Interpretation of P53 Immunohistochemistry in Endometrial Carcinomas: Toward Increased Reproducibility

Martin Köbel, M.D., Brigitte M. Ronnett, M.D., Naveena Singh, M.D., Robert A. Soslow, M.D., C. Blake Gilks, M.D., and W. Glenn McCluggage, M.D.

Summary: P53 immunohistochemistry has evolved into an accurate surrogate reflecting the underlying TP53 mutation status of a tumor, and has utility in the diagnostic workup of endometrial carcinomas. Recent work predominantly carried out in tubo-ovarian high-grade serous carcinoma has revealed 4 main patterns of p53 staining (normal/wild-type, complete absence, overexpression, and cytoplasmic); the latter 3 patterns are variably termed abnormal/aberrant/mutation-type and are strongly predictive of an underlying TP53 mutation. The aim of this review is to provide practical advice to pathologists regarding various aspects of p53 immunohistochemical staining. These include laboratory methods to optimize staining, a description of the different patterns of staining, advice regarding the interpretation, and reporting of p53 staining and practical uses of p53 staining in endometrial carcinoma diagnosis. Illustrations are provided to aid in the interpretational problems. Key Words: Endometrial carcinoma—p53—TP53—Immunohistochemistry—Interpretation.

The mutational status of TP53 is the single most important molecular factor, which predicts prognosis in endometrial carcinomas, with the presence of a TP53 mutation being associated with an unfavorable outcome (1,2). The TP53 mutation status may be used clinically in different ways such as aiding in the distinction between serous and endometrioid histotype (3,4), predicting outcome within a given histotype (1,5) or predicting outcome across several histotypes (2). The value of p53 in these scenarios is discussed in several other papers in this issue. As most pathologists do not have access to TP53 sequencing, they use p53 immunohistochemistry, which is quick, easy to perform, and inexpensive, as a surrogate for TP53 mutational analysis. Hence, p53 immunohistochemistry is very commonly utilized on endometrial carcinoma samples.

The aim of this review is to provide practical advice to pathologists regarding various aspects of p53 immunohistochemical staining. These include laboratory methods to optimize staining, a description of the different patterns of staining, advice regarding the interpretation, and reporting of p53 staining and practical uses of p53 staining in endometrial carcinoma diagnosis.

DIFFERENT PATTERNS OF P53 STAINING

It has long been recognized that nonsynonymous TP53 missense mutations result in nuclear accumulation of p53...
protein that can be detected as overexpression by immunohistochemistry. This is in the form of diffuse strong nuclear positivity involving at least 80% of the tumor cells but usually almost 100%. With increased refinement of immunohistochemistry, we, and others, observed other abnormal p53 expression patterns that correlate with the presence of a TP53 mutation. Although most of this work has been performed in tubo-ovarian high-grade serous carcinomas, these patterns are also found in other tumor types (6,7) and we believe that identical staining protocols and interpretational cut points apply for endometrial carcinomas. However, validation studies in endometrial carcinomas are necessary.

A 3-tier system has been recommended for p53 immunohistochemistry interpretation with overexpression and complete absence (which requires the presence of a positive internal control with staining of non-neoplastic cells such as lymphocytes, fibroblasts, or endothelial cells) both interpreted as abnormal/aberrant/mutation-type, in contrast to the normal/wild-type pattern with p53 expression levels in between these extremes (6,7). Wild-type staining is characterized by an admixture of negative cells, weakly and strongly positive cells. Subsequent studies using ovarian carcinomas validated that optimized immunohistochemistry agrees extremely well (specificity up to 100%) with the underlying TP53 mutation status (8–10). In other words, if the p53 staining pattern is abnormal (aberrant/mutation-type) there is almost certainly an underlying TP53 mutation. Notably, some splice site mutations or truncating mutations (the latter characterized by c-terminal stopgain) can result in detectable (but non-functional) p53 protein yielding a normal wild-type staining pattern. This occurs in ~5% of tubo-ovarian high-grade serous carcinomas. With optimized immunohistochemistry, a fourth uncommon p53 staining pattern was observed. This cytoplasmic pattern is characterized by an unequivocal cytoplasmic staining, which is accompanied by a variable nuclear staining. Strong diffuse nuclear overexpression with low-intensity cytoplasmic background should be interpreted as overexpression and not cytoplasmic. In tubo-ovarian high-grade serous carcinomas, the cytoplasmic pattern is associated with mutations disrupting the nuclear localization domain of the p53 protein (9). Thus, there are currently 4 patterns of p53 staining (Fig. 1), which correlate with the TP53 mutational status, resulting in 2 main corresponding interpretational categories (Table 1). Table 1 shows the percentages of the different staining patterns seen in tubo-ovarian high-grade serous carcinomas, where TP53 mutations are ubiquitous.

While immunohistochemistry can accurately predict the TP53 mutation status and high interobserver agreement can be achieved with training (11), significant variation regarding the interpretation of p53 immunohistochemistry is still observed in practice. In the following sections, we review difficult areas in the interpretation of p53 immunohistochemistry, including interpretational difficulties between the normal wild-type staining versus the 3 abnormal staining patterns. We also discuss problems with heterogenous staining (defined as subclonally abnormal staining within wild type staining) that can sometimes be seen in endometrial carcinomas. We recommend that p53 staining should not be reported as positive or negative as this is confusing and ambiguous terminology; rather the pattern of staining should be reported as wild-type or abnormal/aberrant/mutation-type and the pattern of the latter described.

INTERPRETATIONAL DIFFICULTIES: NORMAL WILD-TYPE VERSUS ABNORMAL OVEREXPRESSION

Currently the main use of p53 immunohistochemistry is to predict the presence or absence of TP53 mutation rather than a specific group of mutation, which may become of interest in the future when certain TP53 mutations may become targetable (12). The normal wild-type pattern can show a significant range of staining from only very few tumor cell nuclei positive to the majority of nuclei being positive. The level of wild-type p53 expression is dependent on the cellular state of differentiation and related to the proliferative activity. Tumors with a higher proliferation index often show more p53 staining and tumors with so-called “high” wild-type staining may be confused with overexpression. Figures 2A and B show examples of tumors that were stained with a high p53 antibody concentration (see below). To guide interpretation, the p53 expression levels should be compared with the internal control (these cases show clear p53 staining in normal stromal fibroblasts, endothelial cells, and lymphocytes), and also to the expected overexpression pattern for this protocol (these cases, eg, stain weaker compared with Fig. 1B). In these tumors, the intensity of the nuclear staining is variable with a few nuclei exhibiting strong staining, most moderate to weak staining and some being negative. Taken together, the cases in Figures 2A and B are interpreted as wild-type, albeit quite “high” wild-type.

In contrast, some cases with nonsynonymous TP53 mutations can show a lesser degree of p53 staining than expected for overexpression. Figures 2C and D show 2 endometrial serous carcinomas with areas of lower p53

| Interpretable | TP53 Mutation Status | Interpretation |
|---------------|----------------------|----------------|
| Wild-type     | Negative             | Normal         |
| Abnormal/mutation-type | Positive             | Abnormal/mutation-type |
| Overexpression | More than 80%         | Overexpression |
| Nuclear      | Partial               | Partial Nuclear |
| Cytoplasmic  | Minimal               | Minimal Cytoplasmic |

Table 1: Staining patterns of p53 in high-grade serous carcinomas.

Int J Gynecol Pathol Vol. 38, No. 1 Supplement 1, January 2019
Preanalytical factors (such as delayed fixation resulting in antigen degradation, which is more common in hysterectomy than biopsy specimens) are the presumed cause for areas with lower expression in these cases. This should not be interpreted as heterogenous expression, as defined below, or wild-type staining. In general, stronger p53 staining is more resilient against staining compared with the remainder of the tumor shown in the inset. Preanalytical factors (such as delayed fixation resulting in antigen degradation, which is more common in hysterectomy than biopsy specimens) are the

**TABLE 1.** p53 immunohistochemical staining patterns observed in tubo-ovarian high-grade serous carcinoma

| Staining pattern | TP53 status | P53 IHC interpretation | % in tubo-ovarian high-grade serous carcinoma |
|------------------|-------------|------------------------|---------------------------------------------|
| TP53 mutation absent | No mutation | Normal/wild-type | 0 |
| Wild-type | Nonsynonymous missense mutation | Abnormal/aberrant/mutation-type | 66 |
| TP53 mutation present | Loss of function mutation | Abnormal/aberrant/mutation-type | 25 |
| Complete absence | Loss of function mutation disrupting nuclear localization domain | Abnormal/aberrant/mutation-type | 4 |
| Cytoplasmic | Truncating mutation | Normal/wild-type | 5 |

IHC indicates immunohistochemistry.
fixation issues and usually provides the same staining when comparing endometrial biopsy with hysterectomy specimens. Conceivably, real molecular alterations could also explain these “mosaic” patterns, for example splice site mutation which may have an unpredictable effect on expression from 1 tumor cell to another, or low allelic frequency of TP53 mutation in some tumor areas; however, such changes appear to be rare.

INTERPRETATIONAL DIFFICULTIES: NORMAL WILD-TYPE VERSUS ABNORMAL COMPLETE ABSENCE AND NORMAL WILD-TYPE VERSUS ABNORMAL CYTOPLASMIC

The distinction of wild-type versus complete absence does not usually result in problems in interpretation as long as the tissue is well fixed and the assay sufficiently optimized to consistently stain normal cells. It is important to see adequate staining of internal controls (fibroblasts, endothelial cells, or lymphocytes) before making a diagnosis of abnormal complete absent p53 staining. Cases without a positive internal control are regarded as uninterpretable, and this may be more problematic on scant biopsies with minimal tissue represented. We also draw attention to an artifact, that is, encountered occasionally with the more sensitive p53 immunostaining methods; in some instances, a nonspecific nuclear blush can be present, which could be misinterpreted as wild-type in cases of true complete absence (Figs. 3A, B).

The cytoplasmic pattern of p53 staining has only recently been recognized as a distinct abnormal expression pattern (aberrant/mutation-type), which is occasionally seen with optimized immunohistochemistry, and experience with this pattern of staining is

![Image of biological samples](image_url)
limited. The identification of this pattern of staining is very dependent on the immunohistochemical protocol because it is probably not seen with weaker staining (see below). We have encountered occasional cases with variable nuclear expression and a cytoplasmic blush where the distinction between wild-type and CY staining can be difficult (Figs. 3C, D). Truly abnormal CY staining should be definite, and not an equivocal blush, which can be ignored.

Interpretational difficulty may also arise within the various abnormal p53 staining patterns, although it seems impossible to confuse overexpression with complete absence. Of note, we have seen 1 tubo-ovarian high-grade serous carcinoma that showed overexpression on a pretreatment biopsy and a combination of complete absence and overexpression on the surgical specimen indicating either different clonal origin or tumor progression with acquisition of a second loss of function TP53 mutation that resulted in a changed immunostaining pattern (13).

Although there are undoubtedly challenges with interpretation of p53 immunohistochemistry, equally there are also issues around quality assurance for genetic testing such that TP53 mutation testing may not be totally reliable. For example, in external proficiency testing for KRAS mutations in colorectal carcinoma, which is much less technically challenging than TP53 mutation analysis, >25% of laboratories had errors in 1 or more of 10 test samples (14).

**HETEROGENOUS P53 EXPRESSION**

In contrast to endometrial serous carcinomas, where TP53 mutation is the early founder mutation and

---

FIG. 3. (A, B) Endometrial serous carcinoma with complete absence pattern of abnormal p53 expression stained on 2 different platforms. (A) Nonspecific nuclear staining interpreted as wild-type pattern; (B) shows complete absence of nuclear staining but a weak cytoplasmic blush indicating staining bordering on too strong. (C) Endometrial endometrioid carcinoma with wild-type staining with slight cytoplasmic blush on the left and true abnormal cytoplasmic staining on the right (compare with low-power view in Fig. 4C). The true abnormal cytoplasmic staining is accompanied by a variable nuclear staining of similar intensity but not strong diffuse. (D) Endometrial endometrioid carcinoma with wild-type pattern showing weak cytoplasmic staining probably due to too strong staining. This should not be interpreted as abnormal cytoplasmic staining.
therefore present in any subclone, there are some endometrioid carcinomas with a mutator phenotype (either \textit{POLE} ultramutated or mismatch repair deficient hypermutated) that can acquire a \textit{TP53} mutation later in the course. Such a subclonal \textit{TP53} mutation may result in heterogenous p53 expression characterized by

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig4.jpg}
\caption{(A) Endometrial endometrioid carcinoma showing a combination of wild-type pattern on the left and overexpression on the right (normal + abnormal = heterogenous). (B) Endometrial endometrioid carcinoma with heterogenous staining (normal wild-type pattern + abnormal overexpression and complete absence). (C) Endometrial endometrioid carcinoma with heterogenous staining (normal wild-type pattern and abnormal overexpression and cytoplasmic staining). (D) Endometrial endometrioid carcinoma with heterogenous staining (normal wild-type pattern and abnormal overexpression and complete absence). (E) High power from (C) showing transition from overexpression to wild-type pattern. (F) Endometrial endometrioid carcinoma with variable wild-type pattern (not heterogenous), “high” wild-type on left versus “low” wild-type on the right.}
\end{figure}
areas of normal wild-type and areas with abnormal mutation-type staining patterns (Fig. 4). Traditionally, some of those tumors have been diagnosed as “mixed” endometrial carcinomas but they are probably better characterized as endometrioid carcinomas, particularly if endometrioid-like alterations (mismatch repair deficiency, loss of PTEN, or ARID1A) are detected (15). Interpretable difficulties in p53 staining can arise in determining whether these subclonal foci are indeed a distinct pattern (Fig. 4E) or whether this represents variability in the intensity of wild-type staining not reaching the threshold for abnormal overexpression (i.e. areas of “high” wild-type) (Fig. 4F) or variability in overexpression (Figs. 2C, D). As a potential pitfall, we have seen cases in which the abnormal component of heterogeneous staining was present on 1 tumor section but not another. This raises the possibility that such a case may be erroneously classified as a serous carcinoma while in fact it may be a POLE mutated endometrioid carcinoma. Hence, if morphologic features suggestive of POLE mutations are seen and p53 staining is abnormal, it may be useful to stain >1 tumor section (16,17).

**RECOMMENDATION FOR p53 IMMUNOHISTOCHEMISTRY OPTIMIZATION**

The DO7 clone is the most widely used p53 antibody but there are several other high-quality p53 antibodies available. Each laboratory must establish an immunohistochemical protocol with high sensitivity and specificity using appropriate controls. For p53, these include both external and internal controls. As external controls, a “low expressor” positive control to assess the lower limit of detection along with a “high expressor” positive and a negative tissue control are recommended by immunohistochemistry proficiency testing programs (eg, NordiQC, UK NEQAS, CIQC) (18). The typical “high expressor” positive control is a high-grade serous carcinoma with overexpression. Tonsil or colon (serving both as a “low expressor” positive and a negative control) allow gauging of immunohistochemical protocols and should be considered for inclusion as on-slide controls. In the tonsil, there is variably intense nuclear staining of scattered keratinocytes in the lower third of the squamous epithelium and in the germinal center B cells (≥20%), whereas the upper squamous layers should be negative and only occasional cells of the mantle zones of secondary follicles (<10%) should stain. In the colon (as well as appendix), there is variably intense nuclear staining in scattered epithelial cells in the basal crypts but no staining of the luminal epithelial cells. An advantage of p53 immunohistochemistry is that an internal “low expressor” positive control is present in almost any tissue (fibroblasts, endothelial cells, or lymphocytes), with variably intense nuclear staining in scattered cells. This internal control is invaluable as it provides information not only about analytical but also preanalytical factors. As discussed previously, consistent staining of the internal control is a prerequisite for interpretation of the complete absence pattern.

In Figure 5, we illustrate the influence of different immunohistochemical protocols on the staining results. Each column represents the same case stained with 4 different immunohistochemical protocols using the same DO7 antibody on the same Dako Omnis platform but varying primary antibody dilution and amplification steps (for details please see Fig. 5 legend).

Protocols #1 and #2 show sufficient staining of the “low expressor” positive control (>20% staining of the normal tonsillar germinal center B cells). In contrast, protocols #3 and #4 show a weak staining intensity and a reduced proportion of normal tonsillar germinal center B-cell staining. The effect on the p53 expression patterns of 5 tumors are illustrated next. The overexpression case with protocol #4 (too weak) shows a similar intensity compared with the high wild-type case stained with protocol #1 (too strong). External proficiency testing program runs have demonstrated that insufficient p53 staining was mostly due to staining being too weak (61%-84% of insufficient cases in the NordicQC run 38 and CIQC run 42) (19). Typically, these weak staining results were caused by a too low concentration of the primary antibody, including ready to use protocols not properly calibrated by the vendors (protocol #3 is a vendor protocol). The complete absence case with protocols #3 and #4 was rendered uninterpretable due to the lack of an intrinsic positive control. The cytoplasmic case is potentially misinterpreted as wild-type with protocols #3 and #4. Although the overexpression case with protocol #3 seems to stain sufficiently, protocol 3 is inferior in recognizing complete absence and cytoplasmic patterns, again highlighting the limitation on reliance of a “high expressor” sample as the sole control.

In a minority of cases, errors occur as a result of too strong staining, typically caused by an inappropriately high concentration of the primary antibody in combination with inappropriate amplification steps. This can result in a low-grade endometrioid carcinoma, and sometimes even a benign lesion, being interpreted as abnormal overexpression when it is wild-type. However, with protocols generally changing to a
stronger staining for consistent detection of the internal controls, the interpretation threshold also needs to be adapted. Cut-off recommendations for overexpression versus wild-type staining are difficult to make, whereas p53 staining intensity varies across laboratories. One of the authors (M.K.) routinely utilizes a very strong protocol (#2), which is more resilient against pretreatment influence. As a consequence the staining intensity between paired endometrial biopsies and hysterectomy specimens is similar. In cases of overexpression, this results in strong staining in virtually 100% of tumor cell nuclei in a well-fixed case and at least 80% of tumor cell nuclei in a less well-fixed case. Increased cytoplasmic background in an overexpression case is generally an indication that the staining is becoming too strong, while lack of consistent detection of the internal control is an indication of too weak staining.

PRACTICAL USES OF P53 IMMUNOHISTOCHEMISTRY

The value of p53 staining in a diagnostic sense in endometrial carcinomas is discussed in several other papers in this review and only a few brief points are made here. In endometrial carcinomas, p53 immuno-histochemistry may be useful in diagnosing a high-grade carcinoma associated with unfavorable outcome and can be used as part of a panel for histotyping. However, 1 point worth mentioning is that a small percentage of low-grade endometrioid adenocarcinomas contain TP53 mutations and exhibit mutation-type immunoreactivity. However, that being said, mutation-type p53 staining may be helpful in avoiding underdiagnosis of a serous carcinoma with intermediate-grade nuclear features as grade 1 or 2 endometrioid carcinoma (3,20). Abnormal p53 staining alone is not sufficient for the differential diagnosis of endometrioid from serous carcinomas as a significant minority of grade 3 endometrioid carcinomas with mutation-type p53 expression have a worse prognosis than grade 3 endometrioid carcinomas with wild-type expression (22,23). Mutation-type p53 immunostaining serves as an indicator of the Cancer Genome Atlas (TCGA)-based molecular subtype of endometrioid carcinoma with the worst prognosis, especially when applied as part of a diagnostic algorithm (1,2).

Given that we know that the sensitivity of p53 immunohistochemistry in detection of TP53 mutation is not 100% (see earlier), there will be a small percentage of
morphologically prototypical endometrial serous carcinomas that exhibit a wild-type pattern of p53 immunoreactivity but still harbor a TP53 mutation (eg, truncating), and the diagnosis of serous carcinoma can be made in a tumor with wild-type p53 staining. In conjunction with mismatch repair de
tumor with wild-type p53 staining. In conjunction with and the diagnosis of serous carcinoma can be made in a morphologically prototypical endometrial serous carcino-
have encountered 1 case of a staining makes a diagnosis of dedifferentiated/undiffer-
endometrioid carcinoma that underwent dedifferentiation and subsequent management.

CONCLUSION

In conclusion, p53 is perhaps the single most important immunohistochemical stain used in the pathologic workup of endometrial carcinomas. Careful attention to laboratory protocols, including adequate controls, and training in interpretation is needed to achieve high interobserver consistency to make this a reliable test informing endometrial carcinoma diagnosis and subsequent management.

Acknowledgments: Dr Köbel was supported by Calgary Laboratory Services Research support (RS14-525i). The authors thank Shuhong Liu and Young Ou for performing p53 immunohistochemistry. Dr Soslow is supported in part by the MSK Cancer Center Support Grant of the National Institutes of Health/National Cancer Institute under award number P30CA008748.

REFERENCES

1. Stelloo E, Nout RA, Osse EM, et al. Improved risk assessment by integrating molecular and clinicopathological factors in early-stage endometrial cancer-combined analysis of the PORTEC cohorts. *Clin Cancer Res* 2016;22:4215–24.
2. Talhouk A, McConkey MK, Leung S, et al. Confirmation of ProMisE: a simple, genomics-based clinical classifier for endometrial cancer. *Cancer* 2017;123:802–13.
3. Chen W, Husain A, Nelson GS, et al. Immunohistochemical profiling of endometrial serous carcinoma. *Int J Gynecol Pathol* 2016;36:128–39.
4. Altman AD, Ferguson SE, Atenafu EG, et al. Canadian high risk endometrial cancer (CHREC) consortium: analyzing the clinical behavior of high risk endometrial cancers. *Gynecol Oncol* 2015;139:268–74.
5. Fadare O, Gwin K, Desouki MM, et al. The clinicopathologic significance of p53 and BAF1-250a (ARID1A) expression in clear cell carcinoma of the endometrium. *Mod Pathol* 2013;26:1101–10.
6. Köbel M, Reuss A, Bois A du, et al. The biological and clinical value of p53 expression in pelvic high-grade serous carcinomas. *J Pathol* 2010;222:191–8.
7. Mccluggage WG, Soslow RA, Gilks CB. Patterns of p53 immunoreactivity in endometrial carcinomas: ‘all or nothing' staining is of importance. *Histopathology* 2011;59:786–8.
8. Yemelyanova A, Vang R, Kshirsagar M, et al. Immunohistochemical staining patterns of p53 can serve as a surrogate marker for TP53 mutations in ovarian carcinoma: an immunohistochemical and nucleotide sequencing analysis. *Mod Pathol* 2011;24:85-1248–53.
9. Köbel M, Piskorz AM, Lee S, et al. Optimized p53 immunohistochemistry is an accurate predictor of TP53 mutation in ovarian carcinoma. *J Pathol Clin Res* 2016;2:247–58.
10. Cole AJ, Dwight T, Gill AJ, et al. Assessing mutant p53 in primary high-grade serous ovarian cancer using immunohistochemistry and massively parallel sequencing. *Sci Rep* 2016;6:26191.
11. Singh N, Parry S, Won J, et al. Technical and interpretive performance characteristics of p53 immunostaining: British Association of Gynaecological Pathologists (BAGP) and United Kingdom National External Quality Assessment Service (UKNEQAS) collaborative project. *Virchows Arch* 2017;471 (suppl 1):1.
12. Lehmann S, Bykov VIN, Ali D, et al. Targeting p53 in vivo: a first-in-human study with p53-targeting compound APR-246 in refractory hematologic malignancies and prostate cancer. *J Clin Oncol* 2012;30:3635–40.
13. Casey L, Köbel M, Ganesean R, et al. A comparison of p53 and WT1 immunohistochemical expression patterns in tubo-ovarian high-grade serous carcinoma before and after neoadjuvant chemotherapy. *Histopathology* 2017;71:736–42.
14. Tembusyer L, Ligtengrel MJL, Normanno N, et al. Higher quality of molecular testing, an unfilled priority: results from external quality assessment for KRAS mutation testing in colorectal cancer. *J Mol Diagn* 2014;16:371–7.
15. Köbel M, Meng B, Hoang LN, et al. Molecular analysis of mixed endometrial carcinomas shows clonality in most cases. *Am J Surg Pathol* 2016;40:166–80.
16. Hussein YR, Weigelt B, Levine DA, et al. Clinicopathological analysis of endometrial carcinomas harboring somatic POLE exonuclease domain mutations. *Mod Pathol* 2015;28:505–14.
17. Bakhsh S, Kinloch M, Hoang LN, et al. Histopathological features of endometrial carcinomas associated with POLE mutations: implications for decisions about adjuvant therapy. *Histopathology* 2016;68:916–24.
18. Torlakovic EE, Nielsen S, Vyberg M, et al. Getting controls under control: the time is now for immunohistochemistry. *J Clin Pathol* 2015;68:879–82.
19. Lee S, Piskorz AM, Le Page C, et al. Calibration and optimization of p53, WT1, and Napsin A immunohistochemistry and massively parallel sequencing. *Histopathology* 2015;67:209–21.
20. Nastic D, Shanwell E, Wallin K-L, et al. A selective biomarker panel increases the reproducibility and the accuracy in endometrial biopsy diagnosis. *Int J Gynecol Pathol* 2017;36:339–47.
21. Schultheis AM, Martelotto LG, De Filippo MR, et al. TP53 mutational spectrum in endometrioid and serous endometrial cancers. *Int J Gynecol Pathol* 2016;35:299–300.
22. Bosse T, Nout RA, McAlpine JN, et al. Molecular classification of grade 3 endometrioid endometrial cancers identifies distinct prognostic subgroups. *Mod Pathol* 2017;30:277A.
23. Köbel M, Atenafu EG, Nelson GS, et al. TP53 expression status and association with outcome within grade 3 endome-
24. Hamezis AN, Hoang LN, Coatham M, et al. Loss of switch/sucrose non-fermenting complex protein expression is associ-
ated with dedifferentiation in endometrial carcinomas. *Mod Pathol* 2016;29:302–14.