Invasive Lobular Carcinoma: Histological Diagnosis and E-cadherin Status

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Outlines of my talk

- Definition of ILC according to the WHO classification 5th edition
- Classic ILC and Variants
- Why do reproducibility issues exist ?
  1. Variants ?
  2. Phenotype ?
  3. *CDH1* gene status?
- Take-home messages and conclusions

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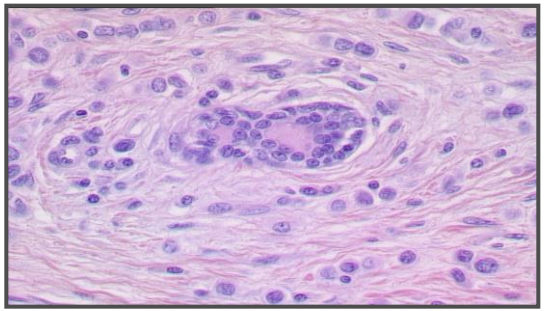
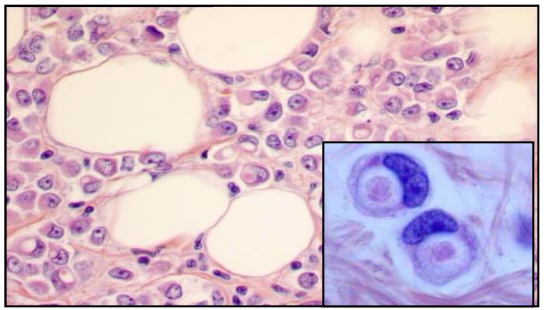
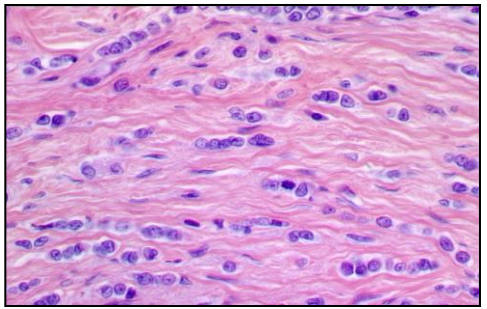
Definition of ILC according to the WHO 5th edition

Definition

Invasive lobular carcinoma (ILC) is an invasive breast carcinoma (IBC) composed of dyscohesive cells that are most often individually dispersed or arranged in a single-file linear pattern.







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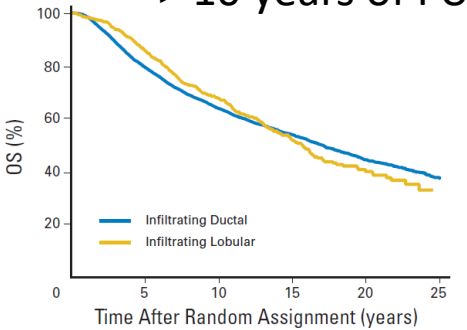
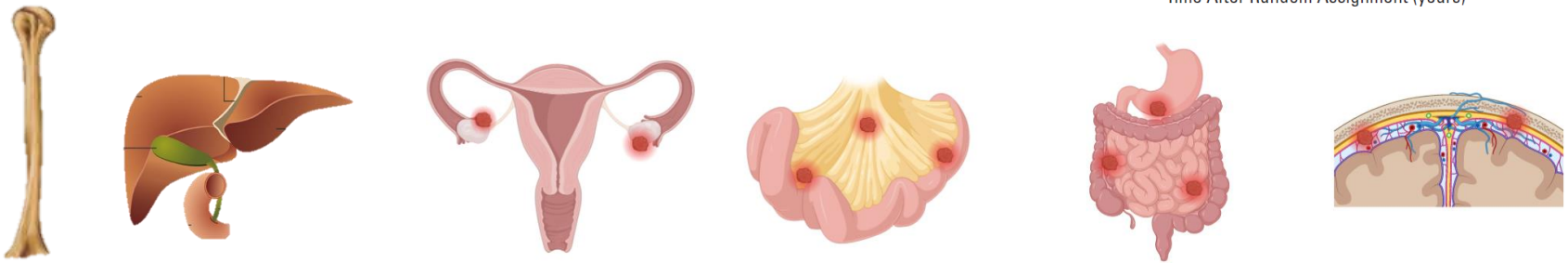
Introduction: Invasive Lobular Carcinoma (ILC)

- 15% of Invasive breast cancers
- Low rate of pCR after neoadjuvant chemotherapy (~ 7%)
- Good response to hormonal treatment (AI)
- No clear benefit of adjuvant chemo for all ILC patients

- Poorer prognosis of ILC vs IDC
  > 10 years of FU



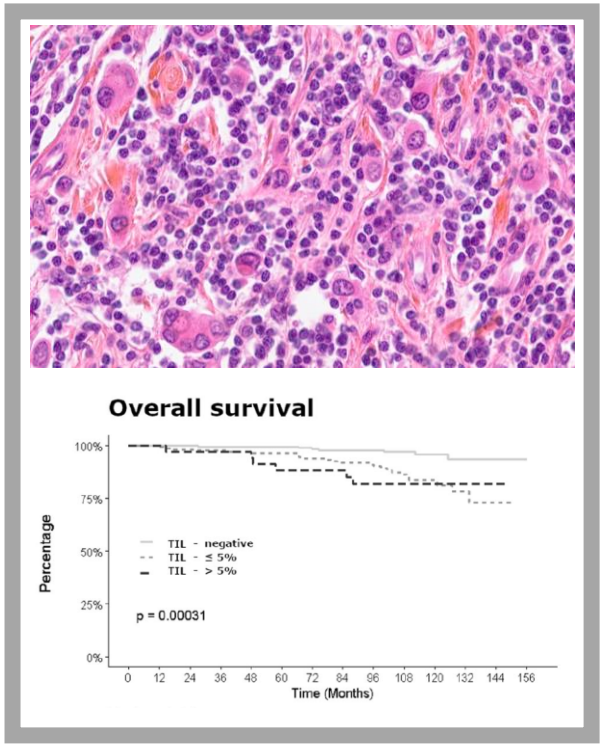
- Metastatic pattern specificities



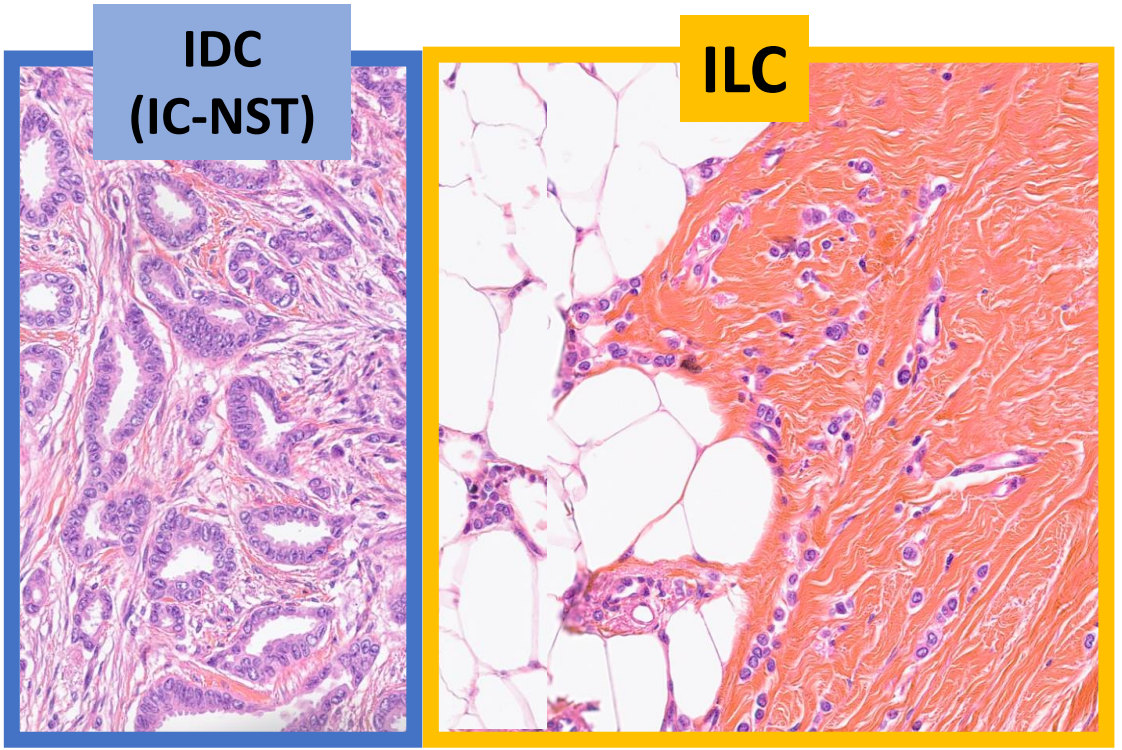
Bone | Liver | Ovaries | Peritoneum | Gastro-intestinal Tract | Meninges

Sastre-Garau *et al*. Cancer 1996; Ferlicot *et al.* Eur. J. Cancer 2004; Pestalozzi et al. JCO 2008; Guiu *et al* Critical Reviews in Oncology/Hematology 2014; Christgen *et al* Cancers 2021; Van Baelen *et al* Annals of Oncol 2022; Cortazar *et al* Lancet 2014; Trapani *et al* Can Treat Reviews, 2021

ILC Tumor Microenvironment Specificities



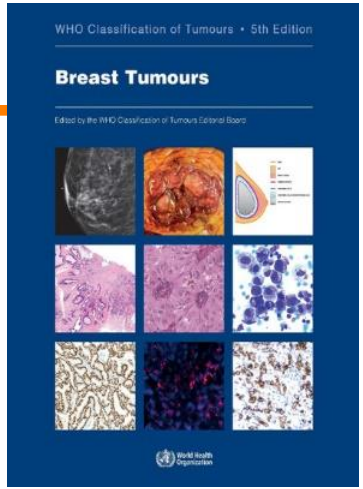
TILs are associated with poor survival in ILC



Under the microscope, the stroma of ILC is different from that of invasive ductal carcinoma.

Desmedt et al, JNCI 2018; Tille et al, MOD PATH 2020

Inactivating *CDH1* mutations: oncogenic driver of ILC



- **Causative** of the characteristic **lack of cohesiveness** and invasiveness pattern of lobular carcinoma cells.
- **In 50–80% of ILCs, deleterious mutations** of ***CDH1*** often coupled with **loss of heterozygosity** of the wildtype allele (**16q loss**)
- ILCs lacking *CDH1* mutations, **alterations of α-catenin** and potentially other components of the cadherin-catenin family have been reported
- Role of ***CDH1* gene promoter methylation** in the loss of E-cadherin expression in ILCs **remains a matter of controversy**

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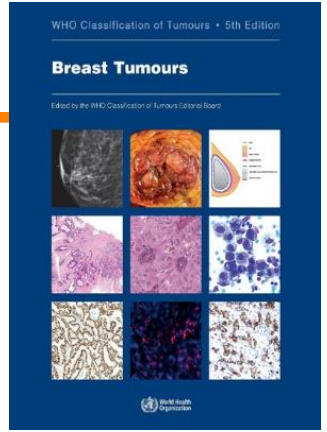
Definition of ILC according to the WHO 5th edition



- *CDH1* gene status not relevant
- E-cadherin expression only a **desirable** diagnostic criteria

Diagnostic molecular pathology

Not clinically relevant

Essential and desirable diagnostic criteria

Classic ILC

Essential: an IBC composed of dispersed or linear dyscohesive cells with low- to intermediate-nuclear-grade morphology and a low mitotic count; ER immunoreactivity is high and HER2 is negative/non-amplified.

Desirable: coexisting lobular neoplasia; E-cadherin loss may be useful.

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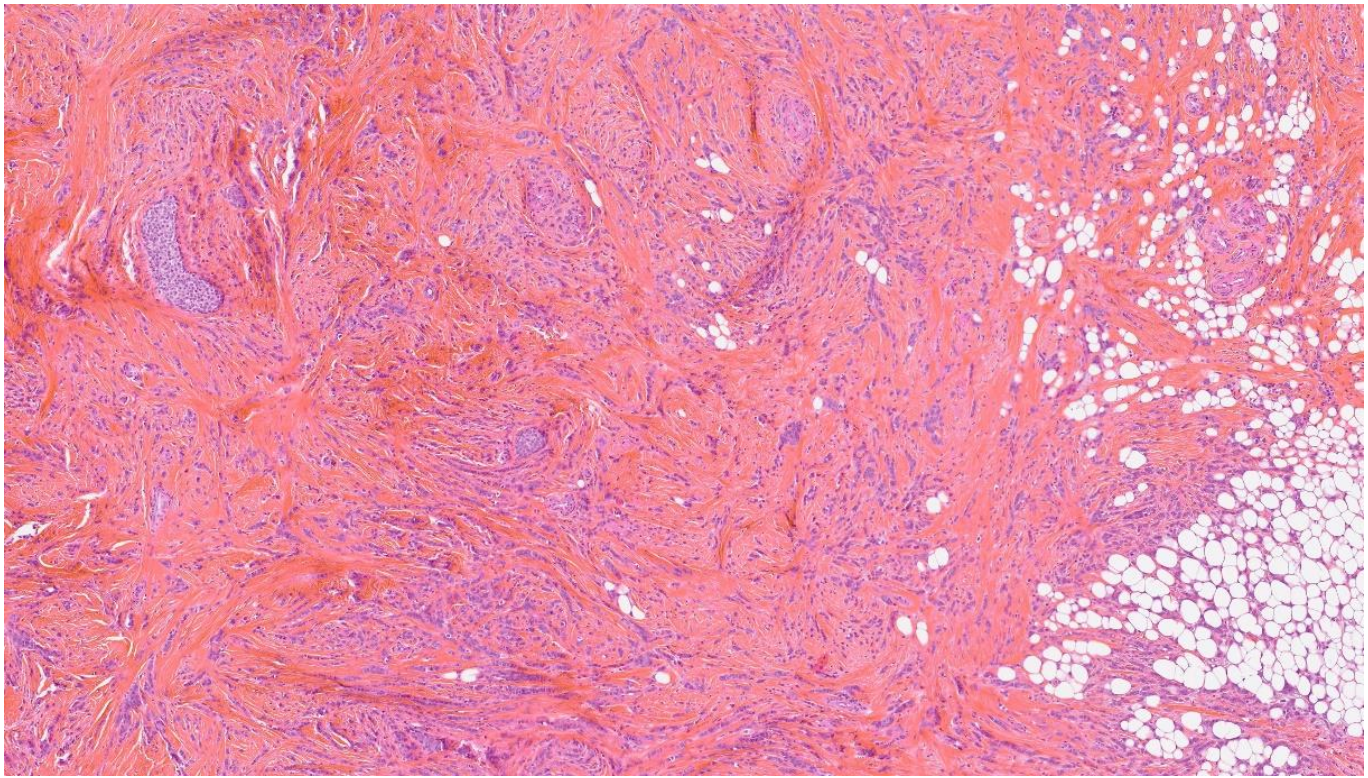
Classic ILC

- **Small cells** that **lack cohesion**
- Individually dispersed throughout a fibrous connective tissue
- **Single-file linear** cords that invade the stroma
- **Round** or notched ovoid **nuclei**
- **Little host reaction** or disturbance of the background architecture
- **ALH and LCIS** associated with ILC in **58–98%** of cases



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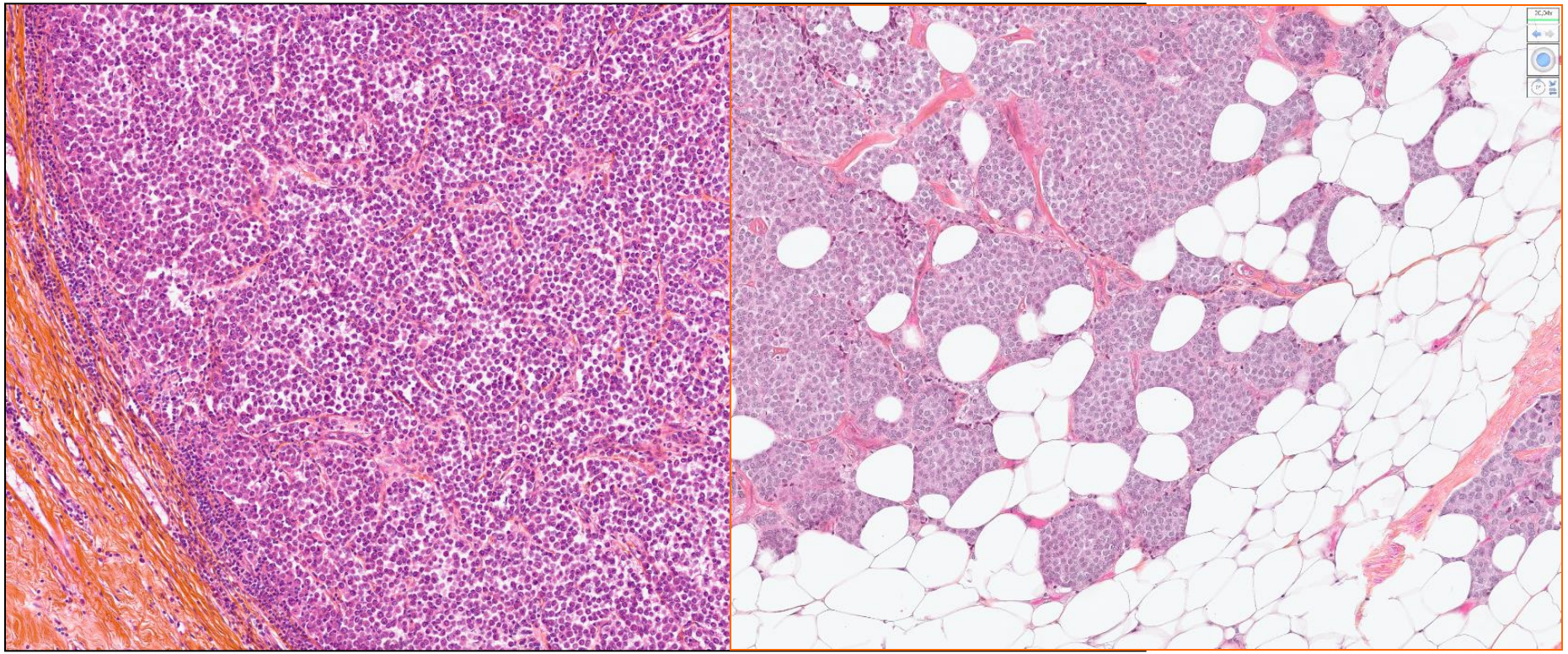
Non classic ILC listed into the WHO (5th edition): Solid variant

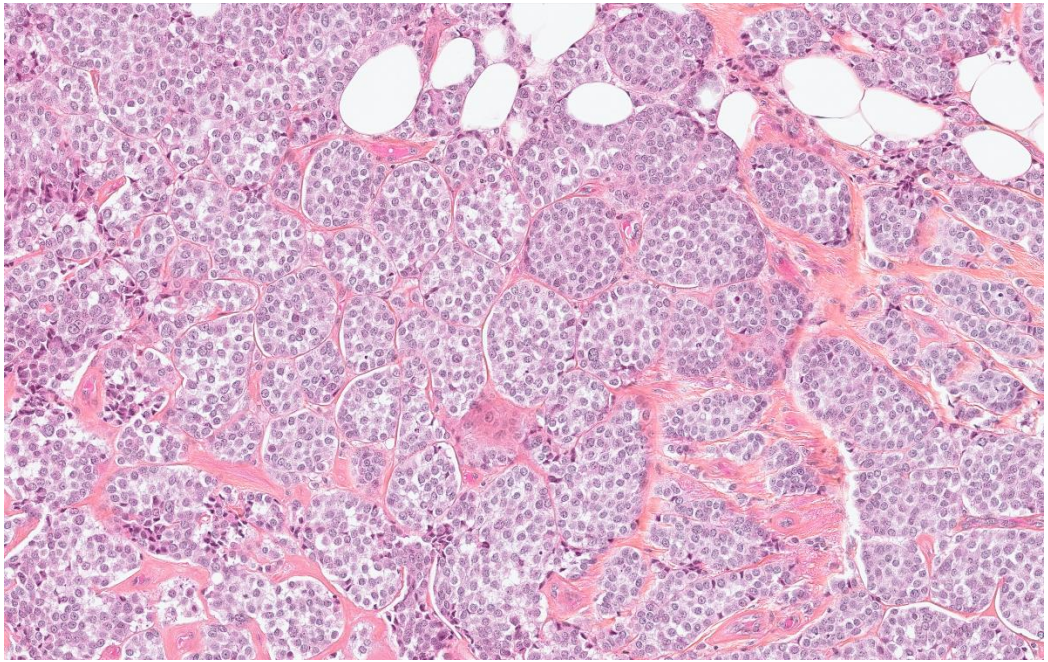
Solid sheets of uniform and dyscohesive cells with more mitoses



WHO 5th edition, 2019

Non classic ILC listed into the WHO (5th edition): Alveolar variant

Globular aggregates of at least 20 cells, that lack cell to cell cohesion, separated by thin bands of stroma



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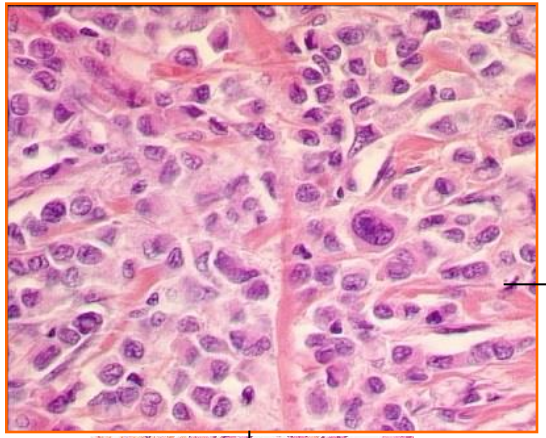
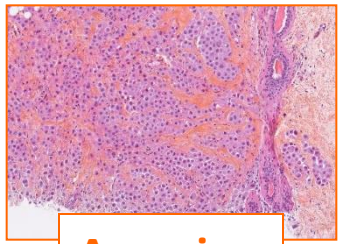
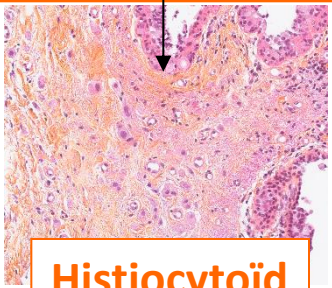
Non classic ILC listed into the WHO (5th edition): Pleomorphic variants

- Larger cells,
- Nuclei > 4 times lymphocytes,
- Higher rate of mitoses

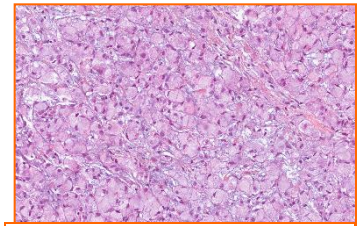




Apocrine



Histiocytoïd



Signet ring cells

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Reproducibility issues in ILC diagnosis

- Central pathology reviews of randomized clinical trials demonstrated **overdiagnosis of ILC in local pathological laboratories**:

  - MINDACT: 60% (395 out of 654)
  - West German Plan B: 66% (253 out of 385)

  of the locally diagnosed ILCs were **confirmed by central pathology review**

Christgen *et al*, J Pathol Clin Res March 2022; 8: 191–205

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Why do these reproducibility issues exist ?

1. Variants (non classic) ?
2. Phenotype ?
3. *CDH1* gene status?

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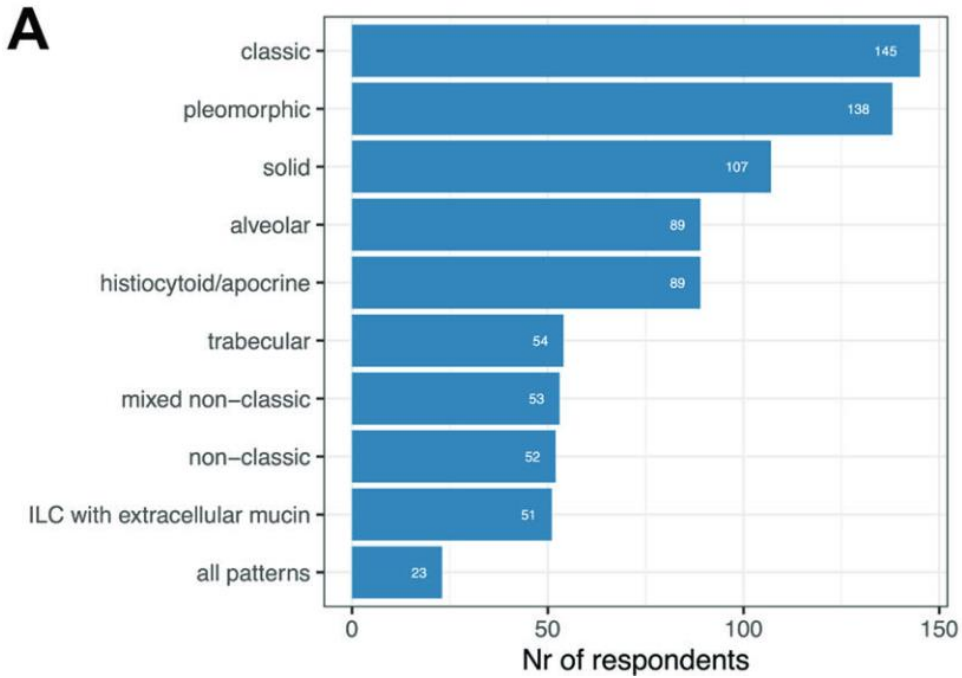
Current practice in ILC diagnostics: reported *variants* of ILC

Among 147 participants to a survey (Eur, Africa, Asia, North and South America, Australia)



De Schepper *et al* Modern Path 2022

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ILC *variants* listed into the WHO (5th edition)

- **In clinical practice and as indicated into the WHO classification:**

  75% of ILC have mixed patterns of **classic ILC + several variants of ILC**

- **Some variants** identified in literature <u>are not listed</u> in the WHO classification

  → trabecular, ILC with tubular elements, with extra-cellular mucin…

Chapter 32, 5th edition of the Rosen's book; Christgen *et al*, Cancers 2021; Soong *et al* Mod Path 2022; Canas-Marques & Schnitt, Histopathology 2016: Rakha *et al* Am J Surg Path 2010

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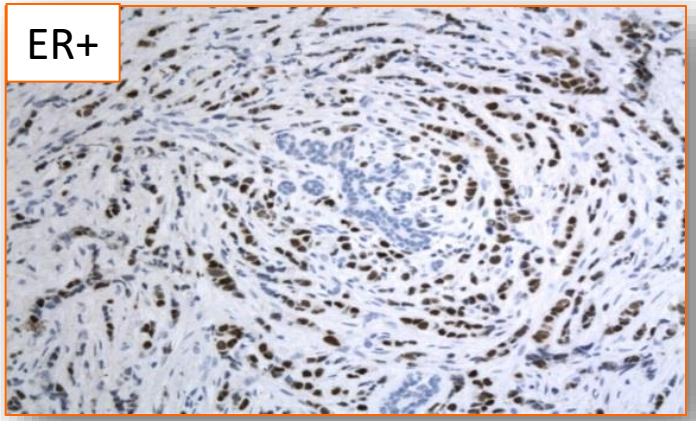
ILC phenotype: ILCs are not « created equal »!

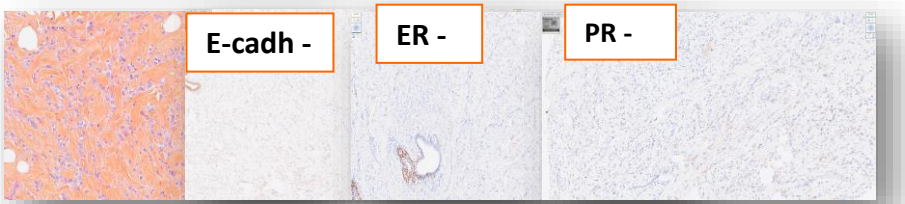
- ER+ > 90% of the cases
  - (ER+ PR- HER2 -): 10% of ILC
- AR + in ~ 70 to 87% of the cases

- HER2 positive: 3 to 13% of the cases
- HER2 low: 33%

- Triple negative ILC: 2 to 9% of the cases

  → AR+ in 94.7%





Rakha et al Am J Surg Path 2010; Aleskandarany et al Br Can Res TTT 2016; Cancello et al Ann of Oncol 2013.
Guiu et al Critical Reviews in Oncology / Hematology, 2014; Iorfida et al Br Can Res TTT 2012; Bergeron et al Mod Path 2021

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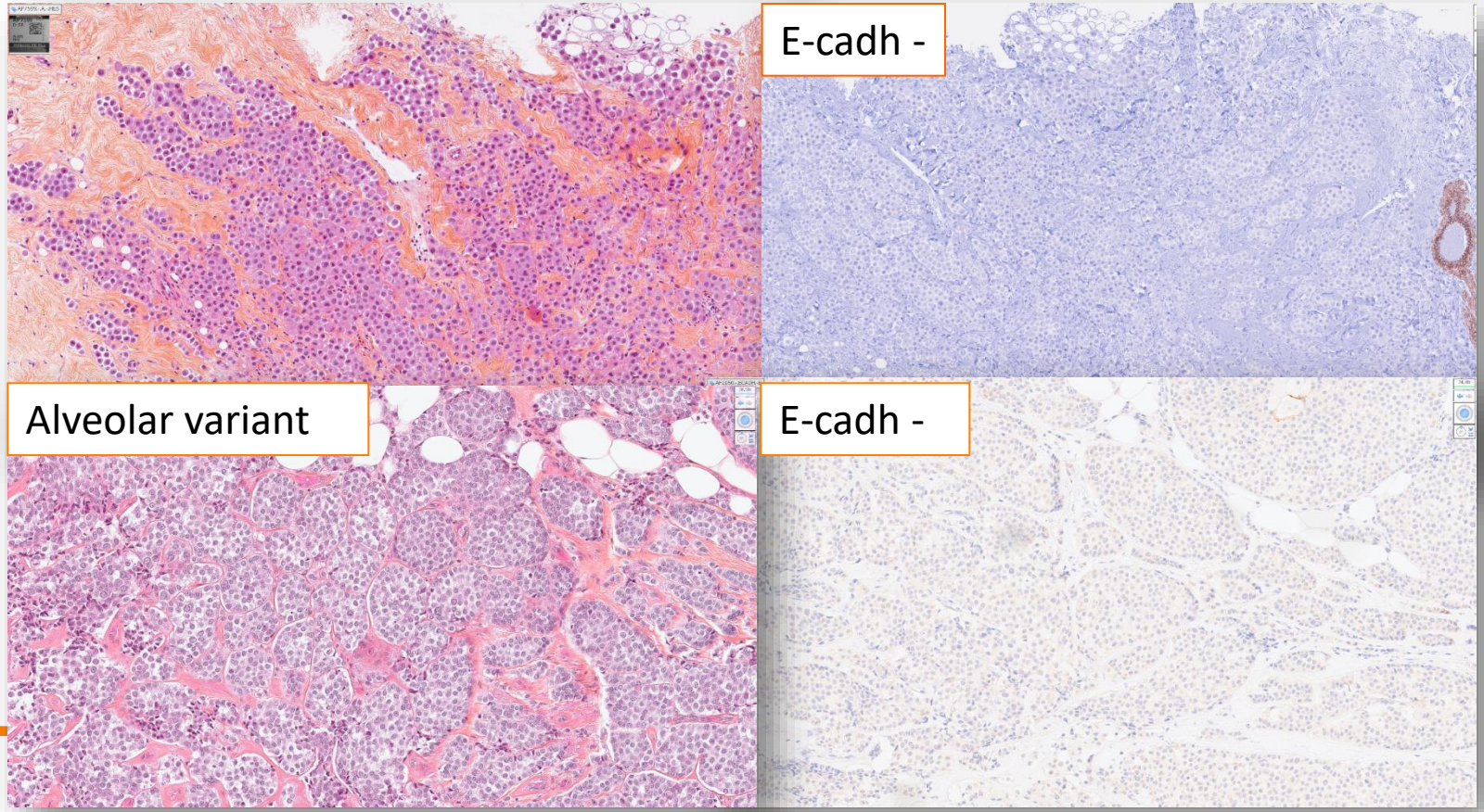
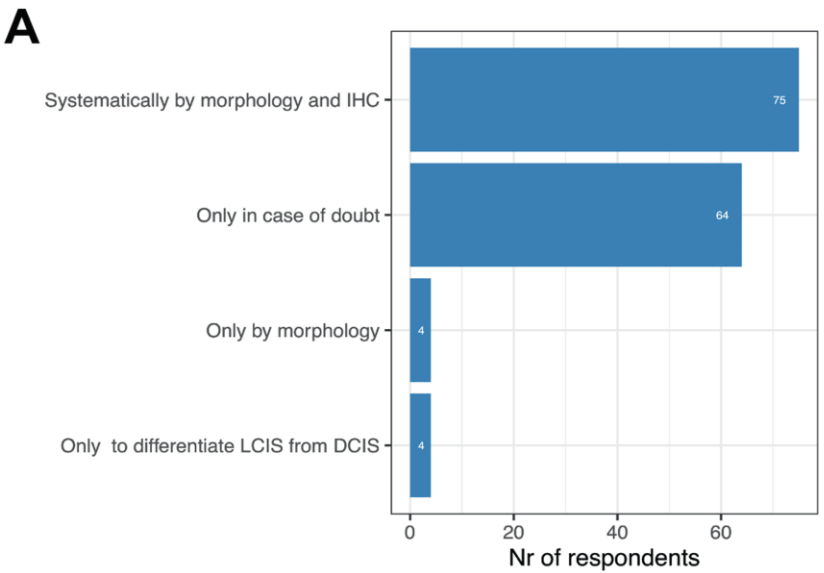
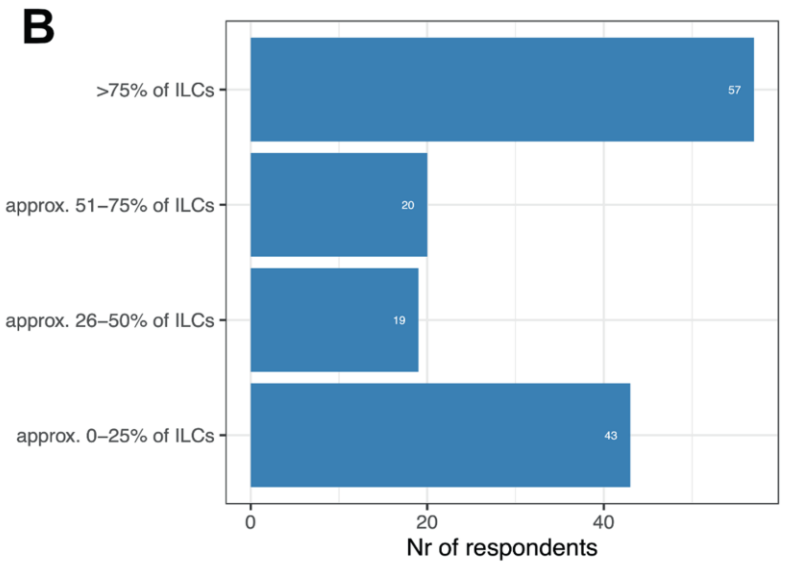
ILC phenotype: E-cadherin loss in 85% of the cases



Use of E-cadherin IHC for ILC diagnostics





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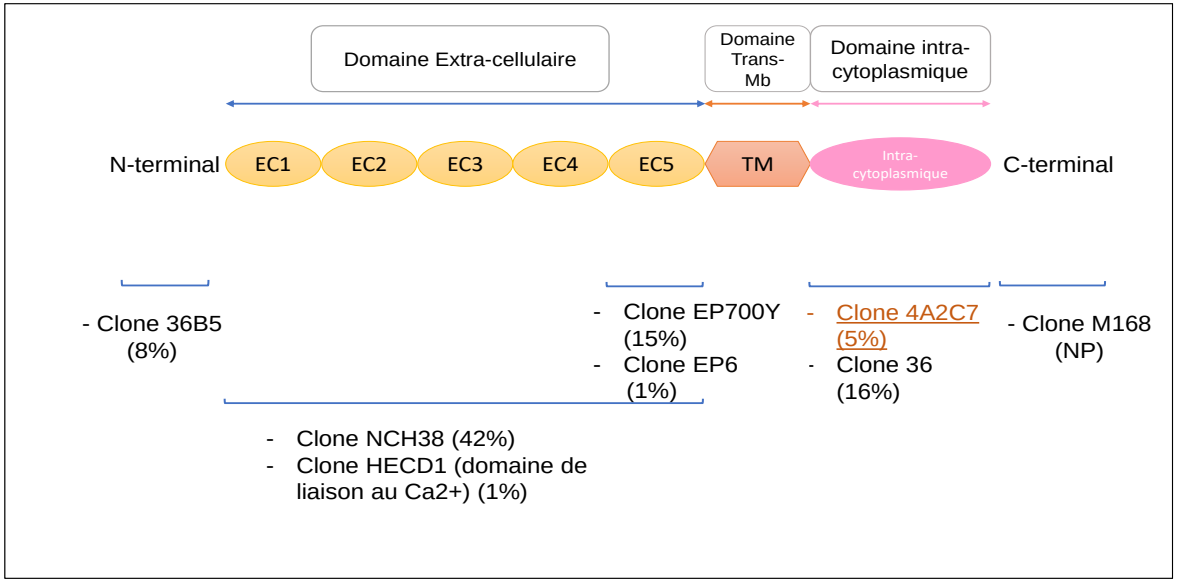
High diversity of E-cadherin antibodies and protocoles

Table 4. Overview of used concentration per most frequently used E-cadherin clone.

Concentration per clone	Number of participants
NCH-38	**38 (42%)**
RTU	12 (13%)
1/100	10 (11%)
1/50	4 (4%)
1/200	3 (3%)
1/300	1 (1%)
1/50 to 1/100	1 (1%)
1/170	1 (1%)
1/25	1 (1%)
Missing data	5 (5%)
Clone 36	**15 (16%)**
RTU	8 (9%)
0,314 µg/ml	2 (2%)
1/200	1 (1%)
Missing data	4 (4%)
EP700Y	**14 (15%)**
1/200	4 (4%)
RTU	5 (5%)
0,314 µg/ml	1 (1%)
1/700	1 (1%)
unknown	1 (1%)
Missing data	1 (1%)
Clone 36B5	**7 (8%)**
RTU	3 (3%)
1/100	1 (1%)
1/40	1 (1%)
Missing data	2 (2%)



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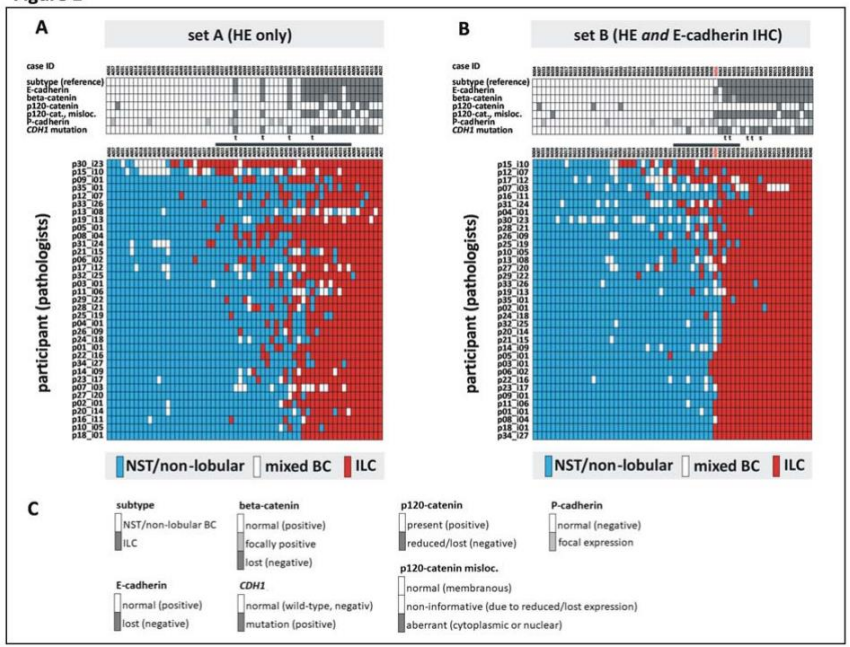
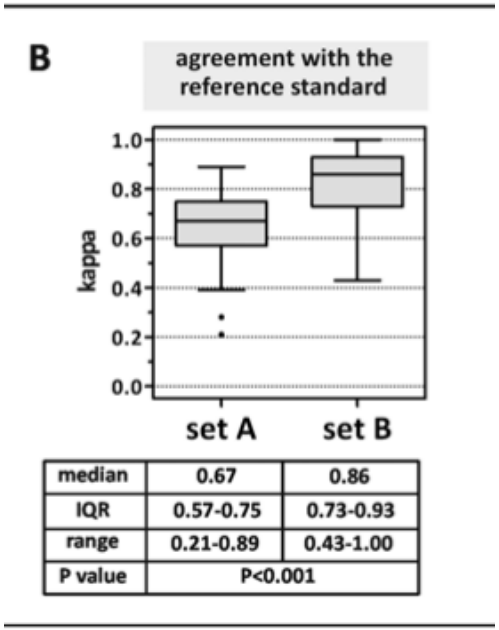
E-cadherin IHC improves diagnosis agreement

Between pathologists

Figure 1



With the referent diagnosis



median	0.67	0.86
IQR	0.57-0.75	0.73-0.93
range	0.21-0.89	0.43-1.00
P value	P<0.001	

Christgen et al, J Pathol Clin Res 2022 Mar;8(2):191-205.

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E-cadherin positive ILC exist

Table 2. Frequency of aberrant E-cadherin expression in invasive lobular carinomas in series with at least 20 cases

First author	No. (%) E-cadherin-positive
Kuroda[19]	0 of 20 (0%)
Siitonen[20]	0 of 55 (0%)
Acs[21]	1 of 42 (2.4%)
Goldstein[22]	5 of 143 (3.5%)
Qureshi[23]	5 of 44 (11.4%)
Da Silva[17]	4 of 25 (16%)
Rakha[18]	38 of 239 (15.9%)
Sarrio[24]	12 of 51 (23.5%)
Total	65 of 619 (10.5%)

- 0 to 23.5% of ILC demonstrate an aberrant E-cadherin expression
- No morphological differences between E-cadherin negative and E-cadherin positive ILC
- E-cadh positivity can be a circumferential membranous complete staining !

Canas-Marquez and Schnitt et al, Histopathology 2016

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E-cadherin positive ILCs: have a *abnormal* expression of E-cadh

MSKCC study

- E-cadh expression:

47 out of 202 ILC cases

- 18 strong/diffuse,
- 22 heterogeneous
- 7 reduced/weak

Inst CURIE study	4A2C7 clone (intra-ɛR)	NCH38 clone (extra-ɛR)
Number of ILCs assessed (/251)	250	247
Pattern of E-cadherin expression:		
Negative (0%)	119 (47.6%)	133 (53.8%)
Focal (1-10%)	63 (25.2%)	74 (30%)
Heterogeneous (11-89%)	48 (19.2%)	17 (6.9%)
Diffuse (≥90%)	20 (8%)	23 (9.3%)
Characteristics of ILCs with diffuse E-cad expression:		
E-cad H-score	241.7 [120-300]	209.6 [96-300]
Type of E-cad membrane staining:		
Continuous	3 (15%)	5 (21.7%)
Discontinuous	17 (85%)	18 (78.3%)

Grabenstetter *et al* Hum Path 2020; Djerroudi *et al* Mod Path 2024

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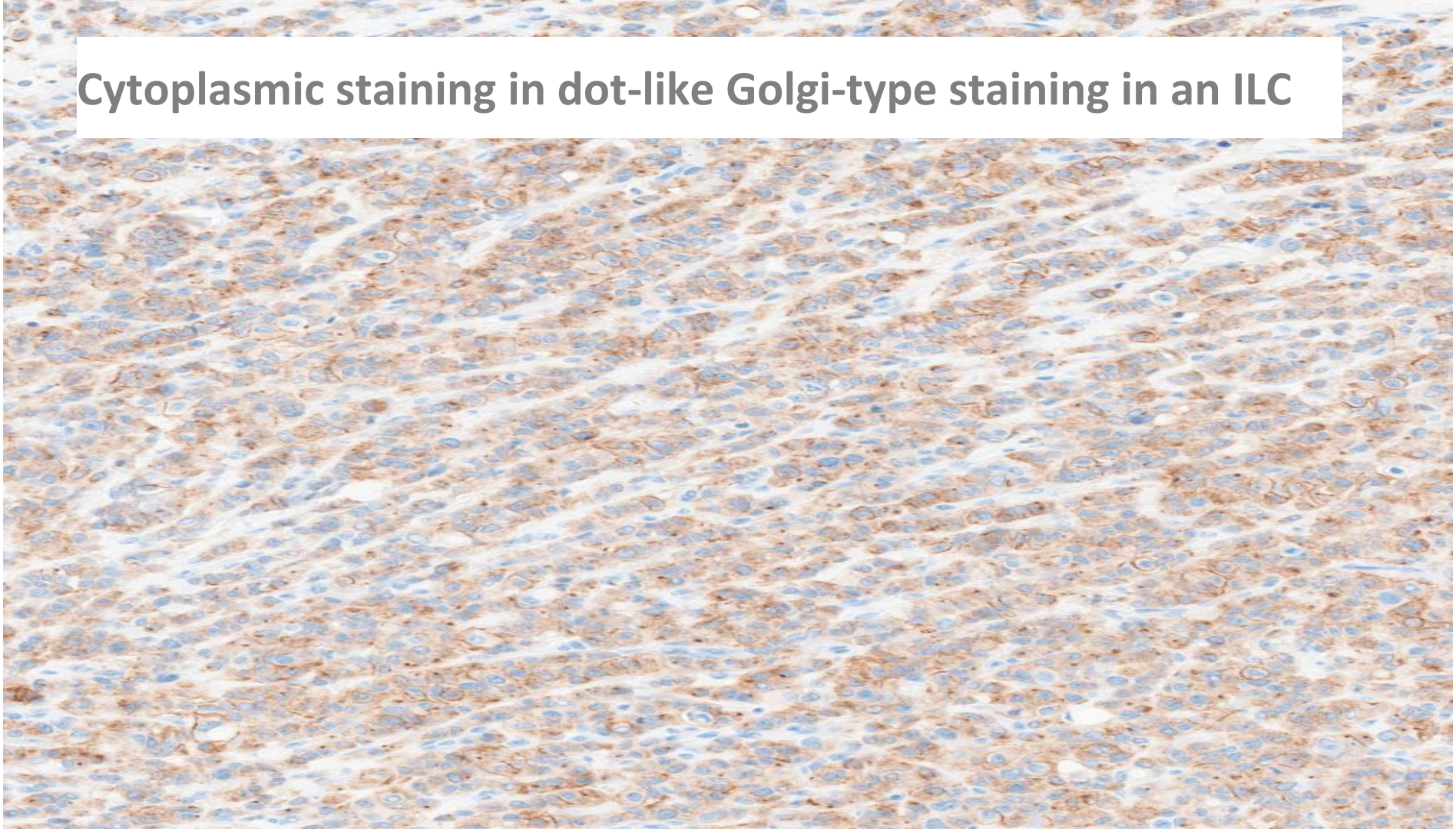
Cytoplasmic staining in dot-like Golgi-type staining in an ILC



E-cadherin, p120 Catenin and β-catenin patterns of expression:

	Normal epithelium	LCIS and ILC	DCIS and IDC
E-cadherin	Membrane staining	Absence of membrane staining	Membrane staining
p120 catenin	Membrane staining	Cytoplasmic staining	Membrane staining
β-catenin	Membrane staining	Absence of membrane staining	Membrane staining

LCIS, Lobular carcinoma *in situ*; ILC, invasive lobular carcinoma; DCIS, ductal carcinoma *in situ*; IDC, invasive ductal carcinoma.

- E-cadherin and β-catenin behave almost the same
- Is it helpful in E-cadh positive ILCs?

Canas-Marquez and Schnitt et al, Histopathology 2016
Petruolo et al, Am J Surg Oncol 2017; Dabbs *et al* Am J Surg Path 2013; Christgen et al J Pathol Clin Res 2022

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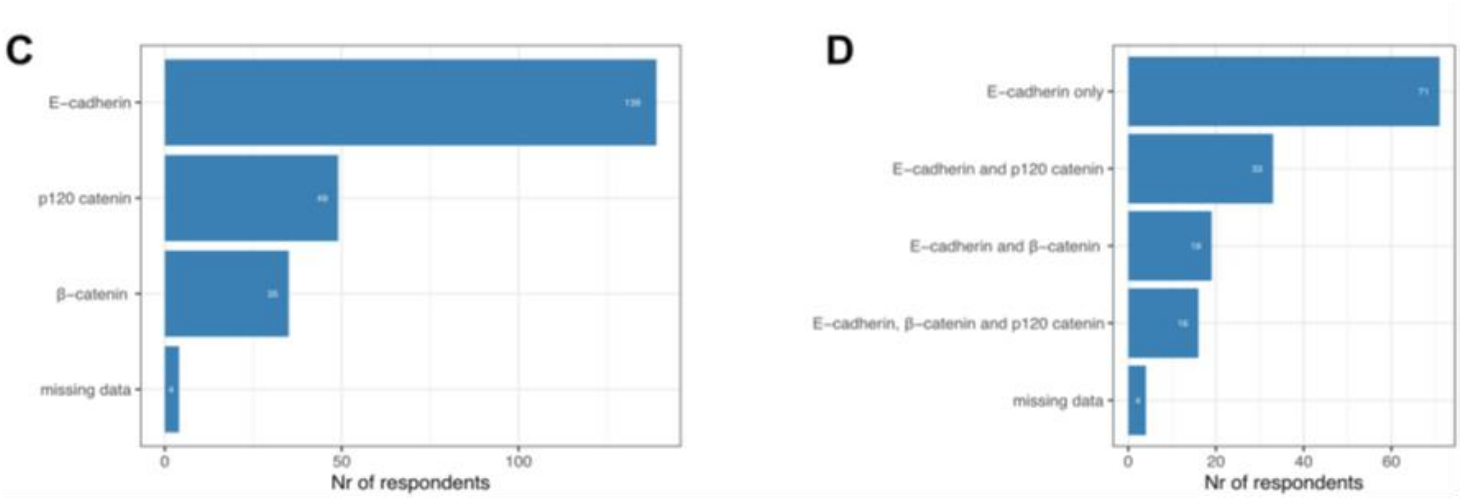
E-cadherin, p120 Catenin and b-catenin IHC use in practice



De Schepper *et al* Modern Path 2022

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Analysis of β-cat and p120-cat expression in E-cadh positive ILCs out of the I Curie series (251 cases)

CDH1 status		β-catenin			p120-catenin			
		Membrane staining			Membrane staining			Cytoplasmic staining
		% of cells	Intensity	Type	% of cells	Intensity	Type	% of cells
CDH1 mutated	Extra-cellular domain	90	weak	incomplete	40	moderate	incomplete	10
		100	strong	incomplete	70	moderate	incomplete	1
		90	moderate	incomplete	90	moderate	incomplete	30
		95	moderate	incomplete	95	strong	incomplete	0
		90	moderate	incomplete	85	moderate	incomplete	5
		100	moderate	incomplete	90	moderate	incomplete	0
		100	strong	incomplete	100	strong	incomplete	0
		90	moderate	incomplete	90	moderate	incomplete	0
		100	moderate	incomplete	90	moderate	incomplete	0
		100	strong	complete	100	strong	complete	0
		100	moderate	incomplete	100	moderate	incomplete	0
		100	moderate	incomplete	80	weak	incomplete	60
	ICD	5	weak	incomplete	60	weak	incomplete	10
No mutation		70	weak	incomplete	40	weak	incomplete	10
		100	moderate	incomplete	70	weak	incomplete	10
		100	moderate	complete	100	strong	Complete	0
		100	strong	incomplete	90	moderate	incomplete	0
		100	strong	incomplete	90	strong	incomplete	0
		100	weak	incomplete	85	weak	Incomplete	5

Djerroudi *et al* Mod Path 2024

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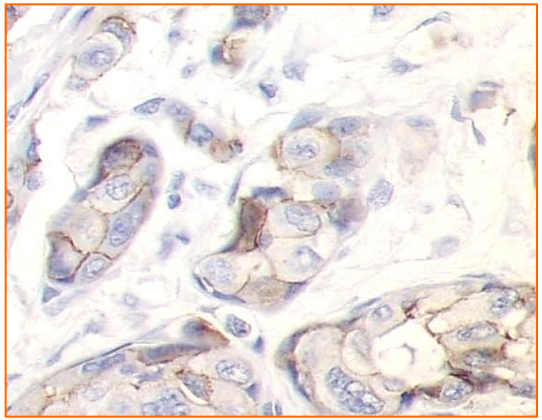
Non lobular carcinomas: E-cadherin negative and *CDH1* alterations

- **Decreased E-cadh expression:** 7,2 to 21% of IC-TNS high grade and TNBC



- **Bi-allelic *CDH1* alterations: 0,11%**

(“ 7 of the 5842 BCs harbored biallelic *CDH1* alterations and lacked lobular features”).

Derakhshan *et al* Hum Path 2020

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Does Sequencing for *CDH1* can help ?

- **In case of normal or discordant features between histological findings and immunohistochemistry,**

- **Sequencing for *CDH1* gene is performed for the definitive diagnosis:**
  - For only 4 out of 120 pathologists
  - In less than 5% of ILC cases

De Schepper *et al* Modern Path 2022

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E-cadherin positive versus negative ILCs have same rates of *CDH1* mutations but less *CDH1* truncating mutations

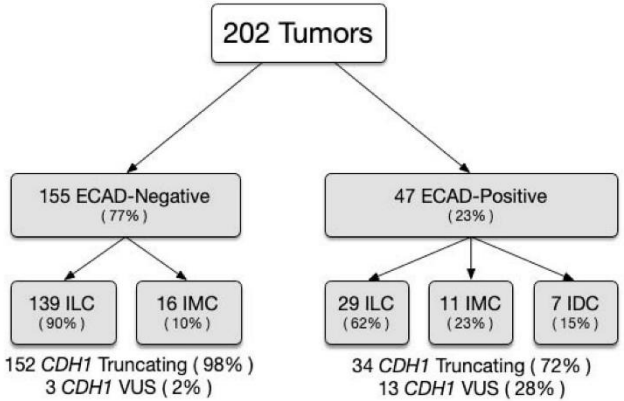

Figure 2:
Study cohort: E-cadherin immunohistochemical staining pattern, *CDH1* alteration and histologic subtype
ECAD – E-cadherin, ILC – invasive lobular carcinoma, IMC – invasive mammary carcinoma with mixed ductal and lobular features, IDC – invasive ductal carcinoma, VUS – variant of unknown significance

"ECAD-positive BC had less *CDH1* truncating mutations, the staining pattern was distributed as follows"

Supplementary Table S1. E-cadherin protein expression by immunohistochemistry in primary invasive lobular carcinomas according to *CDH1* genetic status.

E-cadherin expression	*CDH1* genetic status as per targeted sequencing (MSK-IMPACT)			
	CDH1 biallelic genetic inactivation (n=211)	*CDH1* monoallelic genetic inactivation (n=16)	No *CDH1* genetic alterations (n=18)	Total
Negative	195 (92.4%)	15 (93.8%)	14 (77.8%)	224 (91.4%)
Decreased	6 (2.8%)	0 (0.0%)	2 (11.1%)	8 (3.3%)
Aberrant	10 (4.7%)	1 (6.3%)	1 (5.6%)	12 (4.9%)
Retained	0 (0.0%)	0 (0.0%)	1 (5.6%)	1 (0.4%)
Total	211 (100.0%)	16 (100.0%)	18 (100.0%)	245 (100.0%)

"Aberrant E-cadherin expression was found at a comparable rate (P = 0.52; Fisher's exact test) across ILCs according to their *CDH1* status"

- E-cadh + ILC have:
  - a rate of ***CDH1* mutations (~70%), as comparable to E-cadh negative cases**
  - enriched in ***CDH1* non-truncating** mutations
  - lack signal/pro-peptide domain mutations

Grabenstetter *et al* Hum Path 2020

Dopeso *et al* NPJ Precision Oncology 2024
Djerroudi *et al* Mod Path 2024

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E-cadh positive ILCs without *CDH1* alterations

- ***AXIN2* alterations (fusions n = 2/23; mutation n = 1/23)**
- **1 *CTNND1* (p120 encoding gene) mutation**

Dopeso *et al* NPJ Precision Oncology 2024

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Journal homepage: https://modernpathology.org/

Research Article

Integration of Pathological Criteria and Immunohistochemical Evaluation for Invasive Lobular Carcinoma Diagnosis: Recommendations From the European Lobular Breast Cancer Consortium

Maxim De Schepper[a,b], Thijs Koorman[c], François Richard[a], Matthias Christgen[d], Anne Vincent-Salomon[e], Stuart J. Schnitt[f], Paul J. van Diest[c], Gitte Zels[a,b], Freya Mertens[b], Marion Maetens[a], Isabelle Vanden Bempt[g], Nadia Harbeck[h,i], Ulrike Nitz[h,j], Monika Gräser[h,i,k], Sherko Kümmel[h,l,m], Oleg Gluz[h,i,n], Birgit Weynand[b], Giuseppe Floris[b,*], Patrick W.B. Derksen[c,*], Christine Desmedt[a,*], on behalf of the Pathology Working Group of the European Lobular Breast Cancer Consortium

Recommendations for ILC diagnosis

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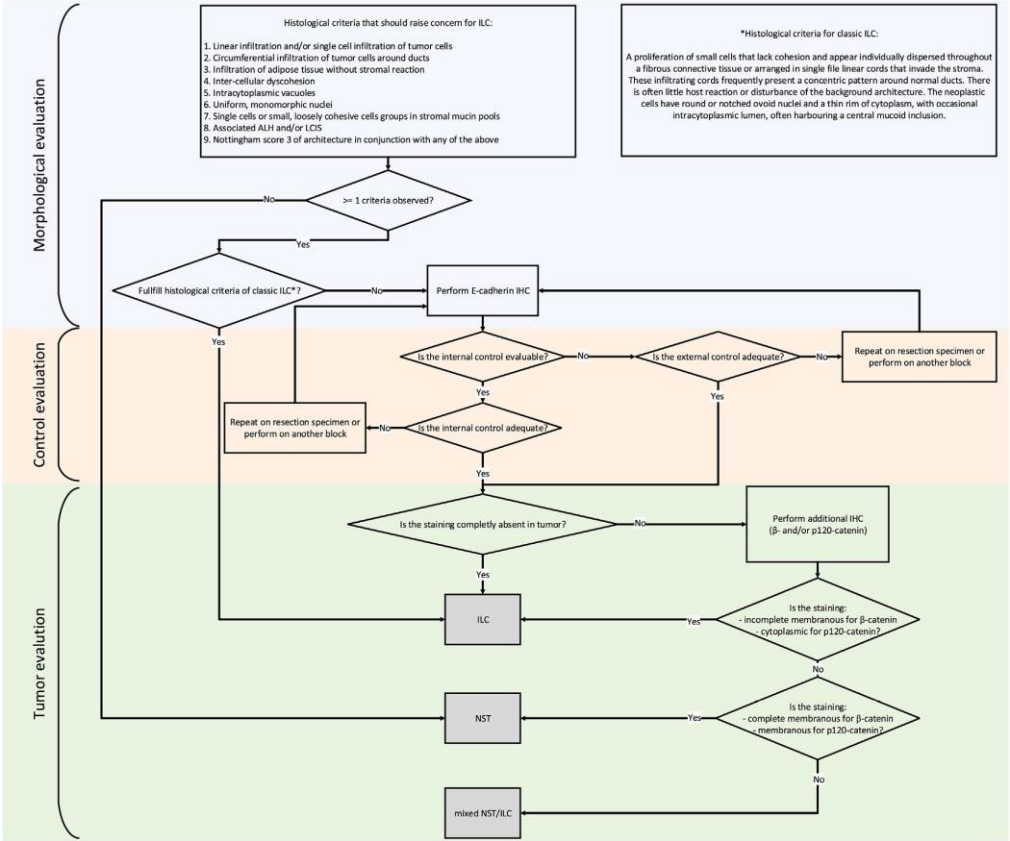
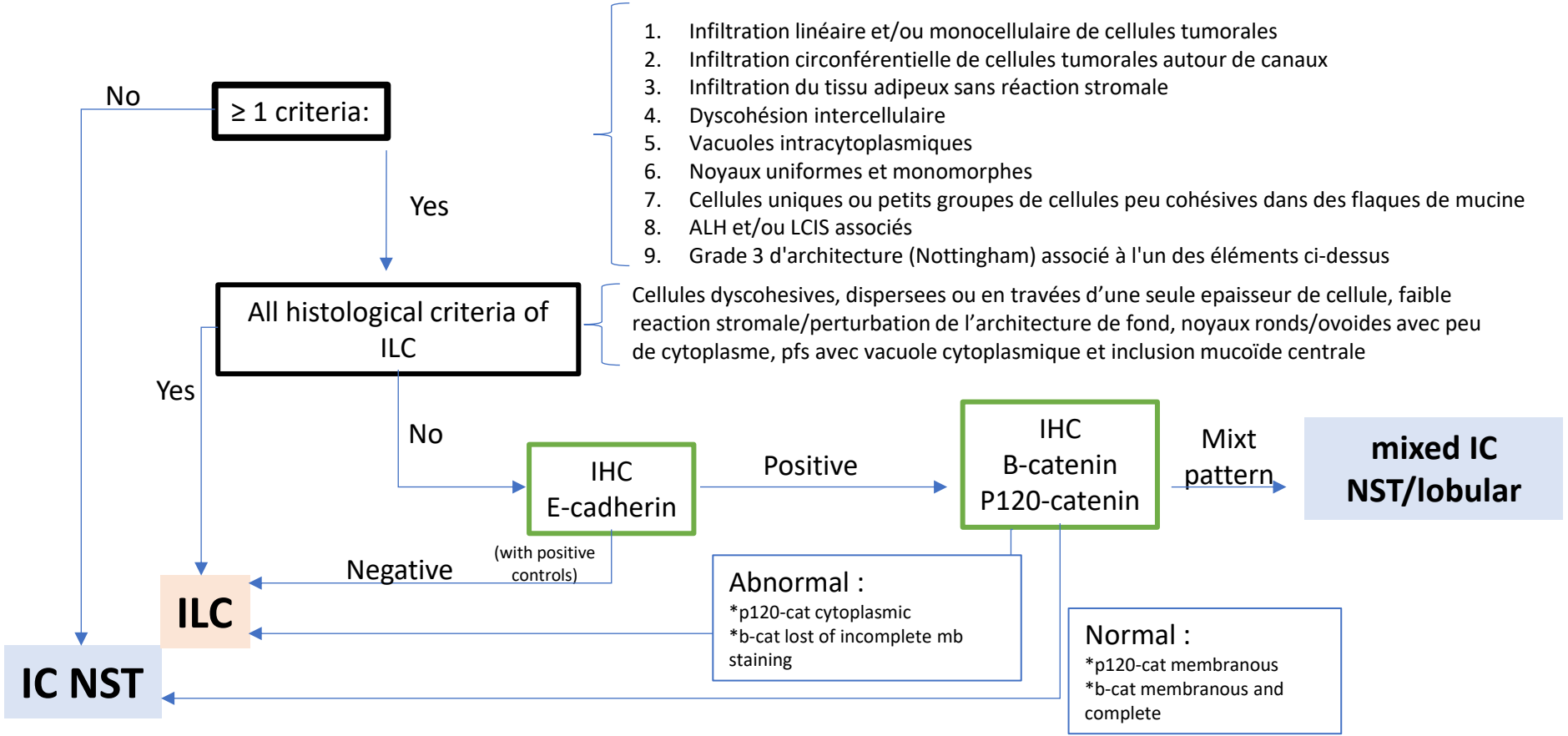


De schepper, Mod Path 2024

Diagnosis of ILC
Recommendations of the ELBCC



Pathology working group

Lobsterpot
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ON GOING WORK

Improve the standardization of ILC Variants diagnosis
CADELAC 2 study.

- **Review of digitalized slides of ILC variants**
- **Establish mandatory criteria for diagnosis of variants**
- **Propose a new classification of ILC variants**

Conclusions and take home messages:

- ILC hallmark = non cohesive cells
- Classic ILC **and** non classic (variants) ILC and their definition needs to be improved
- **E-cadherin loss of expression very helpful to improve diagnosis reproducibility**
- **15%** of ILC are **E-cadh positive** but with an aberrant E-cadherin staining (**decreased intensity & incomplete)**
- p120-cat IHC is helpful for ILC diagnosis **if the expression is intra-cytoplasmic**
- *CDH1* mutations *(> 65% of the cases)*
  - *same rate of CDH1 mutations in E-cadh + and – cases*
  - *E-cadh + ILC enriched in non truncating mutations*
- In ILC **with no** *CDH1* bi-allelic alterations
  - *63% of cases:* ***CDH1 methylation***
  - ***AXIN2*** alterations (fusions n = 2/23; mutation n = 1/23)

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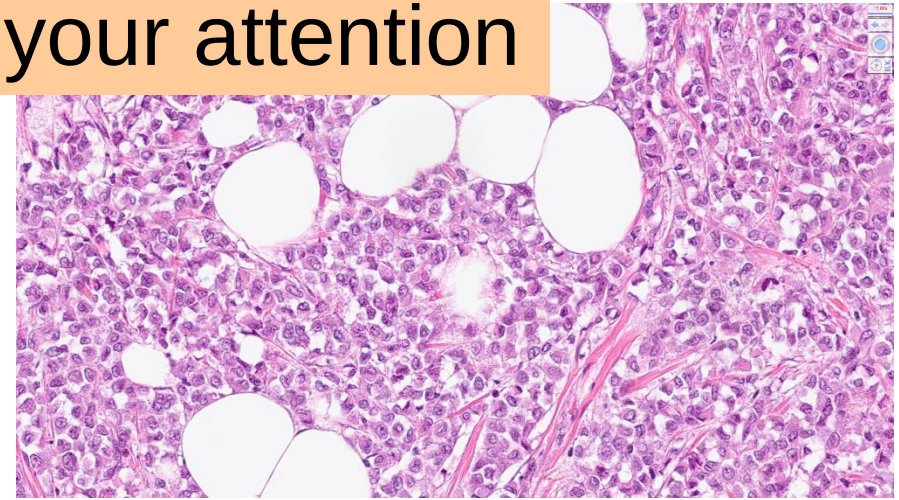
Thank you for your attention



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