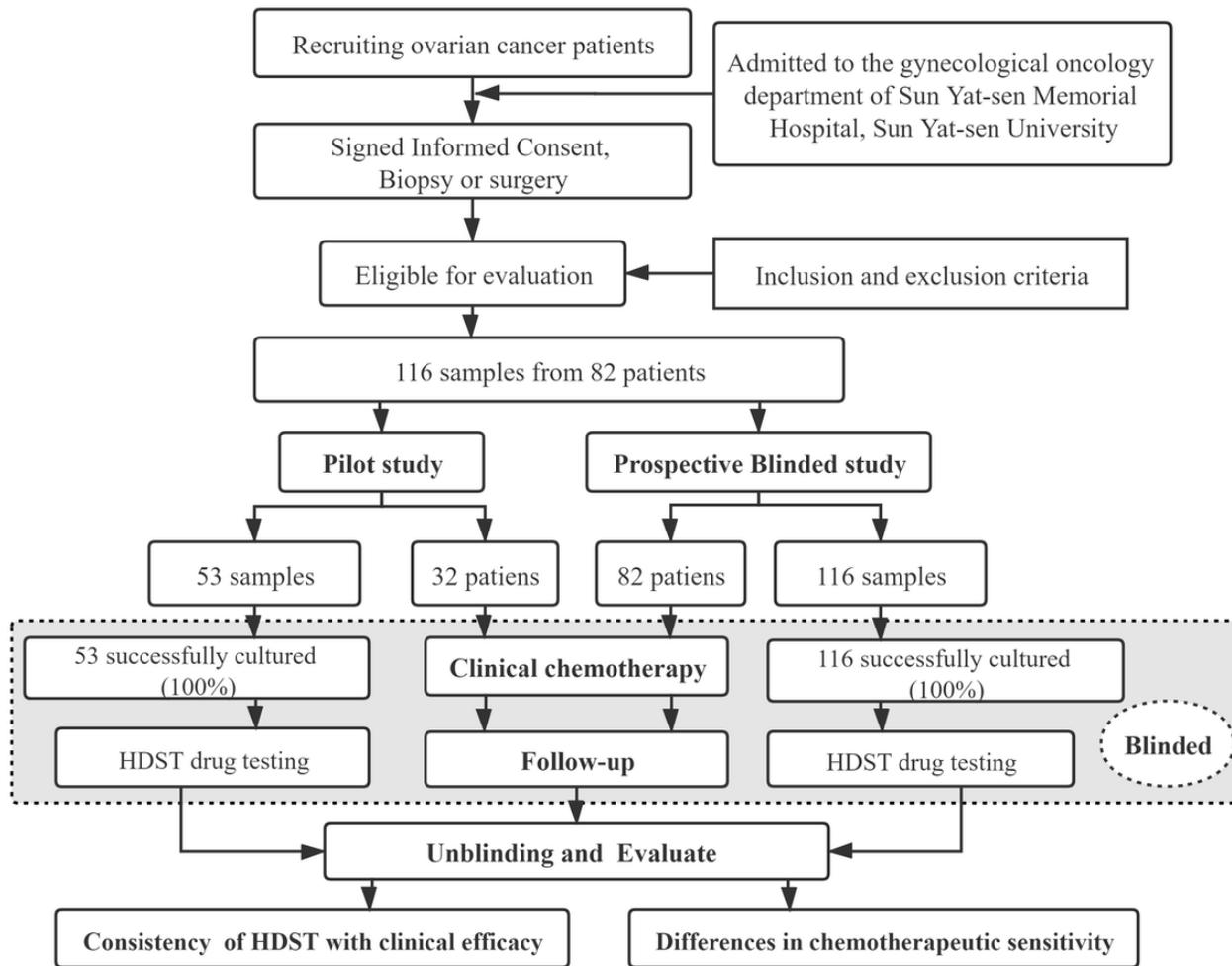


Figure 1

Flowchart of the study method.

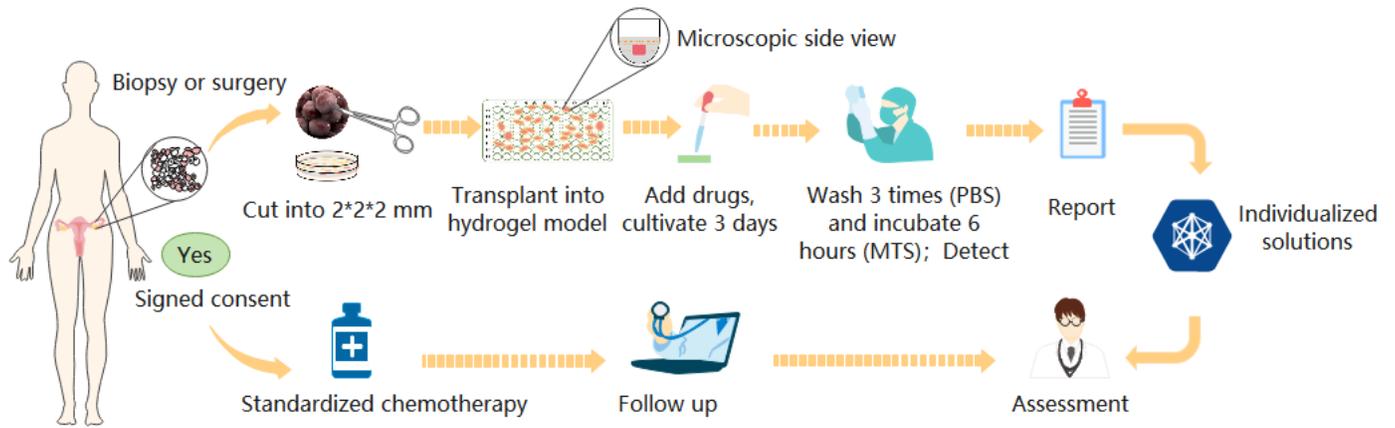


Figure 2

Flowchart of the HDST.

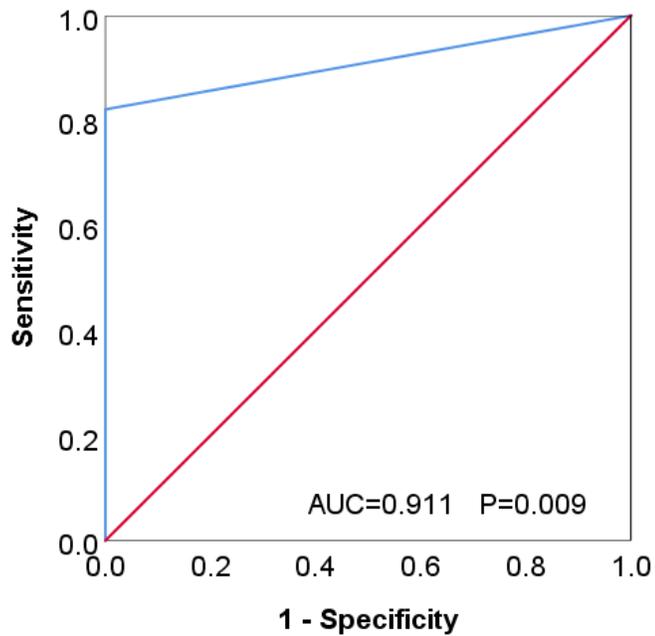
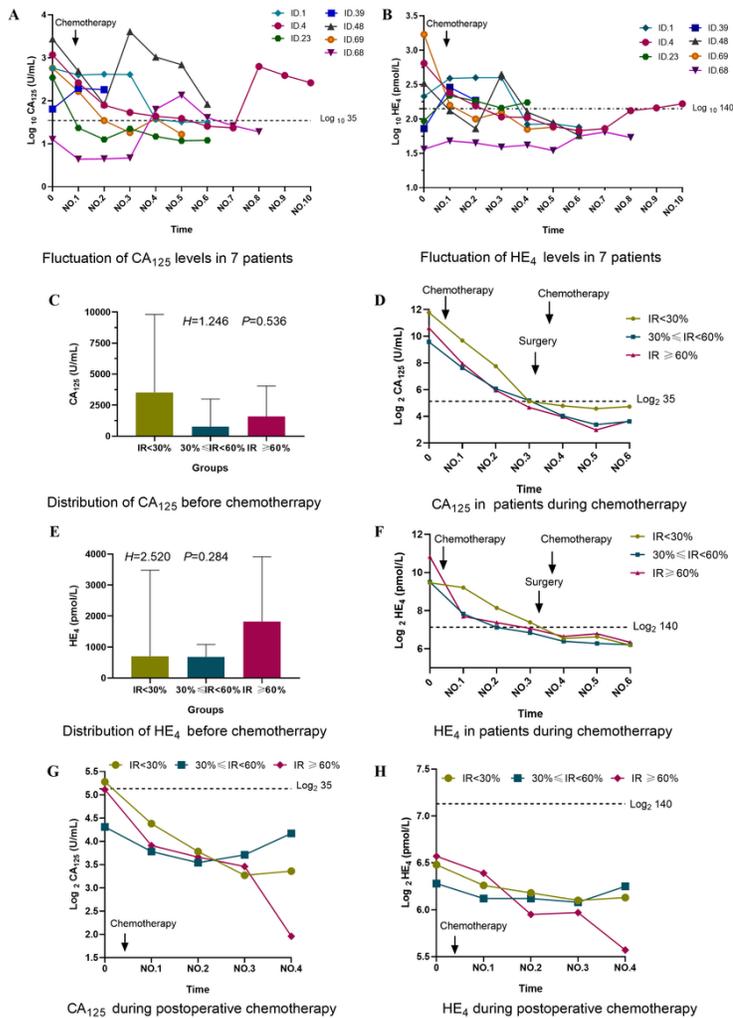


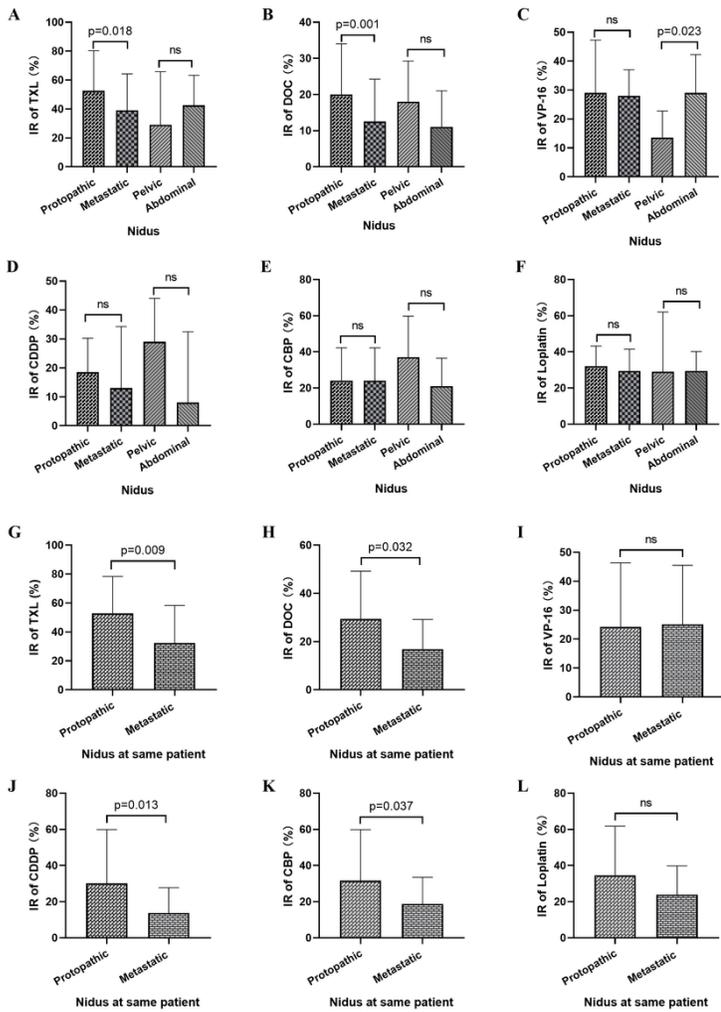
Figure 3

ROC curve for chemotherapy regimens tested in HDST. (AUC was 0.911(95%CI, 0.808-1.000). It is indicated that the judgment result of IR based on HDST is closely related to the clinical response of patients. P=0.009)



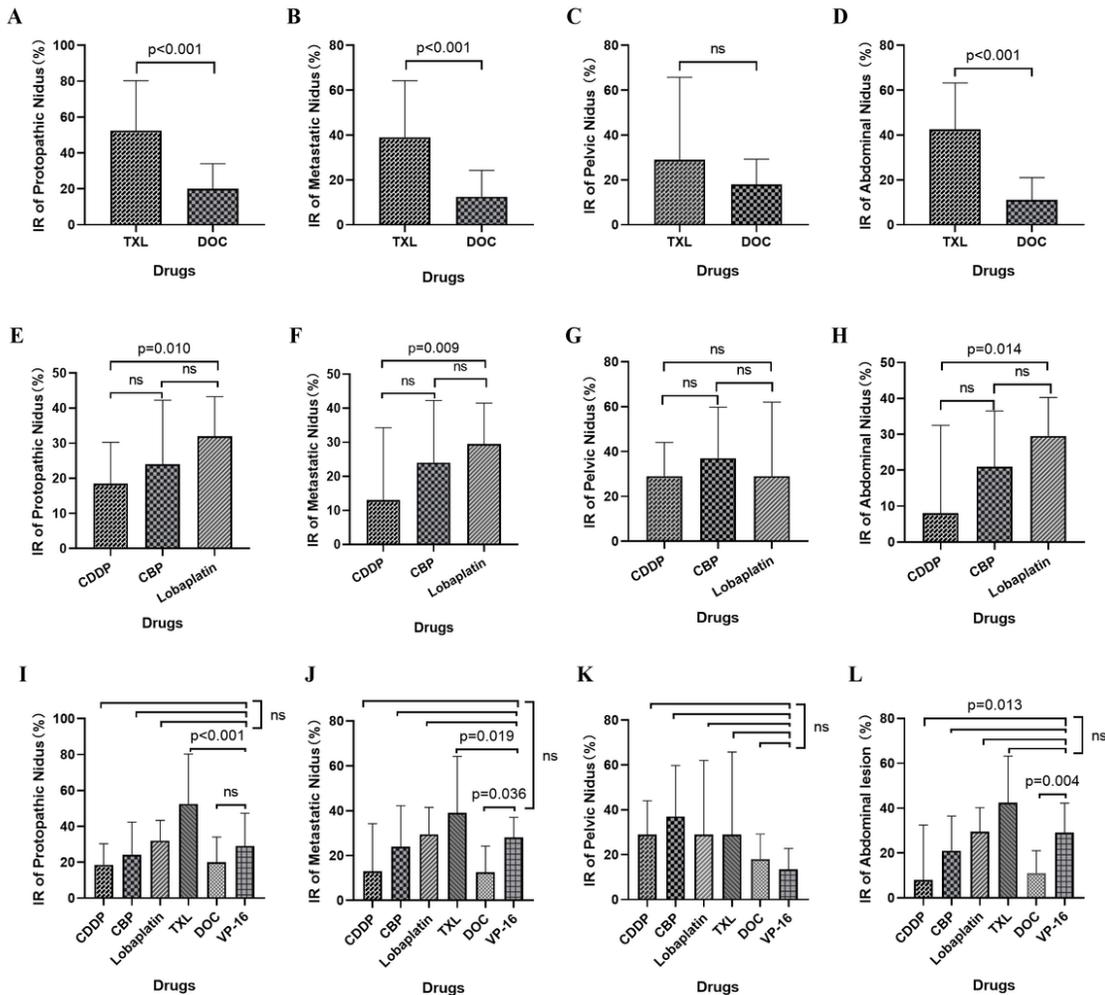
**Figure 4**

Changes of CA125 and HE4 in ovarian cancer with the times of chemotherapy. (A and B were the semi-logarithmic line chart of log<sub>10</sub> CA125 and log<sub>10</sub> HE4 during follow-up, respectively of 7 cases. C and E were the baseline data of CA125 and HE4 before chemotherapy respectively. Median and inter-quartile range are used to describe the data distribution and the Kruskal-Wallis test was used for comparison. The IR results of the drug sensitivity of the patients were divided into 3 groups (IR<30%, 30% ≤IR<60%, IR≥60%). D and F showed the semi-logarithmic line chart of CA125 and HE4 changes in the three groups of patients with NACT during the whole treatment process (n=32). G and H are semi-logarithmic line chart of changes in CA125 and HE4 levels with The Times of chemotherapy during maintenance chemotherapy in 3 groups of patients with different IR levels receiving adjuvant chemotherapy (n=70). Both the abscissas of A, B, D, F, G and H used "NO.n" to indicate the detection of tumor markers after the "times" of chemotherapy. This figure signified that tumor markers returned to normal in all patients after NACT and 3 times of chemotherapy. Among them, the decline rate of patients with IR≥60% level during NACT was higher than that of the other two groups, which generally showed that the decline rate of CA125 and HE4 increased with the increase of IR. Among the 70 patients who receiving postoperative adjuvant chemotherapy, the overall decrease trend of tumor markers was more obvious in the IR≥60% group, and the advantage of continuous decrease could be maintained even after the decrease to the normal range.)



**Figure 5**

IR of the same drug. (Median and inter-quartile range are used to describe the data distribution of 82 cases, and Willcoxon Rank Sum test was used for analysis. Figure A shows the sensitivity of TXL in different nidus (protopathic vs metastatic; pelvic vs abdominal). Figure B shows DOC in different nidus. Figure C shows VP-16, and D, E, F shows CDDP, CBP, Lobplatin, respectively (n=82). Mean and standard deviation are used to describe the self-contrast data distribution, and analysed by Willcoxon Sign-Rank test. Figure G shows the sensitivity of TXL in different nidus at same patient (protopathic vs metastatic). Figure H, I, J, K, L shows DOC, VP-16, CDDP, CBP and Lobplatin, respectively (n=13). Ns means the difference between groups was not statistically significant. This figure signified that IR of CDDP, CBP and Lobplatin was not related to site, Lobplatin was the most stable in different sites. The sensitivity of TXL, DOC and VP-16 in different sites varied to different degrees, and both TXL and DOC were higher in primary sites than in metastatic sites. Further self-matching test showed that TXL and DOC in the same patient had higher IR primary sites than metastatic sites, which was consistent with the results of non-self-matching. In addition, the IR of CDDP and CBP in primary foci was higher than metastatic foci, while the IR changes of Lobplatin and VP-16 in different lesions in the same patient were not significant.)



**Figure 6**

Comparison of IR of drugs in different nidus. (Median and inter-quartile range were used to describe the distribution, Willcoxon Rank Sum test was used for analysis. A, B, C and D shows the IR of TXL and DOC in different nidus. A and B were comparison of the sensitivity of the drugs in the primary and metastases foci (nA=62, nB=54). C and D were the comparison between drugs in pelvic metastasis and abdominal metastasis (nC=12, nD=42), and so on. E to H were the IR of CDDP, CBP and Lobaplatin. Bonferroni was used to compare the drugs pairwise for three drug, and ns means the difference between groups was not statistically significant ( $\alpha=0.017$ ). Furthermore, I to L, VP-16 was used as the control group, and the Dunnett-t test was used to compare the drugs IR pairwise under different conditions ( $\alpha=0.010$ ). Ns means the difference between the groups was not statistically significant. This figure signified that the sensitivity of TXL was significantly higher than DOC, and this difference was not affected by location. There were significant differences in the sensitivity between CDDP and Loplatin in primary metastatic lesions, which was mainly manifested as that Loplatin was higher than CDDP, while there was no significant difference in sensitivity between Loplatin and CBP in different sites. IR of paclitaxel was higher than VP-16 in primary foci; TXL>VP-16>DOC in metastases. In abdominal metastases, the IR of VP-16 was not only higher than DOC, but also higher than CDDP. No matter in the primary or metastatic focus, the IR of VP-16 was similar to that of CBP and Lobaplatin, without statistical significance)

## Supplementary Files

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