Research Article

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Uterine prognostic indicators in low-grade endometrial cancers: anatomical and immunohistochemical findings discovered using an "end-result-driven" statistical approach.

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ABSTRACT

Objectives : It is useful to know which low-grade endometrial adenocarcinomas will likely display evidence of lymph node metastases or extrauterine disease either at hysterectomy or on follow up. In order to model this situation, we used an end-result-driven statistical assessment of pathology findings and related them to known case outcomes with the objective of separating cases with "good" from those with "poor" outcomes, and to determine measurable anatomical/immunohistochemical factors associated with "good" or "poor" prognosis.

Methods : We assessed anatomical, immunohistochemical and clinical data of 150 cancer hysterectomies. 119 women had no lymph node metastasis at the time of cancer hysterectomy and no evidence of recurrent disease on 12-89 months follow-up. 31 women had either positive lymph node dissections at the time of cancer hysterectomy (N = 11) or disease persistence/recurrence over the same follow up interval.

Results : We found 5 significant features ($p<0.05$) that separated "good" from "poor" clinical behavior: (1) Subclonal p53 immunostaining (combination of normal with one or more abnormal IHC-staining patterns, a here-to-fore unrecognized marker of "good-prognosis"), MELF-glands, >70% uterine wall invasion, lymphovascular space invasion (LVI), and stromal tumor-invasion of the uterine cervix. The latter four factors are historically established indicators of "poor-prognosis". When applied as sorting-premises to our data set, these 5 indicators provided a 52%-to-48% split between potentially "good" and "poor" case outcomes, with all positive lymph node dissections and patient deaths relegated to "poor-prognosis" cases.

Conclusions : We conclude that subclonal p53 immunostaining (most likely an indicator of POLε or MMR-deleted cancers), MELF-glands, >70% uterine wall invasion, LVI, and tumoral-invasion of the uterine cervix have significant prognostic value in determining lymph node involvement and clinical case outcome. We argue that these five findings should be routinely listed in the pathology report and that p53 immunohistochemical staining should routinely be used as an adjunct test in the pathological assessment of low-grade endometrial adenocarcinomas.

INTRODUCTION

Endometrial cancer is the most common gynecologic malignancy in the United States and the sixth leading cause of cancer death among women. The most common uterine cancer is endometrioid endometrial adenocarcinoma, which represents about 70% of all uterine cancers [1]. Endometrioid endometrial adenocarcinomas are generally low-grade and low-stage tumors that commonly show good prognosis and survival. However, some women with low-grade uterine cancer are at risk for distant metastasis and unfavorable clinical outcomes.

Historically, Gynecological Oncology Group (GOG) trial 33 determined pathological risk factors in clinical stage I endometrial cancer that would warrant additional treatment. The trial highlighted tumor grade and depth of uterine wall invasion as important factors that stratified women into risk categories for the risk of lymph node metastases. It described the benefit of surgical staging, presenting evidence that some clinical stage I cancers show features justifying adjuvant radiation therapy in 15%-to-25% of women [2]. Subsequently, the Mayo Clinic criteria defined histologically low-grade (G1 or G2), small (< 2 cm), and relatively shallow (<50% depth of invasion) tumors as showing "low-risk" for nodal metastasis. When tested against the Milwaukee model, the Mayo model proved to be the most sensitive in determining which

woman's lymph node dissection could be omitted [3], [4]. Validating these conclusions with SEER registry data, Vargas et al. showed that "low risk" endometrial cancers have a low probability of lymph node metastasis [5].

More recently, Roma et al. led a multi-institutional study that reviewed 589 cases with International Federation of Gynecology and Obstetrics (FIGO) G1 or G2 uterine endometrioid adenocarcinomas to identify risk factors for recurrence and survival. They identified large tumor size, deep myoinvasion, tumor necrosis, one or more foci of lymphovascular invasion (LVI), LVI foci distant/deeper than the invasive tumor front, MELF gland myoinvasion pattern, lower uterine segment or cervical stromal involvement, pelvic and/or para-aortic lymph node metastases at presentation, and a higher tumor grade in metastatic foci as additional features of increased risk for tumor recurrence and decreased survival[6].

In 2013, a tipping point in uterine tumor prognostication occurred with the introduction of molecular classification systems. The Cancer Genome Atlas (TCGA) identified four molecular subtypes of endometrial cancer with distinct prognostic outcomes ranging from very good to very poor, and additional studies led to the design of the PORTEC-4a trial [7], [8]. Unfortunately, in today's practice-and-reimbursement environment, the routine application of molecular studies is cost-prohibitive for the routine assessment of this otherwise common tumor. In certain situations, it is helpful to know which women are unlikely to have evidence of extrauterine disease at the time of diagnosis or on subsequent follow up. In the present study, we propose a simple and easy to execute assessment of morphological findings that blends with it a limited application of immunohistochemistry, and we compare our observations to known case outcomes to determine "low-risk" and "high-risk" prognostic indicators that are ascertainable by a pathological examination of the hysterectomy uterus.

MATERIAL AND METHODS

Our data set consists of 150 uterine cancer hysterectomies from which we obtained detailed anatomical and immunohistochemical data, including lymph node status, and for which we had at least 12 months follow-up (average, 49.6 months) to study "low risk" vs. "high risk" disease outcomes. "Low risk" or "favorable" outcomes are used interchangeably and are defined as: "no lymph node metastasis or extrauterine disease at the time of surgical staging or beyond 12 months follow-up". "High-risk" or "unfavorable" outcomes are defined as: "positive lymph nodes or extrauterine disease found at the time of surgical staging or after more than 12 months follow-up". Anatomical and immunohistochemical data were adjudicated by one pathologist. Follow-up information was obtained from computerized medical records review. Our analysis focuses on discrete data, that is, numerical data that includes whole, concrete numbers with specific and fixed values with outcomes determined by simple "yes" or "no" responses.

Our anatomical and immunohistochemical data set encompassed p53 staining, MELF glands at the invasive tumor interface, >70% tumor invasion of the uterine wall, LVI, cervical stromal invasion by tumor, invasive villoglandular tumor component, squamous differentiation, morular metaplasia, retention of PTEN staining (negative for PTEN deletion), and MMR-deletion (using MLH1, MSH2, MSH6, and PMS2 immunohistochemical staining).

Other patient outcome data included positive lymph node dissections, women with no evidence of disease on follow-up, women alive with disease after at least 12-month follow-up, women dead of disease, and tumor stage. The outcomes of this analysis were matched with information concerning tumor size, depth of tumor invasion, uterine wall thickness, percent of tumor invasion, ER status, PR status, body mass index, age, and months followed.

Tumor immunohistochemistry

We report estrogen receptor-α (ER), progesterone receptor (PR), and p53 staining on a ranked (0-4+, absent-to-strong immunodecoration) scale and semi-quantitatively assign the percent of positive cells as 0%, >0-25% (1+), >25-50% (2+), >50-75% (3+), and >75-100% (4+). ASCO/CAP ER and PR guideline recommendations are used for hormone receptor reporting. A sample is positive for ER or PR if ≥1% of tumor cell nuclei are immunoreactive and negative for ER or PR if <1% of tumor cell nuclei are immunoreactive and if there is evidence that the sample could express ER or PR (positive intrinsic controls are seen, usually in retained stroma) [9]. We evaluated the strongest-staining low-power field using a 4x-lens (surface area ~20 mm2) for stain-scoring. We report PTEN based on "all or none" staining (using an on-slide EIN control) as having either deleted or wild-type pattern. For MMR staining, any staining of tumor cell nuclei is reported as positive, and inflammatory and other benign cells serve as good positive internal controls.

P53 immunohistochemical staining represents a special case. P53-immunostaining patterns of endometrial cancers may be sorted into three broad groups: (1) wild-type, (2) mutated with clonal pattern, and (3) mutated with a "subclonal" pattern.

Correct interpretation of routinely performed p53 immunohistochemistry is essential. The wild-type pattern typically shows from <1% to up to 50% nuclear staining that is of variable intensity and is seen in an on-again/off-again fashion within the tumor's nuclei; and, importantly, without specific geographic concentration. This pattern is taken to imply a functional TP53 gene. A mutated pattern is, in general,

seen throughout all the tumor's cells in an equally intense and homogeneous fashion appearing as either (1) intense and uniform nuclear staining (>75% strong nuclear staining), (2) complete absence (0%) of staining (usually with a commensurate strong and uniform or, rarely, completely absent p16 nuclear and cytoplasmic immunohistochemical decoration of the tumor's cells), or (3) with uniform and typically strong cytoplasmic staining. We recognize that ~5% of p53 mutations are of the truncating type and unfortunately mimic wild type p53 staining. These patterns' uniformity and diffuse distribution are assumed to indicate that a mutation occurred early in tumorigenesis and that it is shared by all the tumor cells.

Recent reports have shown that abnormal p53 expression can also be confined to a distinct geographic area of the tumor, and this pattern has been dubbed "subclonal" p53 expression [10], [11], [12]. In the case of subclonal p53 staining, a well-defined area within a tumor shows an abnormal (mutant-like) p53 immunohistochemical pattern, and the remaining tumor retains wild-type p53 expression. As indicated by Vermij et al., depending on the area of the tumor from which DNA is extracted (for example, for next-generation sequencing), it is conceivable that in tumors with subclonal p53 expression, a TP53 mutation may be missed by sequencing methods [12].

Subclonal immunohistochemical p53 expression in endometrial cancer is understudied. Some have suggested using a threshold of at least 10% abnormal p53 expression to define subclonality, whereas Vermij et al. have reported unequivocal mutant overexpression of p53 in tiny foci (<10%) within an otherwise p53 wild-type tumor. What may hinder the identification of all subclonal p53 cases would be sampling error due to mutation focality or the case where the p53 mutation presents as the complete absence of staining (as seen with loss of function) or as wild type staining (as seen with truncation mutations) [8], [12].

We posit that subclonal p53 staining is seen in a tumor with an otherwise unstable repair mechanism (for example, an MMR-deleted or POLε-mutated tumor) that progressively acquires a p53 mutation and, in that instance, p53 is not likely the primary driver of tumorigenesis, nor is it likely to influence tumor prognosis [8], [13].

RESULTS

First data sort (Table 1)

Of the 150 women studied, 119 had "low risk" or "favorable" outcomes with no evidence of extrauterine disease either at the time of hysterectomy or greater than 12 months follow-up. Thirty-one women showed extrauterine disease (either lymph node, adnexal or other involvement). By sorting these groups into the desired end-results of "no extrauterine disease" vs. "extrauterine disease," we found that (1) microcystic elongated and fragmented (MELF)-glands at the invasive tumor interface, (2) >70% uterine wall invasion, (3) LVI, and (4) tumor invasion of the cervix were poor prognostic indicators, and that (5) subclonal p53 immunostaining was a good prognostic indicator.

Thus, our primary anatomical and immunohistochemical data predicting either a "low-risk" or "high-risk" outcome included: MELF-gland invasion, deep (>70%) myometrial invasion, LVI, cervical stromal invasion, and subclonal p53 staining and these became the premises of our second sort of the same data set.

Table 1: Data sort on known outcomes (first sort).

UTERINE FINDINGS	Total		Actual Good Prognosis		p<	Actual Poor Prognosis	
Number of women	150		119	79.3%		31	26.1%
Subclonal p53 staining	7	4.7%	7	5.9%	0.01	0	0.0%
MELF-gland invasion	51	34.0%	35	29.4%	0.03	16	51.6%
>70% tumor invasion	22	14.7%	8	6.7%	0.00	14	45.2%
Angiolymphatic invasion	33	22.0%	16	13.4%	0.00	17	54.8%
Cervical stromal invasion	11	7.3%	5	4.2%	0.05	6	19.4%
Average tumor size mm	39		36.7		0.02	47.8	
Average depth of invasion mm	6.7		5.7		0.01	10.5	
Average percent invasion	35%		30%		0.00	53%	
Squamous differentiation	19	12.7%	13	10.9%	0.28	6	19.4%
Villoglandular component	55	36.7%	40	33.6%	0.15	15	48.4%

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Morular metaplasia	10	6.7%	8	6.7%	0.96	2	6.5%
Diffuse p53 staining	2	1.3%	0	0.0%	0.16	2	6.5%
PTEN-wild-type	33	22.0%	24	20.2%	0.33	9	29.0%
MSI-high	44	29.3%	35	29.4%	0.97	9	29.0%
OUTCOME/DEMOGRAPHICS							
Number of women	150		119	79.3%		31	26.1%
Positive LN dissections	11	7.3%	0	0.0%	0.00	11	35.5%
No evidence of disease	119	79.3%	119	100.0%	0.00	0	0.0%
Alive with disease	28	18.7%	0	0.0%	0.00	28	90.3%
Dead of disease	3	2.0%	0	0.0%	0.08	3	9.7%
ER (quartile)	3.4		3.5		0.28	3.2	
PR (quartile)	3.2		3.3		0.41	3.1	
Age	61.6		62.4		0.04	58.7	
Months followed if < stage III	49.6		49.9		0.43	45.1	
Average body mass index	34.7		34.8		0.54	33.4	

Grey bands indicate statistically significant findings: T-test at $p<0.05$.

Second data sort (Table 2)

The same data set was then sorted using the five products of the first sort serving as premises of a "second sort." We wanted to see if these five products could successfully divide women into categories of either "favorable" or "unfavorable" outcomes as defined above. Women with known outcomes were again split, now into potentially "favorable" (78 women, 52%) and potentially "unfavorable" (72 women, 48%) groups, as determined by the five products: firstly, subclonal p53 followed by MELF-gland invasion, deep (>70%) myometrial invasion, LVI, and cervical stromal invasion.

These products successfully placed all women without positive lymph nodes into a "favorable" category. Two women with proven extrauterine disease were sorted into this category: one who showed extrauterine tumor in her initial hysterectomy (Stage IIIA, adnexal involvement that may also have represented independent stage IA ovarian cancer and would be classified as Stage IA3 according to the 2023 FIGO system) and one who initially presented with stage IA endometrial cancer that showed only 10% myometrial invasion but that recurred as a higher-grade tumor in the retroperitoneum after 34 months follow up—a situation described by Roma et al in their risk factor analysis of low-grade endometrial cancers [6].

This exercise showed that sorting our data first on "subclonal-p53-positive" followed by the presence of MELF-glands at the invasive tumor interface, >70% uterine wall invasion, LVI, and tumor invasion of the cervix (1) successfully separated women with negative lymph node dissections from women with positive lymph node dissections; and (2) in 76 of 78 cases, separated women with negative lymph nodes at the time of hysterectomy and no evidence of disease on follow up from those with potentially "unfavorable" clinical outcomes. The five products used in the second sort acted fairly well in separating women into "favorable" and "unfavorable" categories. The "favorable" category showed no lymph node metastases, one instance of local (adnexal only) disease at the time of hysterectomy (that would have been removed with total abdominal hysterectomy and bilateral salpingo-oophorectomy and may well have represented a simultaneous and independent Stage IA endometrioid ovarian carcinoma) and presently would be classified as FIGO IA3 disease, and one instance of retroperitoneal disease (one stage IA tumor that showed a higher-grade retroperitoneal recurrence) on longitudinal follow up. This latter situation was recognized as a failure of otherwise successful group separation.

The "unfavorable" group of the second sort showed, at the $p<0.05$ level: squamous differentiation, at least 10% villoglandular growth pattern in the invasive tumor, lower ER and PR quantitation, greater tumor size, and greater average depth of tumor invasion. The "unfavorable" group included all three women who died of their disease. Compared to our known outcomes, 26 (36%), as opposed to two (3%) patients, were alive with their disease, and 43 (60%) as opposed to 76 (97%) patients, had no evidence of disease. The outcome of these first and second sorts are shown in Tables 1 and 2.

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Table 2: Data sort on outcomes predicted by Subclonal p53 staining followed by MELF-gland invasion, >70% tumor invasion, Angiolymphatic invasion, and Cervical stromal invasion.

UTERINE FINDINGS	Total		Predicted Good Prognosis		p<	Predicted Poor Prognosis	
Number of women	150		78	52.0%		72	48.0%
Subclonal p53 staining	7	4.7%	7	9.0%	0.01	0	0.0%
MELF-gland invasion	51	34.0%	2	2.6%	0.00	49	68.1%
>70% tumor invasion	22	14.7%	0	0.0%	0.00	22	30.6%
Angiolymphatic invasion	33	22.0%	2	2.6%	0.00	31	43.1%
Cervical stromal invasion	11	7.3%	0	0.0%	0.00	11	15.3%
Average tumor size mm	39		35.6		0.03	42.7	
Average depth of invasion mm	6.7		4.5		0.00	9.1	
Average percent invasion	35%		24%		0.00	47%	
Squamous differentiation	19	12.7%	4	5.1%	0.01	15	20.8%
Villoglandular component	55	36.7%	18	23.1%	0.00	37	51.4%
Morular metaplasia	10	6.7%	6	7.7%	0.60	4	5.6%
Diffuse p53 staining	2	1.3%	0	0.0%	0.16	2	2.8%
PTEN-wild-type	33	22.0%	17	21.8%	0.95	16	22.2%
MSI-high	44	29.3%	22	28.2%	0.75	22	30.6%
OUTCOME/DEMOGRAPHICS							
Positive LN dissections	11	7.3%	0	0.0%	0.00	11	15.3%
No evidence of disease	119	79.3%	76	97.4%	0.00	43	59.7%
Alive with disease	28	18.7%	2	2.6%	0.00	26	36.1%
Dead of disease	3	2.0%	0	0.0%	0.08	3	4.2%
ER (quartile)	3.4		3.6		0.01	3.2	
PR (quartile)	3.2		3.4		0.03	3.01	
Age	61.6		60.8		0.26	62.5	
Months followed if <stage III	49.6		49.4		0.92	49.8	
Average body mass index	34.7		35.6		0.10	33.2	

Grey bands indicate statistically significant findings: T-test at p<0.05.

DISCUSSION

Data from the SEER registry indicates that women are at low risk for nodal endometrial cancer metastasis if their tumors are histologically low grade (G1 or G2), myometrial invasion is less than 50%, and tumor size is equal to or less than 2 cm. Women not meeting these criteria are considered at high risk for nodal involvement [5]. If we apply the SEER recommendations (i.e., Mayo Clinic criteria) to our data set, 20 of 150 (13%) cases would have qualified as being at "low risk" for nodal disease, and 130 of 150 (87%) of cases would have been considered as "high risk" cancers. In addition, among the 20 "low risk" cases, there would have been one stage IIIC1 tumor (that showed diffuse p53 nuclear staining and 1 of 18 lymph nodes positive for metastatic adenocarcinoma) and one case that recurred after 28 months follow-up.

Roma et al. led a multi-institutional study and reviewed 589 patients with International Federation of Gynecology and Obstetrics grades 1 or 2 endometrioid endometrial adenocarcinomas. They used a Cox proportional hazard analysis to identify univariate and multivariate risk factors for recurrence and survival. Features of tumors that recurred (at sites other than vagina) included large size, deep (at least 75%) myometrial invasion, tumor necrosis, one or more foci of angiolymphatic invasion, angiolymphatic-foci distant/deeper than invasive tumor front, MELF gland myoinvasion pattern, low uterine segment or cervical stromal involvement, pelvic and/or para-aortic lymph node metastases at presentation, and higher-grade tumor in metastatic foci [6].

Our study was designed to "back into" significant anatomical/immunohistochemical findings by enumerating observations that were subsequently benchmarked against outcomes defined as "evidence of disease" vs. "no evidence of disease" at the time of the index hysterectomy or on greater than 12 months follow up. Our first sort determined five "first derivative observations" or premises/products that, when tested against the same population with known outcomes, separated patients into 52%-to-48% groupings that favored "no evidence of disease" in 52% of cases. Only one of the asserted "good prognosis" patients showed a poor clinical outcome.

We postulate five anatomical/immunohistochemical prognostic indicators for low-grade uterine adenocarcinomas that were derived from data-sorting based on known case outcomes: (1) subclonal p53 immunostaining, (2) MELF-glands at the invasive tumor interface, (3) >70% tumor invasion of the uterine myometrium, (4) lymphovascular (angiolymphatic) invasion, and (5) tumor invasion of the cervical stroma. These indicators of disease would be especially useful in cases where the lymph node status was unknown, such as in women with incidental cancer found after hysterectomy, in women with multiple comorbidities limiting the ability to do a lymph node dissection, or for women with unsuccessful sentinel lymph node dissections. Additionally, these indicators may guide practitioners who need to defer lymphadenectomy if an acceptable frozen section is unavailable.

p53 immunostaining (Figure 1)

The absence of functional p53 results from genetic mutation and increases cancer risk. Among our low-grade endometrial uterine cancers, nine (6%) women showed abnormal p53 immunohistochemical staining. In two cases, positive p53 immunostaining uniformly involved all tumor cells; and this staining pattern is consistent with a driver-type mutation, and these women would now be classified as well differentiated "copy number high" tumors. Not surprisingly, both women showed extrauterine disease (stages IIIC1 and IVB). It should be noted, however, that in endometrial cancer, the accumulation of p53 protein may be associated with not only gene mutations but also dysregulation of factors such as ERβ and MDM2 [14].

The dysfunction of p53 in endometrial cancer is closely associated with diffuse/clonal TP53 mutations. Driver-type TP53 mutations are detected in about 25% of all endometrial cancer patients, with their frequency being less than 5% in low-grade tumors and nearly 100% in typical serous carcinomas. In our data set, even among tumors that were sorted into the "unfavorable" category, diffuse p53 nuclear staining was uncommon and was seen in only two instances. Broaddus and Kurnit found TP53 gene mutations in 16 of 175 (8.4%) low-grade endometrial cancer patients, and they reported that, after multivariate analysis, the presence of TP53 mutation was associated with significantly worse recurrence-free survival [15].

In our data set, the two women with diffusely abnormal p53 were high stage at the time of discovery (IIIC1 and IVB) but showed only 13% and 25% uterine wall invasion. Although our findings do not reach a level of statistical significance, we posit that driver-type p53-mutations, although uncommon among low-grade endometrial cancers, presage an "unfavorable" clinical outcome.

In the remaining seven cases, the p53 mutation was "subclonal," meaning that it uniformly involved only parts of the tumor and represented a mutation that likely occurred during disease evolution [13], [16]. None of these seven cases showed either lymph node metastases or evidence of disease on follow-up (that averaged 51 [range, 20-to-58] months) and on the first sort, paradoxically, were sorted into the "favorable" tumor category. Six had a villoglandular component, and five (83%) were also MMR-deleted. The MMR-intact villoglandular tumor (that showed MELF-glands and angiolymphatic invasion) occurred in a 51-year-old woman (average age, 61.7) who had a BMI of 29 (average BMI, 34.6) and who had a PTEN-deleted tumor and showed increased lymphocytes at the invasive tumor interface, all begging the question of POLε ultramutation. This was confirmed with follow up genetic testing of the tumor.

One case that showed no specific morphological features occurred in a 67-year-old woman with a BMI of 28 who was followed for 58 months. She had a PTEN deleted tumor that showed 65% tumor invasion, whose molecular analysis demonstrated a pathogenic PIK3CA mutation. In the TCGA database, subclonal p53 mutations are most often seen in POLε ultramutated and MMR-deleted tumors that likewise often show PTEN deletion. Thus, p53-abnormal along with p53-wild type areas within the same tumor, especially in the face of PTEN deletion, support the concept that endometrial cancer can be comprised of different subclones of the primary tumor that, by extension, may offer increased tumor antigenicity [13], [17].

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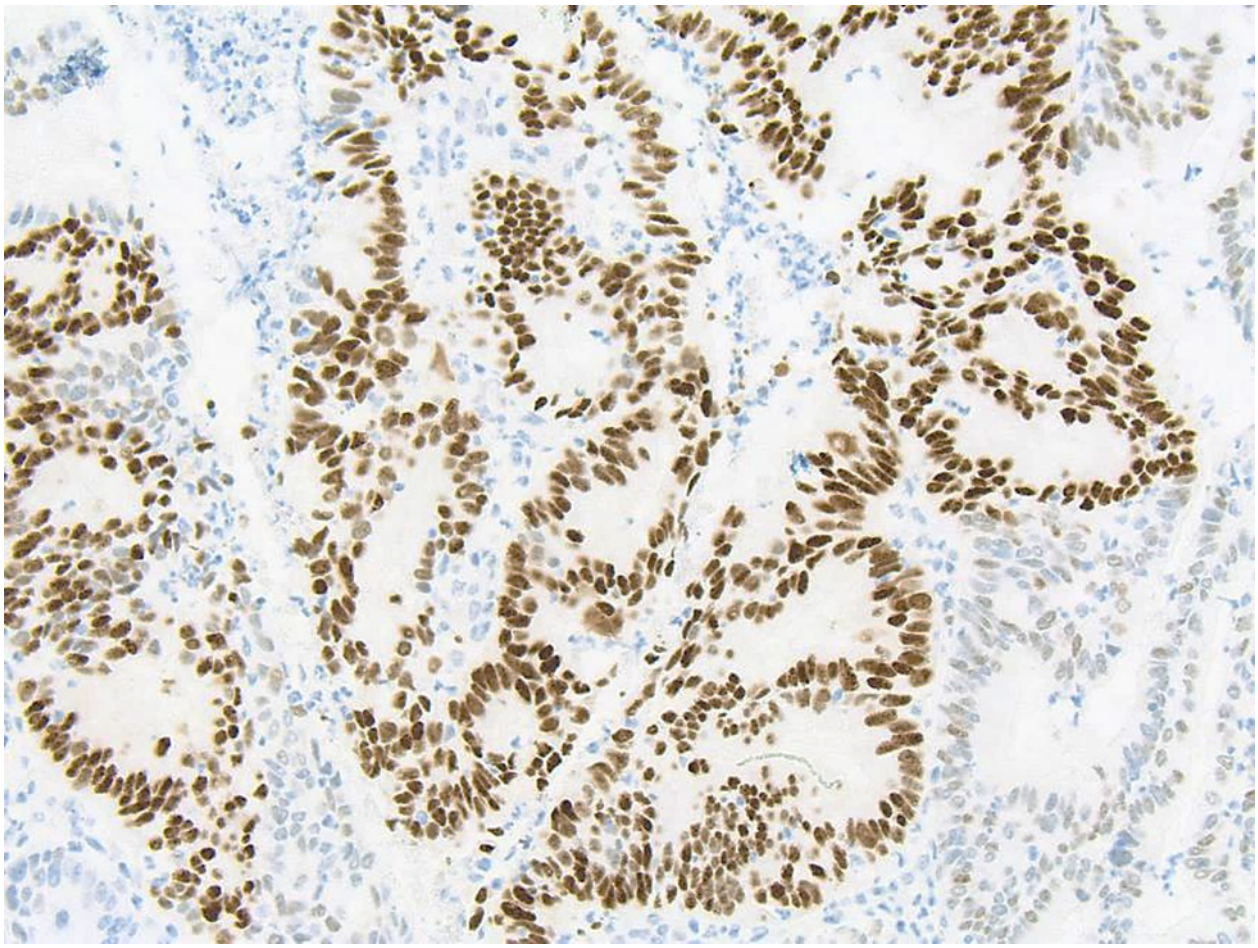
Figure 1



Subclonal p53 staining pattern. Defined as the combination of normal with one or more abnormal patterns. Its recognition in immunohistochemical staining is limited to tumors with overexpression or cytoplasmic expression of a p53 mutation, including only ~70% of cases. There is a juxtaposition of mutated and wild type p53 immunohistochemical staining. The central portion of the image shows mutated pattern p53 staining whereas the periphery of the image shows a wild-type staining pattern. Although not previously used in tumor risk-stratification, this event has recently been described in POL and MMR-defective endometrial cancers, where the p53 mutation seems to have no effect on tumor outcome. In our series of patients, this staining pattern uniformly correlated with "low risk" cancers.

MELF-glands at the invasive tumor interface (Figure 2)

In our series, MELF-glands were seen in 29% of patients with no evidence of extrauterine disease at greater than 12 months follow-up, and in 52% of patients with extrauterine disease. As a predictor of disease, MELF-glands occurred in 3% of patients predicted to have a good outcome and in 68% of patients predicted to have a poor outcome. MELF-gland positive cancers, when compared to MELF-gland negative cancers, show increased angiolymphatic invasion (45% vs. 10% in our series), deeper myometrial and percent invasion (48% vs. 28% in our series), as well as more frequent MMR-deletions (41% vs. 23% in our series). It is generally accepted that MELF-gland positive cancers represent a more aggressive subset of endometrial carcinomas [18,], [19]. Women with low-grade carcinomas are classically considered to have early-stage disease and if staging lymphadenectomy is not performed, we strongly advise routinely reporting MELF-gland histology to identify individuals at higher risk of metastatic disease.

It is well known that immunophenotypic changes of MELF-glands include loss or reduction of CD147, MMP2, E-cadherin, and Galectin-3, which are all indicative of epithelial mesenchymal transition. This results in loss of cell-cell adhesion and polarity, endowing cells with migratory and invasive properties, histiocyte-like morphology, and potentially "stemness." Furthermore, the cellular disintegration seen with these glands produces cytoplasmic fragments that we posit may represent extracellular tumor cell microvesicles that participate in cell-to-cell communication, conditioning non-tumor tissue to be receptive to invasion and metastasis [20]. In Roma et al.'s series, MELF pattern myometrial invasion was a significant predictor of tumor recurrence, primarily when it occupied the invasive tumor front. Likewise, they speculated that MELF pattern (as well as an

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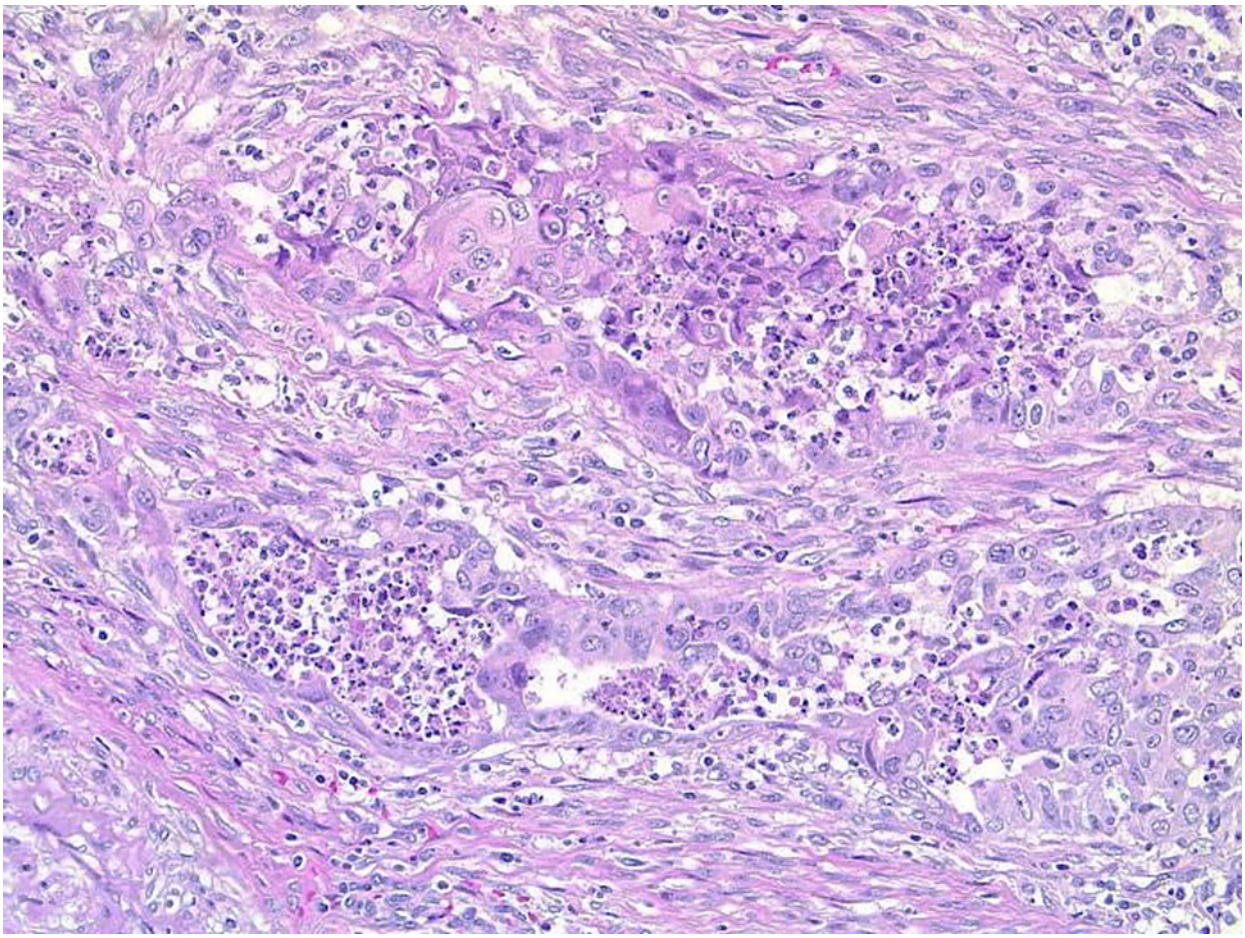
increased amount of single cell invasion, consistent with tumor budding) could indicate epithelial mesenchymal transition, which potentiates tumor cell infiltration into the surrounding stroma and enhances tumor progression [6].

Figure 2



Microcystic, elongated, and fragmented (MELF) glands. Early stage low-grade FIGO endometrial carcinomas generally represent good prognosis disease; however, a subset remains aggressive. Microcystic, elongated, and fragmented (MELF) glands are a myometrial invasion pattern associated with LVI and lymph node metastasis. In our series, MELF-glands were seen with 29% of tumors with no evidence of disease at greater than 12 months follow up, and with 52% of tumors with disease. This image shows a MELF-gland with an isolated focus of squamous metaplasia.

>70% tumor invasion of the uterine myometrium

Our 70% depth of invasion limit was arbitrarily chosen. When factors other than depth of invasion were used to sort our data set, five cases with extrauterine disease fell out of the high-risk data set. One individual (who was alive with disease) showed 10% invasion, and one individual (who initially had stage IIIA disease) showed 43% invasion. Neither would have been discovered using the traditional >50% depth of invasion. The remaining three women showed 75%, 94%, and 96% invasion. Therefore, >70% was chosen as a reasonable cutoff value that would not have overpacked the high-risk data set. This limit corresponds surprisingly well to observations made by Cox Bauer et al. and Roma et al., who analyzed risk factors for recurrence in low-grade endometrial adenocarcinoma and found their cutoff points for percent invasion differed from those established by current literature. Specifically, >66% and >75% (as opposed to the more traditional >50%) myometrial invasion predicted recurrence in their series, which we argue supports our choice of >70% myometrial invasion as an appropriate cutoff point for our series [3], [6].

Lymphovascular (angiolymphatic) invasion (Figure 3)

In our series, LVI was seen in 13% of patients with no evidence of extrauterine disease at greater than 12 months follow-up and in 55% of patients with extrauterine disease. Also, as a predictor of disease, LVI occurred in 3% of patients predicted, on the basis of our second sort, to have a “favorable” outcome and in 43% of patients predicted to have an “unfavorable” outcome.

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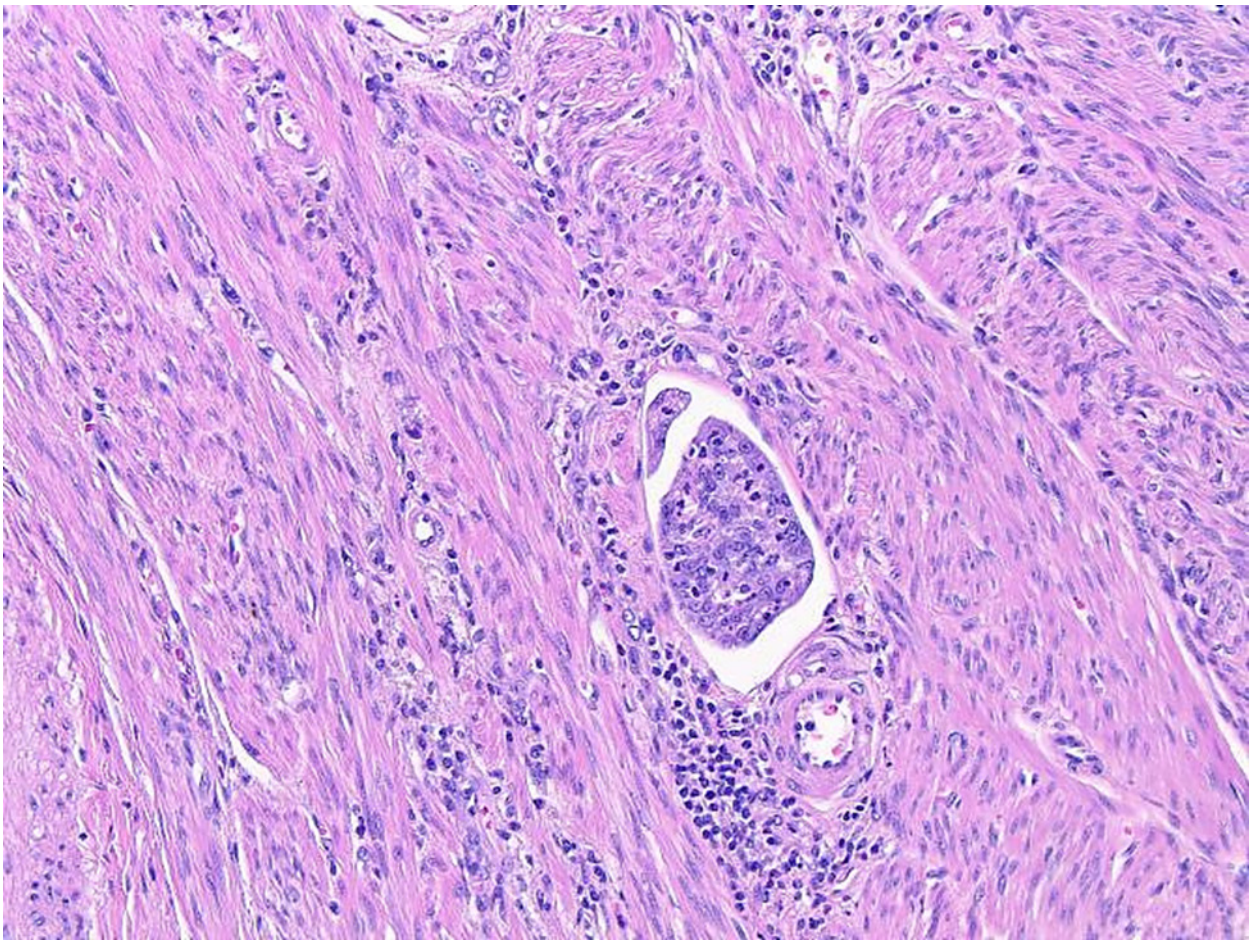
LVI is defined as the presence of tumor cells within endothelial-lined spaces within the uterine wall outside the primary tumor, and it is an independent poor prognostic factor in early-stage endometrial cancer due to its association with nodal metastasis and disease recurrence [21]. Roma et al. showed that the presence of LVI, regardless of the number of foci, was significantly associated with recurrence [6]. It is associated with a worse prognosis in otherwise low-risk endometrial cancer patients. LVI status is generally not appreciated until after the hysterectomy has been processed for histology. It needs to be included in the final pathology report because it represents information pertinent to the decision process as whether to do pelvic and/or para-aortic lymphadenectomy or deliver adjuvant vaginal cuff or whole abdomen radiation after hysterectomy.

Figure 3



Lymphovascular invasion (LVI). This is defined as the presence of tumor cells within endothelial-lined spaces within the uterine wall outside the main tumor, and it is an independent poor prognostic factor in early-stage endometrial cancer due to its association with nodal metastasis and disease recurrence. This image shows LVI that has occurred at a distance from the invasive portion of the tumor. In our series, LVI was seen with 13% of tumors with no evidence of disease at greater than 12 months follow up, and with 55% of tumors with disease.

Tumor invasion of the cervical stroma

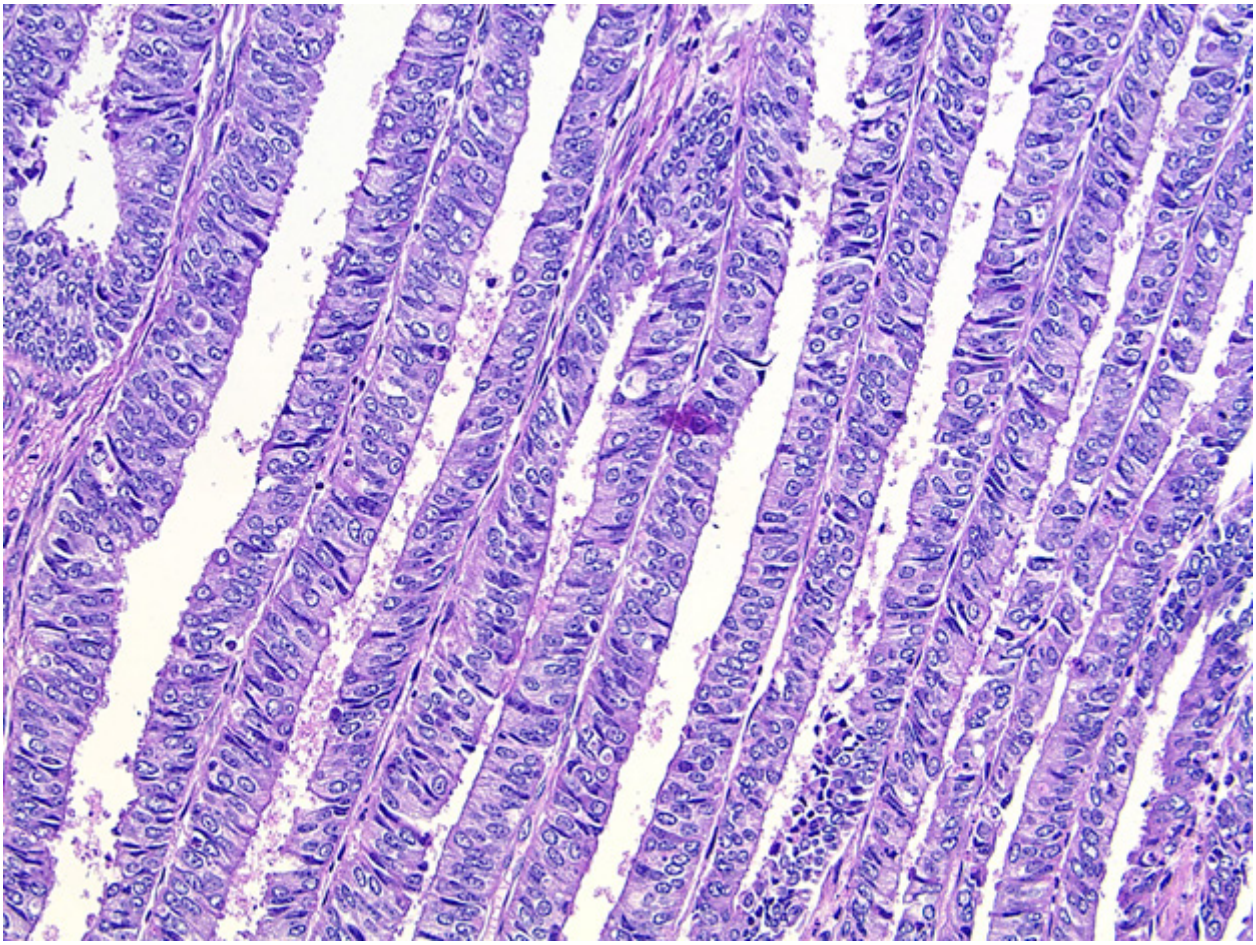
In our series, invasion of cervical stroma was seen in 4% of patients with no evidence of extrauterine disease at greater than 12 months follow up, and in 19% of patients with extrauterine disease. Also, as a predictor of disease, invasion of the cervical stroma occurred in no patients predicted, on the basis of our second sort, to have a “favorable” outcome and in 15% of patients predicted to have an “unfavorable” outcome. The presence of cervical stromal invasion upstages endometrial cancer. However, since 2009, involvement limited to cervical epithelium is no longer considered FIGO stage II (previously as IIA) as it has been shown that patients with cervical epithelial involvement have identical outcomes to those with stage I tumors. Cervical stromal invasion correlates with recurrence. The practical problem is that boundaries between lower uterine segment and upper endocervix are poorly defined and the distinction of endocervical gland involvement from stromal invasion may be challenging. Although no clear boundary exists, it is suggested that the uppermost mucinous endocervical gland be taken as

the boundary between the low uterine segment and endocervix [22]. Roma et al. observed that both cervical and low uterine segment stromal invasion portend extrauterine disease [9], [6]. This may be due to common vascular inosculations that exist between both these uterine structures and the vessels of the pelvic floor musculature.

>10% villoglandular growth pattern in the invasive tumor component (Figure 4)

Patients showing >10% villoglandular growth pattern in their invasive tumor component had a more frequent subclonal p53 staining (11% vs. 1%), more frequent MELF-glands (62% vs. 18%), and more frequent vascular invasion (40% vs. 12%), but less frequent cervical involvement (2% vs. 11%). Moreover, when comparing outcomes of disease for women "with" vs. those "without" a >10% villoglandular growth pattern in their invasive tumor component, populations of women who died of disease, were alive with disease, or showed no evidence of disease, did not differ from each other at a $p<0.05$ level. Therefore, it is difficult to say whether villoglandular tumors fare worse than or are equivalent to other endometrial cancers and villoglandular growth in the invasive tumor component remains a topic for additional study. Historically, Ambros et al. and Zaino et al. debated this issue, presenting opposing views concerning the prognostic significance of villoglandular differentiation [23], [24]. Without further analysis, our data does not provide a clear resolution to this debate.

Figure 4



The villoglandular growth pattern of endometrioid adenocarcinoma. This consists of finger-like projections lined by tall columnar cells with bland nuclei whose cells are supported on a delicate vascular stroma. Focal invasive villoglandular changes were seen in about 37% of our endometrioid carcinomas. When compared to other cancers in our series, cancers with >10% villoglandular growth pattern in their invasive tumor component showed more frequent subclonal p53 staining (11% vs 1%), more frequent MELF-glands (62% vs 18%), and more frequent vascular invasion (40% vs 12%), but less frequent cervical involvement (2% vs 11%). It is difficult to say whether villoglandular tumors fare worse than or equivalent to other endometrial cancers, and this question remains a topic for additional study.

Direct-from-gland squamous (but not morular) growth pattern (Figure 5)

When we sorted on predicted indicators of disease, 12.7% of all patients had direct-from-gland squamous differentiation.

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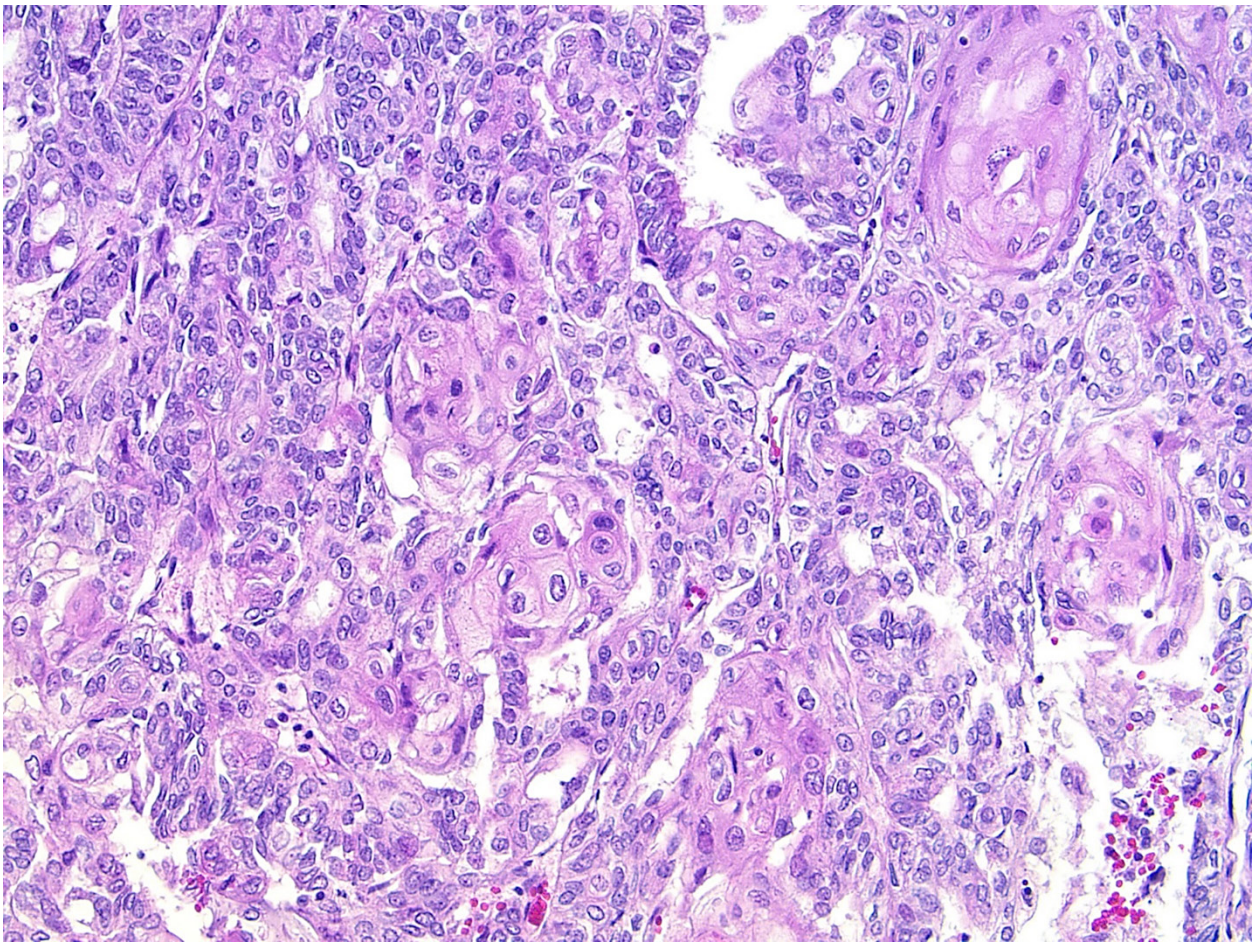
Although a statistically significant conclusion could not be drawn from this small number of cases, 21% of cases predicted, on the basis of our second sort, to have an "unfavorable" outcome showed direct-from-gland squamous differentiation, whereas this was seen in only 5% of cases predicted to have a "favorable" outcome.

Presently, squamous and morular differentiation in endometrial cancer is considered a prognostically neutral finding, although this has not always been the case. Demopoulos et al. found that women with "adenoacanthomas" (referring to the well-differentiated end of the spectrum of adenosquamous carcinomas) persisted in having a worse prognosis when compared to grade and stage-matched adenocarcinomas without squamous differentiation [25]. More recently, de Andrade et al. showed that squamous differentiation portends poor prognosis in low- and intermediate-risk endometrial cancers, carrying a 5.6-fold increased risk for recurrence [26]. Using genetically engineered mice, Reske et al. showed that invasive endometrial adenocarcinoma with mutant ARID1A exhibited ATF3 induction, reduced apoptosis, TP63+ squamous differentiation (which is not the case with morular metaplasia) and invasion [27]. In this animal model, ARID1A loss promoted both squamous differentiation and the acquisition of invasive properties.

Figure 5



Direct from gland squamous differentiation without interposed morular metaplasia. Presently, squamous differentiation in endometrial cancer is considered a prognostically neutral finding, although past studies and few present studies including animal modeling seem to show an association between squamous metaplasia and poor tumor prognosis (even when tumors are corrected for grade of the adenomatous component.)

What surprised us was the absence of prognostic effect with morular metaplasia. Morular metaplasia in endometrial cancer is associated with CTNNB1 (exon 3) mutation and β-catenin immunohistochemical staining. Niu et al. found a strong positive association between morules and glandular β-catenin nuclear staining whereas there was no association between morules and glandular PAX2 or PTEN aberrant expression or squamous differentiation. Tumors with β-catenin mutations usually occur in younger age women, tend to have low-grade histology, low rates of myometrial invasion and low rates of lymphovascular space invasion—all traditional indicators of "good prognosis". Paradoxically, β-catenin mutated endometrial cancers have been shown to have worse outcomes with a significantly increased rate of disease recurrence and lower overall survival

when compared to other grade, age, and stage-matched tumors. A summary of published data on clinical outcomes of patients with CTNNB1 (exon 3)/β-catenin mutations indicates that they show worse recurrence free survival and that β-catenin mutations may be prognostic for distant metastasis, leading some experts to conclude that they make up a fifth TCGA molecular subgroup. That is, a break-away group from NSMP (non-specific molecular profile) tumors that are characterized by a low number of somatic copy number alterations, low mutational burden and high levels of estrogen and progesterone receptor (ER/PR) expression. [28], [29], [30] The poor outcomes that we expected to see with morular metaplasia were not apparent in our morphological evaluation of cases; and without, perhaps, a larger data set and/or β-catenin immunohistochemical staining of cases, we cannot provide a clear answer to this contradiction.

CONCLUSION

By assessing anatomical, immunohistochemical and follow-up data, we found five potential prognostic indicators for low-grade uterine adenocarcinomas that would serve to predict "low risk" or "favorable" vs. "high risk" or "unfavorable" clinical behavior regarding lymph node involvement. Most of these are recognized today. Subclonal p53 immunostaining appears unique to this analysis but is recognized in TCGA studies as being associated with POLε and MMR-deleted tumors. Our indicators have been established by examining results limited to the hysterectomy-uterus. In our data set, they afford a 52%-to-48% split between potentially "favorable" and "unfavorable" outcomes. Four are negative prognostic indicators (MELF-glands, >70% invasion, angiolymphatic invasion, and cervical involvement) and one is a favorable prognostic indicator (subclonal-pattern p53 staining). We have too little data to say much about whether clonal pattern p53 staining, villoglandular growth in the invasive component of the tumor, or direct-from-gland squamous differentiation also function as poor prognosis indicators. However, we feel that their presence deserves to be mentioned in the pathology report.

Average tumor size, average depth of invasion, average percent invasion, positive lymph node dissections, individuals dead of disease or alive with disease, and age were all found to be associated with the first sorting that was based on the absence or presence of extrauterine or lymph node disease and was used to discover our five prognostic indicators. Average tumor size, average depth of invasion, average percent invasion, direct from gland squamous differentiation, the presence of >10% villoglandular component in the invasive tumor, positive lymph node dissections, individuals dead of disease or alive with disease, and the intensity of ER and PR immunohistochemical staining were all found to be associated with the second sorting that was based on the presence of our five prognostic indicators.

We conclude that these five indicators have prognostic value and deserve to be routinely reported with low-grade endometrial cancer hysterectomies because of their value in predicting lymph node metastasis and subsequent disease, possibly without relying on more expensive molecular testing. This can be especially useful in certain clinical situations where it may augment risk stratification, improve operative planning, and possibly avoid overtreatment. Finally, we recommend that, in addition to MMR immunohistochemistry, p53 immunohistochemical staining should be used as a routine adjunct test in the pathological assessment of low-grade endometrial adenocarcinomas.

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