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**p53 immunohistochemistry is an accurate surrogate for *TP53* mutational analysis in endometrial carcinoma biopsies**

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**Abstract**

*TP53* mutations are considered a surrogate biomarker of the serous-like “copy number high” molecular subtype of endometrial carcinoma (EC). In ovarian carcinoma, p53 immunohistochemistry (IHC) accurately reflects mutational status with almost 100% specificity but its performance in EC has not been established. This study tested whether p53 IHC reliably predicts *TP53* mutations identified by next generation sequencing (NGS) in EC biopsy samples for all EC and as part of a molecular classification algorithm after exclusion of cases harbouring mismatch repair defects (MMRd) or pathogenic DNA polymerase epsilon exonuclease domain mutations (*POLE*mut). A secondary aim assessed inter-laboratory variability in p53 IHC.

From a total of 207 cases from 5 centres (37–49 cases/centre), p53 IHC carried out at a central reference laboratory was compared with local IHC (n=164) and curated tagged-amplicon NGS *TP53* sequencing results (n=177).

Following consensus review, local and central p53 IHC results were concordant in 156/164 (95.1%) tumours. Discordant results were attributable to both interpretive and technical differences in staining between the local and central laboratories. When results were considered as any mutant pattern versus wildtype pattern staining, however, there was disagreement between local and central review in only one case. The concordance between p53 IHC and *TP53* mutation was 155/168 (92.3%) overall, and 117/123 (95.1%) after excluding MMRd and *POLE*mut EC. Three (3/6) discordant results were in serous carcinomas with complete absence of p53 staining but no detectable *TP53* mutation. Subclonal mutant p53 IHC expression was observed in 9/177 (5.1%) cases of which 4 were either MMRd or *POLE*mut. Mutant pattern p53 IHC was observed in 63/63 (100%) serous carcinomas that were MMR proficient/*POLE* exonuclease domain wildtype.

Optimised p53 IHC performs well as a surrogate test for *TP53* mutation in EC biopsies, demonstrates excellent inter-laboratory reproducibility and has high clinical utility for molecular classification algorithms in EC.

**Keywords:** endometrial carcinoma, p53, TP53, The Cancer Genome Atlas, molecular classification

**Introduction**

Immunohistochemical staining of human tumours for p53 has a long and interesting history (1). The accumulation of p53 protein in the nuclei of some tumours initially led to the belief that p53 is an oncogene, but it was soon established that p53 is a tumour suppressor and the accumulation of p53 protein occurs secondary to gene mutations (2). More recently the complexity of the relationship between p53 protein expression and *TP53* mutation has become better understood (3). Non-synonymous missense mutations in *TP53* usually result in a mutant protein that resists degradation; the mutant protein accumulates in tumour cell nuclei. Stop-gained and splice site mutations are associated with complete absence of p53 protein in tumour cells. Finally, some C-terminal mutations can result in disruption of the nuclear localisation signal and accumulation of p53 in the cytoplasm of tumour cells. With recognition of these abnormal/mutant patterns of p53 expression as being indicative of an underlying *TP53* mutation, immunohistochemistry (IHC) has emerged as a reliable surrogate test for *TP53* mutation status in ovarian carcinoma, with close to 100% specificity and 96% sensitivity (3).

The accuracy of p53 IHC as a surrogate for *TP53* mutation in endometrial carcinoma (EC) has not been established and, given the genomic differences between EC and ovarian carcinoma (OC), it is important not to extrapolate the test performance of p53 immunostaining from OC to EC. In high-grade serous tubo-ovarian carcinoma, mutations in *TP53* are ubiquitous early driver events (4) and give highly reproducible and clonal p53 immunostaining patterns (3). The same interpretation guidelines developed for OC could, in theory, be applicable to EC (5, 6), but EC has subtypes with a high mutational burden that can result in secondary or passenger *TP53* mutations that lack the same biological significance as *TP53* driver mutations in OC (7, 8), the expression patterns of which have not been well studied. The Cancer Genome Atlas (TCGA) identified four molecular subtypes of EC based on genomic alterations: 1) ultramutated/DNA polymerase ε mutated (*POLE*mut), 2) hypermutated/microsatellite instability, 3) low copy number abnormalities (CN-low), and 4) high copy number abnormalities (CN-high) (7). Pathogenic variants in the exonuclease domain of *POLE* are used as a surrogate marker for the ultramutated group, while abnormal expression of mismatch repair proteins, recognised through loss of expression on immunostaining (mismatch repair deficient or MMRd), is a widely used clinical marker of the hypermutated/microsatellite instability group (9–17). The presence of *TP53* mutation characterises the CN-high group. High numbers of somatic copy number abnormalities are a feature of serous carcinomas of the endometrium and a subset of endometrioid carcinomas that have been described as “serous-like”. Grade 3 endometrioid carcinomas with abnormal p53 expression (p53abn) have a worse prognosis than grade 3 p53 wild-type endometrioid carcinomas (18). CN-high EC with *TP53* mutations correspond broadly to the Type II tumours described by Bokhman, whereas the CN-low EC correspond to Type I endometrial carcinomas (19). It is important to note that *TP53* mutations/mutant pattern p53 expression can occur in *POLE*mut or MMRd EC (7) *TP53* mutations in ultra- or hypermutated tumours are not associated with the poorer prognosis seen in EC with only *TP53* mutations, and are therefore considered passenger mutations (8). In other words, the behaviour of tumours with both *POLE*mut and p53abn, or MMRd and p53abn is that of EC with *POLE*mut or MMRd, respectively. Therefore, *TP53* mutation is only a marker of CN-high EC after *POLE*mut and MMRd subtypes have first been excluded.

The aims of this study were: First, to assess inter-laboratory variation in reporting p53 immunostaining in EC biopsies; Second to determine the agreement between p53 protein expression and *TP53* mutation status in EC, in endometrial biopsy specimens, using a molecular classification algorithm, to account for MMRd and *POLE*mut EC, and thirdly, to further characterise EC with discordant p53 immunoexpression and *TP53* mutation status.

**Methods**

This study was sponsored by Queen Mary University of London (ReDA 011470) following ethical approvals (London-Brent NHS Research Ethics Committee, reference 17/LO/0155, 20/01/2017) and NHS Health Research Authority (IRAS 209675, 06/02/2017).

Five histopathology laboratories each identified cases of EC in endometrial biopsy specimens, selecting cases with known MMR status and sufficient tumour to allow performing additional IHC and molecular testing. Case selection included serous:non-serous high grade:G1-2 endometrioid EC in an approximately 2:1:1 ratio with the goal of identifying 200 cases with >50% prevalence of *TP53* mutation. For each case p53 IHC was carried out locally with the protocol used for clinical purposes. Unstained sections were sent to the University of Calgary which acted as a reference site using p53 IHC protocols validated for ovarian carcinoma (20). Details of the immunostaining protocols used are provided in supplementary material, Table S1. Locally performed p53 IHC was interpreted by the local study pathologist (NS, TB, MJ-L, CBG), while slides submitted to the central reference laboratory were interpreted independently by a single study pathologist (MK).

Criteria for interpretation of the 4 categories of p53 immunostaining were used as described (21). Briefly, strong diffuse staining of 80–100% of tumour cell nuclei is abnormal/mutant (overexpression), and complete absence of staining of tumour cell nuclei, in the presence of positive internal control staining, is abnormal (complete absence). As noted in our previous study (3), a weak blush of nuclear staining, much less than that seen in benign internal control cells, should also be interpreted as abnormal/complete absence. Abnormal (cytoplasmic) staining is defined as significant cytoplasmic staining i.e. more than a faint blush, in the presence of variable and less than strong and diffuse nuclear immunoreactivity. Staining of 1–80% of nuclei, with variable intensity of staining, was considered normal (wildtype) p53 expression. For the purposes of this study, subclonal mutant p53 immunostaining was defined as the combination of more than one pattern of staining (typically wild type plus one or more mutant patterns or two different mutant patterns), with each present in at least 5% of tumour cells.

Results of p53 IHC were compared for local versus central/reference staining. For discordant cases, the slides were scanned and reviewed independently by all study pathologists (NS, TB, MJ-L, CBG, MK) with a review interpretation arrived at by consensus, i.e. when 4 or 5 of 5 reviewing pathologists agreed on the interpretation.

DNA was isolated locally from each case. Extracted DNA samples were sent to the Cancer Research UK Cambridge Institute for mutational analysis. The coding regions of *TP53*, *PTEN* and hot spots for *EGFR*, *PIK3CA*, *KRAS, BRAF, POLE, CTNNB1*were sequenced using tagged amplicon deep sequencing (TAm-Seq) as described [19] using Fluidigm 48.48 Access Array platform [Fluidigm, San Francisco, USA] on an Illumina MiSeq or HiSeq4000 using PE-150bp protocol [Illumina, San Diego, USA].

 *[EdQ: please provide* ***city****,* ***state or province****,* ***country*** *details for each supplier of equipment or reagents or software* ***at first mention;*** *thereafter use only the supplier name]* Sequencing data and variant verification were performed using an in-house analysis pipeline and IGV software as described previously (22). All called *TP53* mutations were assessed for their presence in publicly available databases: ClinVAr and COSMIC (23, 24). Algorithms predicting the functional impact were used: SIFT and Polyphen (25, 26). Rare SNV with potential tolerated or benign predictions were further characterized using the IARC (WHO) *TP53* mutation database (27) (supplementary material, Table S2).

Results of the central reference laboratory p53 IHC were compared to results of *TP53* mutation status for all cases and after removal of *POLE*mut and MMRd tumours (28). Binary analysis was carried out to assess concordance between p53 IHC and *TP53* mutation by combining overexpression, complete absent, cytoplasmic patterns into a single abnormal/mutant (p53abn) score. A further analysis was carried out to correlate p53 IHC staining pattern with the type of *TP53* mutation; for this complete absence/cytoplasmic staining, overexpression and wild type staining were compared with stop gained/splicing mutations, non-synonymous missense mutations, and absence of mutation, respectively.

Sensitivity, specificity and agreement or accuracy were calculated. All primary analyses were performed blinded.

**Results**

**Study Groups**

We selected 207 endometrial carcinomas for study (Table 1): 164 cases were included in the analysis of interlaboratory reproducibility of p53 IHC comparing local and central IHC results (this analysis did not include 43 local cases from the reference site). Agreement between central IHC and *TP53* sequencing results was assessed on 177 samples because *TP53* sequencing failed in 24 samples and there was insufficient tumour cellularity to proceed to mutational analysis in 6 cases.

**Interlaboratory reproducibility of p53 IHC**

In 11 of 164 (6.7%) cases the local interpretations of the staining patterns were discordant with those of central review (Table 2A). In order to address whether the discordant results were due to differences in interpretation or differences in staining protocols, the 11 discrepant cases were re-evaluated by all study pathologists. This resulted in three groups (Table 3). First, concordance was reached in 3 of the 11 cases, hence, these were either local or central interpretational or data entry errors. Second, no consensus in interpretation was reached in 5 cases either on the locally stained section (n=4) or the centrally stained section (n=1), indicating ambiguity in interpretation due to limited experience with the interpretation of uncommon patterns or technical limitations of p53 IHC, resulting in equivocal staining results (3 cases showed subclonal expression, and 1 case showed cytoplasmic expression). Third, for the 3 remaining cases consensus was reached for local and central results but the paired results remained discordant indicating technical differences between assays. Two of these three also showed subclonal or cytoplasmic expression. Of note, after consensus re-evaluation, only one case had disagreement between p53abn (any pattern of abnormal staining) versus wild-type expression (overexpression on local section and wild type pattern on central section) (Table 2B).

**Agreement of p53 IHC with presence/absence of *TP53* mutation**

Deleterious *TP53* mutations were detected in 102 of 177 (57.6%) cases and mutant or subclonal p53 expression (using central p53 IHC data after consensus re-evaluation) was also present in 102 of 177 cases (Figure 1). However, the agreement was not perfect (Table 4). Because the study was not designed to detect subclonal *TP53* mutations, i.e. scoring of tumour tissue was not targeted to areas with mutant pattern p53 staining, and the clinical significance of subclonal mutations is not known, we decided to exclude the 9 cases with subclonal staining from the agreement analyses, and these will be discussed separately below. The agreement analysis on the remaining 168 cases was performed as a binary test (presence of any pathogenic *TP53* mutation versus any p53abn mutant staining pattern), for all cases and after exclusion of MMRd (n=39) or *POLE*mut (n=6) EC because of their hyper-/ultramutator genotype (Table 4). A separate analysis was carried out to correlate the specific mutant staining patterns with the type of *TP53* mutation. For cases showing discordant p53 IHC and *TP53* sequencing results, NGS was repeated on tumour DNA extracted from matched biopsy and corresponding hysterectomy samples.

On analysis of all cases, p53 IHC showed an accuracy of 92.3% (95% CI 87.1% to 95.8%) for the presence of *TP53* mutation (sensitivity: 90.8 [95% CI 83.3% to 95.7%] and specificity: 94.3% [95% CI 86.0% to 98.4%]); Table 5A). Confining the analysis to MMRd or *POLE*mut cases improved the accuracy to 95.1% (95% CI 89.6% to 98.2%) (sensitivity: 97.7% [95% CI 91.9% to 99.7%] and specificity: 88.9% [95% CI 73.9% to 96.9%]; Table 5B).

There were 9 tumours with false-negative IHC with normal/wildtype immunostaining but *TP53* mutation detected, of which 7 were either *POLE*mut (n=3) or MMRd (n=4) EC (supplementary material, Table S2). Removing the *POLE*mut and MMRd cases from the analysis reduced the number of false-negative cases to 2, and increased sensitivity from 90.8% to 97.7% (Table 5B).

There were 4 tumours with false-positive staining with p53abn IHC and no *TP53* mutation detected. It is noteworthy that 3 of 4 these tumours showed complete absence of p53 staining, and none of the 4 cases was MMRd or *POLE*mut. Excluding *POLE*mut and MMRd cases from the analysis therefore did not change the false-positive rate but reduced specificity from 94.3% to 88.9% by decreasing the total number of cases with no mutation detected (Table 5B).

The overall accuracy of p53 immunostaining for predicting *TP53* mutation increased from 92.3% to 95.1% when *POLE*mut and MMRd cases were excluded (Table 5B).

After exclusion of MMRd and *POLE*mut cases, and cases showing subclonal expression, there were, 6 tumours in which there was disagreement between p53 IHC and *TP53* mutational analysis. Of the two cases with wild type IHC and a clearly detected *TP53* mutation, one was a grade 1 endometrioid carcinoma whereas the other was a carcinosarcoma. Of the four cases showing p53abn IHC with no detectable *TP53* mutation, all had serous histology with three showing complete absence of p53 protein and one showing p53 overexpression. Based on the prevalence of p53 abnormalities in serous carcinoma (see below), it appears that p53 IHC may more accurately reflect p53 functional abnormalities in these latter cases, compared to mutational analysis.

**Agreement of p53 IHC with type of *TP53* mutation**

The p53 IHC pattern was correlated with the type of *TP53* mutation (supplementary material. Tables S3A,B). Analysis was carried out for complete absence/cytoplasmic staining, overexpression and wild type staining compared with stop gained/splice site mutations, non-synonymous missense mutations, and absence of mutation respectively, for all cases and also after exclusion of MMRd and *POLE*mut tumours. The accuracy of the IHC pattern for predicting mutation category increased from 87.5% to 89.4% after exclusion of the MMRd and *POLE*mut tumours (supplementary material, Table 3B). While there were no cases with a non-synonymous missense mutation that showed complete absence or cytoplasmic staining, there were 7 cases (two with splice site mutations and three with the same stop gained mutation p.R342\*) that resulted in overexpression (supplementary material, Table S2).

Cytoplasmic p53 expression was observed in 5/177 cases (2.8%). All 5 cases harboured a *TP53* mutation including one with a splice site mutation, and 4 mutations predicted to result in a p53 protein length of 298 to 345 aa (supplementary material, Table S2) with two mutations causing a stop signal at 345 aa (G266Dfs\*79, p.N310Tfs\*35). As noted previously these 5 cases with cytoplasmic expression included 2 of the 8 tumours where there was discrepancy between local and central IHC interpretation.

***TP53* mutation status in tumours with subclonal p53 IHC staining**

Subclonal p53 expression was observed in 9 of 177 (5.1%) tumours (Figure 2). *TP53* mutations were detected in only 4 of these cases, however DNA extraction from microdissected areas was not carried out as part of this study. Eight cases showed a combination of wild type with one or more of the mutant patterns and one case showed a combination of 2 mutant patterns. Four of the 9 tumours with subclonal expression were either MMRd (n=1) or *POLE*mut (n=3); all of these were of endometrioid histotype and showed wild type pattern in the majority of the tumor cells, with mutant pattern staining in less than 10%. None of these 4 cases showed a detectable *TP53* mutation and their molecular subtype assignment, using an algorithmic approach to classification, is independent of *TP53* mutation status (8). The second group consisted of MMR proficient and *POLE* wild type serous (n=2), mixed serous/endometrioid (n=1), grade 2 endometrioid (n=1) and grade 1 endometrioid (n=1) carcinomas; these harboured *TP53* mutations in 4 cases. One serous carcinoma showed 75% complete absence and 25% overexpression and harboured a splice site mutation consistent with sampling from the dominant area showing complete absence of p53 expression. One grade 2 endometrioid carcinoma showed two mutant patterns in approximately equal distribution accounting for 95% tumour area, together with a 5% focus of wild type pattern; this case harboured two separate non-synonymous missense *TP53* mutations (p.Y163C and p.G245A). Mutations were detected in two of the other cases with mutant areas occupying 50% or 95% of the tumour (one USC and one mixed) but not in the case with only 33% of the tumour showing mutant expression (G1EEC). Thus, in MMR proficient/*POLE* wildtype EC, subclonal staining patterns appear to correlate with *TP53* mutation.

**Abnormal p53 IHC/*TP53* mut and EC histotype**

The results of p53 IHC and *TP53* mutation analysis for the tumours based on histotype, together with MMR and *POLE* mutation status are shown in Table 6. As expected, most serous, mixed serous/endometrioid and carcinosarcomas showed both p53abn IHC and a *TP53* mutation (70/85, 82.4%). After exclusion of the MMRd and *POLE*mut cases, all of the serous and mixed serous/endometrioid carcinomas showed p53abn IHC (70/70, 100%), as did 6/7 (85.7%) carcinosarcomas. Within MMR proficient and *POLE* wild type endometrioid carcinomas, a significant portion also show p53abn staining accompanied by a *TP53* mutation (11/86, 12.8%), with most being grade 3 (7/11, 63.6%). Notably, p53abn IHC expression accompanied by *TP53* mutation was also present in 3 of 40 (7.5%) grade 1 endometrioid carcinomas. Also notable was the inclusion of 5 clear cell carcinomas of which 4 showed p53abn IHC accompanied by *TP53* mutation.

**Discussion**

Our study shows that p53 IHC has high accuracy in predicting *TP53* mutations in endometrial carcinomas, comparable to that in ovarian carcinomas. Importantly, inter-laboratory agreement in assessment of p53 IHC was excellent, with the notable exception of tumours showing subclonal p53 staining, which was observed in 5% of cases and caused many of the discordant interpretations. Interpretation of p53 immunostaining on well-fixed biopsy specimens may be more difficult as they have consistently stronger staining than is seen in hysterectomy specimens. To improve the wider recognition and interpretation of p53 EC immunostaining, we have created a tutorial/self-assessment web resource with scanned slides from this study (<http://www.gpec.ubc.ca/p53>).

The accuracy of p53 IHC for predicting the presence of a *TP53* mutation in all EC cases was 92% which is lower than the 97% observed in ovarian carcinoma. Once MMRd and *POLE*mut are excluded, however, the accuracy was 95% and the sensitivity increased to 97.7%, equivalent to ovarian carcinoma interpretation. The rationale for excluding MMRd and *POLE*mut cases is based on TCGA data, where it was observed that 35% of *POLE*mut cases harboured a *TP53* mutation but that these were commonly non-hotspot mutations (29). Furthermore, the outcome of patients with *POLE*mut/p53abn or MMRd/p53abn EC are significantly better than would be expected if they harboured driver mutations in *TP53* (8), providing further evidence that these *TP53* mutations are likely secondary events. In the molecular classification of EC, the presence of *TP53* mutation or p53abn IHC is only a surrogate for CN-high molecular subtype if the *POLE*mut and MMRd tumours are excluded (7,14,17). In our series there were 9 false negative cases; notably, 7 of the 9 false negative cases were either MMRd or *POLE*mut.

After exclusion of *POLE*mut and MMRd tumours, there were two false negative and four false positive IHC results. The false-negative cases were one grade 1 endometrioid carcinoma while the other was a carcinosarcoma. In the latter tumour it is likely that p53 IHC was indeed a false-negative result and that the p.R249S *TP53* mutation detected on sequencing was biologically and clinically relevant, given the strong association of carcinosarcomas with mutant *TP53*. There were four cases of false-positive p53 IHC (i.e. p53abn IHC with no detectable *TP53* mutation), and all four were serous carcinomas, an EC histotype characterized by *TP53* mutations. In 3 of these tumours there was complete absence of p53 expression. Tumours with complete absence of p53 staining harboured a mutation in only 13/16 (81%) cases, which is lower compared to other patterns of mutant p53 IHC (81/82; 98.8%). In our previous study using the same sequencing approach we reached a specificity of 100% as every tumour with mutant pattern expression had a detectable mutation. In EC, we may speculate on possible explanations for the absence of protein expression with no detectable mutation. Firstly, this could be the result of differences in the tumour cellularity in the sections used for IHC and for extracting DNA. Secondly, this could be related to difficulties in detection of stop gained/splicing mutations and large deletions or insertions (more than 30bp) using the amplicon deep sequencing method we applied to all samples, especially in endometrial biopsy samples where there may be large numbers of admixed normal cells. Alternatively, it is possible that alterations in non-coding regulatory regions, *MDM2* amplification or other as yet undetermined mechanisms may account for p53 loss in some EC, without underlying *TP53* mutation, even though such a phenomenon was not identified in tubo-ovarian high-grade serous carcinoma. Thus, it is possible that in half or more of the 6 cases of EC with discrepant IHC and mutational assessment, the IHC may more accurately reflect p53 functional status in the tumour.

Although subclonal p53 staining has been recognized as an uncommon finding, it was found to be significant in EC (5%). The association with *POLE*mut in 3 cases and MMRd in one indicates firstly that a subclonal p53 IHC pattern suggests an underlying MMRd or a *POLE*mut tumour; all these cases were of endometrioid histotype. Thus, many instances of subclonal p53 immunostaining are attributable to MMRd or *POLE*mut, and the subclonal nature of expression likely reflects the acquisition of *TP53* mutations as a later event during tumour progression. Further, independent data showing that the outcome is driven by the underlying MMRd or *POLE*mut status suggests that these subclonal mutations will not impact on molecular subtype assignment, as these EC would be considered MMRd or *POLE*mut, respectively. The mutant staining areas within this group were focal and the fact that no *TP53* mutation was detected in these tumours may be attributable to the lack of targeting of the small mutant areas. The second group of MMR proficient/POLE wildtype tumours (n=5) contained two carcinomas with 2 mutant patterns suggesting two independent subclones or even primaries. The other 3 cases suggest the possibility that rare EC can acquire a *TP53* mutation as part of tumour progression, in the absence of MMRd or *POLE*mut. Importantly, the tumours with subclonal p53 expression accounted for some of the inter-laboratory disagreements in p53 IHC results, and this issue is much reduced if MMR immunostaining and *POLE* extranuclease domain sequencing are done as part of the molecular categorization, as many EC with p53 heterogeneity belong to the MMRd or *POLE*mut molecular subtypes. The remaining MMR proficient/*POLE* wildtype EC with subclonal staining patterns had demonstrable *TP53* mutations in most cases; although we have no information about the natural history of such tumours, the serous histology and presence of p53abn IHC/*TP53* mutation supports classifying these tumours with the p53abn molecular subtype of EC at present. However, future studies are needed to establish whether this is the correct approach.

Histotype strongly correlated with p53abn IHC and the presence of *TP53* mutations, as expected (30). Apart from a single example each of MMRd and *POLE*mut EC diagnosed morphologically as serous, in which *TP53* mutations were not detected, all serous carcinomas showed p53abn immunostaining (63/63, 100%), and 59/63 contained *TP53* mutations. The four serous carcinomas with p53abn IHC and no mutation detected are the 4 false-negative cases discussed above. However, p53abn is not specific for serous carcinoma because a significant portion of endometrioid, particularly grade 3, also harboured a mutation. There were 4 MMR proficient/*POLE* wildtype low-grade endometrioid EC showing p53abn immunostaining and harbouring *TP53* mutations; such cases have recently been reported to show poor survival, further supporting the importance of the *TP53* status even in apparent low-grade cases (31). Notably 5 clear cell carcinomas were included in this study, 4 of which showed concordant p53abn IHC and *TP53* mutation; this is considered a rare and poorly reproducible histotype of EC (21) and its heterogeneous molecular nature is just beginning to be unravelled, with roughly half of cases reported to show p53abn IHC or *TP53* mutation (32).

An important observation was the fact that consensus in interpretation was not achieved in 5 cases, based on local p53 IHC compared with the centrally stained slide, illustrating the importance of optimized and quality assured p53 IHC protocols with adequate attention to controls and external proficiency testing, as has been previously emphasized (33) (3). *TP53* sequencing is valuable in these difficult cases with equivocal p53 IHC staining, but these are uncommon if an optimized protocol is used. p53 IHC is more accessible, less expensive, quicker and performs with comparable accuracy in EC biopsies, compared to *TP53* sequencing. It can also be performed on smaller samples, with fewer failed analyses, an important consideration in assessment of endometrial biopsy specimens.

**Conclusions**

This study confirms that p53 IHC is an accurate predictor of *TP53* mutation status in endometrial carcinoma, similar to results obtained in ovarian carcinoma. There was excellent agreement between p53 protein expression as determined by IHC and *TP53* mutation status, based on endometrial biopsy specimens, if MMRd and *POLE*mut EC were excluded from consideration (95% agreement). There was also excellent agreement in inter-laboratory assessment of p53 IHC. p53 IHC is widely available and inexpensive, compared to sequencing, and also has a short turnaround time allowing the results to be incorporated in the surgical pathology report for the endometrial biopsy specimen. It also is applicable to samples with small numbers/low percentages of tumour cells. For these reasons, p53 IHC currently has significant advantages over sequencing in testing for the CN-high, serous-like molecular subtype of EC. *TP53* sequencing is valuable in the minority of cases presenting difficulties in p53 IHC interpretation.

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**Author contributions statement**

Study conception and design, NS, CBG, MK, and JDB. Study set-up, funding and approvals, NS. Case selection and review, NS, MJL, BR, CBG, TB, MK, and CBG. Immunohistochemistry central review, MK. Molecular analysis: AP and JDB. Data analysis, JDB, MK, and NS. Manuscript preparation, all authors.

**List of Abbreviations [EdQ; please treat Abstract and remaining text separately and ensure that abbreviations are given in full at first use. Repetition in the table footnotes is OK]**

CCC: Clear cell carcinoma

EC: Endometrial carcinoma

EEC: Endometrioid endometrial carcinoma

G1/2/3: Grade 1/2/3

IHC: Immunohistochemistry

MMR: DNA Mismatch repair protein

MMRd: Mismatch repair deficient

p53abn: Abnormal or mutant pattern p53 IHC

*POLE*: gene encoding DNA polymerase ε

*POLE*mut: Case harbouring pathogenic variant in *POLE* exonuclease domain

*TP53*mut: Case harbouring pathogenic mutation in *TP53*

USC: Uterine serous carcinoma

**Figure legends**

**Figure 1.** Expression patterns of p53 immunostaining. (A–C) examples of p53abn overexpression, complete absence, and cytoplasmic patterns. (D) an example of normal (wildtype) expression.

**Figure 2.** Subclonal p53 immunostaining. (A,B,D) cases showing a combination of normal (wildtype) and p53abn expression (overexpression in A and D; cytoplasmic in B). (C) an example of two p53abn patterns, overexpression, and complete absence.

**Table 1.** Case characteristics

|  |  |  |  |
| --- | --- | --- | --- |
| Histotype of endometrial carcinomaa | Total, N | N for paired local and central p53 IHCb | N for paired central p53 IHC and *TP53* sequencing resultsc |
| Serous | 72 | 65 | 67 |
| Endometrioid, grade 3 | 32 | 18 | 25 |
| Endometrioid, grade 2 | 26 | 21 | 21 |
| Endometrioid, grade 1 | 49 | 38 | 40 |
| Clear cell | 6 | 3 | 5 |
| Carcinosarcoma | 11 | 10 | 8 |
| Mixed | 10 | 8 | 10 |
| Undifferentiated | 1 | 1 | 1 |
| TOTAL | 207 | 164 | 177 |

aNo central review of H&E sections was undertaken

bCases from the central reference laboratory (University of Calgary, n=43) were excluded from the comparative analysis of local versus central p53 IHC

cCases with insufficient tumour (n=6) and those that failed sequencing (n=24) were excluded from analysis of *TP53* sequencing versus p53 IHC

**Table 2.** Results of local and central p53 IHC interpretation: (A) before and (B) after consensus re-evaluation of discordant cases.

A

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | CENTRAL |  |
|  |  | Normal | Abnormal-OE | Abnormal -CA | Abnormal -CY | Subclonal | TOTAL |
| LOCAL | Normal | **67** | 1 | 0 | 1 | 3 | 72 |
| Abnormal -OE | 2 | **68** | 1 | 1 | 0 | 72 |
| Abnormal -CA | 1 | 0 | **14** | 1 | 0 | 16 |
| Abnormal -CY | 0 | 0 | 0 | **1** | 0 | 1 |
| Subclonal | 0 | 0 | 0 | 0 | **3** | 3 |
|  | TOTAL | 70 | 69 | 15 | 4 | 6 | 164 |

B

|  |  |  |  |
| --- | --- | --- | --- |
|  |  | CENTRAL AFTER CONSENSUS EVALUATION |  |
|  |  | Normal | Abnormal - OE | Abnormal - CA | Abnormal - CY | Subclonal | No consensus | TOTAL |
| LOCAL AFTER CONSENSUS EVALUATION | Normal | **68** | 0 | 0 | 0 | 0 | 1 | 69 |
| Abnormal - OE | 1 | **69** | 0 | 1 | 0 |  | 71 |
| Abnormal - CA | 0 | 0 | **14** | 0 | 0 |  | 14 |
| Abnormal - CY | 0 | 0 | 0 | **1** | 0 |  | 1 |
| Subclonal | 1 | 0 | 0 | 0 | **4** |  | 5 |
| No consensus | 1 |  |  | 1 | 2 |  | 4 |
|  | TOTAL | 71 | 69 | 14 | 3 | 6 | 1 | 164 |

OE= Overexpression

CA=Complete absence

CY=Cytoplasmic

**Bold font**=cases showing complete concordance between local and central evaluation following consensus review

|  |
| --- |
| [EdQ: please explain use of **bold** font] |

**Table 3.** Details of review of locally and centrally stained p53 slides for the 11 discordant cases

|  |  |  |  |
| --- | --- | --- | --- |
| Histotype | Local p53 IHC | Central p53 IHC | Final agreement(local versus central) |
|   | Original | Review (majority) | Original | Review (majority) |   |
| USC | Mutant (over expression) | Subclonal | Mutant (complete absence) | Subclonal | Concordant |
| CCC | Wild type | Wild type | Mutant (cytoplasmic) | Wild type | Concordant |
| EEC | Wild type | Mutant (over expression) | Mutant (over expression) | Mutant (over expression) | Concordant |
| EEC | Wild type | No majority | Subclonal  | Subclonal | No consensus in interpretation |
| EEC | Wild type | No majority | Subclonal  | Subclonal  | No consensus in interpretation |
| EEC | Wild type | Wild type | Subclonal | No majority | No consensus in interpretation |
| Mixed E and S | Mutant (complete absence) | No majority | Mutant (cytoplasmic) | Mutant (cytoplasmic) | No consensus in interpretation |
| EEC | Mutant (over expression) | No majority | Normal; wild type |  Normal; wild type | No consensus in interpretation |
| USC | Mutant (over expression) | Mutant (over expression) | Mutant (cytoplasmic) | Mutant (cytoplasmic) | Discordant regarding specific mutant pattern |
| USC | Mutant (complete absence) | Subclonal  | Normal; wild type | Wild type | Discordant regarding subclonal |
| Undiff | Mutant (over expression) | Mutant (over expression) | Normal; wild type | Normal; wild type | Discordant regarding binary interpretation |

CCC=clear cell carcinoma

EEC=endometrioid endometrial carcinoma

Mixed E and S=mixed endometrioid and serous carcinoma

USC=uterine serous carcinoma

Undiff=undifferentiated carcinoma

**Table 4.** Comparison of p53 IHC and *TP53* mutation status

|  |  |  |
| --- | --- | --- |
| **Reference p53 IHC** | **Deleterious *TP53* Mutation** | **TOTAL** |
| **Present** | **Absent** |
| All | **Excluding MMRd and *POLE*mut** | All | **Excluding MMRd and *POLE*mut** | All | **Excluding MMRd and *POLE*mut** |
| Wild type | 9 (9%) | **2 (2%)** | 66 (94%) | **32 (89%)** | 75 (45%) | **34 (28%)** |
| Mutant (overexpression) | 71 (72%) | **67 (77%)** | 1 (1%) | **1 (3%)** | 72 (43%) | **68 (55%)** |
| Mutant (complete absence/null) | 13 (13%) | **13 (15%)** | 3 (4%) | **3 (8%)** | 16 (10%) | **16 (13%)** |
| Mutant (cytoplasmic) | 5 (5%) | **5 (6%)** | 0 (0%) | **0 (0%)** | 5 (3%) | **5 (4%)** |
| TOTAL | 98 | **87** | 70 | **36** | 168 | **123** |
| Subclonal | 4 | **4** | 5 | **1** | 9 (5%) | **5** |

**Bold font**=Results of p53 IHC and *TP53* mutation after exclusion of MMRd and *POLE*mut cases, ie performance of p53 IHC as a surrogate for *TP53* mutation when utilised as part of an algorithm17.

[EdQ: please explain use of **bold** font]

**Table 5.** Comparison of p53 IHC and *TP53* mutation status in: (A) all tumours and (B) with exclusion of MMRd and *POLE*mut tumours.

A

|  |  |  |
| --- | --- | --- |
|  |  | **TP53 mutation** |
|  |  | Present | Absent |
| **IHC** | **Abnormal** | 89 | 4 |
| **Normal/ wild type** | 9 | 66 |
| Sensitivity: 90.82 (95% CI 83.28% to 95.71%) |
| Specificity: 94.29% (95% CI 86.01% to 98.42%) |
| Accuracy: 92.26% (95% CI 87.13% to 95.82%) |

B

|  |  |  |
| --- | --- | --- |
|  |  | **TP53 mutation** |
|  |  | Present | Absent |
| **IHC** | **Abnormal** | 85 | 4 |
| **Normal/ wild type** | 2 | 32 |
| Sensitivity: 97.70% (95% CI 91.94% to 99.7%) |
| Specificity: 88.89% (95% CI 73.94% to 96.89%) |
| Accuracy: 95.12% (95% CI 89.68% to 98.19%) |

**Table 6.** Results of p53 IHC, *TP53*, MMR and *POLE* status for different histotypes

|  |  |  |  |
| --- | --- | --- | --- |
|   |   | ***TP53* mutation present** |  ***No TP53* mutation detected** |
|   |   | MMRp, *POLE*wt | MMRd, *POLE*wt | MMRp, *POLE*mut | MMRp, *POLE*wt | MMRd, *POLE*wt | MMRn, *POLE*mut |
| **Histotype** | **p53 IHC** |   |   |   |   |   |   |
| USC (n=67) | Wild type IHC pattern |   |   |   |   | 1 | 1 |
| Mutant IHC pattern | 59 |   |   | 4 |   |   |
| Subclonal | 2 |   |   |   |   |   |
| EEC G1 (n=40) | Wild type IHC pattern | 1 |  2 | 1 | 15 | 17 |  |
| Mutant IHC pattern | 3 |   |   |   |   |   |
| Subclonal |   |   |   | 1 | 1 | 1 |
| EEC G2 (n=21) | Wild type IHC pattern |   | 2 |   | 8 | 7 |   |
| Mutant IHC pattern | 1 |   | 1 |   |   |   |
| Subclonal |  1 |   |  |   |   | 1 |
| EEC G3 (n=25) | Wild type IHC pattern |   |   | 2 | 7 | 7 | 1 |
| Mutant IHC pattern | 7 |   |   |   |   |   |
| Subclonal |   |   |   |   |   | 1 |
| Mixed USC-EEC (n=10) | Wild type IHC pattern |   | 1 |   |   | 1 |   |
| Mutant IHC pattern | 4 | 3 |   |   |   |   |
| Subclonal | 1 |   |   |   |   |   |
| CaSa (n=8) | Wild type IHC pattern | 1 |   |   |   |   |   |
| Mutant IHC pattern | 7 |   |   |   |   |   |
| CCC (n=5) | Wild type IHC pattern |   |   |   | 1 |   |   |
| Mutant IHC pattern | 4 |   |  |   |   |   |
| Undiff (n=1) | Wild type IHC pattern |   |   |   | 1 |   |   |
| Mutant IHC pattern |   |   |   |   |   |   |

MMRp=Mismatch repair proficient

MMRd=Mismatch repair defective

*POLE*wt=*POLE* wild type

*POLE*mut=*POLE* mutant (harbouring pathogenic variant in *POLE* extranuclease domain)

CCC=clear cell carcinoma

EEC=endometrioid endometrial carcinoma

USC=uterine serous carcinoma

Undiff=undifferentiated carcinoma

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SUPPLEMENTARY MATERIAL ONLINE

**Table S1.** Laboratory protocols for p53 Immunohistochemistry

**Table S2.** Clinicopathological and *TP53* mutation data for 177 cases included in p53 IHC versus *TP53* mutation analysis **[EdQ: the file appears to rely on external links - please ensure that none exist]**

**Table S3.** Concordance of p53 IHC (central interpretation after consensus re-evaluation) with: (A) *TP53* mutation status; (B) *TP53* mutation status, excluding dMMR and POLE EDM cases.