Uterine artery pulsatility index: a predictor of methotrexate resistance in gestational trophoblastic neoplasia

R Agarwal*1,2, V Harding1,2, D Short1, RA Fisher1, NJ Sebire1, R Harvey1, D Patel1, PM Savage1, AKP Lim1 and MJ Seckl*1,2

1Department of Medical Oncology, Charing Cross Gestational Trophoblastic Disease Centre, Charing Cross Hospital, Imperial College Healthcare NHS Trust, Fulham Palace Road, London W6 8RF, UK

BACKGROUND: Neo-angiogenesis is a hallmark of cancer. The aim of this study was to test the hypothesis, in a prospective patient cohort, that in low-risk gestational trophoblastic neoplasia (LR-GTN) the uterine artery pulsatility index (UAPI), a measure of tumour vascularity, can predict resistance to methotrexate chemotherapy (MTX-R).

METHODS: 286 LR-GTN patients (Charing Cross Hospital (CXH) score 0–8, or FIGO score 0–6) were treated with methotrexate between January 2008 and June 2011 at CXH. During staging investigations, patients underwent a Doppler ultrasound to assess the UAPI.

RESULTS: 239 patients were assessable for both UAPI and MTX-R. The median UAPI was lower (higher vascularity) in MTX-R compared with MTX-sensitive patients (0.8 vs 1.4, P<0.0001). In multivariate logistic regression, UAPI ≤ 1 predicted MTX-R, independent of both CXH and FIGO scores. The risk of MTX-R in patients with a FIGO score of 6 and UAPI ≤ 1 was 100% vs 20% in patients with UAPI > 1 (χ² P<0.0001).

CONCLUSION: UAPI represents an independently validated clinically useful predictor of MTX-R in LR-GTN. Further, consideration of whether to incorporate UAPI into the FIGO scoring system is now warranted so that patients with a score of 6 and a UAPI ≤ 1 might be upstaged and offered combination chemotherapy rather than MTX.

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Gestational trophoblastic neoplasia (GTN) is one of the few malignancies that can be cured by cytotoxic chemotherapy, even when widespread disease is present. The GTN encompasses a spectrum of histological entities including invasive mole, choriocarcinoma, placental site trophoblastic tumour and the extremely rare epithelioid trophoblastic tumour (Shih Ie, 2007; Seckl et al., 2010). Commonly these arise from molar pregnancies, although they can arise from any type of pregnancy including term deliveries, ectopic pregnancies and miscarriages. Molar pregnancies are classified as either partial (PHM) or complete hydatidiform moles (CHM) (Kajii and Ohama, 1977; Szulman and Surti, 1978a, 1978b). In UK, the incidence of CHM is about 1 out of 1000 pregnancies with PHM more common (3 out of 1000). The risk of persistent GTN is about 15% with CHM and 0.5–1% with PHM (Smith, 2003). In UK, all patients diagnosed with molar pregnancies are registered in one of the three centres (Dundee for Scotland, Sheffield for Northern England and London for the rest of the UK) to enable centralised pathological review and surveillance using serial human chorionic gonadotrophin (hCG) measurements according to an established protocol (Seckl et al., 2010).

If a rise or plateau in hCG is detected during surveillance, this indicates the likely onset of malignant change requiring chemotherapy. Additional criteria for commencing chemotherapy in these patients, which include a histological diagnosis of choriocarcinoma and the presence of metastases to the brain and gastrointestinal tract, are shown in Supplementary Table 1.

The chemotherapy used is based on the FIGO prognostic scoring system, and has been in use in its present form since 2002 (Supplementary Table 2). It has super-ceded previous scoring systems, including the Charing Cross Hospital (CXH) scoring system developed by Bagshawe (Bagshawe, 1976). A FIGO score of 7 (CXH score of 9) or more denotes disease that is at high risk of failure to single-agent chemotherapy, and these patients are treated from the outset with etoposide, methotrexate and Dactinomycin alternating weekly with cyclophosphamide and vincristine (EMA/CO) (Bower et al., 1997; Aghajanian, 2011). A FIGO score of ≤ 6 (CXH score 8) denotes disease that has a ‘low risk’ of developing resistance to single-agent therapy with methotrexate or Dactinomycin (McNeish et al., 2002; Aghajanian, 2011). However, about 30% of these low-risk patients become resistant to single-agent chemotherapy and require combination chemotherapy most commonly in the form of EMA/CO (Table 1) (McNeish et al., 2002; Seckl et al., 2010; Aghajanian, 2011). The majority of the low-risk patients are salvaged and the overall survival in low-risk
patients is now virtually 100% (McNeish et al., 2002). However, switching chemotherapy following development of resistance to single-agent methotrexate (MTX-R) prolongs the overall duration of chemotherapy and can cause considerable psychological distress to patients. Consequently, there is a need to further refine the scoring system so that we can more accurately identify the 30% of patients destined to become resistant to single-agent therapy.

Neo-angiogenesis, the formation of new blood vessels, is a critical step in tumourogenesis (Hanahan and Weinberg, 2011). Neo-angiogenesis is associated with increased tumour growth, acquisition of metastatic potential, drug resistance and poor prognosis in a number of solid tumours such as breast, lung and ovarian cancer (Weidner et al., 1992; Weidner, 1993; Fontanini et al., 1995; Hollingsworth et al., 1995; Siddiqui et al., 2010). Histological assessment of microvessel density (MVD) with CD34 immunostaining is used to assess angiogenesis in these tumours (Weidner, 1993). In contrast, MVD assessment is not possible in GTN as biopsy is frequently contraindicated because of the risk of precipitating life-threatening haemorrhage in this highly vascular disease. Instead, the diagnosis is usually made on the basis of rising hCG levels post-molar pregnancy. We previously proposed the use of Doppler ultrasonography as a non-invasive alternative to assess tumour vascularity in GTN, using the uterine artery pulsatility index (UAPI) (Agarwal et al., 2002). The UAPI is inversely proportional to tumour vascularity, and a low UAPI is indicative of increased arteriovenous shunting, a feature of the abnormal neo-angiogenesis characteristic of tumours. In that study of 164 patients with GTN, a UAPI of ≤ 1 was shown to be an independent predictor of MTX-R and in combination with the CXH scoring system improve prediction of MTX-R. In particular, the risk of MTX-R in patients with medium-risk CXH scores of 6–8 with a UAPI of ≤ 1 was increased to 72.7% from a baseline risk of 56.3% in this group. Our findings suggested that UAPI might be a useful additional variable to incorporate into the prognostic scoring systems to help refine which patients might be treated with EMA-CO chemotherapy upfront (Agarwal et al., 2002).

The aim of this study was to test this hypothesis in an independent patient cohort, and demonstrate that the UAPI should be used in addition to the FIGO score for patient stratification for chemotherapy in GTN.

### METHODS

**Patients**

All patients (n = 286) treated upfront with single agent MTX-R for GTN between January 2008 and June 2011 were identified from the Charing Cross Hospital Trophoblastic Disease Database (CXH-TDD). We reviewed the patient characteristics including age, hCG level, number and location of metastatic disease at presentation, histological diagnosis and CXH and FIGO scores. Those patients that were switched to a second-line chemotherapy (either single agent Dactinomycin or EMA-CO) were considered resistant to methotrexate if the change was the result of a plateaued or rising hCG. Patients who were switched due to drug side-effects (serositis or allergy) were not considered to be resistant. In the CXH prognostic scoring system, a patient with a score between 0 and 5 is at low risk of MTX-R. Patients scoring 6 to 8 are at medium- and high-risk of MTX-R, respectively. Only patients with CXH scores ≤ 9 or FIGO scores ≤ 7 at baseline are treated with single-agent methotrexate (18) and were therefore included in this study; high-risk patients were excluded. The details of individual prognostic factors, CXH and FIGO prognostic scores, and treatment details were obtained for all patients in the study from the CXH-TDD.

Patients underwent a single pelvic ultrasonographic examination before chemotherapy. The results of Doppler assessments were obtained from the original US reports. These reports were missing, or inadequately documented in 47 patients. Because our intention in this study was to examine the relationship between tumour vascularity measured by Doppler US and MTX-R, these patients were excluded from subsequent analysis.

**Uterine artery pulsatility index**

The total uterine volume and the UAPI were measured as described previously. Doppler US took an average of 15–20 min to complete per patient. Doppler assessments were performed using an Aplio XG ultrasound scanner (Toshiba Medical Systems, Nasu, Japan) with a 2–5 MHz curvilinear array probe. Uterine volume

| Table 1 | Patient characteristics |
|---------|-------------------------|
|----------|-------------------------|
|          | All | Yes | No | χ² |
| Age (years) | Median | 33 | 32 | 33 | 15–52 | 15–55 | 0.379* |
| Age score | 0 | 189 | 94 | 83 | 95 | 75 | 0.139 |
| 1 | 50 | 19 | 17 | 31 | 25 |
| Pregnancy score | 0 | 226 | 106 | 94 | 120 | 95 | 0.835 |
| 1 | 8 | 4 | 4 | 4 | 3 |
| 2 | 5 | 3 | 3 | 2 | 2 |
| Interval score | 0 | 222 | 105 | 93 | 117 | 93 | 0.996 |
| 1 | 15 | 7 | 6 | 8 | 6 |
| 2 | 2 | 1 | 1 | 1 | 1 |
| hCG score | 0 | 46 | 14 | 12 | 32 | 25 | 0.011 |
| 1 | 67 | 29 | 26 | 38 | 30 |
| 2 | 120 | 65 | 58 | 55 | 44 |
| 4 | 6 | 5 | 4 | 4 | 1 |
| No. of metastases score | 0 | 214 | 100 | 88 | 114 | 90 | 0.617 |
| 1 | 25 | 13 | 12 | 12 | 10 |
| Site of metastasis score | 0 | 239 | 113 | 100 | 126 | 100 | NA |
| Large mass score | 0 | 112 | 48 | 42 | 64 | 51 | 0.256 |
| 1 | 74 | 35 | 31 | 39 | 31 |
| 2 | 53 | 30 | 27 | 23 | 18 |
| FIGO score | Median | 3 | 3 | 0–6 | 2 | 0–6 | 0.026* |
| 0–4 | 213 | 97 | 86 | 116 | 92 | 0.234 |
| 5–6 | 24 | 14 | 12 | 10 | 8 |
| 0–3 | 169 | 76 | 67 | 93 | 74 | 0.364 |
| 4–6 | 68 | 35 | 31 | 33 | 26 |
| CXH score | Median | 3 | 3 | 0–8 | 3 | 0–8 | 0.039* |
| 0–5 | 226 | 104 | 92 | 122 | 97 | 0.103 |
| 6–8 | 13 | 9 | 8 | 4 | 3 |

Abbreviations: CXH = Charing Cross Hospital; FIGO = International Federation Gynecology and Obstetrics; hCG = human chorionic gonadotrophin; MTX-R = methotrexate chemotherapy. *Mann–Whitney U test.
was calculated using the prolate ellipsoid formula: uterine volume \( (\text{cm}^3) = L \times (\text{cm}) \times AP \times (\text{cm}) \times W \times (\text{cm}) \times 0.523 \), where \( L \) is length, \( AP \) is maximum antero-posterior diameter, and \( W \) is maximum width (1 cm\(^3\) = 1 ml).

UAPI was chosen to assess blood flow in this study, because it is independent of the angle of insonation, and this angle cannot reliably be estimated for uterine arteries because of their small diameter and tortuosity. The UAPI is given by the formula: \( \text{UAPI} = (A - B)/\text{mean} \), 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. The UAPI was calculated by averaging the values from a minimum of three 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. Using power Doppler, the uterine arteries were located before spectral Doppler analysis, and both uterine arteries were examined. The lowest UAPI from either uterine artery was used for assessment, because it is a reflection of the maximal deviation from the normal impedance.

Chemotherapy and response evaluation

Patients were initially treated with fortnightly cycles of 50 mg of methotrexate i.m. on days 1, 3, 5 and 7, with 15 mg of oral folic acid rescue on days 2, 4, 6 and 8 (the MTX regimen) (McNeish et al., 2002). Response to chemotherapy was monitored by twice weekly serum hCG measurements. Methotrexate chemotherapy was defined by a plateau or a rise in two consecutive hCG concentrations (McNeish et al., 2002). Patients with MTX-R were changed to Dactinomycin 0.5 mg i.v., daily for 5 days every 2 weeks, if their hCG concentration at resistance was \( \leq \) 300 IU\(^{-1}\). Otherwise, patients were treated with EMA/CO given intravenously as a weekly alternating schedule (Bower et al., 1997; McNeish et al., 2002). Treatment was continued in all patients for 6 weeks beyond the fall of the hCG to normal (\( \leq 4 \text{IU}^{-1}\)).

Statistical analysis

SPSS V17.0 (SPSS, Chicago, IL, USA) was used for statistical analyses. Univariate analyses were performed using the Mann–Whitney U test or \( \chi^2 \) test. UAPI was subdivided into high- and low-risk categories for MTX-R using a cut of \( \leq 1 \) and \( > 1 \), defined in our previous study based on ROC curve analysis (Agarwal et al., 2002). These UAPI subgroups were used in multivariate analyses to predict MTX-R, using binary logistic regression with forward stepwise selection. The CXH or FIGO prognostic scores were considered as continuous variables, respectively (Table 3). In all models, the prognostic score was considered as co-variables. In all models, the prognostic score was always included and the additional utility of UAPI assessed. The CXH score was dichotomised into low- (0–5) and medium- (6–8) risk groups as previously published. The equivalent FIGO categories used were 0–5 vs 6, and 0–4 vs 5–6 to assess the impact of UAPI using current FIGO scores.

With 239 patients, and using the pre-defined UAPI cutoff of 1, which was the median in the previous study, and risk of MTX-R of 35% and 65% for patients with an UAPI \( > 1 \) and \( \leq 1 \), respectively (Agarwal et al., 2002), the current study had a power of 99.8% to detect a difference in risk of MTX-R with a two-sided alpha of 5%.

RESULTS

Between January 2008 and June 2011, 286 patients with low-risk GTN were treated with single agent intra-muscular methotrexate. 239 patients with quantitative UAPI results available from baseline pre-chemotherapy assessments were eligible and all were chemonaive at presentation. Three had histologically confirmed chorionicarcomas. The median (range) age of patients was 33 years (15–55 years), serum hCG 11 865 IU ml\(^{-1}\) (12–230 836 IU ml\(^{-1}\)), CXH score 3 (0–8) and FIGO score 3 (0–6; Table 1). The median UAPI was 1 (range 0.3–2.82; Figure 1). In keeping with a previous report demonstrating a rise in MTX-R with the FIGO scoring system (El-Helw et al., 2009), 45% of patients developed MTX-R and switched to either dactinomycin or EMA/CO chemotherapy (Table 1).

In multivariate analyses, UAPI (\( \leq 1 \) vs \( > 1 \)) was an independent predictor of MTX-R with odds ratios (ORs) of 2.82 (\( P = 0.0001 \)) or 2.82 (\( P = 0.0001 \) relative to the CXH score (model A) or FIGO score (model C) considered as continuous variables, respectively (Table 3).
With the CXH score dichotomised into low- (0–5) and medium-risk (6–8) groups as described previously (model B), UAPI remained a significant independent predictor of MTX-R (OR 2.98, P = <0.0001; Table 3). The absolute risk of MTX-R was 100% in patients with CXH scores of 6–8 and UAPI ≤ 1, compared with a risk of 33% in patients with a UAPI > 1 (χ², P = <0.0001; Table 4). The current FIGO system was not designed to divide patients into low and medium risks. Comparing the CXH and FIGO scores among patients, the equivalent FIGO groups used for low and medium risk were 0–4 and 5–6, respectively (data not shown). Using these FIGO score categories (model D), UAPI remained an independent predictor of MTX-R (OR 3.0, P = <0.0001; Table 3). The addition of UAPI to the FIGO score significantly improved prediction of MTX-R, with the absolute risk of MTX-R of 81% in patients with a FIGO score of 5–6 and UAPI ≤ 1, compared with a risk of 33% in patients with a UAPI > 1 (χ², P = <0.0001; Table 4). With FIGO low- and medium-risk groups defined using scores of 0–5 and 6 (model E), respectively, UAPI remained a significant predictor (OR 3.0, P = <0.0001; Table 3) the risk of MTX-R in patients with a UAPI ≤ 1 and FIGO score of 6 was 100%, compared with 20% in patients with a UAPI > 1 (χ², P = <0.0001; Table 4).

**DISCUSSION**

This study demonstrates that increased uterine blood flow, assessed using the UAPI on Doppler sonography, is associated with an increased risk of methotrexate resistance. These results validate our previous study, and establish UAPI as an independent predictor of methotrexate resistance. So how can we use the new information regarding UAPI to select only those patients who will definitely fail single-drug therapy and really need combination agent therapy?

The FIGO scoring system for stratification of patients with GTN to single agent vs multi-agent first-line chemotherapy is an established method for treatment selection (Aghajanian, 2011). However, despite its utility over a third of patients incorrectly receive single-agent MTX-R using this approach, so improvements to this scoring system are still required (McNeish et al, 2002). In our previous study, we found that a UAPI ≤ 1 and CXH score 5–8 was associated with a 73% risk of MTX-R compared with a 56% risk in patients with a UAPI > 1, with an OR of 2.7 (Agarwal et al, 2002). In the current study, we confirmed this increase in risk in patients, 

### Table 2 UAPI and resistance to MTX-R

| MTX-R | n = 239 | UAPI ≤ 1 | UAPI > 1 | χ² |
|-------|---------|---------|---------|-----|
| NO    | 126     | 48      | 78      | 66  | 4 x 10⁻⁵ |
| YES   | 113     | 73      | 60      | 40  | 34       |

**Abbreviations:** MTX-R = methotrexate chemotherapy; UAPI = uterine artery pulsatility index.

### Table 3 Multivariate analysis of independence of UAPI relative to CXH and FIGO scores (CXH or FIGO scores were included in all models and UAPI included if significant by forward selection)

| Model | Variables | Categories | B     | Wald | df | Exp(B) | 95% CI for EXP(B) | Lower | Upper | P    |
|-------|-----------|------------|-------|------|----|--------|-------------------|-------|-------|------|
| A     | CXH score | Continuous | 0.142 | 3.223| 1  | 1.152  | 0.987 – 1.346     | 0.73  |       |
| B     | CXH score | > 1 vs ≤ 1 | 1.037 | 14.593| 1  | 2.821  | 1.657 – 4.804     | <0.0001|       |      |
| C     | UAPI      | > 1 vs ≤ 1 | 1.038 | 14.377| 1  | 2.822  | 1.651 – 4.825     | <0.0001|       |      |
| D     | FIGO score | Low (0–5) vs medium risk (6–8) | 0.350 | 0.599 | 1  | 1.419 | 0.585 – 3.443 | 0.439 |       |      |
| E     | UAPI      | > 1 vs ≤ 1 | 1.079 | 15.756| 1  | 2.941  | 1.726 – 5.009     | <0.0001|       |      |

**Abbreviations:** CI = confidence interval; CXH = Charing Cross Hospital; UAPI = uterine artery pulsatility index. *Reference category.

### Table 4 Risk of resistance to MTX-R by CXH or FIGO score and UAPI

| MTX-R | UAPI ≤ 1 | UAPI > 1 | χ² |
|-------|---------|---------|-----|
| No    | 74      | 66      | 67  |
| Yes   | 38      | 34      | 33  |

**Abbreviations:** CXH = Charing Cross Hospital; MTX-R = methotrexate chemotherapy; UAPI = uterine artery pulsatility index.
The incorporation of UAPI as a marker of tumour vascularity and angiogenesis provides a novel additional biological facet to the FIGO score. The FIGO/CXH scores are essentially based on measures of total tumour burden (e.g., hCG, largest mass and number of metastases) and metastasis (e.g., number of metastases and sites of metastases) (Bagshawe, 1976; Aghajanian, 2011). This is reflected in the strong correlation between serum hCG levels and FIGO/CXH scores (Spearman correlation coefficients 0.63 and 0.70, P < 0.0001). Tumour neo-angiogenesis is a hallmark of solid tumours and critical for tumour growth and metastasis (Hanahan and Weinberg, 2011). Angiogenesis in some cancers can directly enhance tumour aggressiveness and chemoresistance by activating growth, survival, proliferation and anti-apoptotic signalling pathways through angiogenic moieties such as basic fibroblast growth factor, vascular endothelial growth factor and platelet-derived growth factors (Yang et al, 2009; Linderholm et al, 2010; Carmo et al, 2011).

Interestingly, hCG can also act as an angiogenic factor in placental trophoblastic tissues. However, the correlation between UAPI and serum hCG levels was limited in the current study (Spearman correlation coefficients −0.275, P < 0.0001), and suggests that angiogenic factors other than hCG likely drive tumour vascularity in GTN (Zygmunt et al, 2002; Berndt et al, 2006; Cole, 2010). In an ongoing study, we are assessing a panel of known circulating angiogenesis factors and correlating these with the UAPI to establish drivers of angiogenesis, and with MTX-R as alternatives to UAPI. Interestingly, in a recent study Shih le (2011) has shown that choriocarcinomas despite their apparent vascular-ity, lack endothelial-lined intra-tumoural blood vessels, and instead exhibit vascular mimicry. Consequently, it is plausible that the biological drivers of this may be distinct from classical angiogenic factors (Shih le, 2011). In the current study, only three patients had choriocarcinomas. Exclusion of these patients did not significantly alter our results, and due to the small numbers we were unable to assess the impact of vascular mimicry on UAPI in choriocarcinomas.

In conclusion, this study validates the utility of UAPI as a non-invasive marker of tumour vascularity, and combined with the previous study, confirms in a total cohort of over 400 patients, the clinical utility of UAPI for MTX-R prediction in LR-GTN, independent of CXH/FIGO scores. These results suggest that upfront EMA/CO chemotherapy could be considered for patients with GTN with CXH scores 6–8 or FIGO score 6, and a UAPI ≤1, respectively. In the future, this might be achieved by adding one point for a UAPI ≤1 to the FIGO score.

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REFERENCES
Agarwal R, Strickland S, McNeish IA, Patel DC, Foskett M, Boultree JE, Newlands ES, Seckl MJ (2002) Doppler ultrasonography of the uterine artery and the response to chemotherapy in patients with gestational trophoblastic tumors. Clin Cancer Res 8: 1142 –1147

Aghajanian C (2011) Treatment of low-risk gestational trophoblastic neoplasia. J Clin Oncol 29: 786 –788

Bagshawe KD (1976) Risk and prognostic factors in trophoblastic neoplasia. Cancer 38: 1373 –1385

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British Journal of Cancer (2012) 106(6), 1089 – 1094
Berndt S, Perrier d’Hauterive S, Blacher S, Pequeux C, Lorquet S, Munaut C, Applanat M, Herve MA, Lamande N, Corvol P, van den Brule F, Frankenne F, Poutanen M, Huhtaniemi I, Geenen V, Noel A, Foidart JM (2006) Angiogenic activity of human chorionic gonadotropin through LH receptor activation on endothelial and epithelial cells of the endometrium. *FASEB J* 20: 2630–2632

Bower M, Newlands ES, Holden L, Short D, Brock C, Rustin GJ, Begent RH, Bagshaw KD (1997) EMA/CO for high-risk gestational trophoblastic tumors: results from a cohort of 272 patients. *J Clin Oncol* 15: 2636–2643

Carmo CR, Lyons-Lewis J, Seckl MJ, Costa-Pereira AP (2011) A novel requirement for Janus kinases as mediators of drug resistance induced by fibroblast growth factor-2 in human cancer cells. *PLoS One* 6: e19861

Cole LA (2010) Biological functions of hCG and hCG-related molecules. *Reprod Biol Endocrinol* 8: 102

El-Helw LM, Coleman RE, Everard JE, Tidy JA, Horsman JM, Elkhenini HF, Hancock BW (2009) Impact of the revised FIGO/WHO system on the management of patients with gestational trophoblastic neoplasia. *Gynecol Oncol* 113: 306 – 311

Fontanini G, Bigini D, Vignati S, Basolo F, Mussi A, Lucchi M, Chine S, Angeletti CA, Harris AL, Bevilacqua G (1995) Microvessel count predicts metastatic disease and survival in non-small cell lung cancer. *J Pathol* 177: 57 – 63

Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144: 646 – 674

Hollingsworth HC, Kohn EC, Steinberg SM, Rothenberg ML, Merino MJ (1995) Tumor angiogenesis in advanced stage ovarian carcinoma. *Am J Pathol* 147: 33–41

Kajii T, Ohama K (1977) Androgenetic origin of hydatidiform mole. *Br J Cancer* 36: 871–875

Kajii T, Ohama K, Hasegawa N, Tominaga T (1977) Low-risk gestational trophoblastic neoplasia, but hCG levels in excess of 100 000 IU/l. *Br J Cancer* 36: 810 – 814

McNeish IA, Strickland S, Holden L, Rustin GJ, Foskett M, Seckl MJ, Newlands ES (2002) Low-risk persistent gestational trophoblastic disease: outcome after initial treatment with low-dose methotrexate and folinic acid from 1992 to 2000. *J Clin Oncol* 20: 1838 – 1844

Seckl MJ, Sebire NJ, Berkowitz RS (2010) Gestational trophoblastic disease. *Lancet* 376: 717 – 729

Shih Ie M (2007) Gestational trophoblastic neoplasia-pathogenesis and potential therapeutic targets. *Lancet Oncol* 8: 642 – 650

Shih Ie M (2011) Trophoblastic vasculoegenic mimicry in gestational choriovitrocarcinoma. *Mod Pathol* 24: 646–652

Siddiqui GK, Elnasry K, Wong Te Fong AC, Perrett C, Morris R, Crow JC, Maclean AB (2010) Prognostic significance of intratumoral vascular endothelial growth factor as a marker of tumour angiogenesis in epithelial ovarian cancer. *Eur J Gynaecol Oncol* 31: 156 – 159

Smith HO (2003) Gestational trophoblastic disease epidemiology and trends. *Clin Obstet Gynecol* 46: 541–556

Szulman AE, Surti U (1978a) The syndromes of hydatidiform mole. I. Cytogenetic and morphologic correlations. *Am J Obstet Gynecol* 131: 665–671

Szulman AE, Surti U (1978b) The syndromes of hydatidiform mole. II. Morphologic evolution of the complete and partial mole. *Am J Obstet Gynecol* 132: 20 – 27

Weidner N (1993) Tumor angiogenesis: review of current applications in tumor prognostication. *Semin Diagn Pathol* 10: 302–313

Weidner N, Folkman J, Pozza F, Bevilacqua P, Allred EN, Moore DH, Meli S, Gasparini G (1992) Tumor angiogenesis: a new significant and independent prognostic indicator in early-stage breast carcinoma. *J Natl Cancer Inst* 84: 1875–1887

Yang J, Liu X, Nyland SB, Zhang R, Ryland LK, Broeg K, Baab KT, Jarbadan NR, Irby R, Loughran Jr TP (2009) Platelet-derived growth factor mediates survival of leukemic large granular lymphocytes via an autocrine regulatory pathway. *Blood* 113: 51 – 60

Zygmont M, Herr F, Keller-Schoenwetter S, Kunzi-Rapp K, Munstedt K, Rao CV, Lang U, Preisser KT (2002) Characterization of human chorionic gonadotropin as a novel angiogenic factor. *J Clin Endocrinol Metab* 87: 5290 – 5296

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