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Abstract 477
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Validity of the p53 antibody DO-7 as a surrogate for predicting TP53 mutation status in breast cancer

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Background

In breast cancer (BC), as in other cancers, TP53 mutation portends a poor prognosis. TP53 gene expression is not a part of current prognostic commercial assays like Oncotype DX® and MammaPrint. While Next Generation Sequencing (NGS) is widely considered to be the gold standard for identifying TP53 mutations, it takes time and often comes with great expense. In uterine cancer, pathologists utilize immunohistochemistry (IHC) identifying p53 protein as a surrogate for TP53 mutation status which impacts treatment decisions. Utilizing the well-established p53 antibody DO-7 and interpretative guidelines from uterine cancers, we sought to prove the validity of IHC testing for p53 in BC.

I. In Vitro: Cell Line Data

IA. Methods

Cell lines:
Three BC cell lines with known status:
- Two TP53 mutated: MDA-231, T47D
- One TP53 wildtype: MCF-7

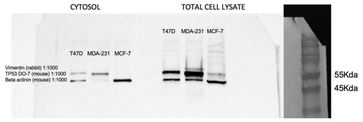
Experiments:
Immunofluorescence (IF)/western blot (WB) staining using BIORAD CAT1707 for p53

IF
- Cells lines were fixed in a crystal coverslip. (formalin 10%, detergent tween 20 at 0.3%).
- The cells were stained with primary-BIORAD CAT1707 and pancytokeratin (in different coverslips).
- A Fluor secondary was used, red 594. Then, DAPI (blue) for nuclear staining was used with mounting media.

WB
- Each cell line was scrapped, centrifuged and lysed.
- A fraction of the total cell lysate was extracted and the rest centrifuged to obtain cytosol and nuclear fractions.
- BioRAD standard methods for WB were used.

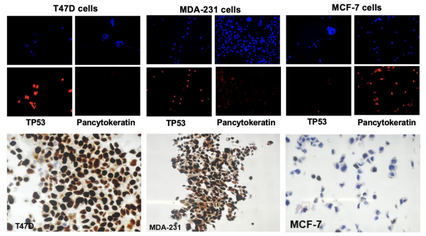
IB. Results

Figure 1. WB analysis using the p53 antibody DO-7 in BC cell lines



As expected: only TP53 mutated cells show cytosolic protein; total cell lysate shows increased TP53 compared to wildtype.

Figure 2. Top: IF analysis using the p53 antibody DO-7 in BC cell lines
Bottom: IHC analysis using the p53 antibody DO-7 in BC cell lines



P53 protein expression using IF and IHC show minimal staining in **wildtype MCF-7** compared to other cell lines with mutated TP53.

II. Patient Samples: Clinical Data

IIA. Methods

Breast cancer patients with NGS were identified by searching both TEMPUS and EPIC databases.

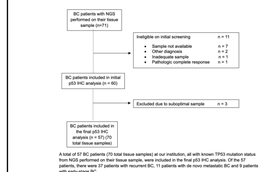
The p53 immunohistochemistry using the DO-7 antibody was performed on 4 µm thick whole slide sections from formalin-fixed paraffin-embedded (FFPE) tumor blocks or cell blocks if cytology.

Assessment of mutational status by level and location of protein expression demonstrated by IHC was performed by two independent pathologists who were blinded to NGS results.

Results were identified as nuclear overexpression, null, cytoplasmic or wild-type according to uterine cancer guidelines.

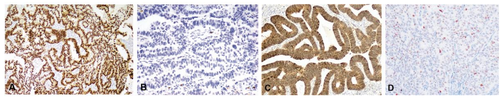
Correlation analysis and sensitivity, specificity testing were performed on the data collected.

Figure 3. Flowchart of cohort selection



A total of 57 BC patients (70 total tissue samples) at our institution, all with known TP53 mutation status from NGS performed on their tissue sample, were included in the final p53 IHC analysis. Of the 57 patients, there were 37 patients with recurrent BC, 11 patients with de novo metastatic BC and 9 patients with early-stage BC.

Figure 4. Representative examples of abnormal p53 IHC staining patterns in uterine cancer[1]



A. Abnormal overexpression with strong diffuse staining greater than 80% of tumor cell nuclei
B. Abnormal complete absence of staining (null pattern)
C. Abnormal cytoplasmic pattern
D. Normal (wild-type) expression

Table 1. Baseline Characteristics of BC patients

	NGS TP53 Mutation Status	
	TP53 Wildtype (n = 33)	TP53 Mutated (n = 24)
Age		
≤ 55	13	13
> 55	20	11
Sex		
Female	31	23
Male	2	1
Ethnicity		
NHB	0	5
NHW	29	14
HW	3	5
HB	1	0

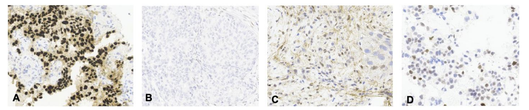
NHB, non-hispanic black; NHW, non-hispanic w hite; HW, hispanic w hite; HB, hispanic black

Median age at the time of diagnosis w as 58 years old (range 29 - 88 years old).

Of the 57 BC patients, 43 w ere w hite (75.4%), 9 w ere hispanic (15.8%), 5 w ere non-hispanic black (8.8%)

IIB. Results

Figure 5. Representative examples of abnormal p53 IHC staining patterns in our BC cohort



A. Abnormal nuclear overexpression pattern. **B.** Abnormal null pattern. **C.** Abnormal cytoplasmic pattern. **D**. Normal (wild-type) expression

Table 2. Concordance between p53 IHC pattern and TP53 mutation status by NGS

	NGS TP53 mutated	NGS TP53 wild-type
IHC p53 mutated	27	0
IHC p53 wild-type	4	39
Total	31	39
Accuracy	94.3% (95% CI 86.0-98.4%)	
Sensitivity	87.1% (95% CI 70.2-96.4%)	
Specificity	100% (95% CI 91.0-100.0%)	

CI, confidence interval

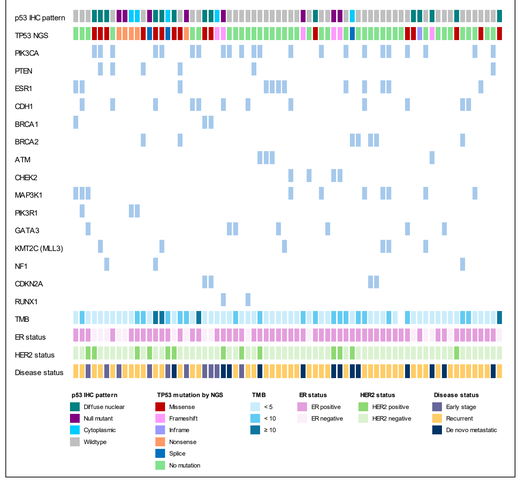
Table 3. Top: p53 IHC pattern and TP53 mutation status by NGS based on disease status at time of NGS
Bottom: p53 IHC pattern and TP53 mutational status by NGS based on subtype at time of NGS

Disease status at time of NGS	Total (n)	WT (n)	Diffuse nuclear (n)	Null (n)	Cytoplasmic (n)	TP53 mutation by NGS (n)	% IHC-NGS concordance
Early stage	10	2	3	3	2	8	100.0
De novo metastatic	13	6	2	4	1	8	92.3
Recurrent	47	35	9	1	2	15	93.6
Total	70	43	14	8	5	31	94.3

Subtype at time of NGS	Total (n)	WT (n)	Diffuse nuclear (n)	Null (n)	Cytoplasmic (n)	TP53 mutation by NGS (n)	% IHC-NGS concordance
ER positive/HER2 negative	45	37	4	1	2	11	91.1
ER positive/HER2 positive	11	3	1	4	3	8	100.0
ER negative/HER2 positive	2	0	2	0	0	2	100.0
Triple negative	12	3	7	3	0	10	100.0
Total	70	43	14	8	5	31	94.3

WT, wild-type

Figure 6. Histopathological, molecular and clinical characteristics of 70 BC samples



Conclusion

- IHC for p53 is a quick, relatively easy to perform and inexpensive surrogate for TP53 mutation status in uterine cancer with treatment implications.
- In our study of 70 BC samples, we had 94% accuracy in predicting TP53 mutation status with no false positive results. This suggests that we can utilize IHC with the DO-7 p53 antibody as a surrogate for TP53 mutation status in BC patients. A larger multi-site validation set should be conducted to confirm our findings.
- Knowing TP53 mutation status, particularly in early-stage hormone receptor positive BC, may elucidate patients that may not respond to traditional endocrine therapy and need more intensive systemic treatment (not specifically chemotherapy) at treatment initiation.

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REFERENCE: 1. Turashvili G, Hanley K. Arch Pathol Lab Med. 2024 Jan 1;148(1):78-98. doi: 10.5858/arpa.2022-0280-RA

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