Targeted therapy for high-risk endometrial carcinoma

Dana M Roque¹, Peter E Schwartz¹ & Alessandro D Santin*¹

Practice Points

- Endometrial cancers can be broadly dichotomized into two types based on histopathologic, clinical and molecular features.
- Type II endometrial cancers constitute a minority of cases but a disproportionate number of deaths and are typified by serous, clear cell or grade 3 endometrioid histology.
- Immunotherapeutic targets for these high-risk endometrial cancers include Her2/Neu, VEGF receptor, epithelial cell adhesion molecule (Ep-CAM), trophoblast cell surface marker (Trop-2), tissue factor (TF) and αV-integrins.
- Immunotherapies may be enhanced by modulation of membrane-bound complement regulatory proteins or costimulation with IL-2.
- Inhibitors of the EGF receptor, FGF receptor and the PTEN/PI3KCA/AKT/mTOR pathway have an emerging role in the treatment of high-risk endometrial cancers.
- Chemoresistance is multifactorial and attributable to a rapid cellular proliferation rate, autocrine secretion of IL-6, as well as upregulation of tubulin-β-III, among other processes.
- Claudin-3 and -4, natural receptors for Clostridium perfringens, may represent an innovative mechanism for targeted drug delivery against chemotherapy-resistant cells and cancer progenitors.
- Epothilones are novel microtubule-stabilizing agents with activity in paclitaxel-resistant disease under clinical investigation for high-risk endometrial cancers.
SUMMARY  Type II endometrial cancers exhibit distinct histopathology, underlying pathogenesis and clinical behavior. These high-risk cancers are associated with an aggressive clinical course and a relatively poor prognosis that underscores the need for the development of novel rational therapeutic strategies that exploit their distinct underlying molecular pathway alterations. In this review, we describe the extent of disease burden and molecular characterization of type II endometrial cancers, summarize the historical development to support current standards of care and delineate the most recent preclinical and clinical advances in immunotherapy, cytotoxic agents and small-molecule inhibitors for this disease.

Disease burden & classification of endometrial cancers
Endometrial cancer is the most common gynecologic malignancy in developed countries, with 47,130 new cases and 8010 deaths projected in the USA alone for 2012 [1]. In total 90% of endometrial cancers arise purely from epithelial glands (adenocarcinomas) [2]. In 8% of cases, malignancy develops from mesodermal components (leiomyosarcoma or endometrial stromal sarcoma) or both mesodermal and epithelial elements (carcinosarcoma) [3]. Endometrial carcinomas may be broadly dichotomized into two classes with distinct underlying molecular pathogenesis, clinical behavior and histopathology [4].

Type I endometrial cancers comprise 80% of cases and are associated with endometrioid histology (grade 1 or 2) [5,6], a history of exposure to unopposed estrogen with retention of estrogen receptor (ER)/progesterone receptor (PR) status [7], and younger age at onset (mean age: 63 years) [8]; deleterious mutations in k-Ras, PTEN or mismatch repair mechanisms predominate [9,10]. Hyperplasia is a common precursor.

Type II endometrial cancers constitute a minority of cases and characterized by serous, clear cell or grade 3 endometrioid histology [11,12], the absence of an antecedent history of unopposed estrogen, higher frequency in black patients, later stage and age at initial presentation [13]; loss of ER/PR [14] as well as E-cadherin [15], aneuploidy, mutations in p53 and HER2/Neu overexpression are common [7,16–18]. Recently, loss of BAF250a, the protein encoded by the chromatin remodeling tumor suppressor gene ARIDIA, was implicated in 39% of grade 3 endometrioid, 26% of clear cell and 18% of high-grade serous cancers of the endometrium as examined by immunohistochemistry (IHC) [19] and has been associated with advanced stage [20]. PPP2R1A, the scaffolding subunit of PP2A holoenzyme, is frequently mutated in uterine serous carcinomas (USCs) [21]. Intraepithelial carcinoma is a recognized precursor. Of note, unlike serous cancers of the endometrium, which demonstrate a genetic signature distinct from serous cancers of the ovary, clear cell carcinomas show remarkable similarity regardless of the organ of origin [22].

Type II endometrial cancers are characterized by an aggressive clinical course with relatively poor prognosis. Though serous, clear cell and grade 3 endometrioid cancers collectively constitute <30% of uterine cancers, they account for 74% of deaths; 5-year disease-specific survival rates for USC, clear cell and grade 3 endometrioid carcinomas are 55, 68 and 77%, respectively [23]. This compares unfavorably to the rate of 89% for grade 1/2 endometrioid cancers [8].

Current standard of care
Treatment for high-risk type II endometrial cancers begins with complete surgical staging with intent for cytoreduction to no residual disease followed by platinum-based combination chemotherapy for advanced disease [24]. Staging should consist of total hysterectomy, bilateral salpingo-oophorectomy, bilateral pelvic/para-aortic lymphadenectomy, omentectomy and peritoneal washings with biopsies [25,26] given that patients with type II disease will exhibit extraterine spread at the time of initial surgery in 52–70% of instances [26,27] and have a much higher likelihood of positive para-aortic lymph node involvement compared with the rate of 4.6% observed in low-grade tumors [28]. CA-125 is the most commonly used clinical biomarker for serous cancers [26] as elevations reflect disease status by means of advanced stage, omental metastases and lymphatic spread, although preoperative levels do not serve as an independent predictor.
for recurrence [29]. The acute-phase reactant serum amyloid A has also been proposed as a novel biomarker given that higher expression is found intracellularly and as a secreted product in vitro in USC cell lines compared with B-cell and cervical cancer controls, as well as in serum of patients with USC compared with healthy volunteers and patients who underwent hysterectomy for benign indications [30]. Also of interest are the trypsin-like serine proteases belonging to the human kallikrein and kallikrien-related peptidase family, which have established clinicopathologic correlations in prostate cancer [31,32]. Human kallikrein 6 and 10 appear to be secreted at higher levels by serous gynecologic tumor cells relative to endometrioid tumor cells in vitro; accordingly, higher plasma levels discriminate patients with USC from patients undergoing hysterectomy for benign indications [33,34].

Modern management algorithms for advanced disease draw largely upon the cumulative experience from five large Phase III studies conducted by the Gynecologic Oncology Group (GOG), each of which enrolled patients with both type I and II disease [35–39]. Historically, GOG 177 established paclitaxel/doxorubicin/cisplatin (TAP) as standard of care for advanced or recurrent endometrioid cancers [36]. GOG 184 subsequently demonstrated equivalent hazard ratios for recurrence or death in patients who received postoperative radiation followed by doxorubicin/cisplatin/paclitaxel versus doxorubicin/cisplatin (0.90; 95% CI: 0.69–1.17, p = 0.21), although there was a 50% reduction in risk of relapse or death with the former regimen in patients with residual disease after debulking at the expense of increased hematologic toxicity [39]. Preliminary analyses of GOG 209 now suggest the noninferiority and favorable side-effect profile of carboplatin/paclitaxel over TAP [40]; this regimen appears to have utility in high-risk disease [41] and is regarded by most as the standard of care.

Fewer data exist regarding treatment of high-risk early-stage disease. Whole-abdominal radiation is of little benefit (GOG–94) [42]. Adjuvant carboplatin/paclitaxel clearly improve recurrence rates, overall survival and progression-free survival [43]. In uterine serous cancers, there is evidence to support the use of vaginal cuff brachytherapy in conjunction with platinum-based chemotherapy. In a retrospective review of 74 patients with stage I uterine serous cancers, no local recurrences occurred in patients who received vaginal cuff brachytherapy, but were diagnosed in six of the 31 patients (19%) who did not. Furthermore, recurrences were far more common in the absence of adjuvant chemotherapy: 6/14 versus 0/7 in stage IA, 10/13 versus 0/15 in stage IB, 4/5 versus 1/7 in stage IC disease [44]. In a similar single-institution review of stage I/II serous cancers, a regimen consisting of six cycles of carboplatin/paclitaxel with vaginal cuff brachytherapy was very well-tolerated and resulted in 5-year progression-free and overall survival rates of 88% [45]. Additionally, there is growing interest in exploring sandwich techniques [46,47].

Data among clear cell cancers is very scant due to the rarity of this entity; even in advanced or recurrent disease, no single Phase III GOG study has enrolled sufficient cases to evaluate the importance of histology [48]. Barney and colleagues published a series of 103 patients with stage I clear cell (pure, n = 21; mixed, n = 8) or serous (n = 74) uterine cancers who received vaginal brachytherapy; approximately 33% also received chemotheraphy [49]. Local, regional and locoregional recurrence rates were 5, 7 and 10%, respectively at 5 years. While some pooled analyses suggest that clear cell or serous histologies have similar initial responses to cytotoxic agents compared with endometrioid cancers, clear cell and serous histology are independent predictors of overall survival; clear cell histology also predicts poorer progression-free survival [50].

Optimal therapy for invasive grade 3 endometrioid carcinoma confined to the uterus is the subject of an ongoing trial (PORTEC-3; NCT00411138) [201]. Alektiar and colleagues found equivalent clinical outcomes in a study of 41 patients with stage I or occult stage II grade 3 endometrioid histology compared with 42 patients with USC or clear cell carcinoma, all of whom received intravaginal brachytherapy after surgical staging and underwent external beam radiotherapy in balanced fashion [51]. Over a median follow-up of 46 months, locoregional control rates were 97 vs 90% (p = 0.2), respectively. 5-year disease-free survival was 79 vs 78% (p = 0.3); 5-year overall survival was 71 vs 79% (p = 0.3). Siow and colleagues reported institutional outcomes of 18 patients with grade 3 endometrioid and ten with grade 3 endometrioid stage IC (according to International Federation of Gynecology and Obstetrics 1988 staging) who underwent total hysterectomy, bilateral
salpingo-oophorectomy and pelvic lymph node dissection (76%) or external beam radiotherapy (24%) [52]. All except one patient received vaginal brachytherapy and eight out of ten patients with endometrioid histology received platinum-based adjuvant chemotherapy. Over a median follow-up of 50.1 months, there were five systemic relapses (17.9%) and one pelvic recurrence (3.6%). The authors concluded that vaginal vault brachytherapy was effective in preventing locoregional recurrences, but argued for consideration for adjuvant chemotherapy in these patients given the high rate of systemic relapse.

Such findings truly underscore the need for development of rational therapeutic strategies that exploit the distinct molecular pathway alterations that underlie these clinically aggressive entities.

**Immunotherapy for type II endometrial cancers: basis & rationale**

Targeted immunotherapy represents a promising strategy for type II endometrial cancers. Monoclonal antibodies result in tumor lysis through antibody-dependent cellular cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). Both pathways begin with recognition and binding of the monoclonal antibodies to tumor antigen. The Fc region may then be recognized by Fc receptors located on natural killer cells, monocytes, macrophages or granulocytes to initiate ADCC or by C1 (the first component of the complement cascade) to activate the classic pathway of CDC ending in osmotic lysis through the membrane-attack complex.

**Immunotherapy for type II endometrial cancers: Phase II/III data HER2/Neu (ErbB2)**

The human EGF receptor (EGFR) family consists of four members: EGFR (ErbB1), HER2/Neu (ErbB2), HER-3 (ErbB3) and HER-4 (ErbB4). Ligand binding induces hetero- or homo-dimerization and subsequent activation of pathways integral to proliferation pathways [53]. Amplification of HER2/Neu has been documented in 26–62% of USC cases [73,54–58] and as many as 38% of clear cell endometrial carcinomas [59]; positivity by FISH in grade 3 endometrioid endometrial cancers is far less common (3–8%) [60,61]. Overexpression has been linked to poor prognosis not only in endometrial [74,62] but ovarian [63] and breast [64] cancers. Molecular profiling studies have demonstrated ErbB2 to be one of the most overexpressed genes to distinguish uterine serous from ovarian serous tumors [65].

Trastuzumab (Herceptin®, Genentech, CA, USA) is a humanized monoclonal IgG1 antibody that works both through recruitment of natural killer cells and initiation of ADCC as well as abrogation of downstream effectors [66]. It is FDA-approved as an adjunct to cyclophosphamide, paclitaxel and doxorubicin in the treatment of early-stage HER2/Neu-positive, node-positive breast cancer and as a single agent for adjuvant treatment of early-stage, HER2/Neu-positive, high-risk ER/PR-negative breast cancers following multi-modality anthracycline-based therapy [67,202]. Based on sound biologic plausibility, there is considerable interest in applications for endometrial cancers. As assessed by standard 32Cr-release assays, trastuzumab results in ADCC in the range of 25–60% against USC that overexpress HER2/Neu; this can be augmented with both IL-2 and simultaneous administration of the heterodimerization inhibitor pertuzumab (Omnitarg®, Genentech)[68,69]. Despite encouraging case reports (Table 1) (70–72), when evaluated as a single-agent, trastuzumab 4 mg/kg in week 1 then 2 mg/kg weekly until disease progression in stage III/IV or recurrent endometrial cancers at the Phase II level in GOG-181B failed to demonstrate significant activity [9]. Interestingly, a large number of clear cell carcinomas in this study overexpressed HER2/Neu. These findings have been criticized as inconclusive given that 45.5% of treated patients did not have definitive HER2/Neu amplification and on the basis of slow accrual leading to premature closure [73]. It has been proposed that inter-individual variation in trastuzumab efficacy may also reflect variable amounts of HER2/Neu extracellular domain (ECD) shedding [74]. In a study of 38 USC patients versus 19 controls, serum concentrations of HER2/Neu ECD were higher in patients with tumors expressing HER2/Neu at 3+ by IHC (p = 0.02). HER2/Neu positive primary cell lines also secreted HER2/Neu ECD in vitro; addition of HER2/Neu ECD-containing supernatants decreased ADCC (p = 0.01). A Phase II study of carboplatin/paclitaxel with or without trastuzumab in patients with advanced or recurrent uterine papillary serous carcinoma designated HER2/Neu-positive by 3+ IHC or FISH is currently underway (NCT01367002) [203].
VEGF–A induces pathologic neoangiogenesis in a variety of human cancers [75]. VEGF is a homodimeric glycoprotein that exists in at least four isoforms due to alternative splicing of the primary messenger RNA transcript [76], the most common of which is VEGF-A. In endometrial cancers, VEGF-A expression has been associated with high grade, lymphovascular space invasion, lymphatogenous spread, poor prognosis [77,78] and p53 upregulation [79]. Bevacizumab (Avastin®, Genentech) is a recombinant human monoclonal IgG1 antibody that neutralizes all isoforms of VEGF [80]. In a Phase II study of recurrent endometrial cancer (GOG 229E) [81], bevacizumab 15 mg/kg every 3 weeks produced clinical response rate of 13.5%, including one complete and six partial responses. Median progression-free and overall survival rates were 4.2 and 10.5 months, respectively. Notably, despite representing only 27% of the study population, serous histology was observed in 100% of complete responses and 50% of partial responses. Presently, bevacizumab in combination with paclitaxel and carboplatin is under study for advanced endometrial cancer (NCT00513786) [204]. Another three-arm Phase II trial is investigating carboplatin/paclitaxel/temsirolimus and carboplatin/ixabepilone/bevacizumab (NCT00977574) [205]. VEGF Trap (Afibercept®, Sanofi-Aventis, Paris, France), a fusion protein containing receptor components and fully human immunoglobulin constant region, is also under evaluation (NCT00462826) [206].

### Immunotherapy for type II endometrial cancers: preclinical data
Recently the author’s group has reported the in vitro data to support the candidacy of multiple antigens originally discovered through comparative oligonucleotide microarrays contrasting ten primary USC and five normal endometrial cell lines for targeted immunotherapy [82].

### Table 1. Summary of case reports of clinical activity of trastuzumab in heavily pretreated patients with uterine serous carcinomas.

<table>
<thead>
<tr>
<th>Author</th>
<th>Age (years)</th>
<th>Stage</th>
<th>Histology</th>
<th>Initial treatment</th>
<th>IHC</th>
<th>FISH</th>
<th>Additional regimens</th>
<th>Response</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jewell et al.</td>
<td>72</td>
<td>IIA</td>
<td>Endometrioid grade 2</td>
<td>Surgical staging, whole pelvic RT and vaginal brachytherapy</td>
<td>3+</td>
<td>NR</td>
<td>1st recurrence (pulmonary metastases) at 10 months: weekly paclitaxel then single-agent trastuzumab 100 mg iv. weekly for 7 months</td>
<td>Partial response for 6 months</td>
<td>[71]</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2nd recurrence: weekly paclitaxel and trastuzumab for 2 months then single-agent trastuzumab for 9 months</td>
<td>Stable disease for 11 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Disease progression: addition of docetaxel 40 mg/m² iv.</td>
<td>Stable disease for 15 weeks</td>
<td></td>
</tr>
<tr>
<td>Villela et al.</td>
<td>NR</td>
<td>IVB</td>
<td>USC</td>
<td>NR</td>
<td>3+</td>
<td>+ (&gt;10)</td>
<td>4 mg/kg iv. once, then 2 mg/kg iv. weekly until progression</td>
<td>Complete response for 6 months</td>
<td>[72]</td>
</tr>
<tr>
<td></td>
<td>NR</td>
<td>IIIC</td>
<td>USC</td>
<td>Surgical staging, whole pelvic radiation, doxorubicin 45 mg/m² iv. + cisplatin 50 mg/m² + paclitaxel 160 mg/m²</td>
<td>3+</td>
<td>+ (&gt;10)</td>
<td>4 mg/kg iv. once then 2 mg/kg iv. weekly until progression</td>
<td>Stable disease for 3 months</td>
<td></td>
</tr>
<tr>
<td>Santin et al.</td>
<td>66</td>
<td>IIIA</td>
<td>Endometrioid FIGO grade 3</td>
<td>Surgical staging, whole pelvic radiation, doxorubicin 45 mg/m² iv. + cisplatin 50 mg/m² + paclitaxel 160 mg/m²</td>
<td>3+</td>
<td>+ (2.18)</td>
<td>1st recurrence (pelvic lymphadenopathy); whole pelvic radiation Paclitaxel 175 mg/m² iv. every 21 days + carboplatin AUC 5 iv. every 21 days + trastuzumab 4 mg/kg iv. once, then 2 mg/kg iv. weekly × 6 then trastuzumab 2 mg/kg iv. weekly + carboplatin AUC 5 every 5 weeks × 8</td>
<td>Persistent disease</td>
<td>[70]</td>
</tr>
<tr>
<td></td>
<td>63</td>
<td>IIIC</td>
<td>USC</td>
<td>Surgical staging, whole pelvic/extended-field radiation</td>
<td>2+</td>
<td>+ (3.1)</td>
<td>1st recurrence at 3 months (vaginal cuff); patient declined cytotoxic chemotherapy; trastuzumab 4 mg/kg loading dose then 4 mg/kg biweekly</td>
<td>Partial response for 7 months</td>
<td></td>
</tr>
</tbody>
</table>

---

AUC: Area under the curve; FIGO: International Federation of Gynecology and Obstetrics; IHC: Immunohistochemistry; iv.: Intravenous; NR: Not reported; RT: Radiation therapy; USC: Uterine serous carcinoma.
Epithelial cell adhesion molecule (Ep-CAM, also known as Trop-1, TACSTD1, 17-1A, GA733-2, KSA, KS1/4, 323/A3 and CD326) is a calcium-independent homophilic type I transmembrane glycoprotein molecule of 39–42 kDa, expressed at low levels on most surface epithelial cells. Using IHC and flow cytometry, Ep-CAM can be detected in as many as 96% of USC tissues and expressed superficially in 83% of cell lines [83]. Adecatumumab (MT201; Micromet AG, Munich, Germany), a human monoclonal antibody against Ep-CAM, produced mean ADCC of 33%, despite resistance of these cells to CDC. MT201 has been evaluated at the Phase II level in colorectal carcinoma after complete resection of liver metastases alone and in combination with FOLFOX (5-fluorouracil, leucovorin, oxaliplatin) (NCT00866944) [207], as monotherapy in metastatic breast cancer [84], and prostate cancer [85]. It has also been evaluated at the Phase IB level in conjunction with docetaxel in breast cancer [86], but not in gynecologic malignancies.

Trophoblast cell-surface marker (Trop-2, also known as GA733-1, M1S1 and EGP-1) is 35-kDa transmembrane glycoprotein encoded by the gene TACSTD2 of chromosome 1p32. Trop-2 was originally identified in human trophoblasts [87] and has since been shown to be upregulated relative to normal tissue in both type I [Bignotti et al., Trop-2 protein overexpression as an independent prognostic marker in endometrial endometrial carcinoma (2012), Submitted] and type II endometrial cancers [88,89], as well as other gynecologic malignancies [90–92]. The human monoclonal antibody hRS7 (Immunomedics, Inc., NJ, USA) produces ADCC in the range of 28.2–64.4% in USC [88] and 33.9–50.6% in grade 3 endometrioid cell lines [89]. hRS7 is scheduled to enter clinical testing in solid tumors in late 2012 [208].

Tissue factor (TF) is a transmembrane receptor for coagulation factor VII/VIIa (FVII) that is aberrantly expressed in urogenital, gastrointestinal, hematologic, breast and lung cancers (reviewed by van den Berg et al. [93]). TF overexpression can be observed in 50% of USC cell lines (three of six) as assessed by real-time PCR and flow cytometry; cytoplasmic and/or membrane staining can be documented in 100% (16 out of 16) of tissue specimens analyzed by IHC [94]. Accordingly, in 51Cr-release assays hI-CON-1 (Iconic Therapeutics, Inc., GA, USA), a fusion protein containing a targeting domain against TF and a functional domain consisting of IgG1 Fc, resulted in mean ADCC of 65.6% (range: 57.5–77.0%) in USC cell lines that overexpress TF and was augmented with IL-2. TF overexpression, at least in part, underlies the association of thrombogenesis and cancer (Trouseau’s phenomenon). As clear cell carcinomas are particularly thrombogenic and expression in ovarian clear cell carcinomas appears to exceed that of endometrioid and undifferentiated tumors [95], hI-CON-1 may have therapeutic relevance for clear cell carcinomas of the endometrium. Phase I studies of hI-CON-1 are ongoing for macular degeneration (NCT01485588) [209].

Integrins are glycoproteins that exist in at least 24 unique permutations of heterodimers consisting of one α and one β subunit, allowing for functional variation. Integrins contribute to invasion and metastasis in a variety of human carcinomas including melanoma, breast, prostate [96], oral squamous cell [97] and USC [98]. The αv integrins serve as known receptors for extracellular matrix proteins including vitronectin, tenascin and fibronectin [99]. Of six USC cell lines assessed, 100% expressed at least one isoform of αv integrin, the most common of which were αvβ3 (37.5% of cells, mean fluorescence intensity: 12.3 ± 4.02) and αvβ5 (32.0% cells, mean fluorescence intensity: 17.5 ± 9.23). The human monoclonal antibody intetumumab (formerly CNTO-95, Centocor, Inc., PA, USA) targets αv-integrins and inhibited migration by 17–27% and adhesion by 30–65% at doses of 1.25 µg/ml up to 20 µg/ml when compared with control cells with no monoclonal antibodies added (p < 0.03). Intetumumab has been evaluated at the Phase II level in advanced melanoma [100] and hormone-refractory prostate cancer (NCT00537381) [210], but not yet in gynecologic malignancies.

- Immunotherapy for type II endometrial cancers: modulation through membrane-bound complement regulatory proteins

Despite encouraging preclinical activity, targeted immunotherapies often fail in the clinical arena. Prior chemotherapy and radiation might lead to immunocompromise and limit efficacy in heavily pretreated patient populations [101]. Tumors also evade immune surveillance through antigen evolution and upregulation of
Novel cytotoxic chemotherapies

Over the past quarter of a century, the GOG has evaluated over 25 novel cytotoxic agents at the Phase II level for use in endometrial cancers, and exceedingly few of these have proceeded to Phase III testing [50]. Due to low expression of ER/PR, hormonal modulation is often of little benefit in high-risk endometrial cancers, leaving even fewer options for management. In GOG-81, a study of 299 patients with advanced or recurrent endometrial cancer, Thigpen and colleagues witnessed a response rate of only 8–9% if PR levels were <50 fmol/mg cytosolic protein, compared with 37% in receptor-positive patients [111]. The development of novel cytotoxic chemotherapeutic strategies for recurrent or progressive disease therefore remains tantamount, especially for type II cancers. Several recent promising advances are summarized below.

- Claudin-3 & -4

Claudins are tight junction proteins that regulate paracellular transport and provide cell polarity. In cancer cells, claudin family proteins may contribute to the capacity for invasion and metastasis [112]. While present in few normal tissues, USC [113], as well as prostate cancer cells [114], overexpress claudin-3 and -4; chemotherapy-resistant ovarian cancer cells over-express claudin-4 [115]. These proteins simultaneously serve as low- and high-affinity natural receptors for Clostridium perfringens enterotoxin, the binding of which results in rapid osmolysis, and therefore serve as attractive targets for selective drug delivery [116,117]. In ovarian cancer xenografts, intraperitoneal administration of C. perfringens enterotoxin 7.5–8.5 μg in 500 μl of sterile saline every 72–96 h for a total of eight injections beginning 1 week after tumor establishment prevented detectable tumor growth entirely in ~60% of mice and resulted in statistically significant increases in survival compared with untreated mice who demonstrated hemorrhagic ascites with peritoneal studding by 2–3 weeks and expired within 9 weeks [116].

Although chemotherapy kills the majority of tumor bulk, chemoresistant cancer stem cells frequently remain and contribute to tumor propagation and recurrence [118]. Interestingly, claudin-4 is overexpressed in CD44+/MyD88+ ovarian cancer stem cells. With growing interest in characterization of stem cells within the basalis of the endometrium, which contribute to regeneration across 400 cycles in a typical reproductive lifespan, and cancer stem cells in endometrial cancers [119]. It is conceivable that C. perfringens enterotoxin-based therapies may represent a strategy for destruction of this population in high-risk endometrial cancers as well [120].

- Paclitaxel resistance: IL-6, tubulin-β III & epothilones

Type II endometrial cancers are relatively chemoresistant and any observed responses are generally nondurable [121]. Resistance to cytotoxic chemotherapies appears to be multifactorial. First, a brisk growth rate permits rapid tumor regrowth despite initial intrinsic sensitivity to chemotherapeutic agents [122]. Second, these tumors are associated with a distinct milieu of autocrine factors such as IL-6, which has been shown to prognosticate poor outcome and confer resistance to paclitaxel and cisplatin in gynecologic malignancies [123]. Accordingly, using ELISAs, secreted IL-6 expression was found to be higher in USC compared with endometrioid carcinoma cell lines (mean: 3121 pg/ml, range: 1099–5017 pg/ml/105 cells/48 h vs mean: 88 pg/ml, range: 19–112 pg/ml/105 cells/48 h; p < 0.01) and in the serum from 13 patients with USC compared with 19 women with endometrioid carcinoma (6.1-fold, p < 0.03). By real-time PCR, mean copy number in USC fresh frozen tissues also exceeded that of endometrioid...
Table 2. Selected studies of small-molecule tyrosine kinase inhibitors in patients with persistent/recurrent endometrial cancer.

<table>
<thead>
<tr>
<th>Study</th>
<th>Drug</th>
<th>Target</th>
<th>Study</th>
<th>Dose</th>
<th>Clinical outcome</th>
<th>Translational end points</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gehrig and Bae-Jump (2010)</td>
<td>Lapatinib (Tykerb®, GlaxoSmithKline, PA, USA)</td>
<td>EGFR and HER2/Neu</td>
<td>GOG-229D (NCT00096447), Phase II</td>
<td>1500 mg p.o. daily</td>
<td>Failed to open to second stage of accrual</td>
<td>None</td>
<td>[75]</td>
</tr>
<tr>
<td>Leslie et al. (2009)</td>
<td>Gefitinib (Iressa®, AstraZeneca, DE, USA)</td>
<td>EGFR</td>
<td>GOG-229C (NCT00027690), Phase II</td>
<td>500 mg p.o. daily</td>
<td>Of 26 evaluable patients: one complete response; seven with disease stabilization; insufficient efficacy to continue evaluation</td>
<td>High serum EGFR was associated with overall survival</td>
<td>[143]</td>
</tr>
<tr>
<td>Oza et al. (2008)</td>
<td>Erlotinib (Tarceva®, Genentech, CA, USA)</td>
<td>EGFR</td>
<td>NCIC IN-148, Phase II</td>
<td>150 mg p.o. daily</td>
<td>Of 32 evaluable patients: four partial responses; 15 with disease stabilization</td>
<td>No correlation of response with EGFR mutation or gene amplification by FISH</td>
<td>[144]</td>
</tr>
<tr>
<td>Slomovitz et al. (2007)</td>
<td>Imatinib (Gleevec®, Novartis, Basel, Switzerland)</td>
<td>C-kit, Abl, PDGF receptor B</td>
<td>Phase I, restricted to USC</td>
<td>400 mg p.o. daily in combination with paclitaxel 175 mg/m² every 21 days</td>
<td>Of 11 evaluable patients, dose-limiting toxicities: rash, neutropenia and fatigue</td>
<td>None</td>
<td>[145]</td>
</tr>
</tbody>
</table>

EGFR: EGF receptor; p.o.: Orally; USC: Uterine serous carcinoma.
Overall response rate of 12% was achieved using 40 mg/m² every 21 days; disease stabilization for at least 8 weeks occurred in 60%. Median progression-free and overall survival was 2.9 months and 8.7 months, respectively [142]. Ixabepilone is currently under evaluation as first-line therapy with carboplatin and bevacizumab in stage I/II/IV primary or recurrent endometrial cancers (GOG–86P; [204]).

Small-molecule tyrosine kinase inhibitors & mTOR inhibitors
Small-molecule inhibitors occupy binding pockets in order to block intracellular signaling pathways important to differentiation and proliferation among tumor cells. Tyrosine kinase inhibitors (TKIs) against EGFR have been developed and are under evaluation for efficacy in endometrial cancer (reviewed by Gehrig and Bae Jump [75]) (Table 2) [143–145]. At least in some instances, the dampered response to EGFR inhibitors may relate to overexpression of MDM2, a key inhibitor of the EGFR pathway, and p53, which is commonly mutated in type II cancers and responsible for arrest at the G1/S or G2/M transitions of the cell cycle [146]. TKIs against FGF receptor also show promise. This family consists of four tyrosine kinase receptors and 18 ligands [147], mutations...
of which occur in conjunction with PTEN alterations but appear to be mutually exclusive with KRAS variants \[148\]. FGFR mutations have proven to be oncogenic in endometrial cancers \[149\], but in other contexts may exert tumor suppressive effects \[147\]. In vitro inhibition with pan-FGFR inhibitors results in selective death of endometrial cancer cells that carry activating mutations \[148\]. Various FGFR inhibitors have entered study at the Phase II level in endometrial cancers (TKI258, NCT01379534 \[212\]) and at the Phase I level in other solid malignancies (E–3810, NCT01283945 \[213\]; BGJ398, NCT01004224 \[214\]; AZD4547, NCT00979134 \[215\]).

The PTEN–PI3KCA–AKT–mTOR pathway is also of significance for treatment of type II endometrial cancers. PIK3CA, downstream of EGFR and FGFR, encodes the catalytic p110-α subunit of PI3K, which phosphorylates phosphatidylinositol-3,4,5-trisphosphate to generate phosphatidylinositol-3,4,5-trisphosphate. This subsequently activates the AKT–mTOR oncogenic pathway \[150\]. PIK3CA mutations promote oncogenesis \[151\] and have been observed in as many as 15% of USCs \[142\]. Inhibitors of mTOR, mammalian target of rapamycin, are under evaluation in the treatment of endometrial cancers \[Table 3\] \[152–158\]. Surprisingly, responses do not correlate with PTEN status \[152\] or AKT/mTOR levels \[159\], and most studies find responses across varied histologies including type II disease.

### Conclusion & future perspective

Elucidation of the molecular pathogenesis underlying endometrial cancers remains key in fueling drug discovery. In the near future, whole-genome sequencing will play an increasingly pivotal role in the identification of tumor-specific somatic mutations. A deeper understanding of the mechanisms by which to exploit and augment host immune responses should permit immunotherapy to achieve its intended potential. Tumor-specific drug delivery, small molecule and mTOR inhibitors, and novel cytotoxic agents such as the epothilones are among the most promising developments for this disease and the mature data from their clinical study is eagerly awaited. With growing recognition of the importance of individual tumor biology, in the next 5–10 years we hope that targeted therapies will allow for the genuine practice of personalized medicine.

### Financial & competing interests disclosure

The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. No writing assistance was utilized in the production of this manuscript.

### References

Papers of special note have been highlighted as:

- of interest
- of considerable interest

15. Holcomb K, Delatorre R, Bader Pedemonte B et al. E-Cadherin expression in endometrioid,
Succinct but thorough review of uterine serous carcinoma management.


Targeted therapy for high-risk endometrial carcinoma | Review


Succinct but thorough review of uterine serous carcinoma management.


Document that summarizes all available, albeit scarce, literature dedicated towards...
management of endometrial clear cell carcinoma, written in response to a lack of consensus identified within the Society of Gynecologic Oncologists.


Pooled data analysis of Gynecologic Oncology Group studies designed to examine the association of serous or clear cell histology with respect to chemoresponse and clinical outcome.


First demonstration that uterine serous cancers that overexpress HER2/Neu are exquisitely sensitive to Herceptin-mediated antibody-dependent cellular cytotoxicity.


Santin AD. Letter to the editor referring to the manuscript entitled: “Phase II trial of trastuzumab in women with advanced or recurrent HER2-positive endometrial carcinoma: A Gynecologic Oncology Group Study” recently reported by Fleming et al. (Gynecol Oncol, 116;15-20;2010). Gynecol. Oncol. 98(1), 95–96; author reply 96–97 (2010).


Contemporary and compact review of drug pipeline for the treatment of endometrial cancers.


**Application of microarray technology for discovery of novel molecular targets; this work served as a launchpad for future development of many targeted therapies.**


**Review of antibody-dependent cellular cytotoxicity, complement-dependent cellular cytotoxicity and membrane complement regulatory proteins as a possible etiology for the disappointing clinical efficacy of immunotherapies to date.**

First report to demonstrate that ovarian tumor cells internalize C. perfringens enterotoxin peptide and support the utility of C. perfringens enterotoxin peptide for diagnostic and/or radiometric therapy of gynecologic cancer.

Review of basic differences between tubulin subtypes and their differential tissue expression with an outline of known point mutations, post-translational modifications and microtubule regulatory proteins believed to confer drug resistance.

First demonstration of in vitro and in vivo dose-dependent cytotoxicity of Clostridium perfringens toxin on uterine serous carcinomas cells.

First report to demonstrate that ovarian tumor cells internalize C. perfringens enterotoxin peptide and support the utility of C. perfringens enterotoxin peptide for diagnostic and/or radiometric therapy of gynecologic cancer.

Review of basic differences between tubulin subtypes and their differential tissue expression with an outline of known point mutations, post-translational modifications and microtubule regulatory proteins believed to confer drug resistance.

First demonstration of in vitro and in vivo dose-dependent cytotoxicity of Clostridium perfringens toxin on uterine serous carcinomas cells.

First report to demonstrate that ovarian tumor cells internalize C. perfringens enterotoxin peptide and support the utility of C. perfringens enterotoxin peptide for diagnostic and/or radiometric therapy of gynecologic cancer.

Review of basic differences between tubulin subtypes and their differential tissue expression with an outline of known point mutations, post-translational modifications and microtubule regulatory proteins believed to confer drug resistance.
Targeted therapy for high-risk endometrial carcinoma | Review


Summary of cumulative clinical experience with patupilone through Phase I, II and III testing.


Websites


205 NIH Clinical Trials. Paclitaxel, carboplatin, and bevacizumab or paclitaxel, carboplatin, and temsirolimus or ixabepilone, carboplatin, bevacizumab in treating patients with stage III, stage IV, or recurrent endometrial cancer. http://clinicaltrials.gov/ct2/show/ NCT009157874. (Accessed 8 May 2012)


207 NIH Clinical Trials. Study of adoacetumumab relative to FOLFOX after R0 resection of colorectal metastases (MT201-204). http://clinicaltrials.gov/ct2/show/ NCT00866044 (Accessed 8 May 2012)


NIH Clinical Trials. A study of the safety and effectiveness of CNTO 95 in patients with metastatic hormone refractory prostate cancer.
http://clinicaltrials.gov/ct2/show/NCT00537381
(Accessed 8 May 2012)

NIH Clinical Trials. Patupilone vs doxorubicin in patients with ovarian, primary fallopian, or peritoneal cancer.
http://clinicaltrials.gov/ct2/show/NCT00262990
(Accessed 8 May 2012)

NIH Clinical Trials. A Phase II study to evaluate the efficacy of TKI258 for the treatment of patients with FGFR2 mutated or wild-type advanced and/or metastatic endometrial cancer.
http://clinicaltrials.gov/ct2/show/NCT01379534
(Accessed 22 June 2012)

NIH Clinical Trials. Study of oral E-3810, a dual VEGFR-FGFR tyrosine kinase inhibitor, in patients with solid tumors.
http://clinicaltrials.gov/ct2/NCT01283945
(Accessed 22 June 2012)

NIH Clinical Trials. A dose escalation study in adult patients with advanced solid malignancies.
http://clinicaltrials.gov/ct2/show/NCT001904224
(Accessed 22 June 2012)

NIH Clinical Trials. Study designed to assess the safety and tolerability of AZD4547 at increasing doses in patients with advanced tumours.
http://clinicaltrials.gov/ct2/show/NCT00979134
(Accessed 22 June 2012)