Impact of p16, p53 and Ki67 expression on clinical outcome in specific breast cancer subtypes

Rudi Schmigylski
Dumfries and Galloway Royal Infirmary

Aims

- Four major molecular subtypes of breast cancer; Luminal A, Luminal B, Her-2 Positive and Triple Negative.

- This investigation aimed to use cell cycle markers p16, p53 and Ki67 on archived breast tumour tissue to assess clinical outcomes for each of these subtypes after a 10 year period.

Breast cancer subtypes

Subtype	ER/PR status	Her 2 status	Other notable immunocytochemical expression	Estimated prevalence (percentage of total breast cancers)
Luminal A	+/+	-	Ki67 – (low), luminal marker CK8/18 +	30-70%
Luminal B	+/+	-	Ki67 + (high), luminal marker CK8/18 +	10-20%
Her-2 positive	-/+ -/-	+	N/A	5-15%
Triple Negative	-/-	-	Basal markers CK5/CK14 +, may be EGFR +/-	15-20%

Methods

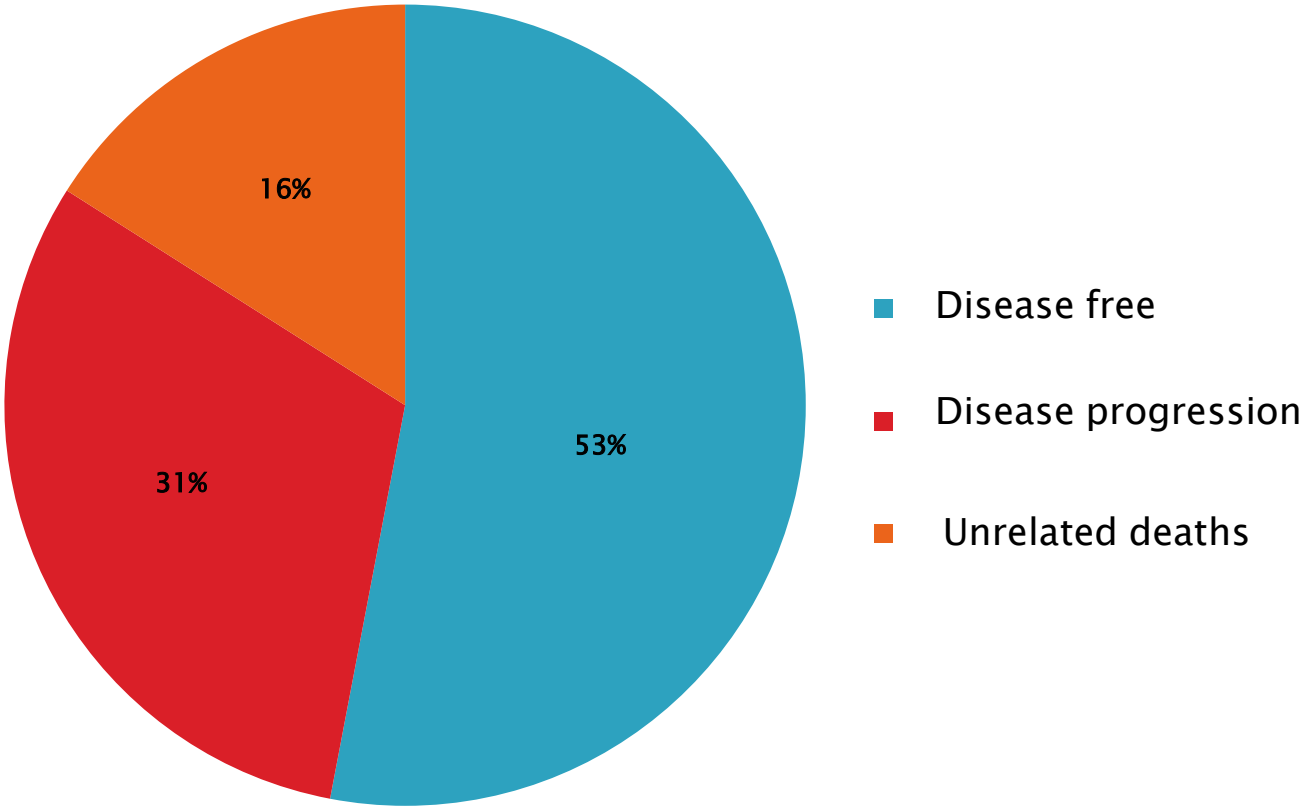
- 76 samples of FFPE tumour resection tissue from female breast cancer patients (2005-2006)
- Patients stratified into subgroups based on previously reported ER, PR and Her-2 results
- Known to be node-negative at time of surgery and had no known pre-surgical chemotherapy

IHC – p16, p53 and Ki67

Leica Bond Max

Primary Antibody	Clone	Provider	HIER	Dilution	Volumes (per 30ml pot)	Control tissue
p16	R19-D	DB Biotech	ER2 for 30 minutes	1:300	64µl in 20ml diluent	Cervical squamous cell carcinoma
p53	DO-7	Dako	ER2 for 20 minutes	1:1500	12µl in 20 ml diluent	Endometrial serous carcinoma
Ki67	MIB-1	Dako	ER2 for 30 minutes	1:100	200µl in 20ml diluent	Normal tonsil

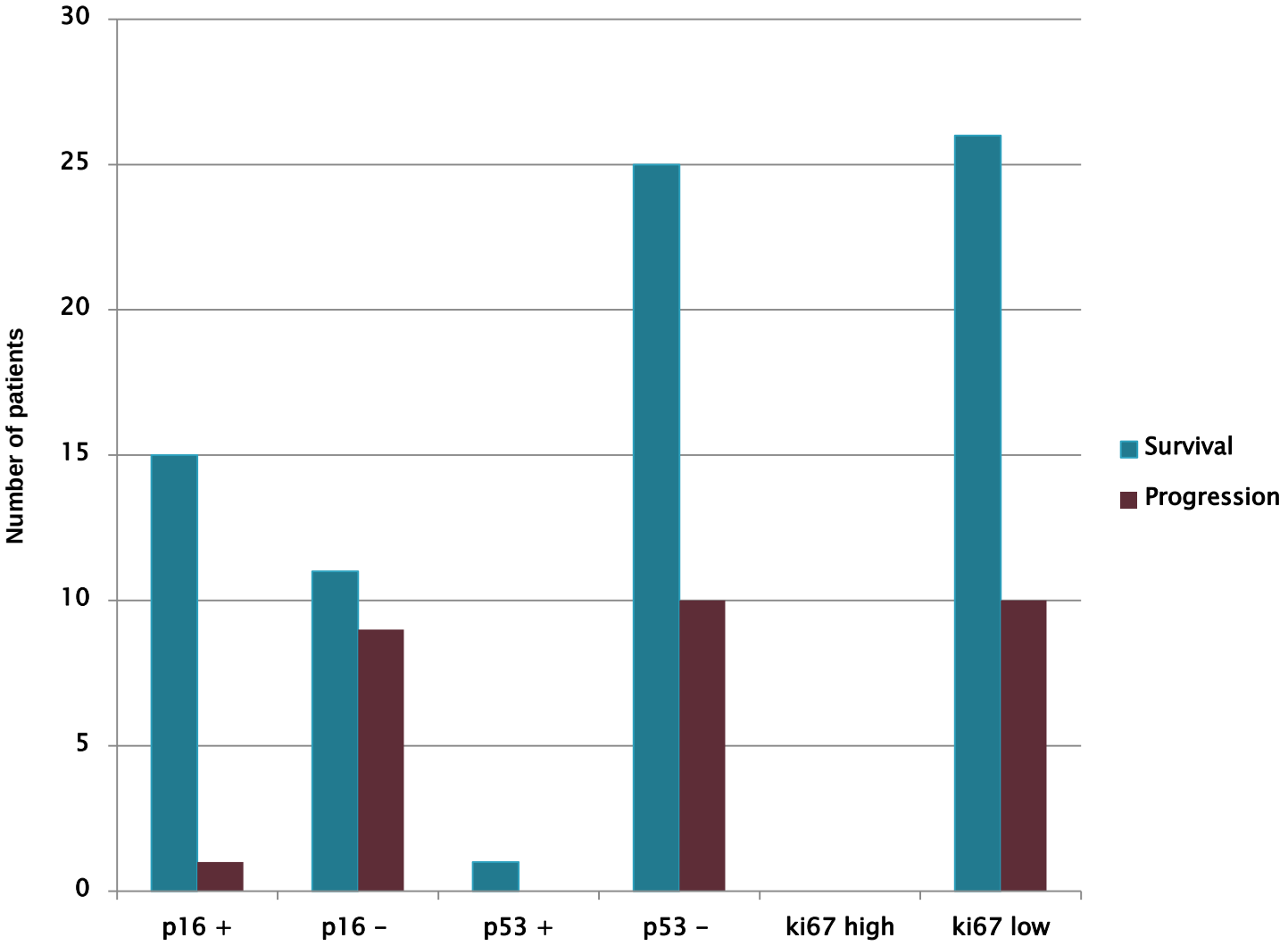
Results


16%
53%
31%
Disease free
Disease progression
Unrelated deaths

Luminal A tumours

- By definition show low Ki67 expression
- Grade I/2
- p16 expression associated with survival
- Malignant phyllodes tumour – only p53 positive – characteristic

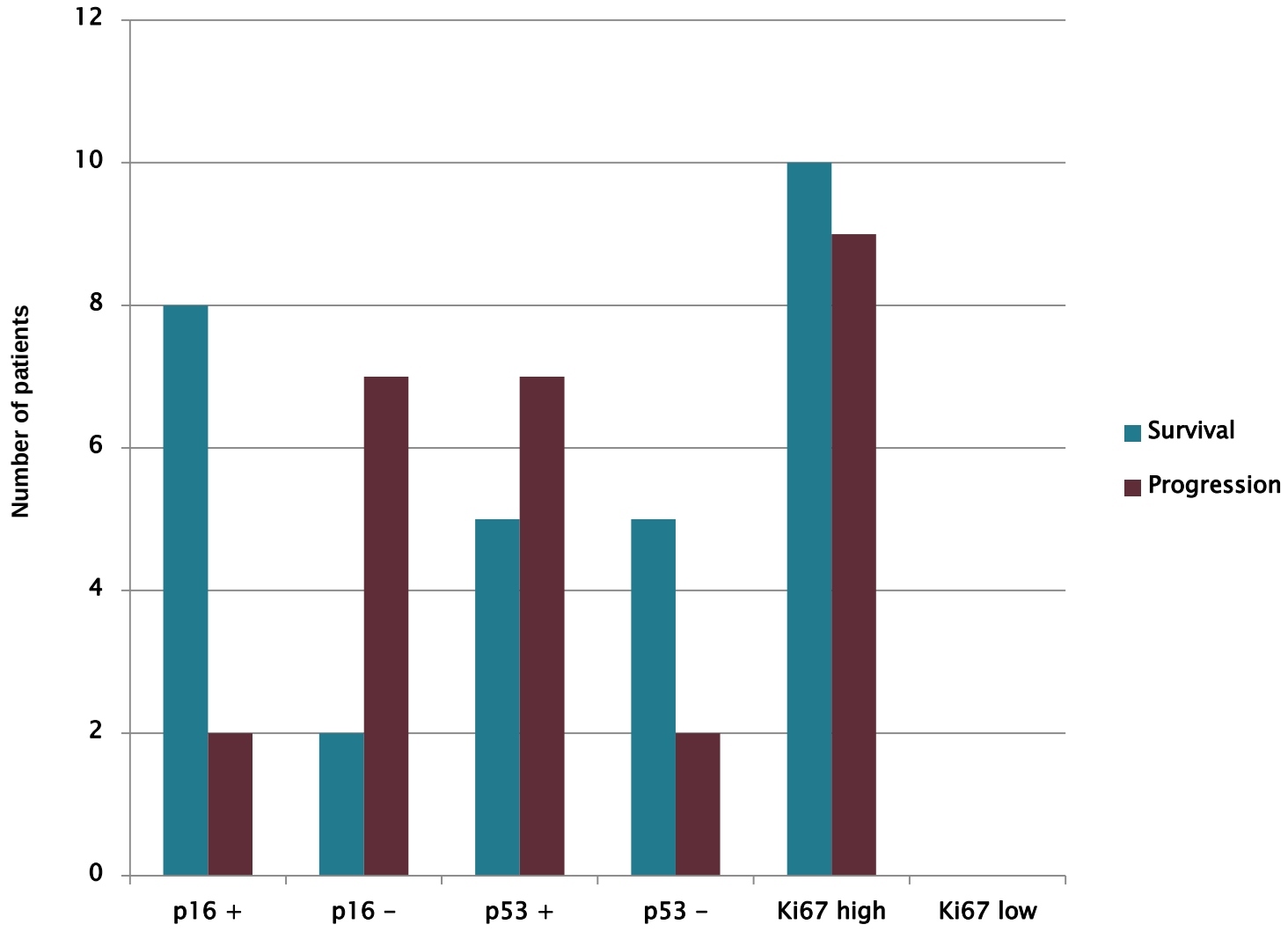
Luminal A tumours



Luminal B tumours

- High Ki67 expression
- p16 strongly associated with survival (80% vs. 20%)
- p53 correlated with progression (78% vs. 50%)
- Tend to be higher grades 2/3

Luminal B tumours



Her-2 positive tumours

- Survival and progression groups showed identical p16 expression – not a good marker for outcome in these tumours
- All p53 positive
- All high Ki67
- 80% Grade 3

Triple Negative tumours

- Majority p16 negative
- Progression mildly associated with p53 negativity – not statistically significant
- p53 positive tumours more likely to respond to nab-paclitaxel – initiates apoptosis via p53 independant pathway
- 92% high Ki67 expression
- Almost 100% grade 3 – aggressive

Hormone positive tumours – Why?

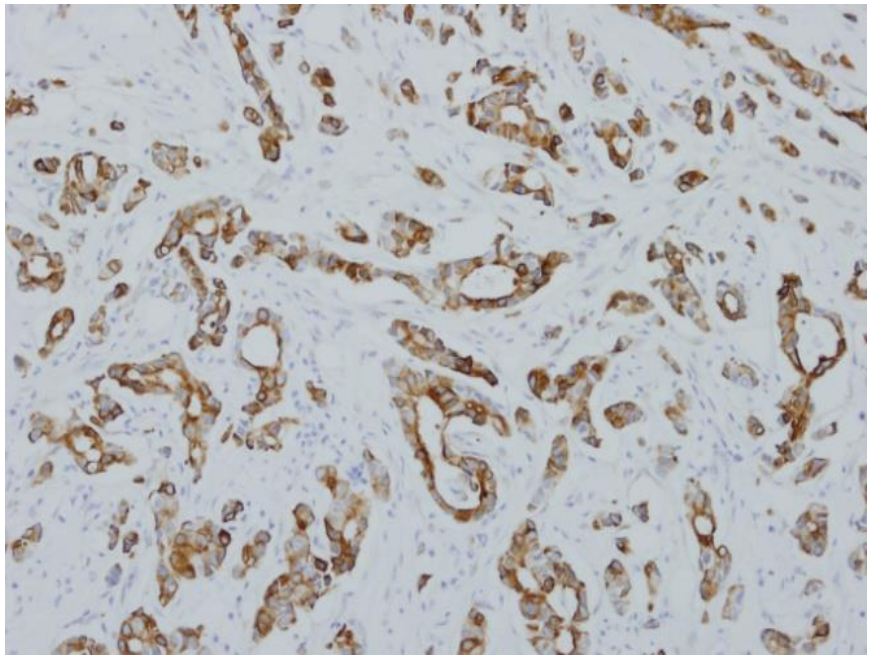
- p16 inhibits **V**ascular **E**ndothelial **G**rowth **F**actor, therefore angiogenesis?
- Overexpression of p16 may be protective
- p53 overexpression in ER/PR positive breast cancers is a contributing factor in hormone therapy resistance
- Mutated p53 may obstruct apoptosis = resistance to Tamoxifen

Impact of findings

- Ki67 important to stratify hormone positive patients into Luminal A and B groups along with tumour grade – needs standardisation for reporting
- p16 – marker of positive clinical outcome in Luminal A and Luminal B patients
- Used in risk assessment of Luminal cancers – p16 negative patients prioritised for follow-up

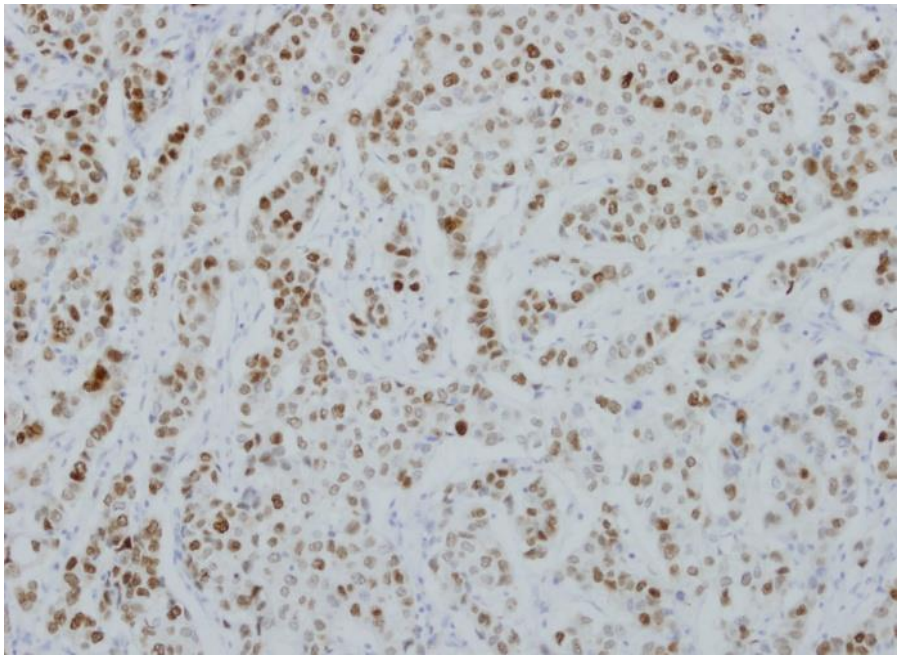
Impact of findings

- Co-expression of p53 and high Ki67 – poor clinical outcome in Luminal B patients
- May benefit from longer therapeutic intervention e.g. longer chemo regimen or increased dosage
- However oncology protocols strictly regulated
- No clinical value in p16, p53 or Ki67 testing in Her-2 positive or TN patients



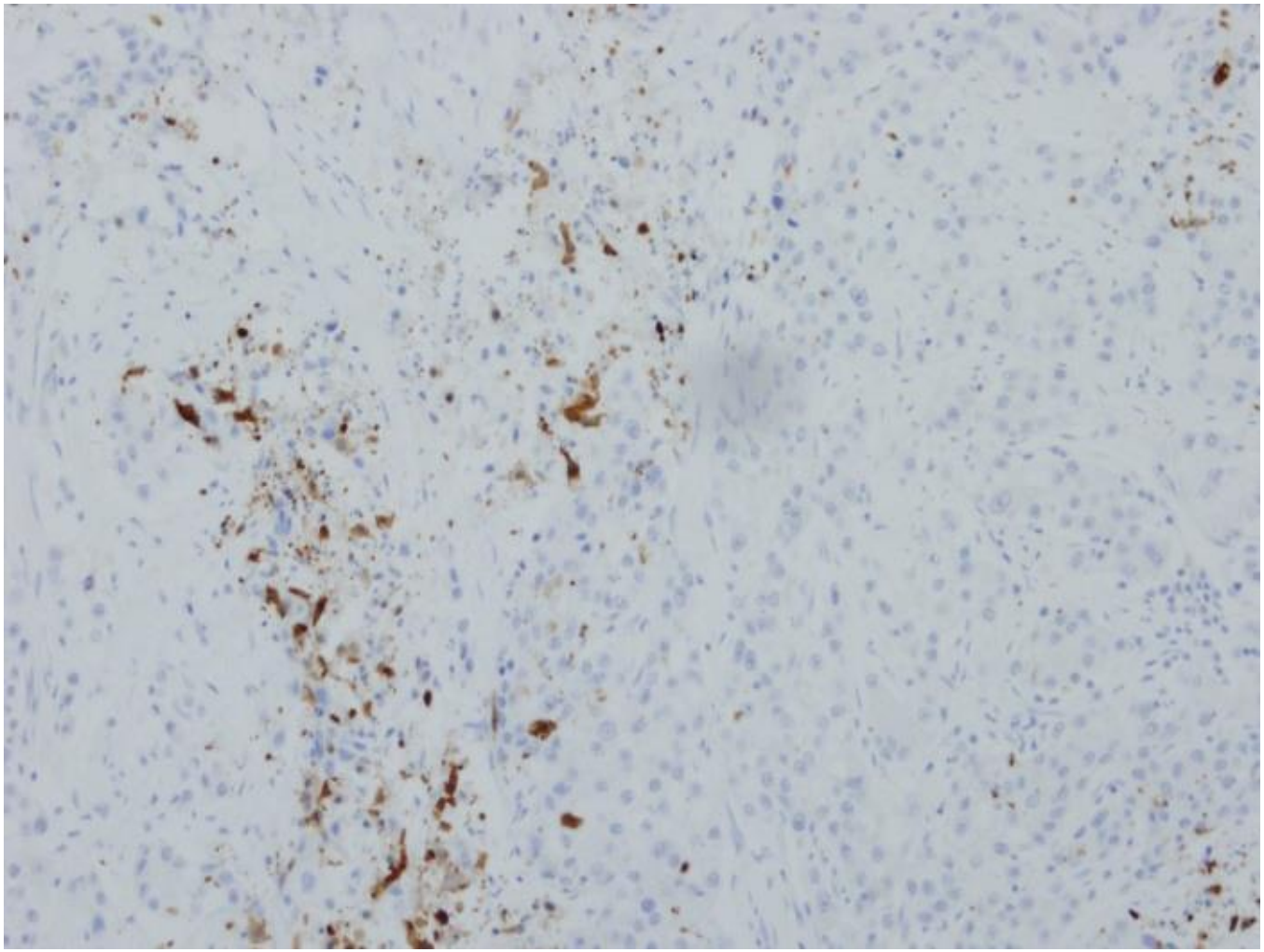
Luminal A p16 positive
(grade 2)

Low risk for
progression



Luminal B p53 positive
(grade 3)

High risk for
progression



p16 staining in spindly myofibroblasts - NOT tumour cells! (TN)