

Interpretation of p53 Immunohistochemistry In Tubo-Ovarian Carcinoma: Guidelines for Reporting

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Background

- p53 immunohistochemistry (IHC) is an accurate, cheap and fast method to investigate *TP53* mutation status [1].
- Historically, p53 IHC has been interpreted as negative or positive based on the percentage of stained tumor cell nuclei using variable cut-offs which range from 5 to 50%.
- More recently, a tripartite interpretation has been suggested [2], wherein an “overexpressed or no expression (all or nothing)” nuclear staining pattern is highly predictive of underlying *TP53* mutation while a normal/wild type pattern is not.
- Of note, rare cases with *TP53* mutation rarely show cytoplasmic staining, and, about 5% cases have a wild type pattern [1].
- As a result p53 IHC can be interpreted as either “Normal” or “Abnormal”, as shown below.

p53 immunohistochemistry pattern and interpretation

Pattern	p53 IHC Interpretation	<i>TP53</i> mutation type	% in HGSC
<i>TP53</i> MUTATION ABSENT			
Wild type	Normal	No mutation	0
<i>TP53</i> MUTATION PRESENT			
Overexpression	Abnormal	Non-synonymous (missense); also in-frame deletion, splicing	66%
Complete absence/null	Abnormal	Indels, stopgains, splicing mutations	25%
Cytoplasmic	Abnormal	Indels and stopgains with disruption of the nuclear localization domain	4%
Wild type	Normal*	Truncating mutation	5%

HGSC- high-grade serous carcinoma

Guidance document: p53 IHC reporting in tubo-ovarian carcinoma version 1.0, dated October 2016



- “Normal” indicates that there is no evidence of an underlying *TP53* mutation, i.e. *TP53* is wild type
 - *exception for normal: truncating mutations can be associated with wild type pattern (see note)
- “Abnormal” indicates that there is evidence of *TP53* mutation.
- Interpretation as negative/positive is ambiguous and therefore **strongly** discouraged.

NOTE: High-grade serous carcinomas (HGSC) ubiquitously (i.e. 100%) harbor *TP53* mutations but only 95% show abnormal p53 staining. In other words, 5% of HGSC show normal p53 immunohistochemistry (wild type pattern) but still harbor a *TP53* mutation [1]. These are truncating mutations, which are characterized by a late stopgain resulting in translation of truncated protein, which can be detected by most N-terminal directed antibodies.

Wild type pattern (Normal p53 IHC)

- The distribution of nuclear staining in a 'wild type' pattern ranges from a few positive cells to almost all cells staining, but with **variable intensity**.
- In general, the intensity and extent of nuclear staining is associated with the proliferation index, eg, basal keratinocytes of normal skin or epithelial cells of colonic crypts bases show variable p53 staining while the mitotically inactive superficial keratinocytes or luminal colonic epithelial cells are negative.
- Stromal fibroblasts and intratumoral lymphocytes also show wild type pattern and are used as intrinsic control (see examples below).

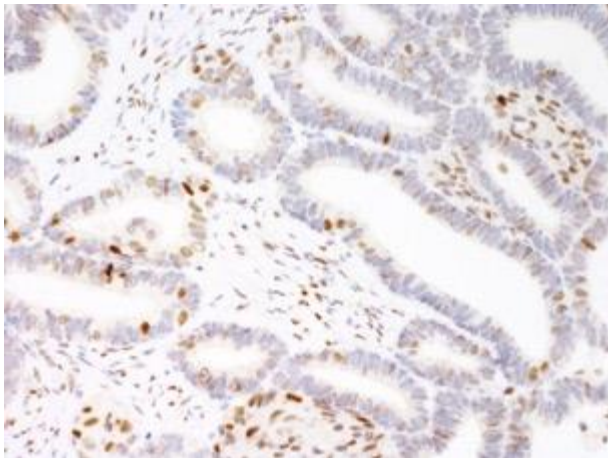


Figure 1a. Wild type pattern in an endometrioid carcinoma with the majority of tumor cells being negative but some showing **variable intensity** (only few strong).

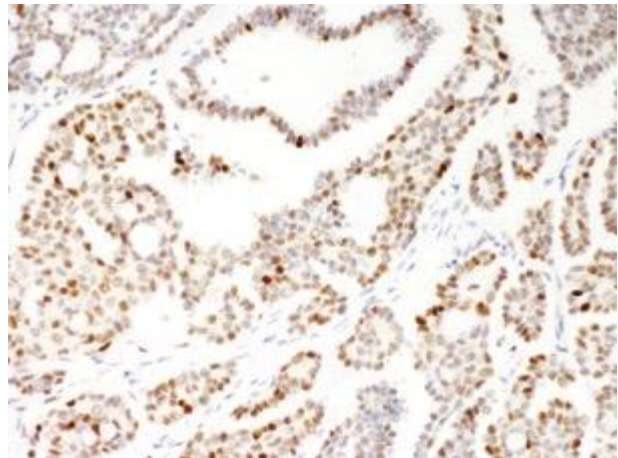


Figure 1b. Wild type pattern in a low-grade serous carcinoma with >80% of tumor cell nuclei staining with **variable intensity** (only few strong). The staining is of similar intensity to that in germinal centers (left Fig. 2).

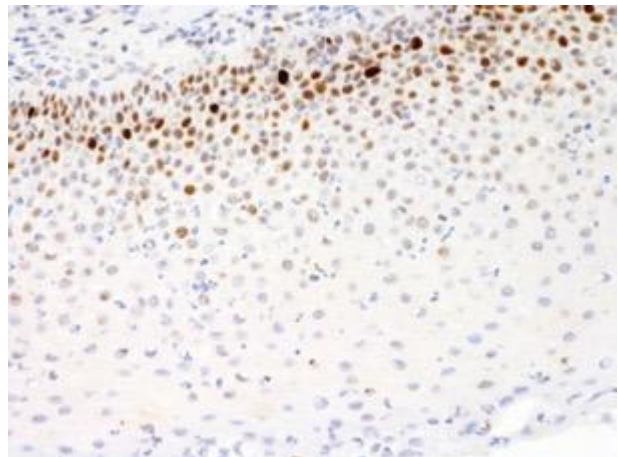
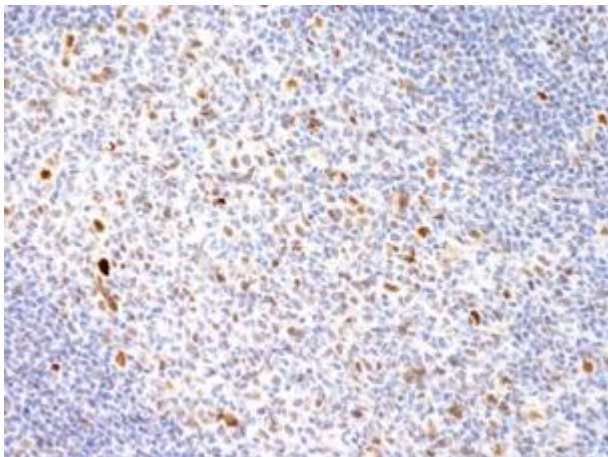


Figure 2a. Wild type pattern in a germinal center B-cells

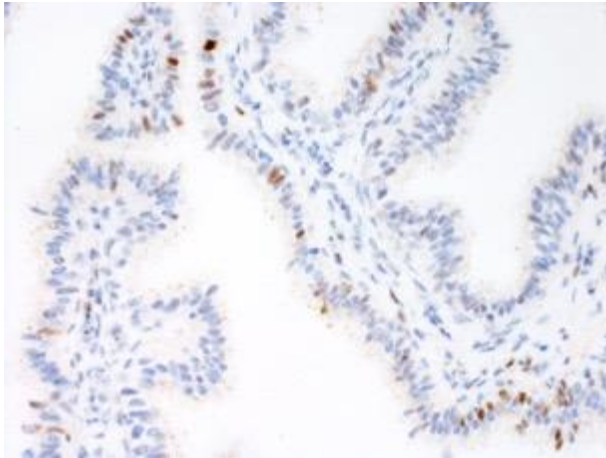


Figure 2b. Wild type pattern in normal squamous epithelium

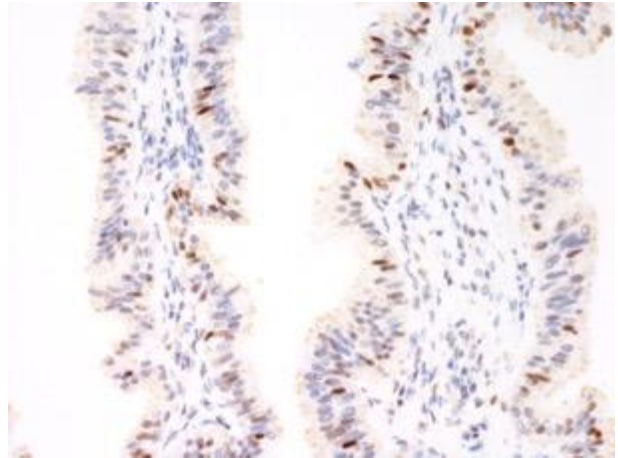


Figure 3. Wild type pattern in fallopian tubes

Overexpression (Abnormal p53 IHC)

- **Overexpression is defined as strong nuclear staining in at least 80% of tumor cell nuclei** (usually 100% tumor cell nuclei, the 80% cut-off accounts for technical artifacts and the rare mosaic pattern, see below).
- Overexpression is the most common (~66%) pattern observed in HGSC, almost certainly signifying an underlying *TP53* mutation.
- p53 overexpression is strongly associated with nonsynonymous (or missense) *TP53* mutations, however, in-frame deletions and splicing mutations can also result in this pattern.
- These mutations interfere with MDM2 mediated ubiquitination resulting in massive nuclear accumulation of the p53 protein.

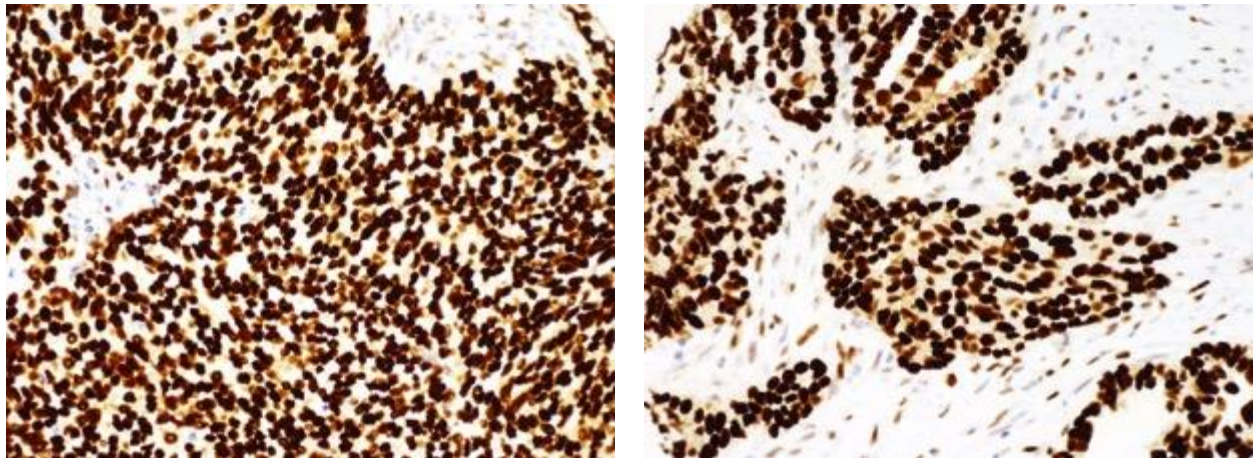


Figure 3. Overexpression seen in two high-grade serous carcinomas. Virtually all tumor cell nuclei show strong staining intensity compared to the intrinsic control (stromal fibroblasts).

Complete absence or null pattern (Abnormal)

- **Complete absence is defined as no staining in tumor cell nuclei in the presence of 'wild-type', ie variable, staining in normal background cells (intrinsic control present).** The interpretation is similar to that of DNA mismatch repair gene protein expression.
- Complete absence is observed in ~25% of HGSC.
- Complete absence is almost certainly indicative of underlying *TP53* mutation.
- The type of mutation includes indels, stopgains and splicing mutations.
- The former two result in a premature stopgain, in which the shorter mRNA is subjected to nonsense mediated decay resulting in no mRNA and therefore no translated protein.

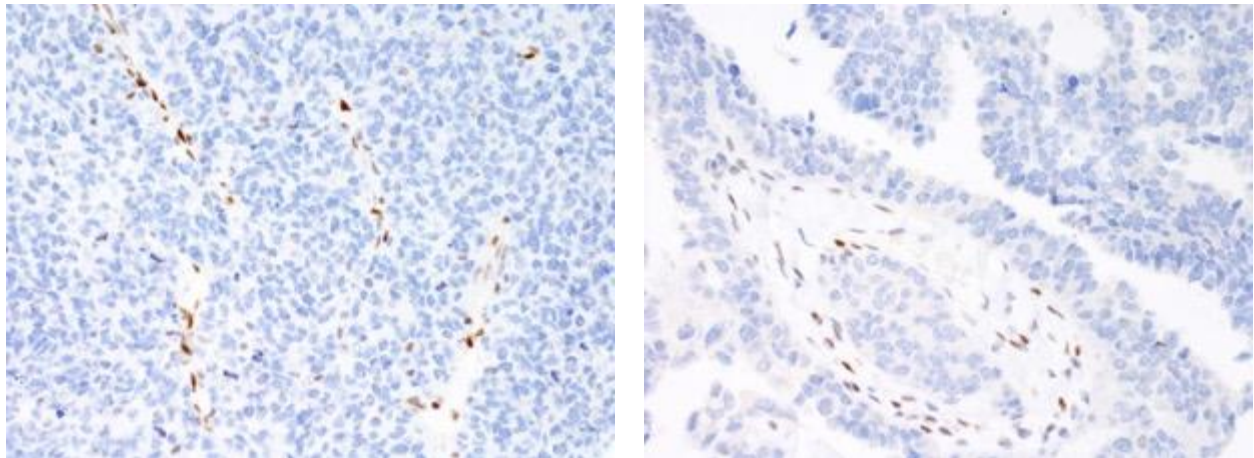


Figure 4. Complete absence in two high-grade serous carcinomas. No staining in tumor cell nuclei but the intrinsic stromal nuclear positive control is clearly visible.

Cytoplasmic (Abnormal)

- **Cytoplasmic staining is defined as predominant cytoplasmic staining in the absence of strong nuclear staining in >80% of tumor cell nuclei, i.e. the nuclear staining can be absent or weak and variable.**
- The cytoplasmic pattern is seen in ~4% of high-grade serous carcinomas and is associated with underlying *TP53* mutation.
- The types of mutation include indel and stopgain with disruption of the nuclear localization domain.

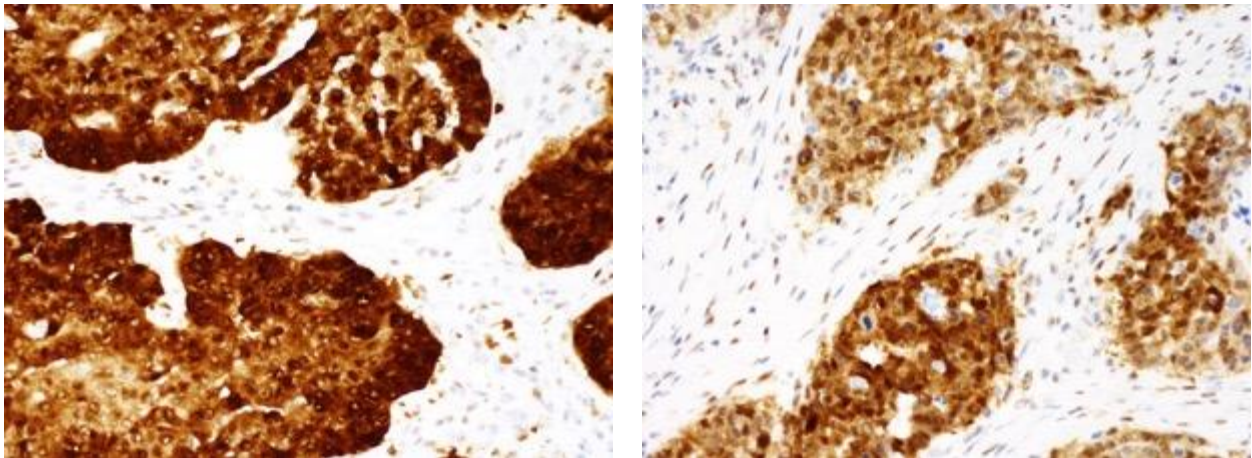


Figure 5. Cytoplasmic staining seen in two high-grade serous carcinomas. Note strong to moderate cytoplasmic staining seen in the epithelial compartment but not in the intrinsic control (stromal compartment).



Problematic Areas in p53 Interpretation

- For diagnostic purposes it is currently more important to distinguish between presence and absence of the *TP53* mutation, i.e. the distinction of normal versus abnormal p53 immunohistochemistry.
- The specific abnormal pattern may become important in the future with p53-targeted therapies entering clinical trials.
- Herein, we focus on interpretational issues that commonly arise in the distinction between normal and the three abnormal patterns:
 1. Wild type pattern versus overexpression
 - a. “High” wild type staining due to high proliferation/strong staining
 - b. “Low” overexpression due to antigen degradation, splicing mutations, or weak staining
 - c. Heterogeneous staining
 2. Wild type pattern versus complete absence
 - a. “Low” wild type pattern due to low proliferation, antigen degradation, or weak staining
 - b. Lack intrinsic control
 - c. Occasional tumor cell staining in otherwise complete absence pattern
 - d. True p53 IHC wild type high-grade serous carcinomas
 3. Wild type pattern versus cytoplasmic
 - a. Threshold for diagnosis of cytoplasmic staining is not well established
 - b. Recognition of spurious cytoplasmic staining

1 - Wild type pattern versus overexpression (Fig. 6)

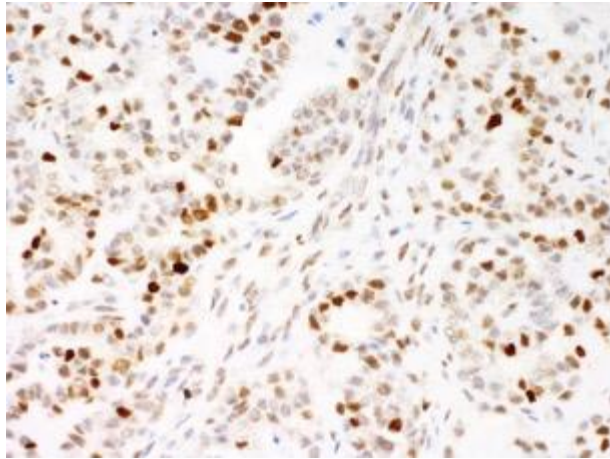


Figure 6a. Reference wild type pattern

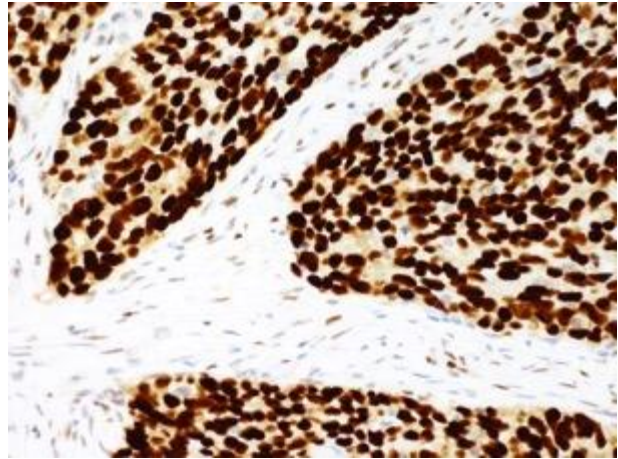
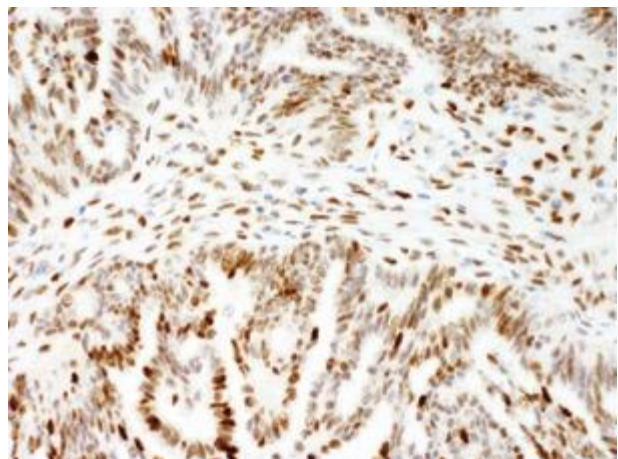
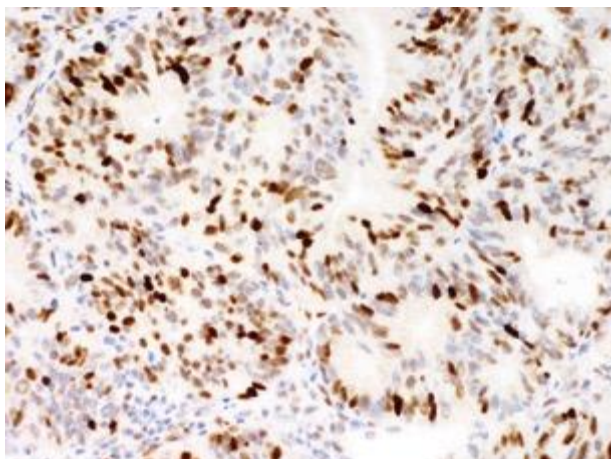


Figure 6b: Reference overexpression

1a. “High” wild type staining due to high proliferation or strong staining (Fig. 7a)

- Staining of overall strong intensity results in a larger proportion of nuclei being strongly stained, with potential for a ‘wild type’ pattern to be interpreted as overexpression.
- Staining remains variable and <80% nuclei should show strong staining.
- The level of staining in the intrinsic control should serve as a rough guide.

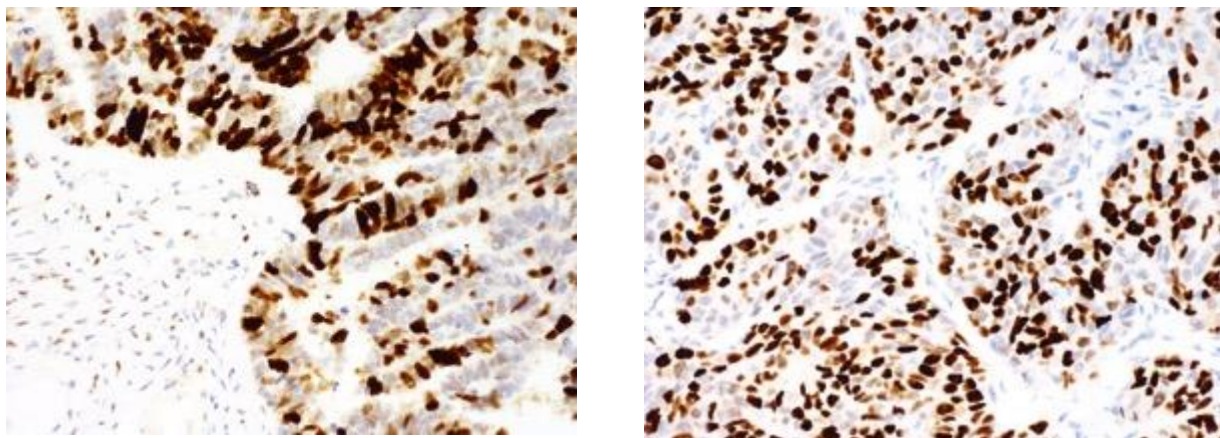
Figure 7a. “High” wild type pattern in an endometrioid and low-grade serous tumor. The staining shows variable intensity. Note, only a minority of nuclei are strongly stained. The intrinsic control in the case on the right is indicating strong staining.



1b. “Low” overexpression due to antigen degradation, splicing mutations, or weak staining (Fig. 7b)

- Staining of overall weak intensity results in a smaller proportion of nuclei being strongly stained, with potential for overexpression to be interpreted as a ‘wild type’ pattern.
- The majority of nuclei stain strongly but a significant proportion stain weakly or are negative
- >80% nuclei should show strong staining.
- The level of staining in the intrinsic control should serve as a rough guide.

Figure 7b. “Low” overexpression in two high-grade serous carcinomas appearing as so called “mosaic” pattern. The majority of nuclei staining strongly but a significant proportion being negative (approaching 80% cut-off)



1c. Heterogeneous staining (Fig 8)

- Heterogeneous staining is defined by the presence of more than one pattern.
- It is rarely seen (<3%) in high-grade serous carcinomas, since *TP53* mutations are an early ancestral event and should be present in all tumor cells [3].
- This is most likely due to poor antigen preservation (i.e. delayed fixation), however, alternative explanations, such as underlying splicing mutation with different effects on p53 expression in different cells of the same tumor cannot not be excluded.
- In contrast, *TP53* mutations are acquired during progression of other histotypes such as mucinous carcinoma [4]; here, heterogeneous p53 expression can be expected and

should be reported as such, e.g. p53 expression abnormal (heterogeneous with areas of overexpression and wild type pattern).

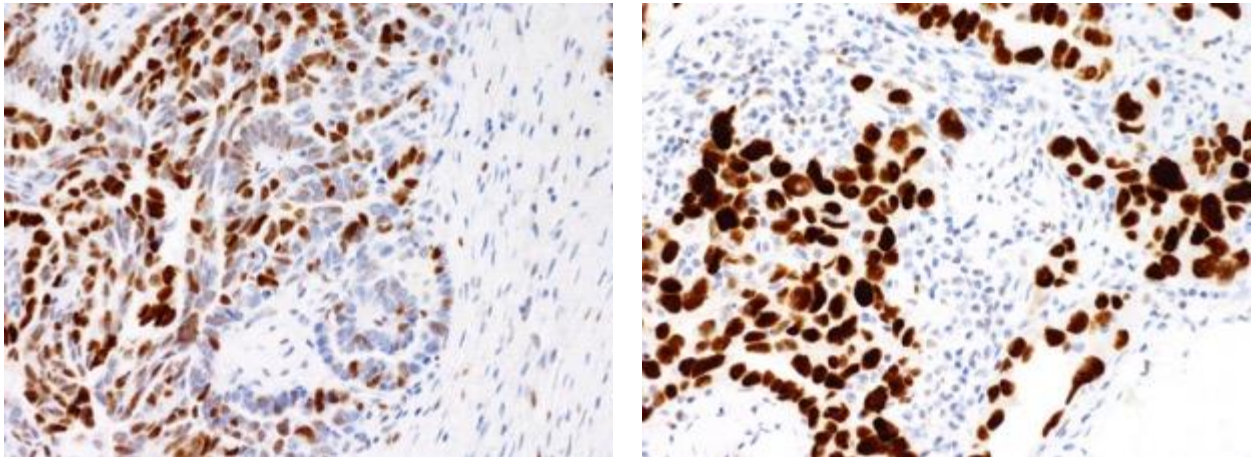


Figure 8. Heterogeneous staining in the same high-grade serous carcinomas with an area bordering wild type pattern (left) and an area of typical overexpression (right).

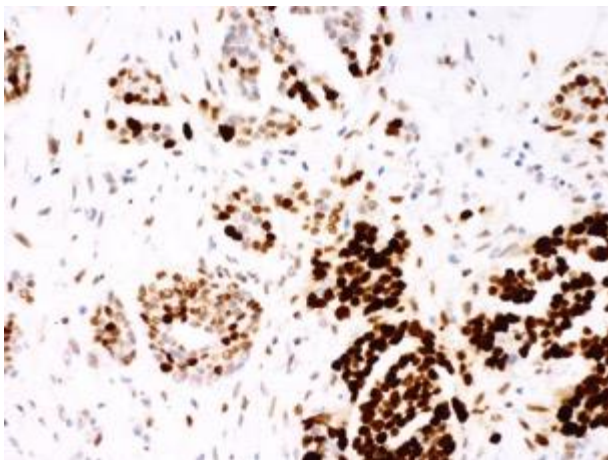


Figure 9a. High-grade serous carcinoma with heterogeneous staining: lower right = overexpression, upper left = wild type pattern.

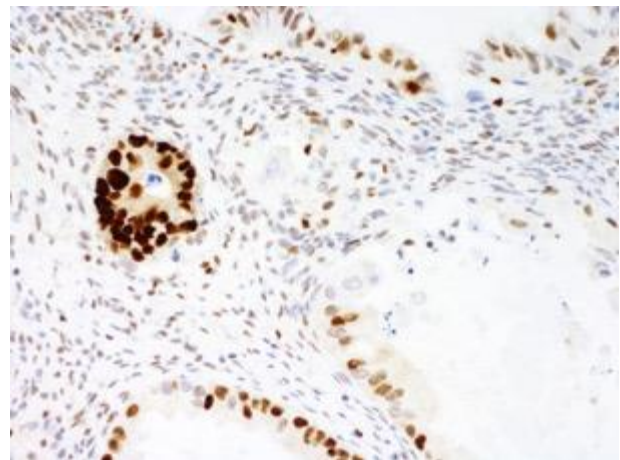


Figure 9b. Mucinous carcinoma with heterogeneous staining.

2. Wild type pattern versus complete absence (Figure 10)

- Staining of overall weak intensity can result in absent nuclear staining in a large proportion or all nuclei, with potential for a 'wild type' pattern to be interpreted as complete absence.
- The level of staining in the intrinsic control should serve as a rough guide.
- A complete absence pattern should *not* be reported in the absence of a positive intrinsic control of stromal cells and lymphocytes with variable but discernible nuclear staining.
- Examples are illustrated below in Figures 11-14.

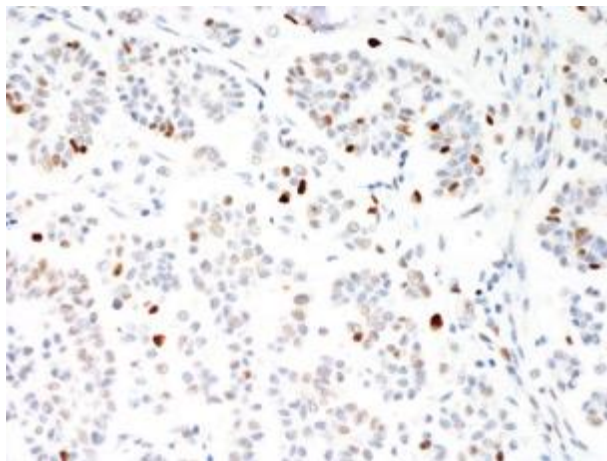


Figure 10a: Reference wild type pattern

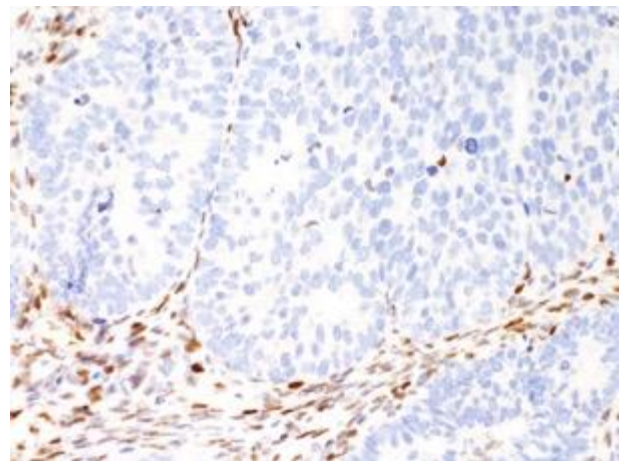


Figure 10b. Reference complete absence

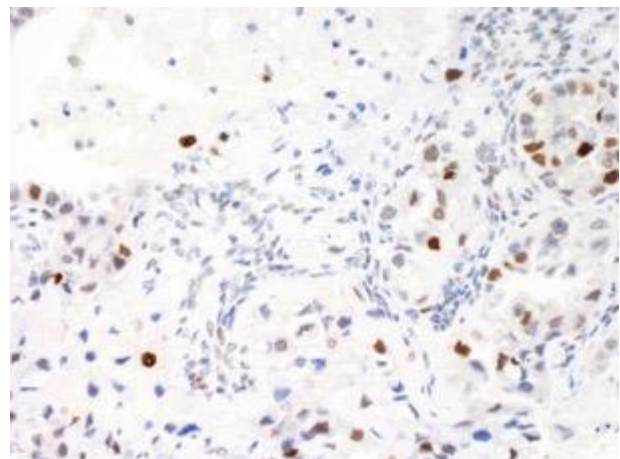
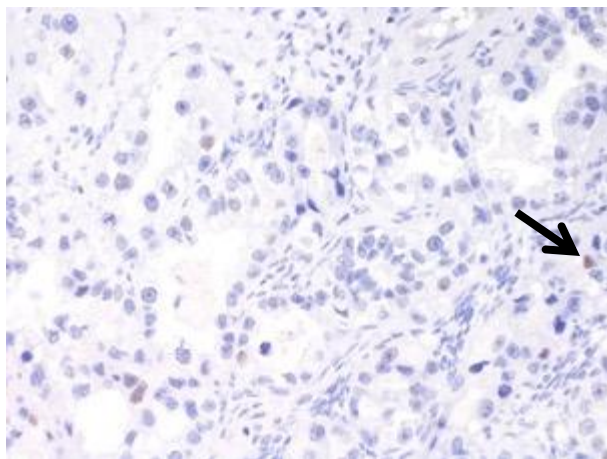


Figure 11 The same case of clear cell carcinoma stained on two platforms. Left: occasional tumor cell nuclei weakly stained (arrow) consistent with wild type pattern. Right: stronger staining with more positivity but variable intensity.

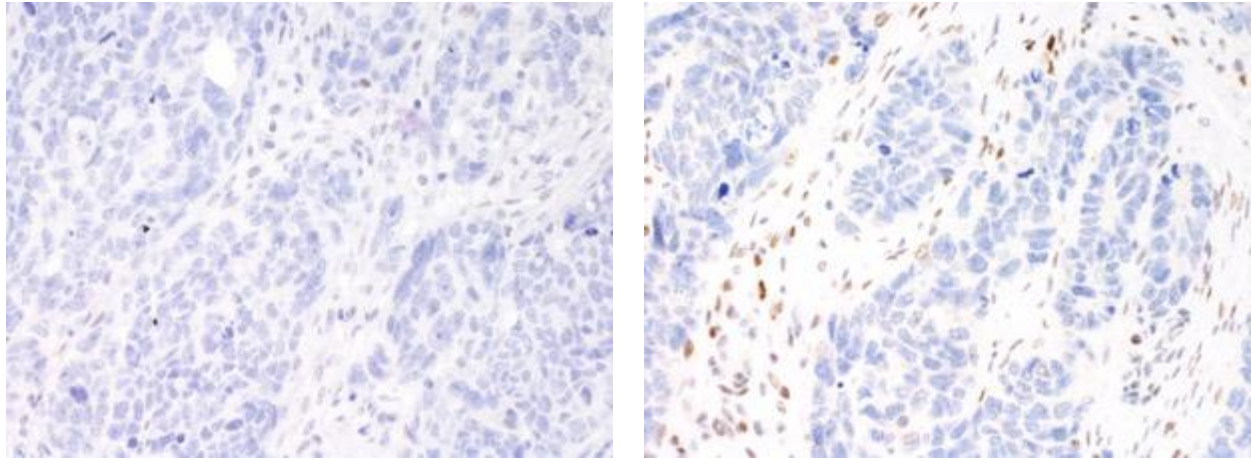


Figure 12. Same high-grade serous carcinoma stained on two platforms. Left, there is no positive internal control, case is non interpretable, right, clear variable staining of internal control clearly interpretable as complete absence

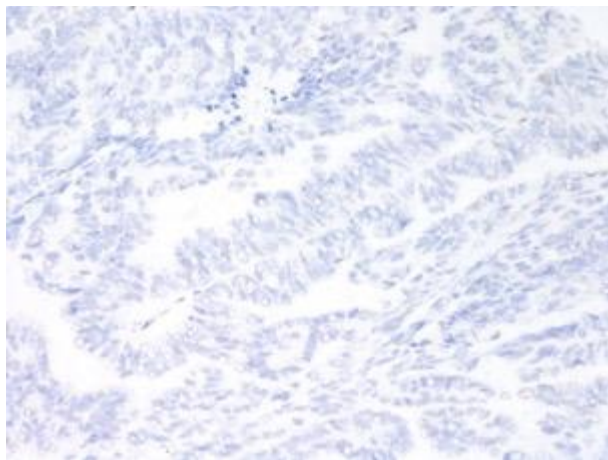


Figure 12a. Non-interpretable due to lack of internal control

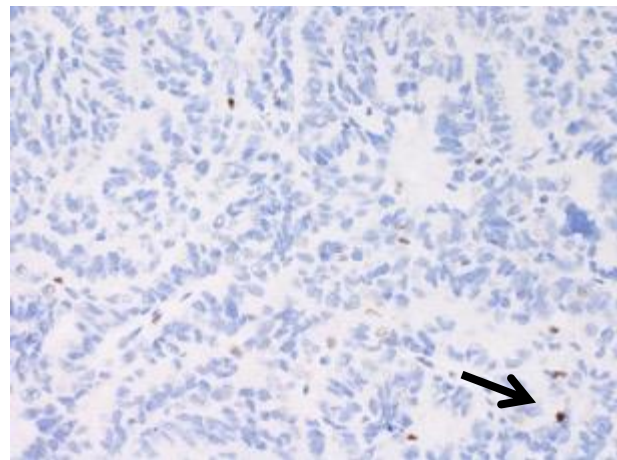


Figure 12a. Complete absence with scant internal control (arrow)

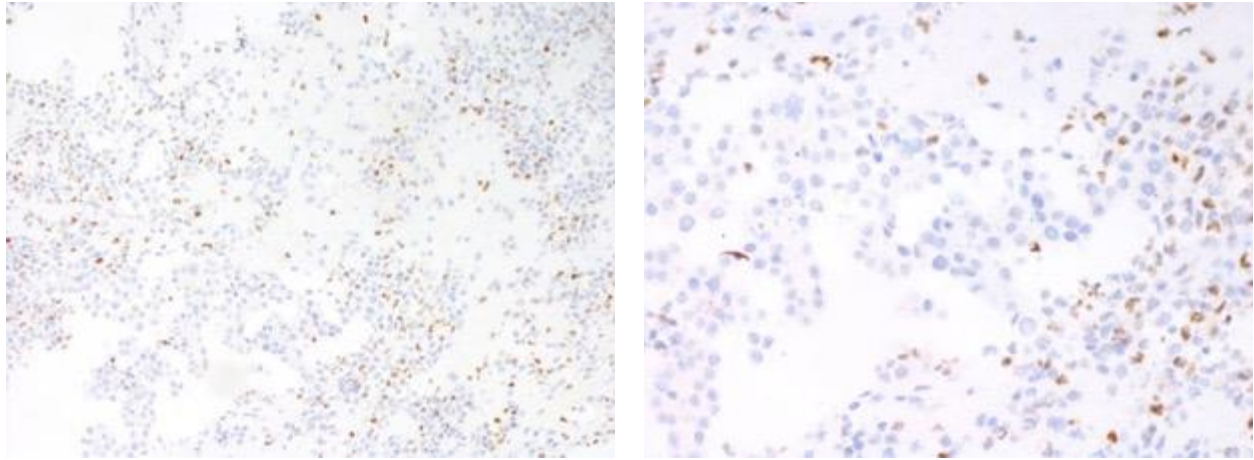


Figure 13. Complete absence in high-grade serous carcinoma from cell block. Left: low power (10x) showing abundance of normal inflammatory cells giving the impression of wild type pattern but on high power (20x, right), the actual tumor cells are completely negative.

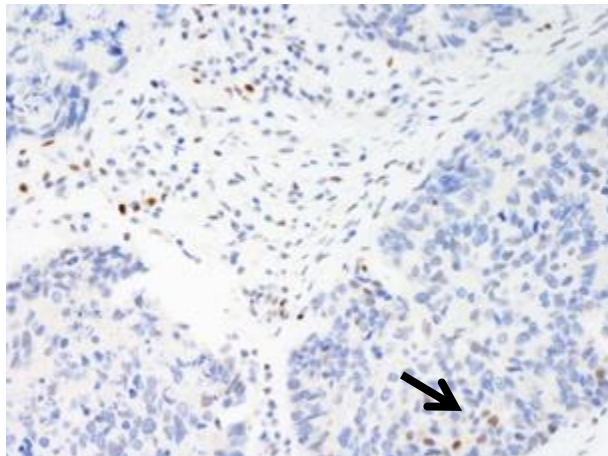


Fig. 14a Occasional tumor cells (arrow) staining in an otherwise complete absence high-grade serous carcinoma. This may be due to non-specific staining and is to be distinguished from the right.

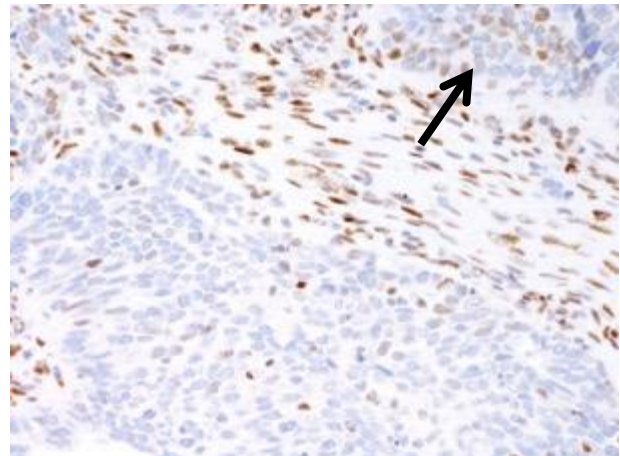


Fig. 14a Occasional tumor cells (arrow) staining in a high-grade serous carcinoma can also be interpreted as wild type pattern. Note, 5% of high-grade serous carcinomas (those with truncating mutations) show p53 wild type pattern.

4. Wild type pattern versus cytoplasmic (Figure 15)

- Weak cytoplasmic staining may be seen in cases with otherwise clearly interpretable nuclear staining.
- In order to reflect an underlying mutation the intensity of cytoplasmic staining should be at least moderate and unaccompanied by any spurious cytoplasmic staining of the background normal cells.
- The nuclear staining intensity in cytoplasmic pattern can be absent or variable but not strong diffuse.

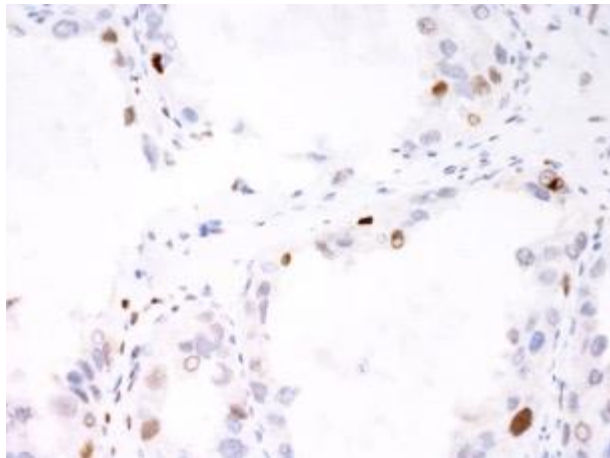


Figure 15a: Reference wild type pattern

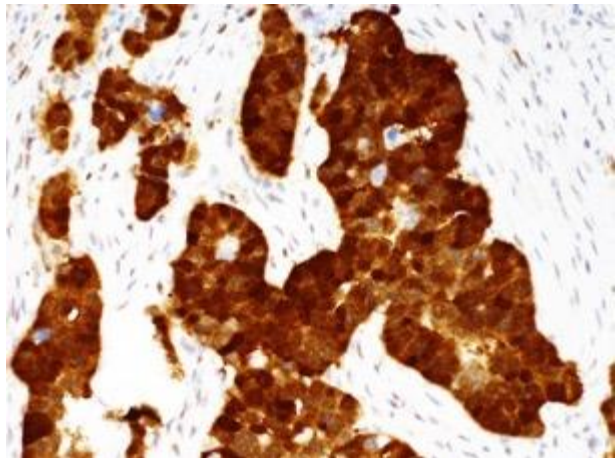


Figure 15b. Reference cytoplasmic

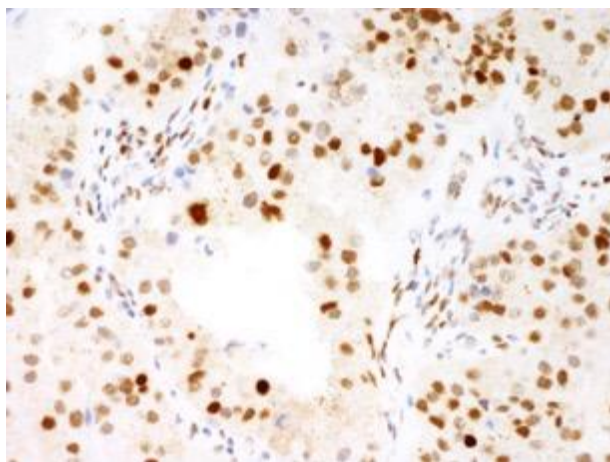


Figure 16a. High wild type pattern with weak cytoplasmic (background) staining but interpretable variable nuclear staining.

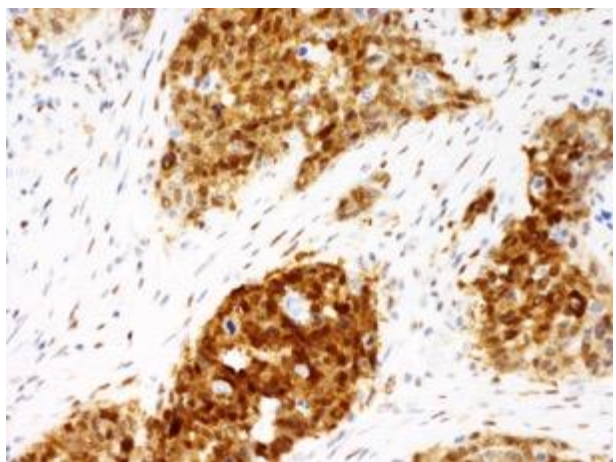


Figure 16b. Cytoplasmic pattern with moderate cytoplasmic staining and nonspecific nuclear staining

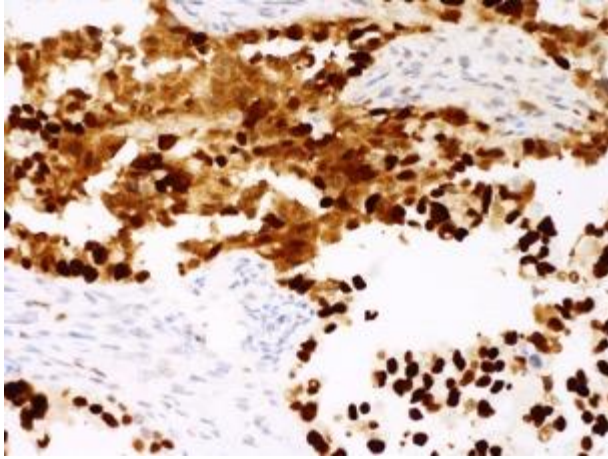


Figure 17a. Overexpression pattern in high-grade serous carcinoma showing an area of spurious artificial cytoplasmic staining (upper left).

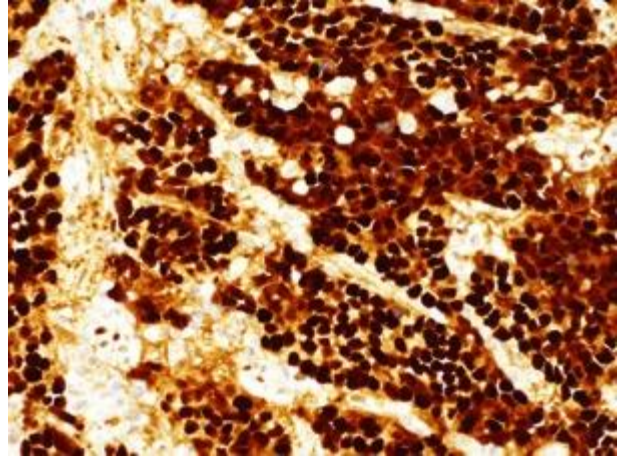


Figure 17b. Overexpression pattern in high-grade serous carcinoma showing an extensive spurious artificial cytoplasmic staining including the stromal component.

References

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