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Uterine artery pulsatility index and serum BMP-9 predict resistance to methotrexate therapy in gestational trophoblastic neoplasia: A cohort study

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A B S T R A C T

Background: Methotrexate is the most common first-line chemotherapy for low-risk gestational trophoblastic neoplasia (GTN). Uterine artery pulsatility index (UAPI) is an ultrasound marker for tumor vascularity that has been associated with an increased risk of methotrexate resistance. The combination of circulating angiogenic factor levels with UAPI data may improve the capacity of this model to predict chemoresistance. *Methods*: This was a single-center cohort study of women newly diagnosed between January 2008 and June 2012 with low-risk GTN during postmolar surveillance and treated with single-agent methotrexate at Charing Cross Hospital, a UK national center for treatment of gestational trophoblastic disease. Two hundred seventeen women underwent an ultrasound for UAPI measurement prior to initiation of chemotherapy. To examine serologic markers of methotrexate resistance among this cohort, we performed a case-control study using archived serum from 76 patients who could be matched based on prognostic risk score. Serum samples were examined by immunoassay to measure 8 different angiogenic factors (VEGF-A, FGF-basic, PLGF-1, PDGF-BB, EGF, ANGPT2, BMP-9, and ENG). Receiver-operator characteristic area under the curve (AUC) values were calculated for the ability of each analyte to correctly classify patients as methotrexate sensitive (MTX-S) or resistant (MTX-R). *Results*: Total human chorionic gonadotropin levels were similar between the MTX-S and MTX-R groups. UAPI values were significantly higher in MTX-S (median 1.30 [interquartile range {IQR} = 0.80-1.90]) compared to MTX-R patients (median 0.875 [IQR = 0.60-1.30]; $P < 0.0001$) with AUC 0.68 (95% confidence interval 0.61-0.76; $P < 0.0001$). In univariate analysis, only BMP-9 concentrations were significantly different between groups, lower among MTX-S (median of 225 ng/L, IQR = 170-287) compared to MTX-R patients (median 280 ng/L [IQR = 200-339]; $P = 0.03$). Combining UAPI with BMP-9 concentration improved prediction for chemoresistance with AUC 0.77 (95% confidence interval 0.66-0.88; $P < 0.0001$). *Conclusion*: Circulating levels of BMP-9 are elevated in newly diagnosed women with low-risk GTN destined to fail primary methotrexate therapy. A combined test using serum BMP-9 plus UAPI might improve prediction of MTX-R in low-risk GTN.

A R T I C L E I N F O

Keywords: Gestational trophoblastic neoplasia; Uterine artery pulsatility index; BMP-9; Methotrexate; Chemotherapy resistance; Prognostic biomarkers

Introduction

Gestational trophoblastic neoplasia (GTN) describes a group of placenta-related malignancies including invasive mole, choriocarcinoma, placental site trophoblastic tumor and the extremely rare epithelioid trophoblastic tumor.[1,2] An antecedent molar pregnancy is the greatest risk factor for GTN. In the United Kingdom, all patients diagnosed with molar pregnancies are registered in 1 of 3 centers (Dundee, Sheffield, or London) to enable centralized pathological review and to ensure surveillance for possible transition from premalignant hydatidiform mole to GTN using serial serum human chorionic gonadotropin (hCG) measurements.[1] After a diagnosis of GTN, but prior to chemotherapy, patients are staged using the International Federation of Obstetrics and Gynaecology (FIGO) prognostic scoring system.[3] In the United Kingdom, most patients have "low-risk" disease as defined by a FIGO score of ≤6 and are treated with single agent methotrexate (MTX).[3-6] Overall 30%-45% of low-risk patients will have disease resistant to first-line single agent chemotherapy, and a considerable proportion require escalation to multi-agent chemotherapy.[5,7,8] Fortunately, most patients are cured with salvage therapy, so overall survival in low-risk patients treated at trophoblastic disease reference centers now approaches 100%.[5,8-10] However, better prediction of MTX resistance (MTX-R) has the potential to reduce exposure to ineffective chemotherapy and allow patients faster resolution of disease and an earlier opportunity to attempt another pregnancy.

The placenta is a highly vascular organ and trophoblastic tumors are similarly characterized by dense vascular networks. In previous studies, we have demonstrated that Doppler ultrasonography to measure the uterine artery pulsatility index (UAPI) represents a noninvasive technique that can estimate tumor vascularity in low-risk GTN and that low UAPI values are predictive for the development of methotrexate resistance.[5,11] An improved understanding of which mediators are key to vascular changes in GTN may allow for a more accurate interpretation of the relationship between UAPI and chemotherapy resistance. Therefore, the objective of this study was to determine whether serum factors that might influence blood vessel growth complement UAPI and might improve prediction of MTX-R among low-risk GTN with similar prognostic risk scores.

Methods

Patients

Patients newly diagnosed between January 2008 and June 2012 with low-risk GTN during postmolar surveillance who had stored serum samples prior to intiation of MTX chemotherapy were identified from the Charing Cross Trophoblastic Disease Unit's database. In total, 217 patients had freezer stored sera taken prior to commencing chemotherapy available for analysis. One hundred two of the 217 MTX treated patients subsequently developed resistance to the monotherapy and were changed to second-line regimens, creating approximately equal cohorts of women with MTX cured (MTX-S) or MTX-R disease. From among these 217 patient samples, equal numbers of MTX-S and MTX-R serum aliquots were randomly selected for multiplexed analysis of circulating biomarkers. The final study cohort was set by matching MTX-S and MTX-R serum aliquots by prognostic risk score in a case-control fashion. Due to limited samply quantitiy, not all biomarkers could be measured in all samples. We have previously reported cytokine data in a select cohort of these patients.[12] In the present study, angiogenic growth factors were measured ($n = 76$). Patient clinical characteristics were abstracted from the Charing Cross Trophoblastic Disease Unit's database, namely, age, gestational age at time of molar evacuation, pretreatment hCG measurement, and FIGO prognostic risk score.

Chemotherapy, response evaluation, and follow-up

All patients were initially treated with fortnightly cycles of 50 mg of intramuscular methotrexate on days 1, 3, 5, and 7, with 15 mg of oral folinic acid rescue on days 2, 4, 6, and 8, a modification of the original treatment protocol devised by Bagshawe et al.[4,5] Response to chemotherapy was monitored by twice weekly serum hCG measurements. MTX-R was defined per FIGO 2002 criteria by a plateau in hCG concentrations in 4 values over 3 weeks or a rise in hCG concentrations in 3 values over 2 weeks.[5] Patients who became resistant at serum hCG concentrations of $\leq$300 IU/L were switched to actinomycin D 0.5 mg intravenously, given daily for 5 days every 2 weeks.[5] All other patients who had failed single agent methotrexate were treated with combination chemotherapy comprising etoposide, MTX, and actinomycin D, and then cyclophosphamide and vincristine (EMA/CO) given IV in a weekly alternating schedule.[10] All patients received consolidation therapy for 6 weeks beyond the fall of the hCG serological concentration to normal ($\leq$4 IU/L).

Uterine artery pulsatility index (UAPI)

Of the 217 patients in the total study population, 189 patients underwent a single transvaginal ultrasonographic examination before chemotherapy, to measure the total uterine volume and UAPI as previously described.[5] Doppler assessments were performed using an Aplio XG ultrasound scanner (Toshiba Medical Systems, Nasu, Japan) with a 2-5 MHz curvilinear array probe.

Using power Doppler, the uterine arteries were located prior to spectral Doppler analysis, and both uterine arteries were examined. The UAPI is given by the formula: UAPI = $(A - B)$/mean, where *A, B*, and the mean are the maximum, minimum, and time averaged Doppler frequency shift of the ultrasound beam after reflection from the moving column of blood in the uterine artery. UAPI is independent of the angle of insonation, which cannot be reliably estimated for uterine arteries due to their tortuosity and narrow diameter. The UAPI was calculated by averaging the values from a minimum of 3 cardiac cycles using the scanner software. The UAPI reflects the impedance to flow distal to the point of sampling; an increase in impedance will result in an increase in the UAPI and vice versa. A low UAPI is indicative of high blood flow through the uterine artery. The lowest UAPI from either uterine artery was used for analysis.

Serum samples

Blood samples for hCG monitoring were collected using serum-separating type blood tubes. Samples were separated by centrifugation at 3000 rpm for 10 minutes and the serum aliquoted into polypropylene tubes and subsequent storage at −20°C until used for further immunoassays.

Angiogenic growth factors

The growth factor assays were performed using a Bio-Rad Bio-Plex 200 analyzer platform (Bio-Rad Laboratories, Hercules, CA), using Millipore's Human Angiogenesis/Growth Factor Magnetic Bead Panel 1 (cat# HAGP1MAG-12K; Millipore Inc, Burlington, MA) for EGF, Angiopoeitin-2, BMP-9, and endoglin and Affymetrix's Procarta Human Immunoassay xMAP kit (Affymetrix Inc, Santa, Clara, CA) for VEGF-A, PLGF-1, PDGF-BB, and FGF-basic. The assays were performed according to the manufacturer's instructions, and plates read within 90 minutes of assay completion. Instruments settings were adjusted to 50 events/bead, 0 minimum events, flow rate at 60 µL/min, and doublet discriminator gates at 7500 and 15,500. Serum concentrations were calculated using a standard curve for each marker. Three separate plates (3 technical replicates) were run of each panel for each patient sample.

Statistical analysis

SPSS (IBM Statistics Version 19, IBM, Armonk, North Castle, NY) was used for statistical analyses. Univariate analyses were performed using the Mann-Whitney U test for continuous variables, and the χ^2 test for categorical variables. Spearman correlation was used to assess the association between continuous variables. P values <0.05 were considered significant. Different markers were combined to determine an optimized serological test for prediction of MTX-R following the method described by Mamtani et al.[13] The performance characteristics of significantly different variables in univariate analysis were assessed by constructing receiver operating characteristic (ROC) curves and calculating the area under the curve (AUC). Correction for multiple testing was performed using the Bonferroni-Šídák test for multiple comparisons. Multivariate logistic regression analysis was performed using the block entry method.

Results

UAPI measurements

In agreement with our previous observations, UAPI values were significantly higher for MTX-S compared to MTX-R patients. MTX-S patients had a median UAPI value of 1.30 (interquartile

Table 1
Characteristics of study population.

Characteristic	Group		
Median (IQR)	MTX-S n = 38	MTX-R n = 38	*P* value[†]
Age (years)	31.9 (26.9-38.2)	32.7 (27.1-36.2)	0.84
GA at Evacuation (weeks)	11.4 (9.5-12.8)	11.1 (9.2-12.9)	0.86
Evacuation to Treatment interval (weeks)	5.36 (4.25-6.96)	5.1 (4.1-6.29)	0.52
FIGO score	3.0 (2.0-3.8)	3.5 (3.0-4.0)	0.05
Total hCG, IU/L	21180 (13532-35581)	27987 (11257-38704)	0.21

[†] Mann-Whitney U test.

Table 2
Angiogenic factor analysis for prediction of methotrexate resistance.

Parameter (units)		Percentiles			*P* value[†]
		25	50 (Median)	75	
BMP9 (pg/mL)	MTX-S	169.62	225.50	286.95	***0.03***
	MTX-R	200.41	280.41	338.78	
Endoglin (pg/mL)	MTX-S	579.51	745.35	886.25	0.07
	MTX-R	672.65	888.90	963.96	
ANGPT2 (pg/mL)	MTX-S	728.11	1112.30	1825.61	0.20
	MTX-R	783.57	1322.39	1894.38	
VEGF-A (pg/mL)	MTX-S	6.62	12.92	45.16	0.35
	MTX-R	2.14	12.68	28.98	
PLGF (pg/mL)	MTX-S	3.61	7.22	16.56	0.92
	MTX-R	4.01	6.52	11.04	
EGF (pg/mL)	MTX-S	5.30	33.86	92.83	0.28
	MTX-R	12.90	73.47	129.38	
FGFbasic (pg/mL)	MTX-S	0.80	4.40	5.52	0.79
	MTX-R	0.80	4.11	5.31	
PDGFBB (pg/mL)	MTX-S	470.58	603.03	1016.18	0.86
	MTX-R	410.34	645.20	1098.91	

IQR, interquartile range; MTR-R, methotrexate resistance; MTX-S, methotrexate sensitive.
[†] Mann-Whitney U test.

range [IQR] = 0.80-1.90, n = 99) vs 0.875 (IQR = 0.60-1.30, n = 90 $P < 0.0001$) for MTX-R patients. The ROC analysis for UAPI yielded an AUC of 0.68 (95% confidence interval [CI] 0.61-0.76; $P < 0.0001$; Figure S1). At the optimal cut-off of ≤1.10 for this study population, UAPI had a sensitivity of 73% (95% CI 63%-82%) and specificity of 57% (95%CI 47%-68%), corresponding to a positive likelihood ratio (PLR) of 1.73 and a negative likelihood ratio (NLR) of 0.46.

Patient characteristics

Among the subset of patients selected for circulating biomarker analysis, patient clinical characteristics including gestational age at time of molar evacuation, pretreatment hCG measurement, and prognostic risk score were similar between the MTX-S and MTX-R groups (Table 1).

Angiogenic factors

In the multiplexed immunoassays of angiogenic growth factors, only BMP-9 concentration differed significantly between the MTX-S (median of 225 pg/mL, IQR = 170-287) and MTX-R groups (median 280 pg/mL, IQR = 200-339, $P = 0.035$; Table 2). BMP-9 alone had a ROC AUC of 0.64 (95%CI 0.51-0.77; $P = 0.01$). When compared to UAPI, FIGO risk score, and total hCG concentration, only the ROC curves for BMP-9 and UAPI reached statistical significance (Table 3).

Table 3
Comparison of prognostic markers for methotrexate resistance.

Parameter (units)		AUC	95%CI		*P* value	Adjusted *P* value‡
			5%	95%		
Lowest UAPI	MTX-S MTX-R	0.74	0.63	0.86	<0.0001	<0.001
FIGO risk score	MTX-S MTX-R	0.63	0.51	0.76	0.03	0.11
hCG (IU/L)	MTX-S MTX-R	0.58	0.45	0.71	0.11	0.37
BMP9 (pg/mL)	MTX-S MTX-R	0.64	0.51	0.77	0.01	0.04

CI, confidence interval; FIGO, International Federation of Gynecology and Obstetrics; hCG, human chorionic gonadotropin; IQR, interquartile range; IU/L, international units per liter; MTR-R, methotrexate resistance; MTX-S, methotrexate sensitive; UAPI, uterine artery pulsatility index.
‡ Bonferroni-Šídák test for multiple comparisons.

Correlations among serum factors

Many of the assayed factors showed strong correlations with each other, consistent with their interrelated regulation of angiogenesis (Table S1). BMP-9 had significant positive correlation with endoglin ($r = 0.337$; $P = 0.003$, $n = 76$). VEGF-A was negatively correlated with hCG ($r = -0.279$, $P = 0.015$, $n = 76$;); VEGF-A was strongly positively correlated with PLGF-1 ($r = 0.399$, $P = 0.0013$, $n = 76$), and FGF-basic ($r = 0.362$, $P = 0.001$, $n = 76$). hCG had a very strong positive correlation with Angiopoeitin-2 ($r = 0.504$, $P < 0.001$, $n = 76$).

Relationship between serum factors and UAPI

UAPI (higher values indicate higher resistance and smaller diameter vasculature) was inversely correlated with hCG levels. UAPI was negatively correlated with BMP-9 and Endoglin with *P* values of 0.013 and 0.001, respectively ($n = 72$). In contrast VEGF-A and PLGF-1 were positively, but not significantly, correlated with UAPI ($r = 0.17$, $P = 0.153$, $n = 72$; $r = 0.189$, $P = 0.112$; Table S1).

Optimization of resistance prediction

The combination of UAPI with BMP-9 increased the AUC of the resistance prediction model to 0.77 (95%CI 0.66-0.88; Fig 1) indicating the benefit of a 2-fold "multimodal" test approach. The regression model derived for the UAPI + BMP-9 combined test, the MTX-R prediction ($MTXR^P$), was $MTXR^P = 0.60 + (0.0006 \times [\text{BMP-9}]) - (0.247 \times \text{UAPI})$. At an optimum cut-off of >0.58 the $MTXR^P$ test had a sensitivity of 62% (95%CI 44-78) and specificity of 82% (95% CI 66-92) with a PLR of 3.35 and NLR of 0.47.

Discussion

Consistent with our prior report, high uterine artery blood flow, as reflected by a low Doppler-derived UAPI, was confirmed to be a predictive marker of MTX-R.[5] Among the serological parameters assessed, BMP-9 and endoglin had the highest predictive value for resistance to MTX chemotherapy, but only BMP-9 levels were significantly higher when the median values between each group were compared. Combining BMP-9 with UAPI to create the test parameter ($MTXR^P$) performed better than either biomarker alone.

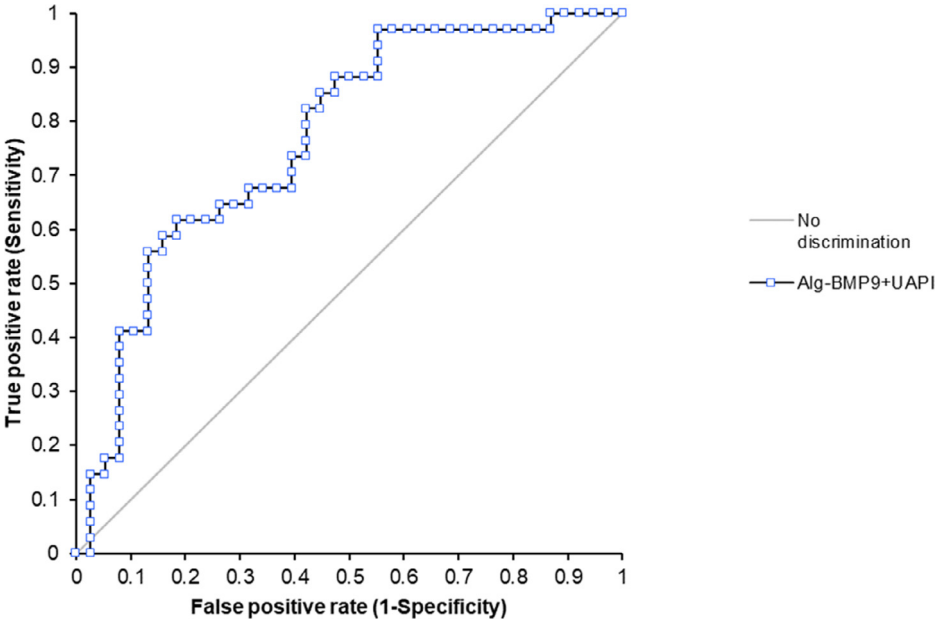

Fig. 1. ROC plot for overall performance of UAPI combined with BMP-9 to discriminate MTX resistance using both datasets. ROC analysis yielded an AUC of 0.77 (0.66-0.88; $P < 0.0001$).

BMP-9 is the natural ligand for endoglin, which itself can exist as either a membrane bound or soluble form. Endoglin is a homodimeric glycoprotein and TGF-β co-factor expressed in vascular epithelium which regulates angiogenesis and endothelial cell migration.[14] Mutations in endoglin cause the disorder Hereditary Haemorrhagic Telangiectasia, characterized by arteriovenous malformations.[15] Together, BMP-9 and soluble endoglin regulate TGF-β signaling in syncytiotrophoblasts and may play a key role in preeclampsia.[16] Our data suggest that BMP-9 similarly contributes to a more aggressive phenotype of GTN. Indeed, a recent report from our group using an antiendoglin therapy to produce a durable remission in a highly chemo refractory case of GTN suggests that this signaling pathway is highly active in chemotherapy resistant GTN.[17] Thus, these data suggest that the vascular features of the uterus at the time of diagnosis of GTN may predict subsequent response to chemotherapy.

Interestingly, while UAPI correlated with higher endoglin and BMP-9 levels, there was an inverse relationship between UAPI and VEGF-A. VEGF-A causes sprouting of new narrow diameter vessels near the tumor site. This would be expected to increase arterial impedance of vessels supplying the area.[18] It appears unlikely therefore that increased neoangiogenesis near the tumor implantation site is the explanation for the reduced UAPI observed in MTX-R low-risk GTN, as this form of neovascularization would cause higher UAPI values. Increased uterine artery blood flow in MTX-R patients instead appear to reflect a decrease in vascular resistance, likely induced by circulating factors such as hCG and potentially BMP-9 rather than tumor neoangiogenic changes.

The strength of this study is that it represents a considerable number of patients for a rare diagnosis. All patients were cared for within a national referral network according to standardized protocols for diagnosis, monitoring, and treatment. The center has extensive experience with measuring and reporting UAPI, and the clinical personnel were consistent during the study period. A criticism of the current work could be that using an optimum cut-off of >0.58, the $MTXR^P$ test had a relatively poor detection rate with a sensitivity of only 62% (specificity of 82%; PLR of 3.35 and NLR of 0.47). While this performance may be viewed as being insufficient for

clinical purposes, it is better than the currently used FIGO risk scoring system.[19] FIGO is a risk scoring system based on hCG measurement, imaging and other clinical findings that is used to stratify GTN patients into low or high risk to determine initial chemotherapy regimen, and as such is expected to have high predictive power for treatment resistance. However, when the FIGO score was combined with either BMP-9 or UAPI, the resulting tests both had lower AUCs than either UAPI or BMP-9 alone, indicating that FIGO's poorer predictive value had diluted their predictive abilities. This stems in large part from the study design as cases and controls were matched by FIGO risk score at the study onset, but the relatively poor predictive value of FIGO may also be explained by the fact that the weighting of individual factors used within the FIGO prognostic scoring system is not statistically derived. Hence the effect of reducing continuous measurements that have predictive value such as hCG concentration to arbitrary or inappropriately weighted discontinuous categories (such as the assignment of scoring points based on hCG concentration thresholds at 1000, 10,000, and 100,000 IU/L, used in FIGO scoring) may have reduced the inherent prognostic value of the component parameters. Accordingly, the most powerful predictive marker of MTX-R in low-risk GTN that has been reported to date, that of the kinetic derived hCG parameter, "residual hCG" (AUC ROC = 0.87, 95%CI 0.61-1) has an estimated prognostic power based on multivariate logistic regression comparison that was 13 times more powerful than FIGO score.[20] However, this parameter can only be assessed once chemotherapy treatment has begun.

In conclusion, UAPI was confirmed to be a predictive marker of MTR-R in low-risk GTN. An improved test for the prediction of MTX-R was derived using UAPI and BMP-9. BMP-9 signaling and its relationship to tumor behavior will be an important future area of study for this disease.

Supporting information

Figure S1. ROC plot for overall performance of UAPI to discriminate MTX resistance using both datasets. ROC analysis yielded an AUC of 0.68 (0.61-0.76; $P < 0.0001$).

Table S1. Table of Spearman Rank correlations between UAPI and angiogenic factors

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Supplementary material

Supplementary material associated with this article can be found, in the online version, at 10.1016/j.currproblcancer.2020.100622.

References

1. Seckl MJ, Sebire NJ, Berkowitz RS. Gestational trophoblastic disease. *Lancet*. 2010;376:717–729.
2. Shih Ie M. Gestational trophoblastic neoplasia – pathogenesis and potential therapeutic targets. *Lancet Oncol*. 2007;8:642–650.
3. Kohorn EI, Goldstein DP, Hancock BW, et al. Workshop report: combining the staging system of the International Federation of Gynecology and Obstetrics with the scoring system of the World Health Organization for Trophoblastic Neoplasia. Report of the Working Committee of the International Society for the Study of Trophoblastic Disease and the International Gynecologic Cancer Society. *Int J Gynecol Cancer*. 2000;10:84–88.
4. Bagshawe KD, Dent J, Newlands ES, et al. The role of low-dose methotrexate and folinic acid in gestational trophoblastic tumours (GTT). *Br J Obstet Gynaecol*. 1989;96:795–802.

5. Agarwal R, Strickland S, McNeish IA, et al. Doppler ultrasonography of the uterine artery and the response to chemotherapy in patients with gestational trophoblastic tumors. *Clin Cancer Res*. 2002;8:1142–1147.
6. Hancock BW, Welch EM, Gillespie AM, et al. A retrospective comparison of current and proposed staging and scoring systems for persistent gestational trophoblastic disease. *Int J Gynecol Cancer*. 2000;10:318–322.
7. El-Helw LM, Coleman RE, Everard JE, et al. Impact of the revised FIGO/WHO system on the management of patients with gestational trophoblastic neoplasia. *Gynecol Oncol*. 2009;113:306–311.
8. Sita-Lumsden A, Short D, Lindsay I, et al. Treatment outcomes for 618 women with gestational trophoblastic tumours following a molar pregnancy at the Charing Cross Hospital, 2000-2009. *Br J Cancer*. 2012;107:1810–1814.
9. Alifrangis C, Agarwal R, Short D, et al. EMA/CO for high-risk gestational trophoblastic neoplasia: good outcomes with induction low-dose etoposide-cisplatin and genetic analysis. *J Clin Oncol*. 2013;31:280–286.
10. Bower M, Newlands ES, Holden L, et al. EMA/CO for high-risk gestational trophoblastic tumors: results from a cohort of 272 patients. *J Clin Oncol*. 1997;15:2636–2643.
11. Sita-Lumsden A, Medani H, Fisher R, et al. Uterine artery pulsatility index improves prediction of methotrexate resistance in women with gestational trophoblastic neoplasia with FIGO score 5-6. *BJOG*. 2013;120:1012–1015.
12. Elias KM, Harvey RA, Hasselblatt KT, et al. Type I interferons modulate methotrexate resistance in gestational trophoblastic neoplasia. *Am J Reprod Immunol*. 2017;77:e12666.
13. Mamtani MR, Thakre TP, Kalkonde MY, et al. A simple method to combine multiple molecular biomarkers for dichotomous diagnostic classification. *BMC Bioinformatics*. 2006;7:442.
14. Nassiri F, Cusimano MD, Scheithauer BW, et al. Endoglin (CD105): a review of its role in angiogenesis and tumor diagnosis, progression and therapy. *Anticancer Res*. 2011;31:2283–2290.
15. Bourdeau A, Dumont DJ, Letarte M. A murine model of hereditary hemorrhagic telangiectasia. *J Clin Invest*. 1999;104:1343–1351.
16. Gregory AL, Xu G, Sotov V, et al. Review: the enigmatic role of endoglin in the placenta. *Placenta*. 2014;35(suppl):S93–S99.
17. Worley Jr MJ, Elias KM, Horowitz NS, et al. Durable remission for a woman with refractory choriocarcinoma treated with anti-endoglin monoclonal antibody and bevacizumab: a case from the New England Trophoblastic Disease Center, Brigham and Women's Hospital and Dana-Farber Cancer Institute. *Gynecol Oncol*. 2017;148:5–11.
18. Eisenbrey JR, Wilson CC, Ro RJ, et al. Correlation of ultrasound contrast agent derived blood flow parameters with immunohistochemical angiogenesis markers in murine xenograft tumor models. *Ultrasonics*. 2013;53:1384–1391.
19. Taylor F, Grew T, Everard J, et al. The outcome of patients with low risk gestational trophoblastic neoplasia treated with single agent intramuscular methotrexate and oral folinic acid. *Eur J Cancer*. 2013;49:3184–3190.
20. You B, Harvey R, Henin E, et al. Early prediction of treatment resistance in low-risk gestational trophoblastic neoplasia using population kinetic modelling of hCG measurements. *Br J Cancer*. 2013;108:1810–1816.