How Many Diseases in Carcinoma in situ?

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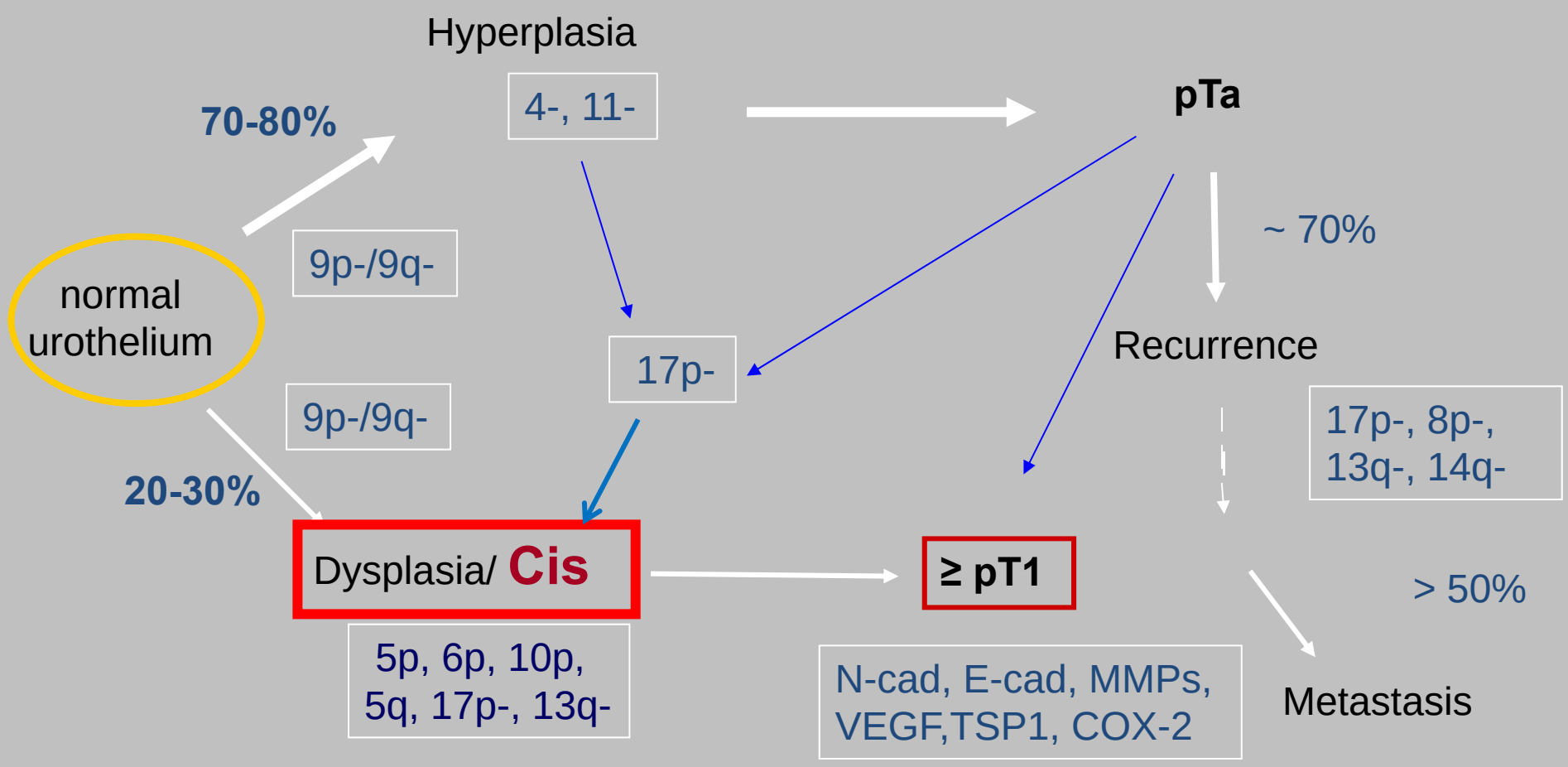
Carcinogenesis of Bladder Cancer (BC)

- BC is a **panurothelial** disease
- +/- Multifocal coexisting tumors
  - Separate tumors +/- same histology
- 2 theories
  - Monoclonal
    - Intraluminal implantation, intraepithelial migration
  - Field effect
    - Genetic alteration at different sites via carcinogenes
    - Multiple genetically unrelated tumors
    - No consensus

Cheng 2010
Hodges 2010
Morton 2007
Wang 2005

Carcinogenesis of BC



Wu ; Nature Review Cancer 2005

Cis

- Prognostic factor
- M = F
  - Age
    - Young age better outcome
  - ↑Risk of Upper urinary tract urothelial carcinoma
    - RR 2.3
  - ↑ Risk of relapse (p=0.045)
  - ↑ Risk of disease specific mortality (p=0.006)

Are there different types of Cis?

Molecular, clinical, histological point of view?

Genetics

Common Cis gene expression signature

- Microarray expression profiling

[CANCER RESEARCH 64, 4040–4048, June 1, 2004]

Gene Expression in the Urinary Bladder: A Common Carcinoma *in Situ* Gene Expression Signature Exists Disregarding Histopathological Classification

Lars Dyrskjøt,[1] Mogens Kruhøffer,[1] Thomas Thykjaer,[1] Niels Marcussen,[2] Jens L. Jensen,[3] Klaus Møller,[4] and Torben F. Ørntoft[1]

[1]Molecular Diagnostic Laboratory, Department of Clinical Biochemistry, Aarhus University Hospital, Skejby, Aarhus N; [2]University Institute of Pathology, Aarhus University Hospital, Aarhus C; [3]Department of Theoretical Statistics, Department of Mathematical Sciences, Ny Munkegade, Aarhus C; and [4]Department of Urology, Aarhus University Hospital, Skejby, Aarhus N, Denmark

- Gene expression patterns in pTa- pT4 UC with surrounding Cis

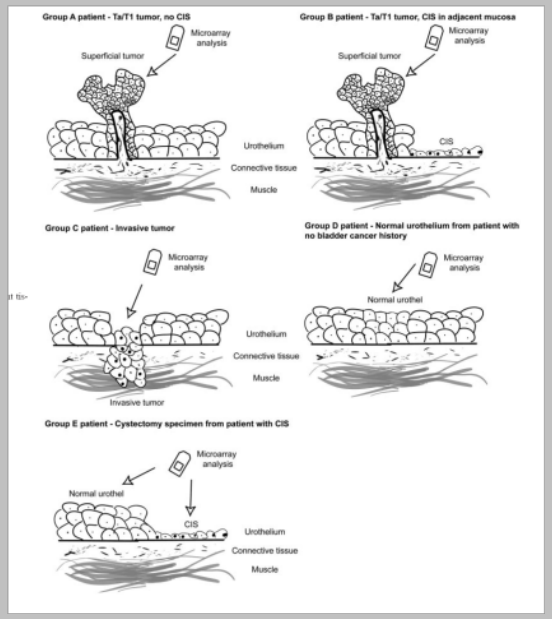
Cancer Res 2004

Common Cis gene expression signature

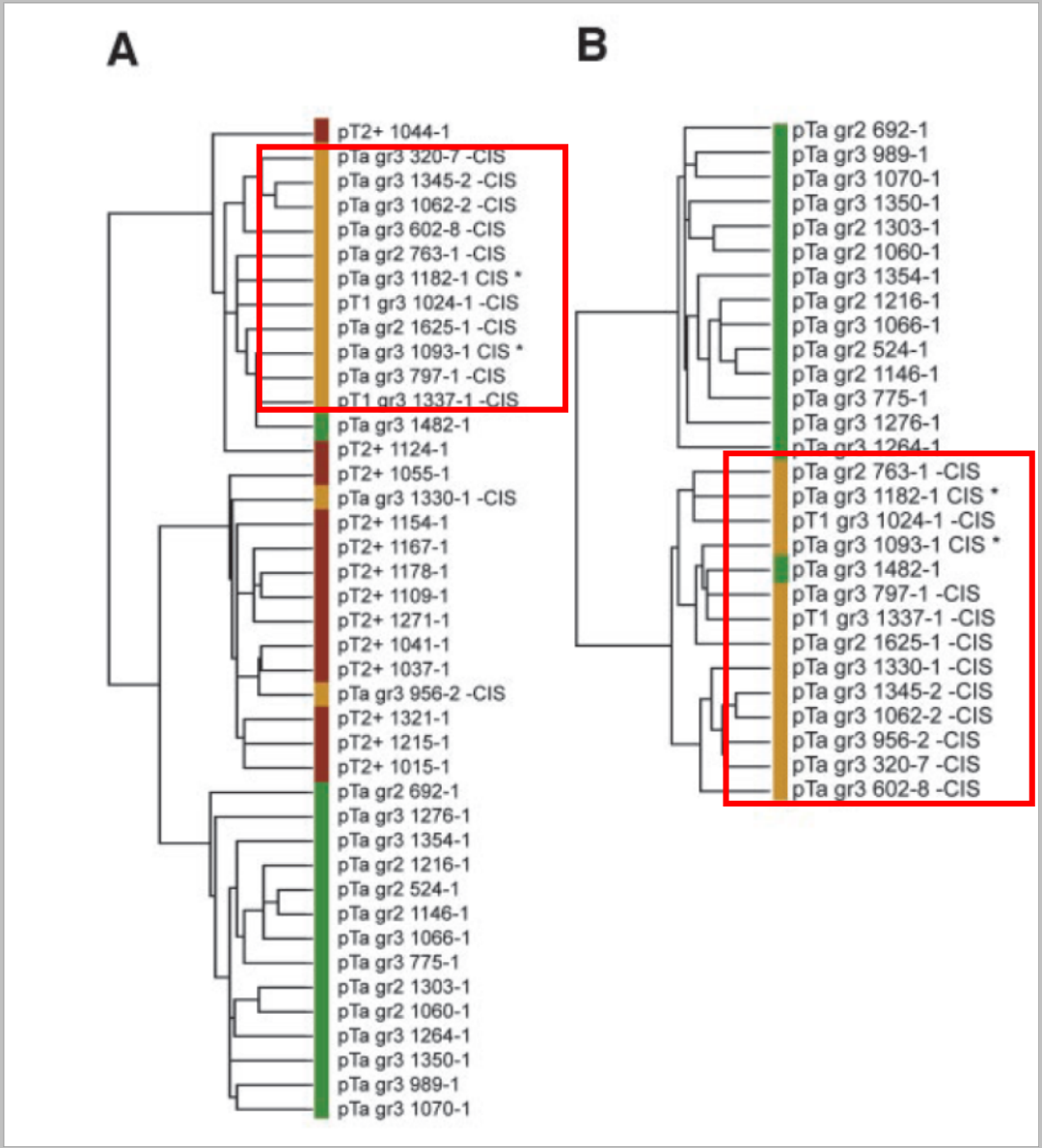
- Cluster analysed +/- Cis

- Normal tissu + Cis + UC
  - Same gene expression in all 3 tissue types
  - Same precursor cell?

- 16 gene-molecular Cis classifier
  - NMIBC classified according to +/- Cis
  - Similar gene expression in MIBC + Cis



Common Cis gene expression signature



Common Cis gene expression signature

- Involved genes
  - *LAMB3, ITGB4* → cell adhesion
  - *FABP4* ↑ non Cis
  - Several genes encoding connective tissue and immune response
- Alterations in the underlining connective tissue
  - Drive expression changes in the cells around Cis
  - Host communication?
  - Span large areas → explain multifocality? *
- Few ressemblance between MIBC and MIBC+Cis
  - Different expression profiles
- Identification of expression signature
  - Guide selection of patients for therapy and follow-up
  - Especially in early BC

*Liotta Nature 2004

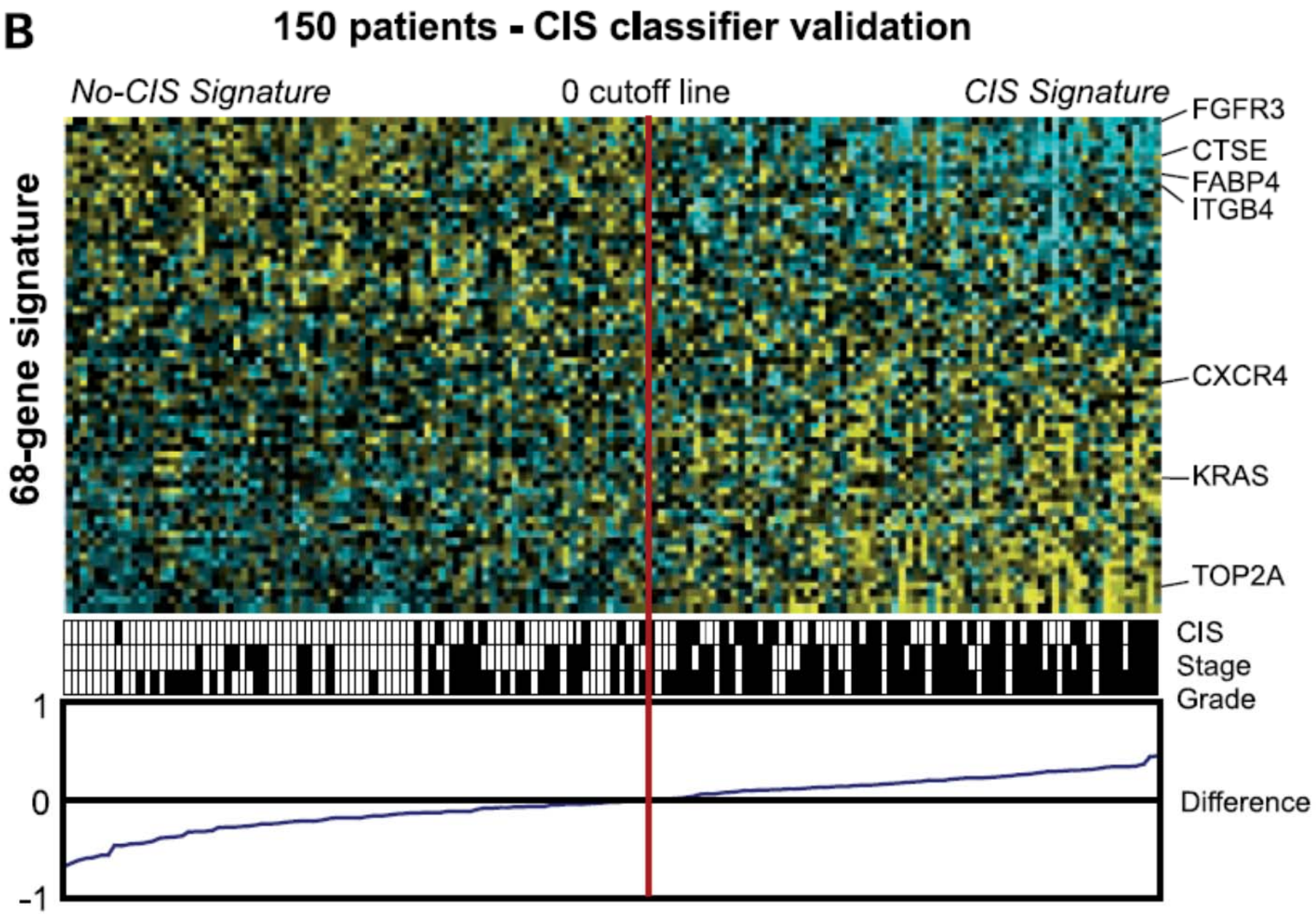
Gene expression signatures predicting outcome

- Gene expression signatures for molecular diagnosis in NMIBC and Cis

Gene Expression Signatures Predict Outcome in Non – Muscle-Invasive Bladder Carcinoma: A Multicenter Validation Study

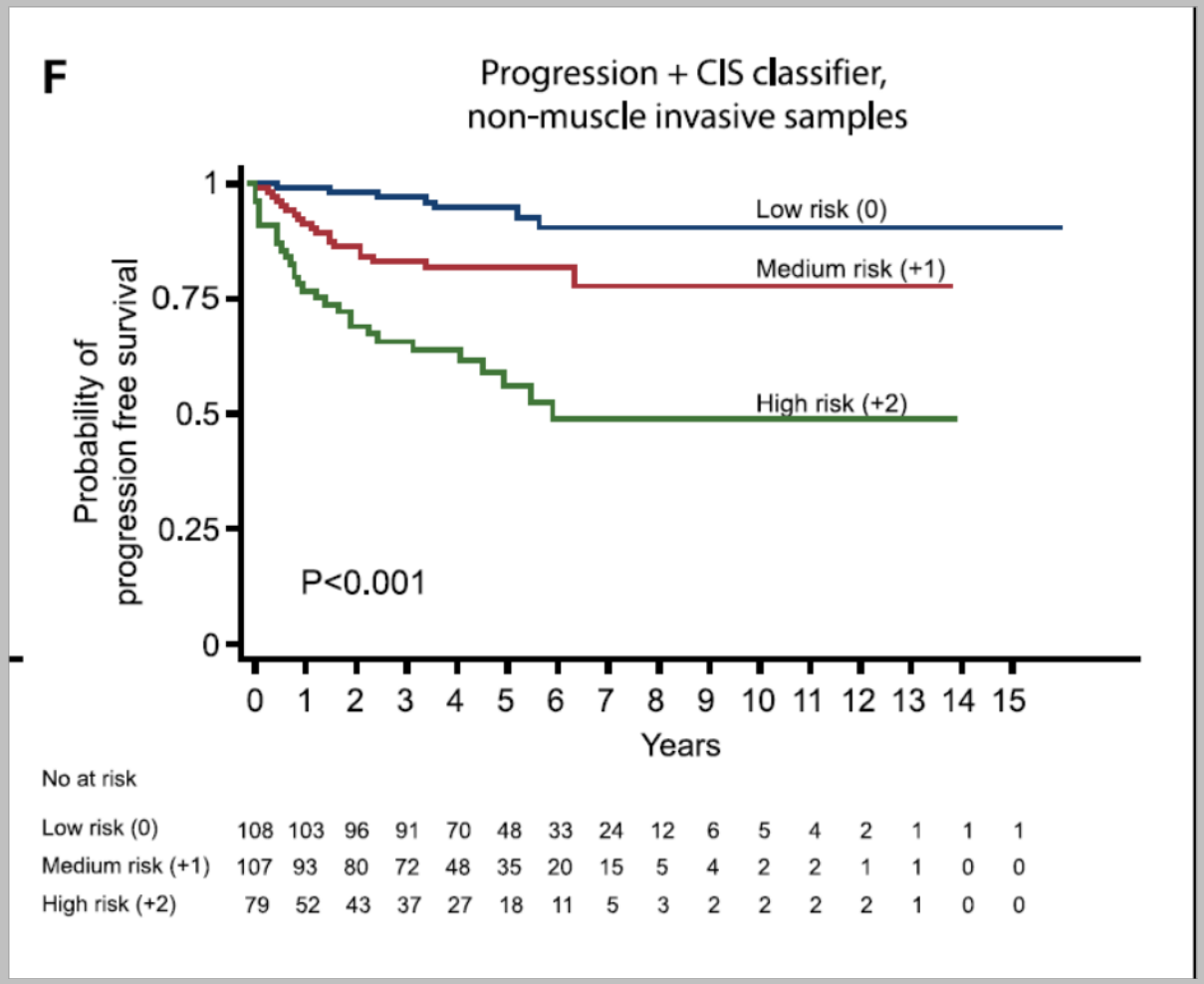
Lars Dyrskjøt,[1] Karsten Zieger,[1,2] Francisco X. Real,[4] Núria Malats,[5] Alfredo Carrato,[6] Carolyn Hurst,[7] Sanjeev Kotwal,[8] Margaret Knowles,[7] Per-Uno Malmström,[9] Manuel de la Torre,[10] Kenneth Wester,[10] Yves Allory,[11] Dimitri Vordos,[11] Aurélie Caillault,[12] François Radvanyi,[12] Anne-Mette K. Hein,[1] Jens L. Jensen,[3] Klaus M.E. Jensen,[2] Niels Marcussen,[13] and Torben F. Ørntoft[1]

Dryskjot Clin Cancer Res 2007


B
150 patients - CIS classifier validation
No-CIS Signature
0 cutoff line
CIS Signature
68-gene signature
FGFR3
CTSE
FABP4
ITGB4
CXCR4
KRAS
TOP2A
CIS
Stage
Grade
1
0
-1
Difference

Cis classifier validation

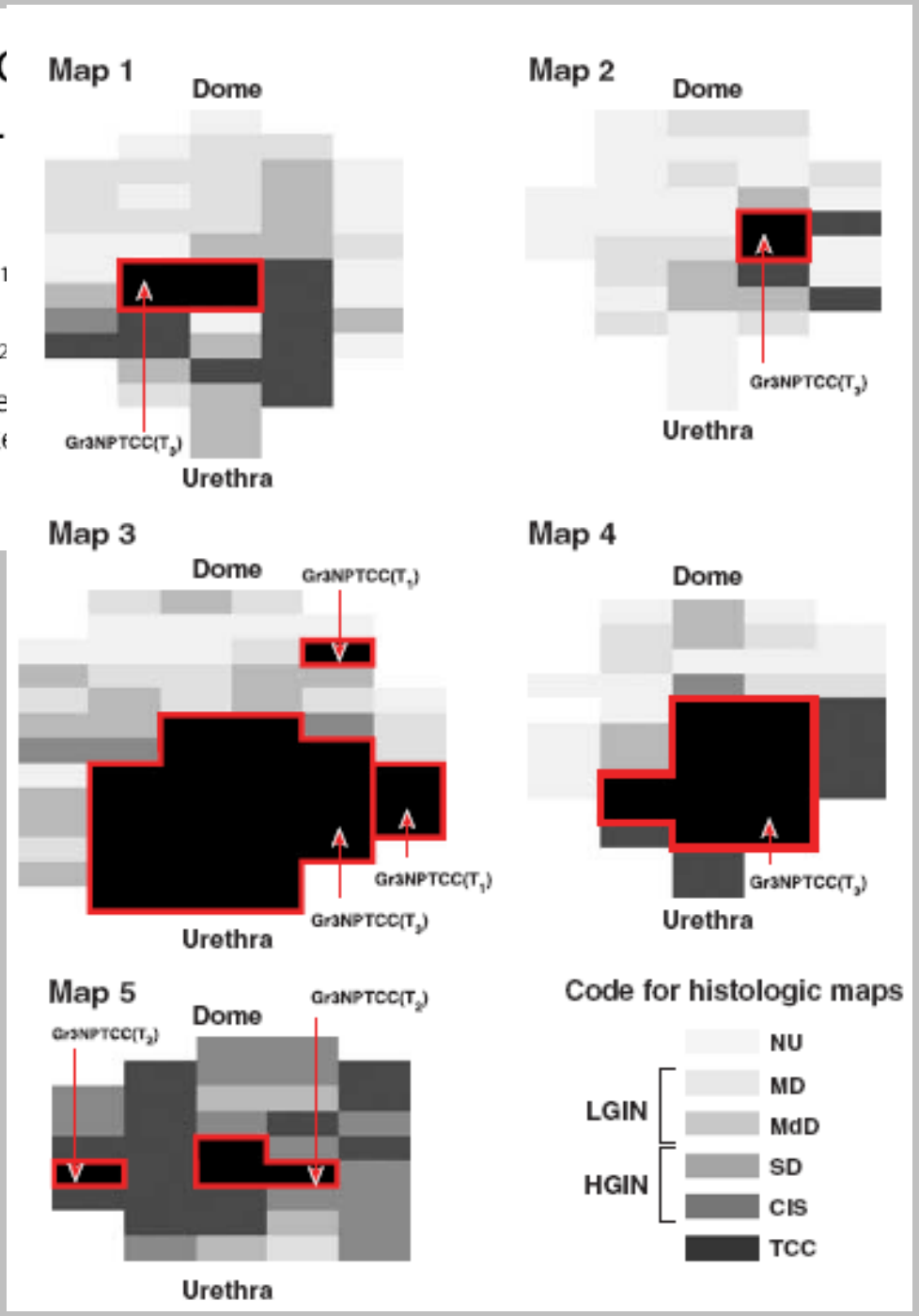
- Validation of classifier
- 68 gene signature
- Correlation with histologic Cis diagnosis
  - 80% sensitvity
  - 68% specifcity
  - OR 5.8, $p<0.001$ multivariate regression analysis
- Cis signature applying on progression of NMIBC
  - 75% sensitvity
  - 55% specifcity



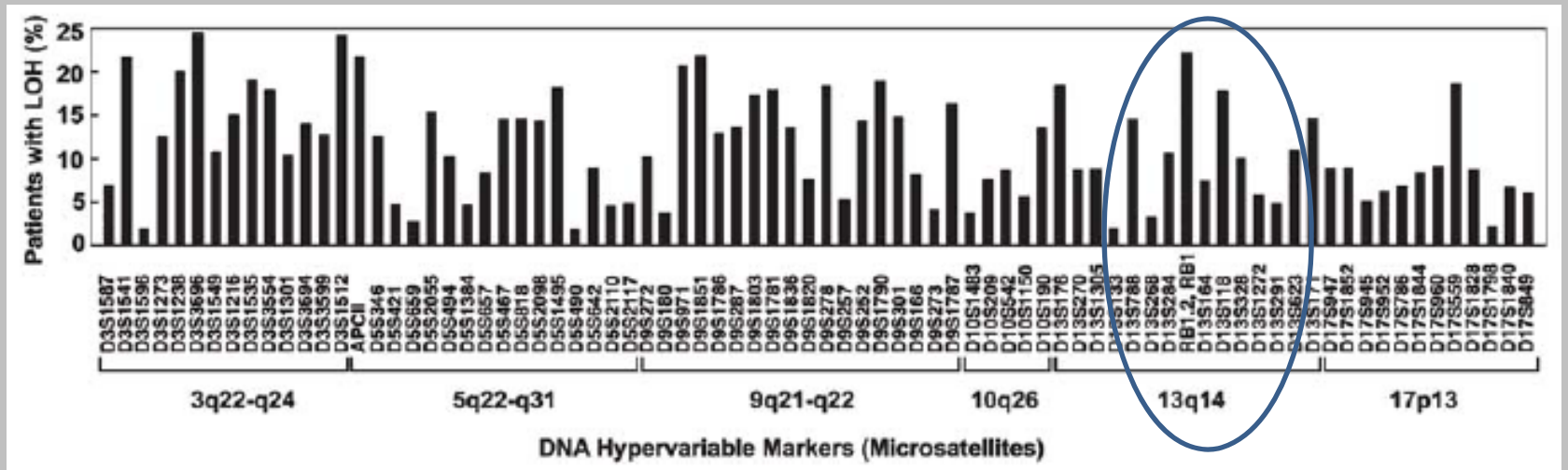
- Combined classification scheme
- Progression- and Cis signature → improve progression prediction
  - p=0.001
  - Hazard ratio 4.6
    - 95% confidence interval 1.87-11.52

Understanding the develo
cancer by using a whole-
strategy

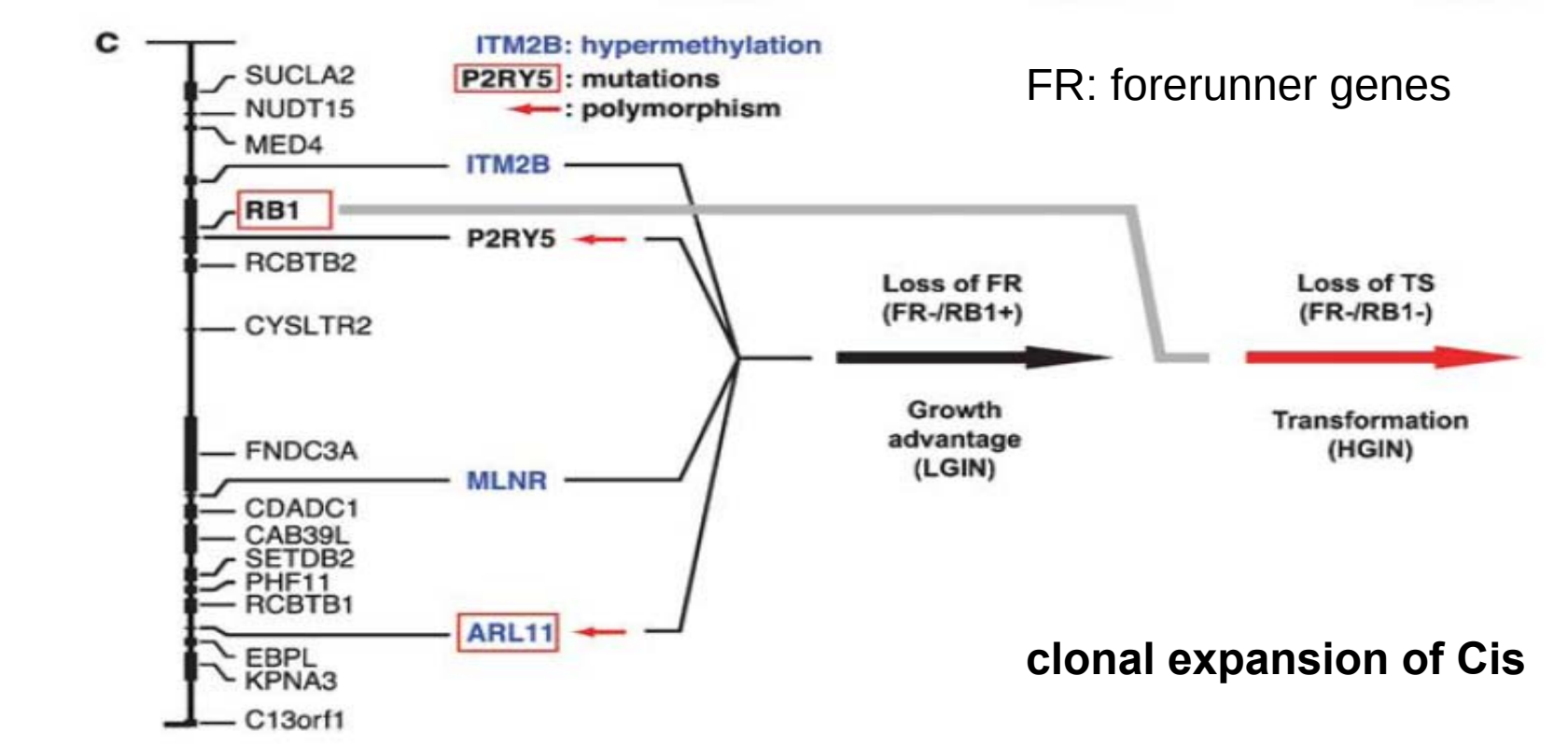
Tadeusz Majewski[1,*], Sangkyou Lee[1,*], Joon Jeong[1]
Tomasz Tuziak[1], Jolanta Bondaruk[1], Sooyong Lee[1],
Lanlan Shen[3], Saira S Ahmed[3], Dennis A Johnston[2]
R Alan Harris[6], Carrie Snyder[7], Slawomir Filipek[8], Ste
Menashe Bar-Eli[11], Xifeng F Wu[12], David J McConke
Steven E Scherer[6] and Bogdan Czerniak[1]

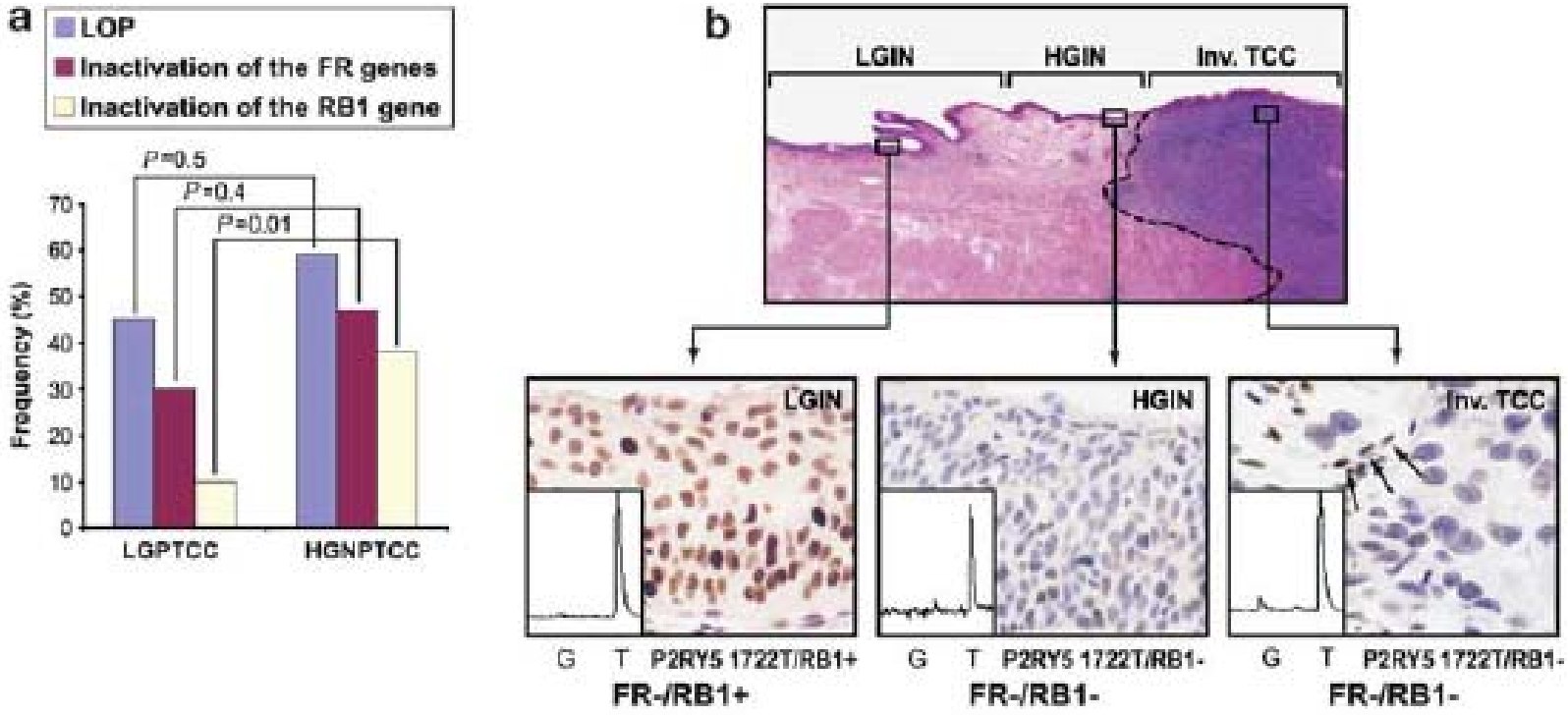


WOHGM
whole-organ histologic and genetic mapping



Identification of six critical chromosomal regions involved in the development of bladder cancer. (a) Genome-wide map of putative





- **Preneoplastic clones develop in normal urothelium**
- **Low grade dysplaisa**
- **Loss of FR genes → ↑ cellular growth**
- **Suppression of *RB1*→ Cis → invasive tumor**

Majewski

Cyclin D3 gene amplification in bladder carcinoma in situ

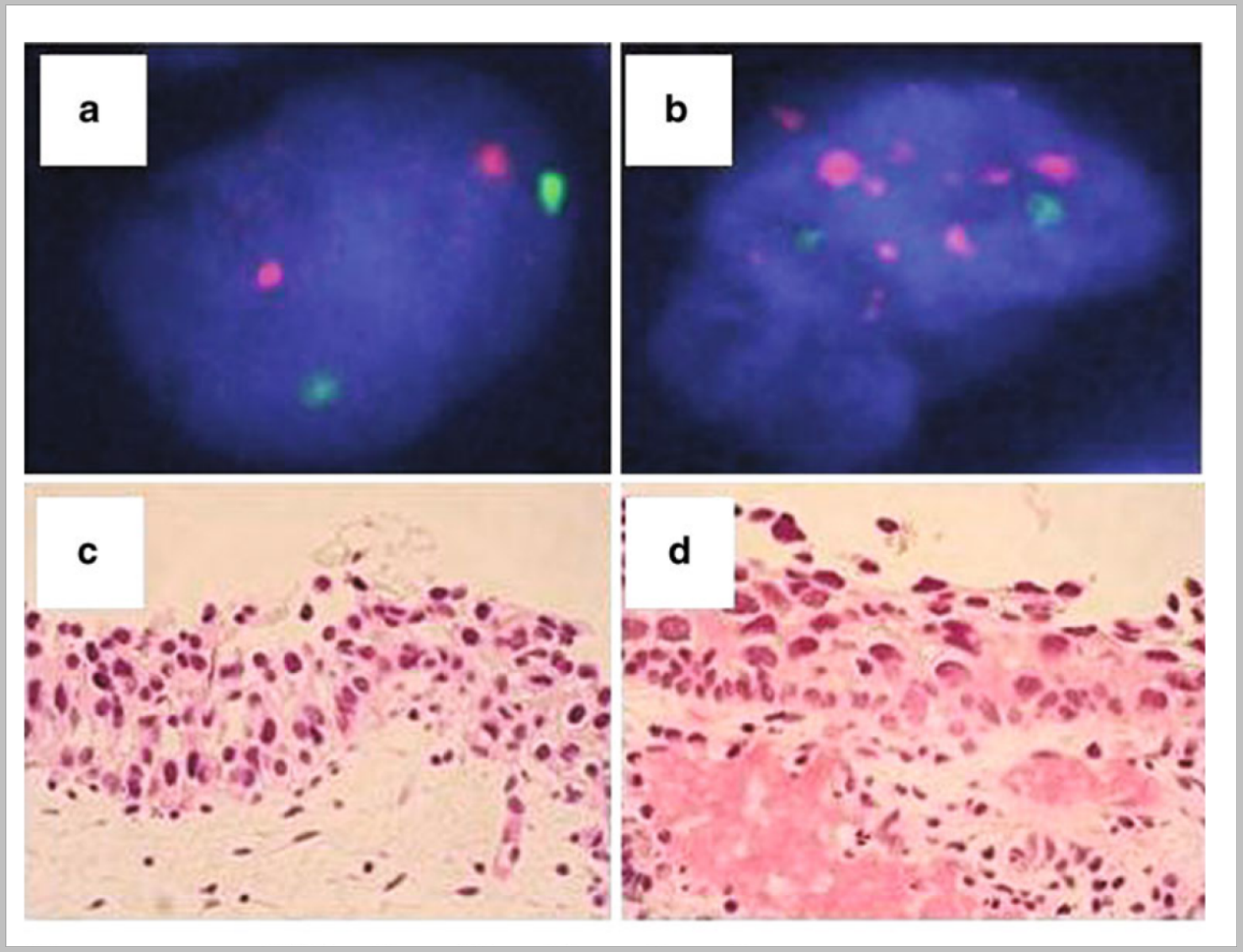
Antonio Lopez-Beltran · Jose L. Ordóñez · Ana P. Otero · Ana Blanca · Vicky Sevillano · Marta Sanchez-Carbayo · Elisa Muñoz · Liang Cheng · Rodolfo Montironi · Enrique de Alava

- Primary/ secondary Cis
- FISH *CyclinD3* amplification
  - Pivotal role G1→ S
  - Deregulated in BC

- Assess patterns of Cyclin D3 gene amplification
  - Cis after BCG-Therapy
  - Correlation with outcome

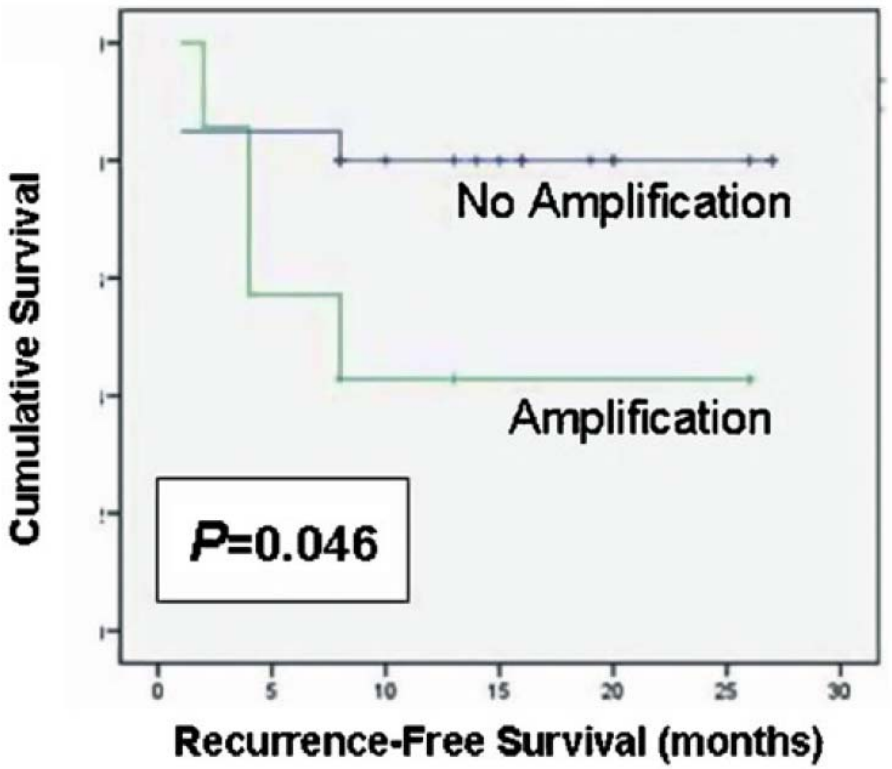
Lopez-Beltran J Pathol 2009
Lopez-Beltran Eur Urol 2004
Bartkova J Cancer 1996

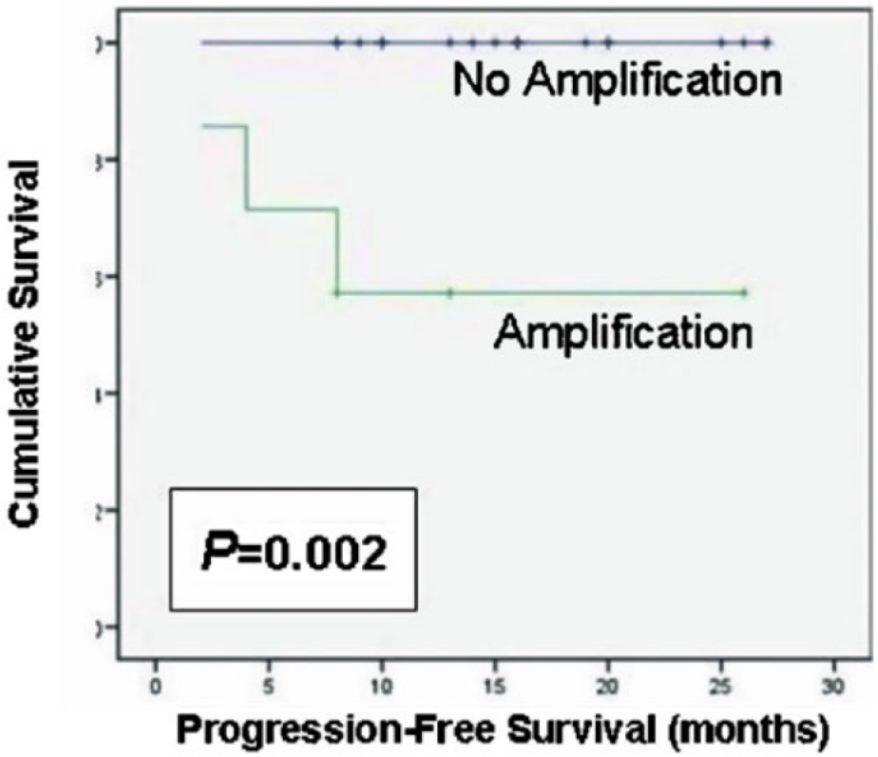
Cyclin D3



Cyclin D3

- No primary Cis with *Cyclin D3* amplification
- Amplification related to
  - Recurrence free and progression free survival





Cyclin D3

- Cyclin D3 independent predictor of progression-free survival

- None of primary Cis recurred

Variable	RR	95% CI	*P* value
Recurrence-free survival			
Cyclin D3 gene amplification	3.159	0.785–12.704	0.105
Type of CIS (primary vs. secondary)	24.535	0.4–154.062	0.057
Progression-free survival			
Cyclin D3 gene amplification	61.503	1.1–274.710	0.041
Type of CIS (primary vs. secondary)	23.945	0.9–240.458	0.065

Clinics

Clinical point of view

- 4 different types of Cis
  - Primary
    - No other tumor, no clinical history
  - Secondary
    - Detection during follow-up
  - Concomitant
    - Association with pTa, pT1
  - Recurrent

EAU guidelines 2013

Primary vs secondary *Cis*

Orozco	*De Novo*	Concomittant *Cis*
Symptoms	45%	62%
Progression	28%	59%
Dead of Disease	7%	45%

Lopez-Beltran : none of primary Cis recurred after BCG

Gofrit: Pure and concomittant Cis similar behaviour
63% vs 54% recurrence-free survival after BCG

Orozco Cancer 1994, Gofrit Urol Oncol 2009, Lopez Beltran Virchow 2010

Clinical Outcome of Primary Versus Secondary Bladder Carcinoma In Situ

Daher C. Chade, Shahrokh F. Shariat, Ari Adamy, Bernard H. Bochner, S. Machele Donat, Harry W. Herr and Guido Dalbagni*

221 primary Cis
255 secondary Cis

Primary better response to BCG, p<0.001

Progression to cT1 43% vs 32%

Progression cT2 17% vs 8%

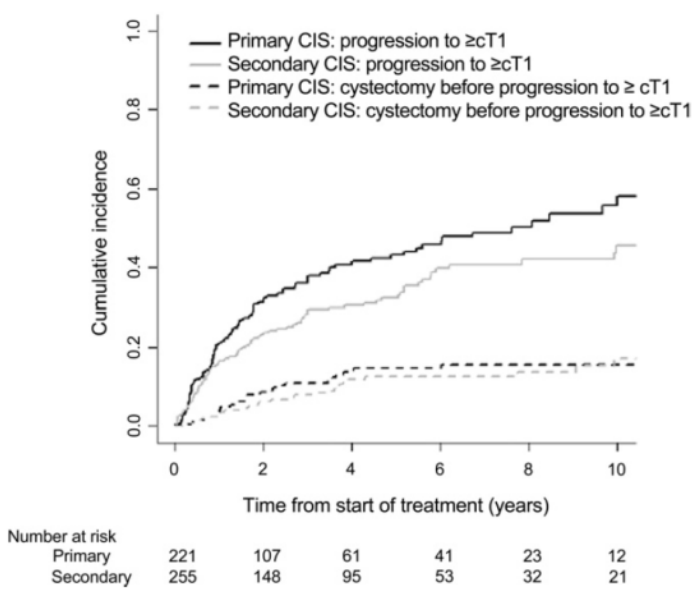


Figure 1. Cumulative incidence of progression to cT1 or higher invasive disease, or RC before progression in patients with primary or secondary CIS.

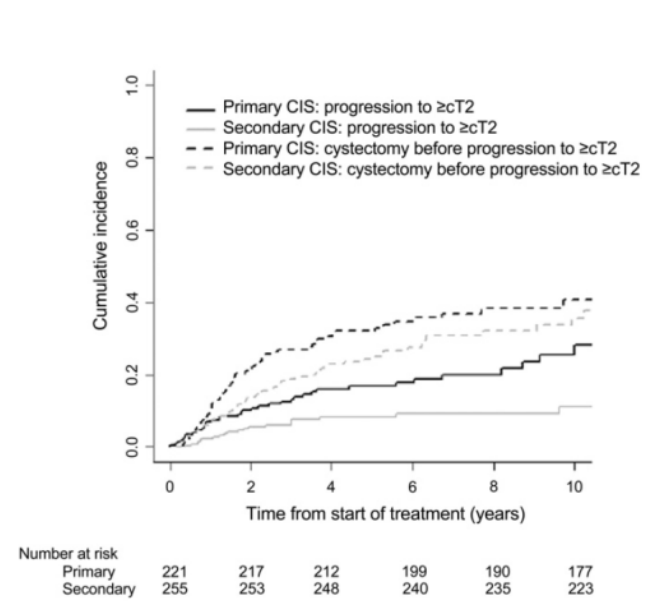


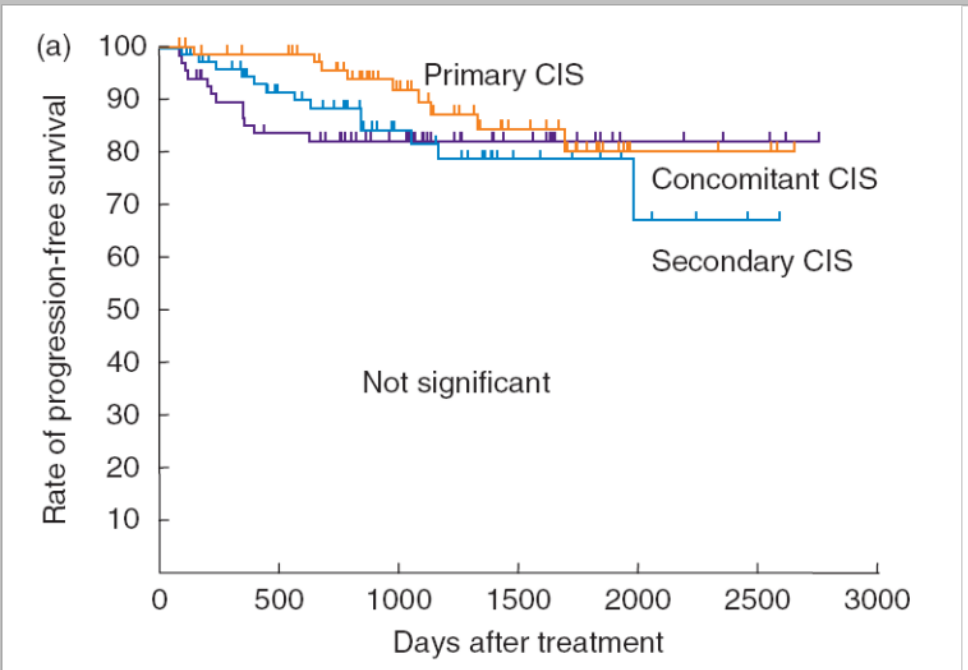
Figure 2. Cumulative incidence of progression to cT2 or higher muscle invasive disease, or RC before progression in patients with primary or secondary CIS.

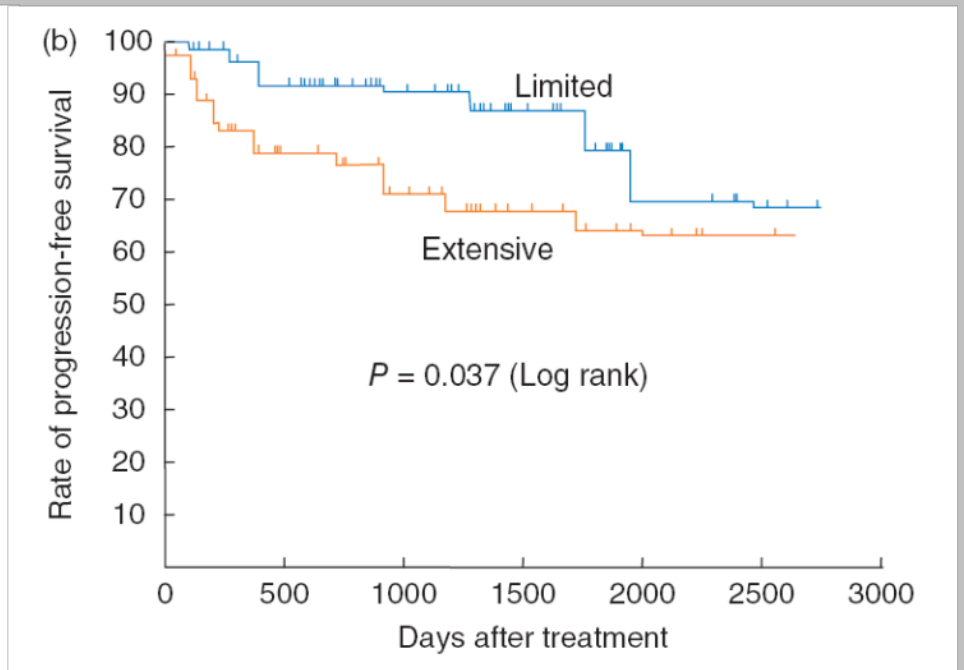
Clinical outcomes of bacillus Calmette-Guérin instillation therapy for carcinoma *in situ* of urinary bladder

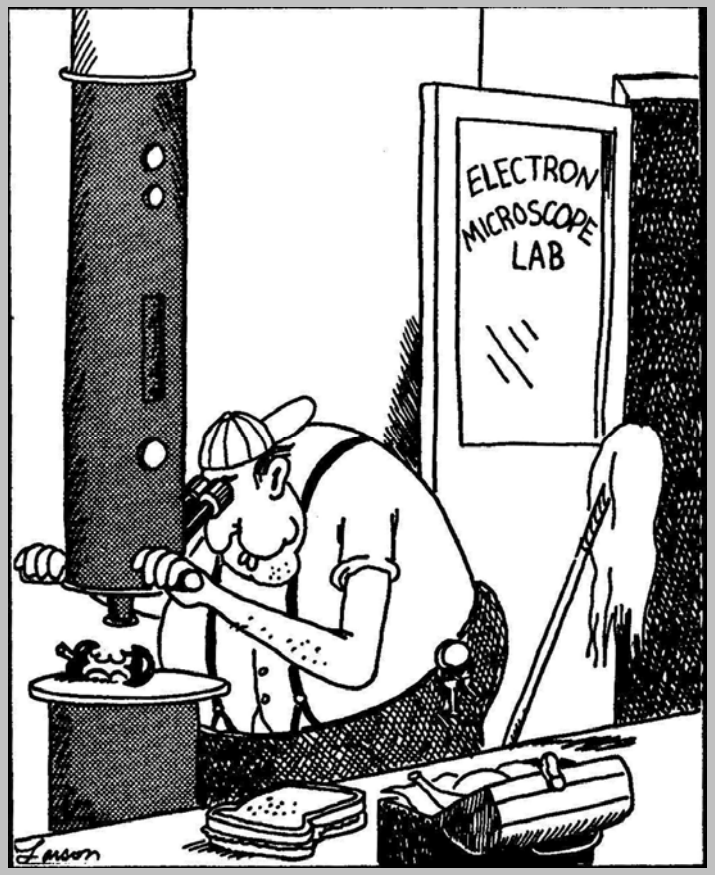
Atsushi Takenaka,[1] Yuji Yamada,[2] Hideaki Miyake,[1] Isao Hara[3] and Masato Fujisawa[1]

[1]Division of Urology, Kobe University Graduate School of Medicine, Kobe, [2]Department of Urology, Hyogo Prefectural Amagasaki Hospital, Amagasaki, [3]Department of Urology, Wakayama Medical University, Wakayama, Japan

- Primary vs secondary or concomittant
  - 5 a recurrence free survival 66%
  - Extent only independent prognostic factor







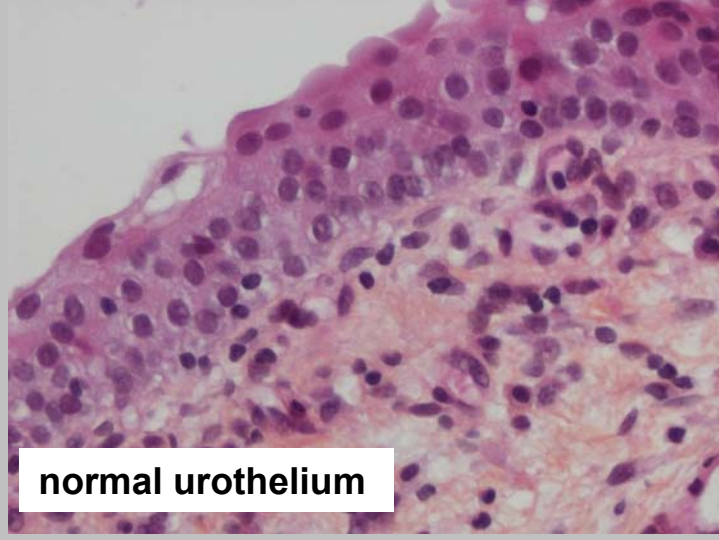
Real life

(Which means pathology)

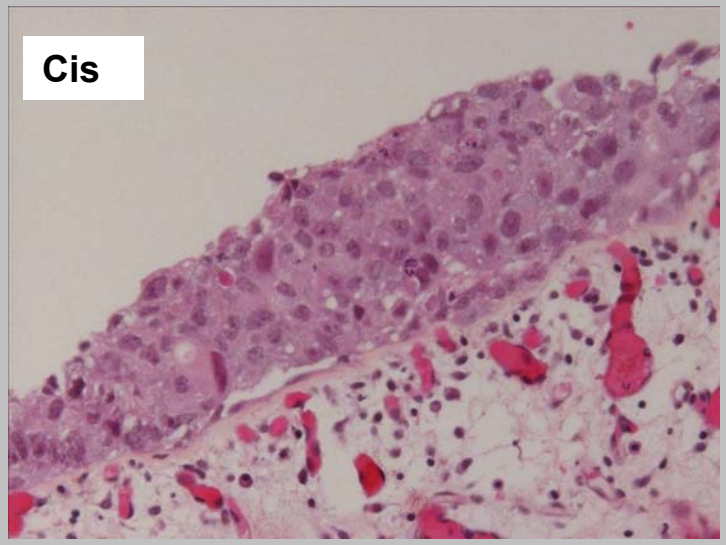
Flat Lesions WHO 2004

- Hyperplasia
- Flat lesions with atypia
- Reactive inflammatory type
- Atypia of unknown significance
- Dysplasia low-grade intraurothelial lesions
- **Carcinoma in situ (high grade intraurothelial lesions)**

Cis

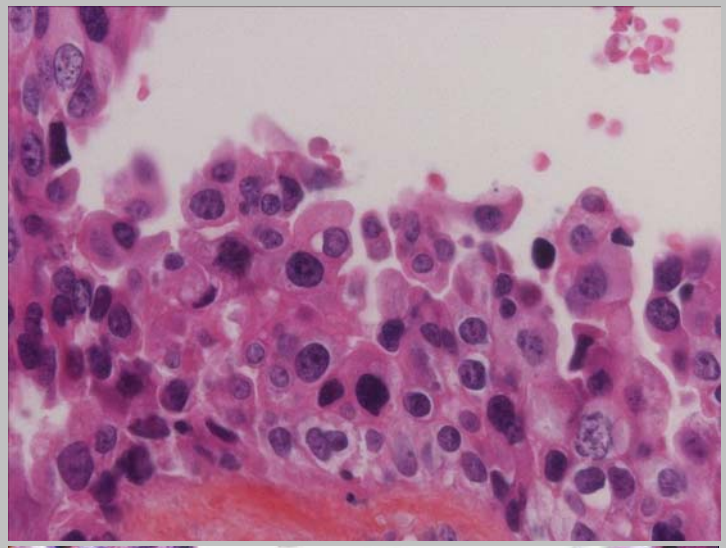


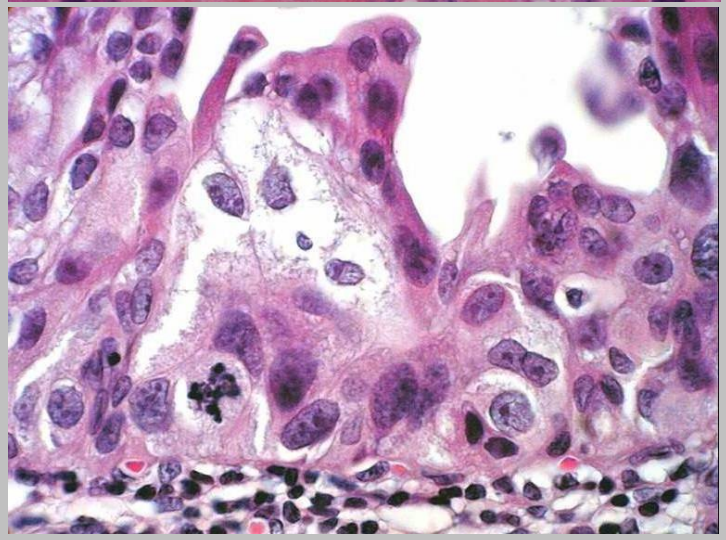
- Malignant flat lesion
  - DNA aneuploidy > 90%
- Does NOT invade underlying tissue
- Precurseur of invasive BC
- Isolated primary Cis in 3%
  - If concomitant worse outcome?
  - 50% association with pT1
  - 60% ≥ pT2

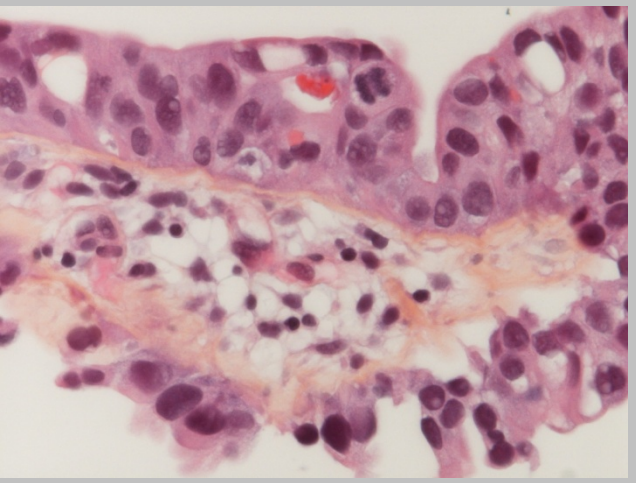
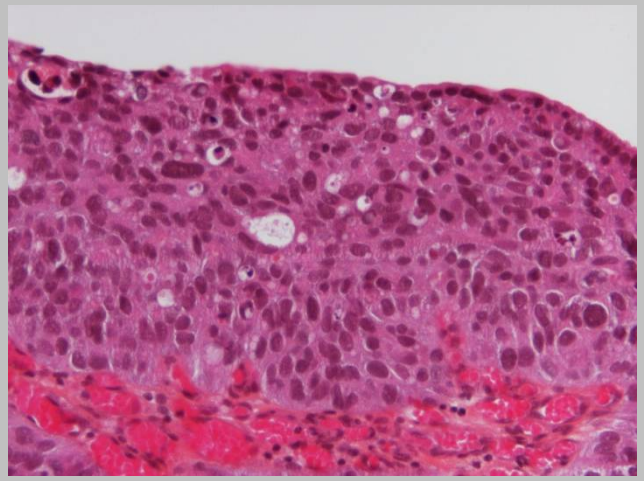
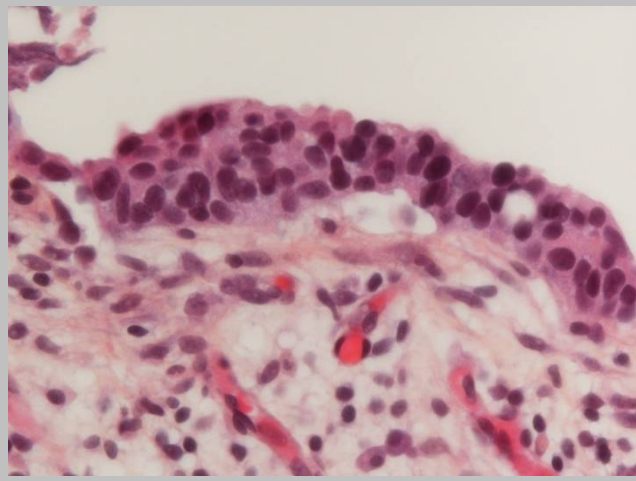


High Grade Dysplasia = C*is*

- Important atypia
- Discohesive
  - Denuded
- Diminished thickness
  - DD : Artifactually?
- Loss of polarity
- Pleomorphism, crowding
- Mitosis

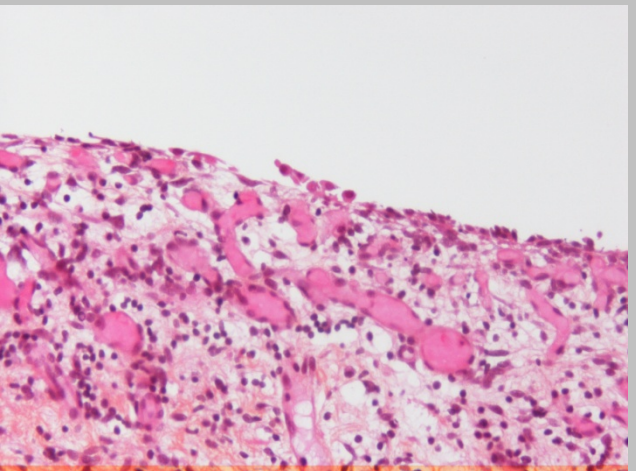
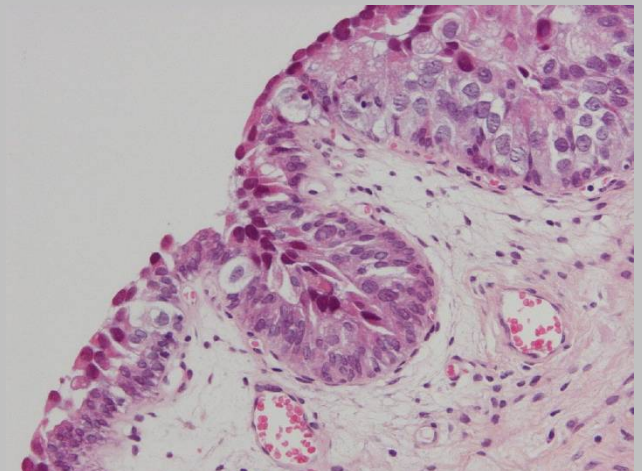
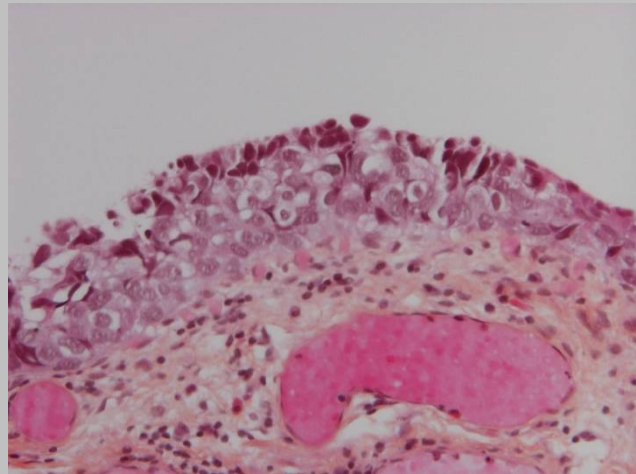




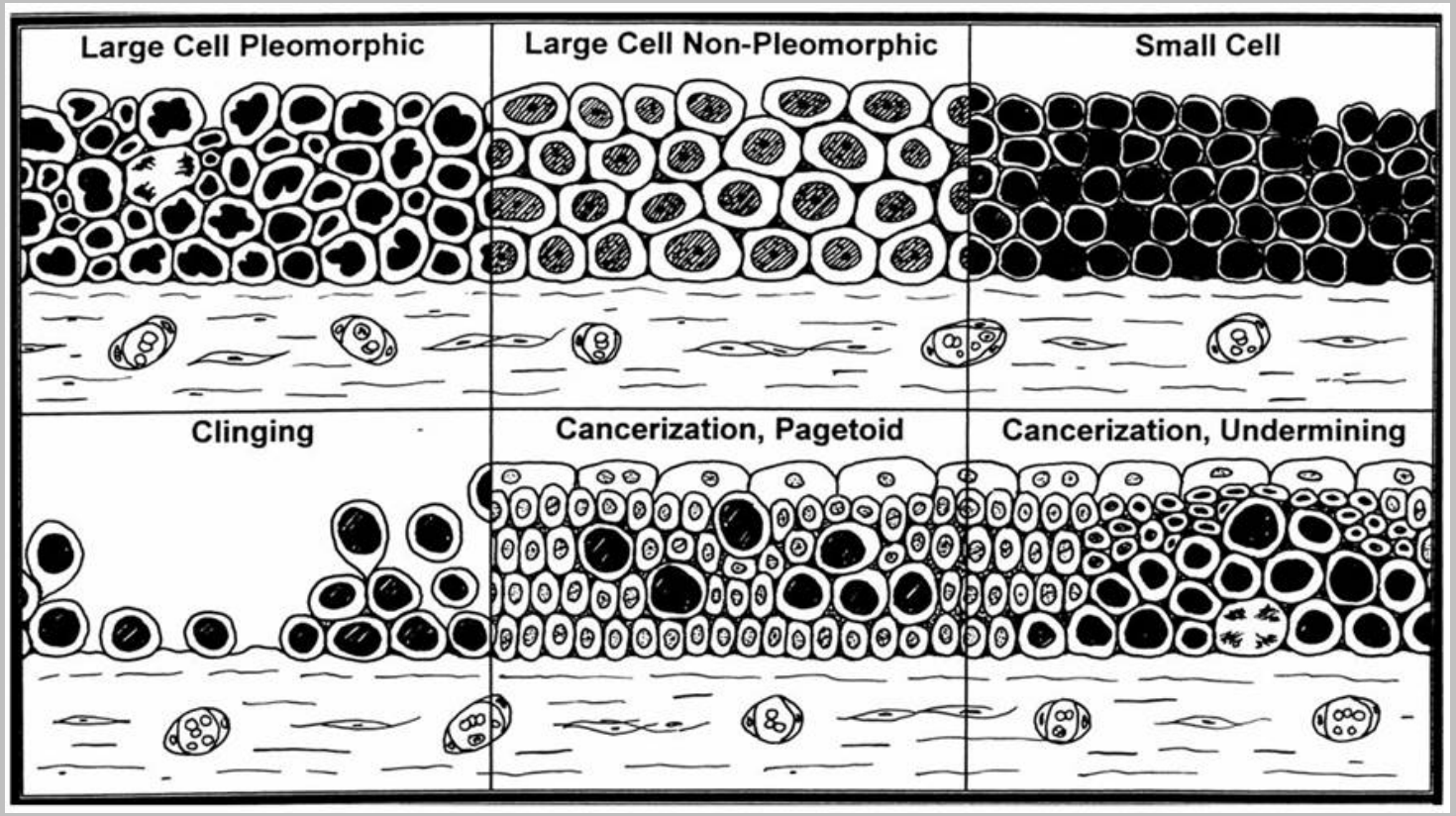




C*is*

Do they all look the same?

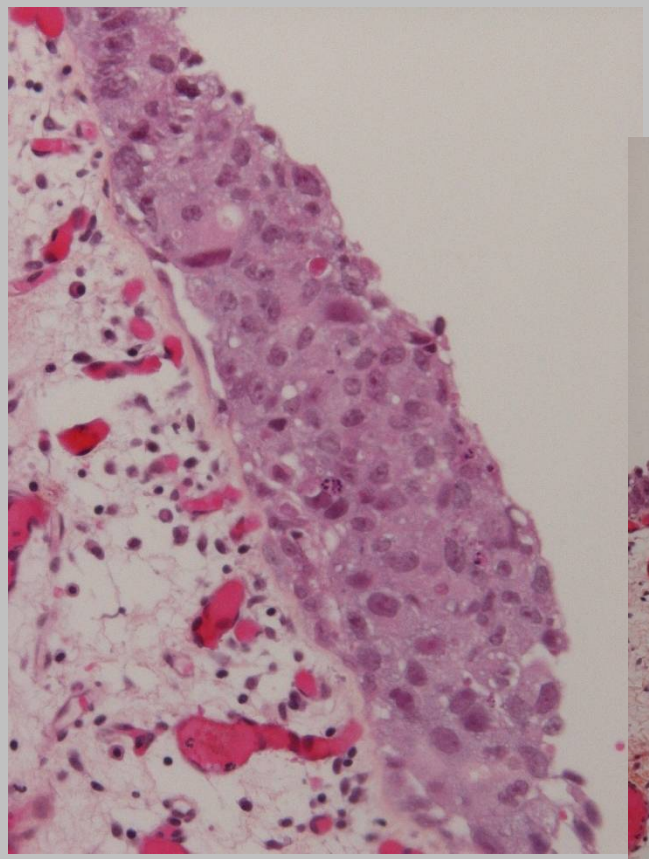




Different Histologic Patterns in Urothelial C*is*

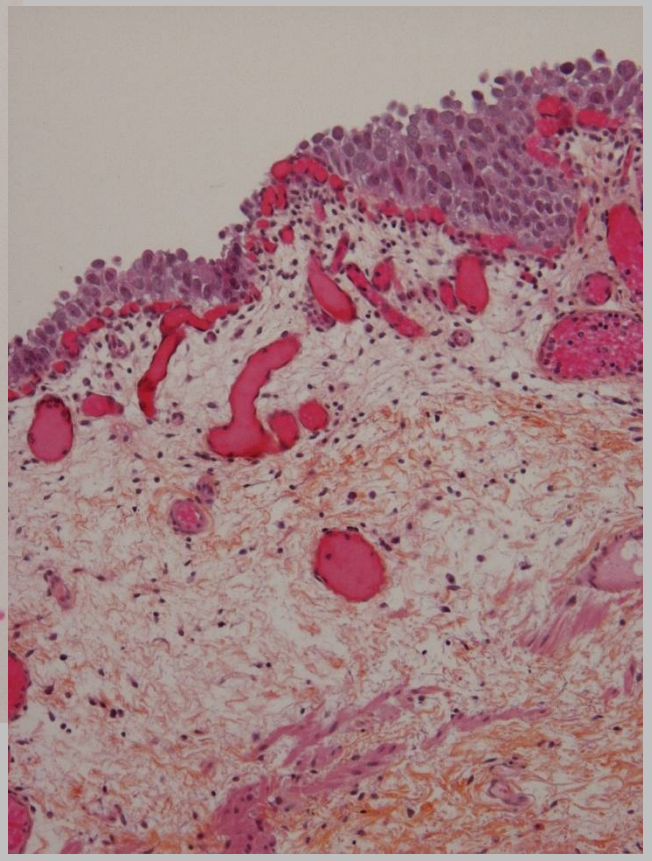


- Often purely descriptive
- Classification for pathologists
- Mixed forms exist

McKenny, 2001, Am J Surg Pathol

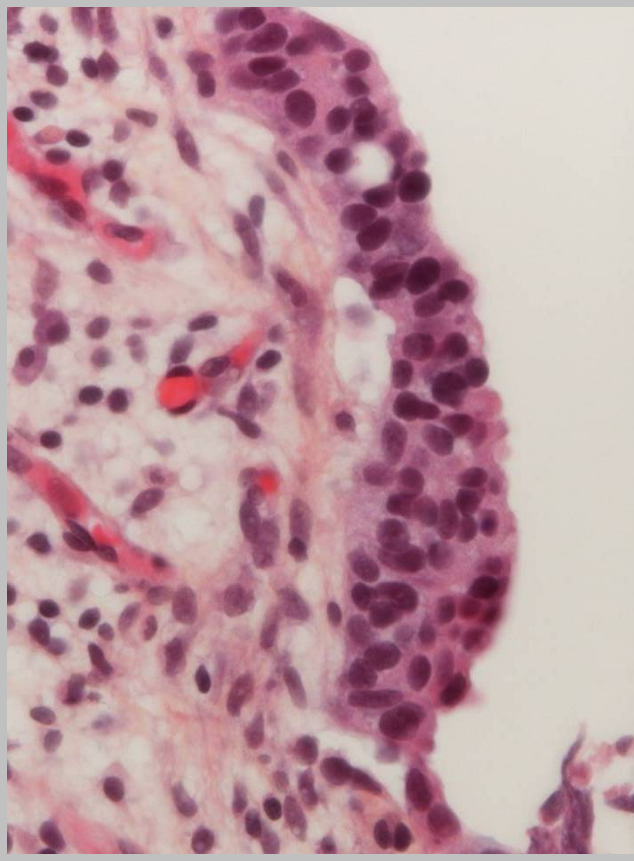


Large cell pleomorphic



Large cell non- pleomorphic

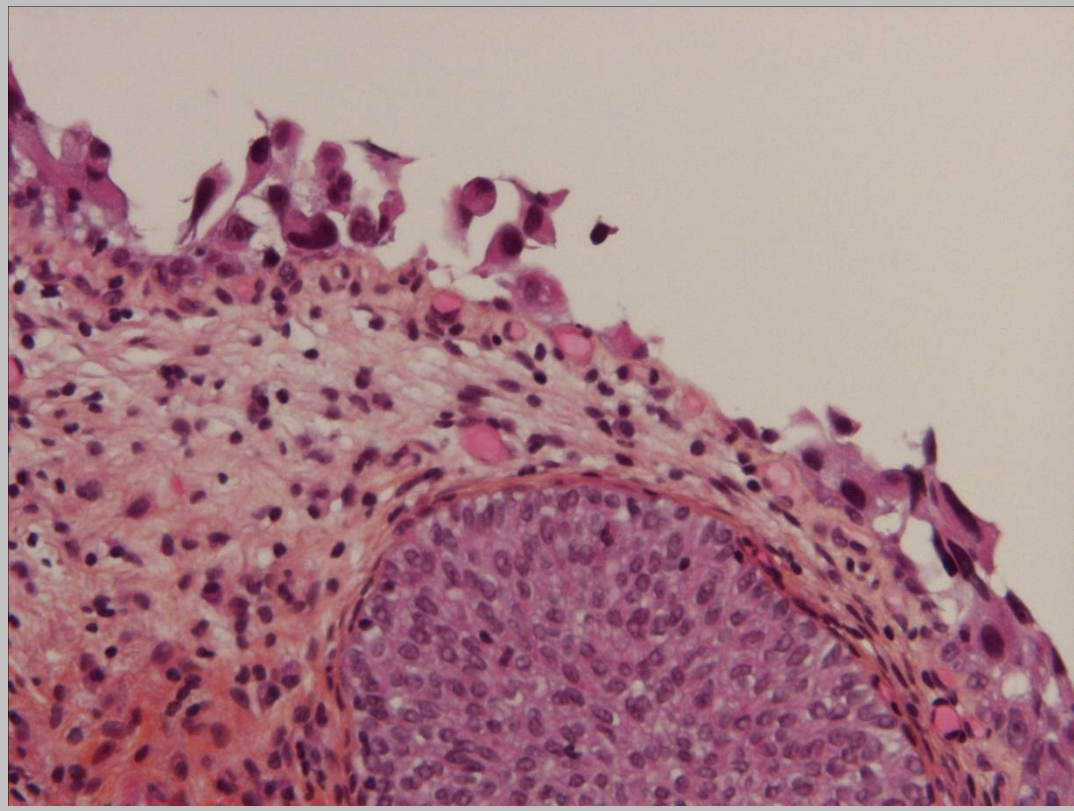
Purely descriptive
No neuroendocrine component
Neuroendocrine staining negative

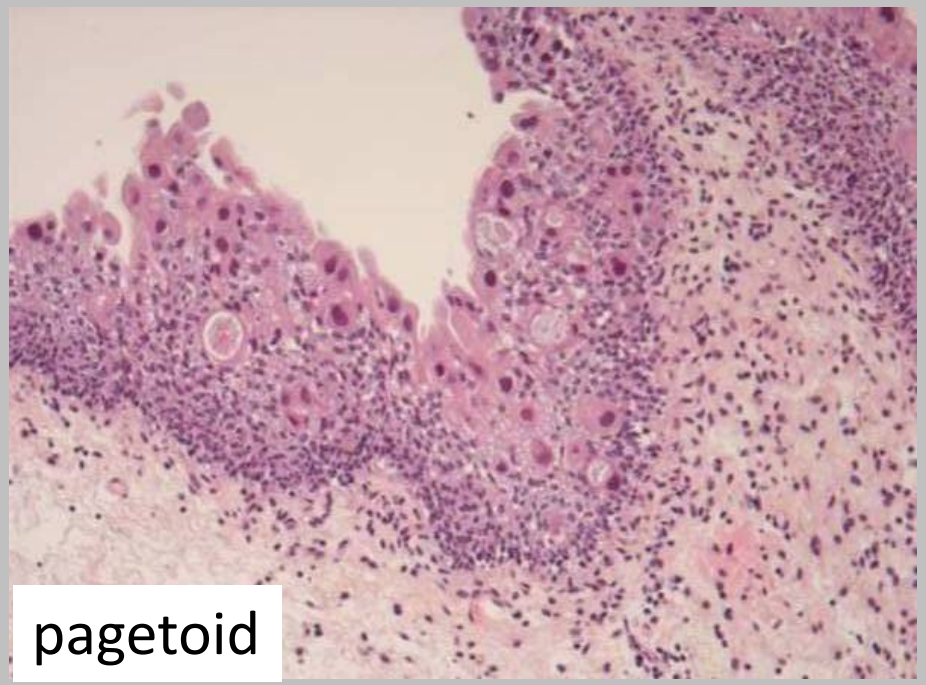


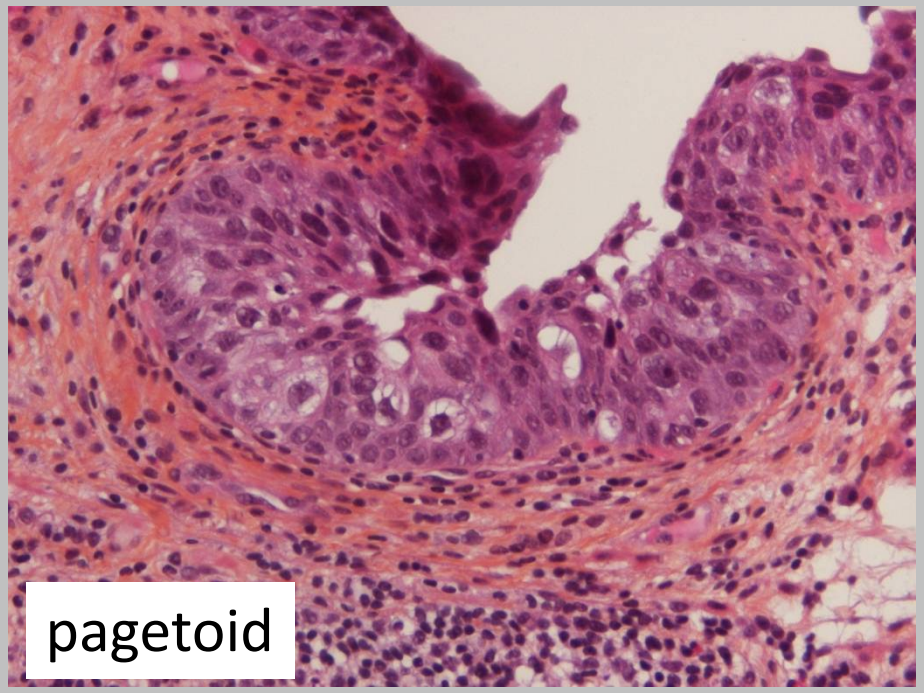
Small cell

Clinging C*is*

- Pb. of denuded epithelium, or 1 layer
- Pb. if inflammation
- Danger of not recognizing
- If no atyipcal cells
  - Cytology




pagetoid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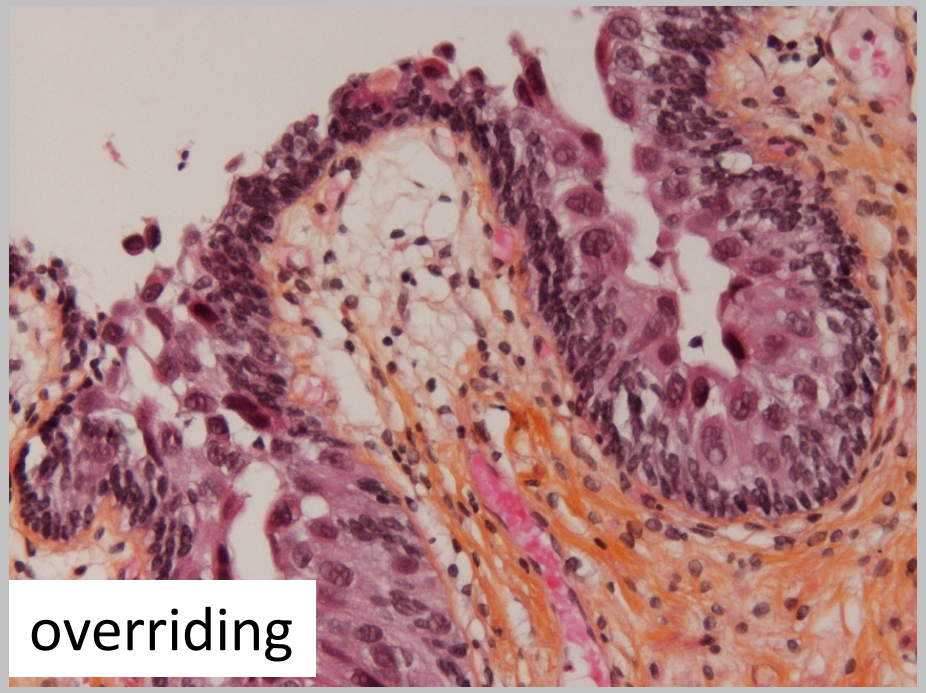
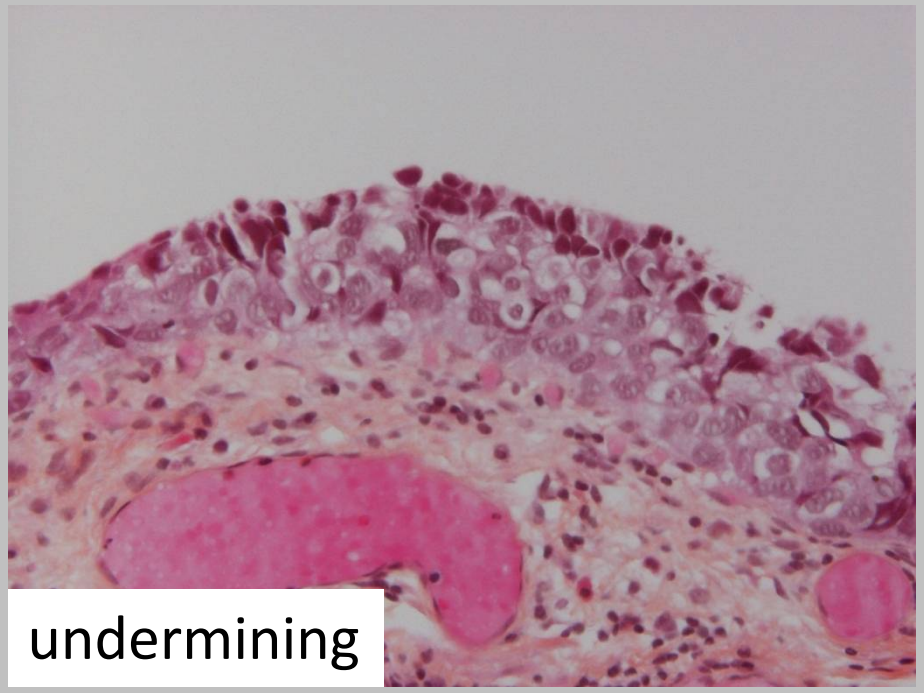
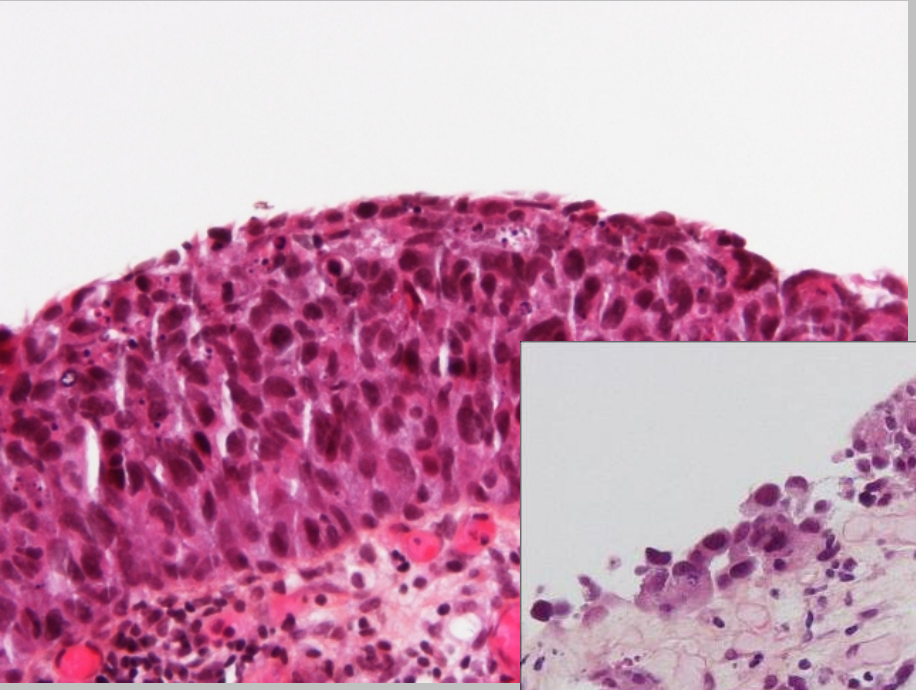
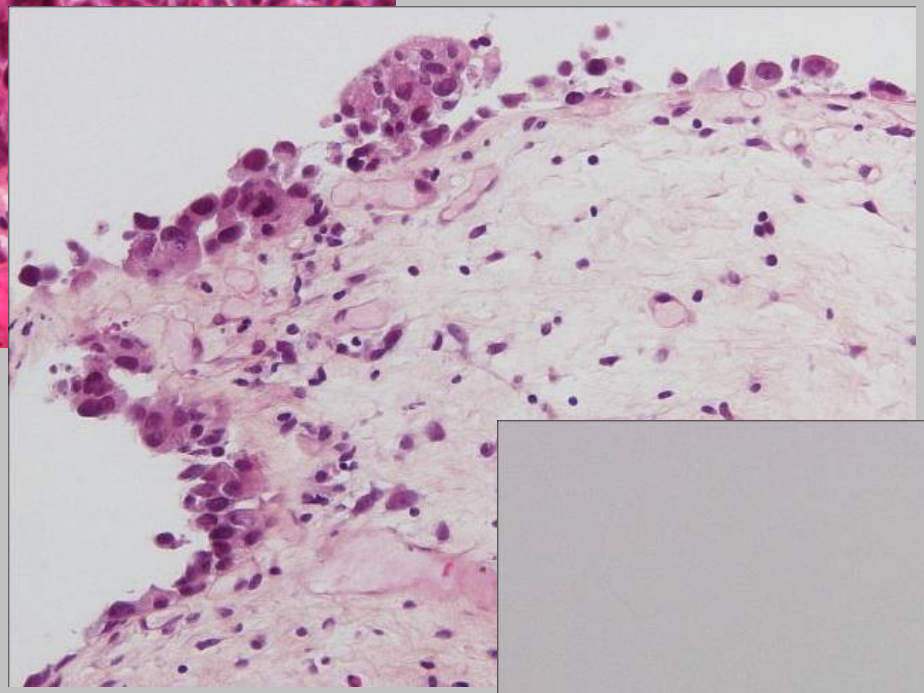
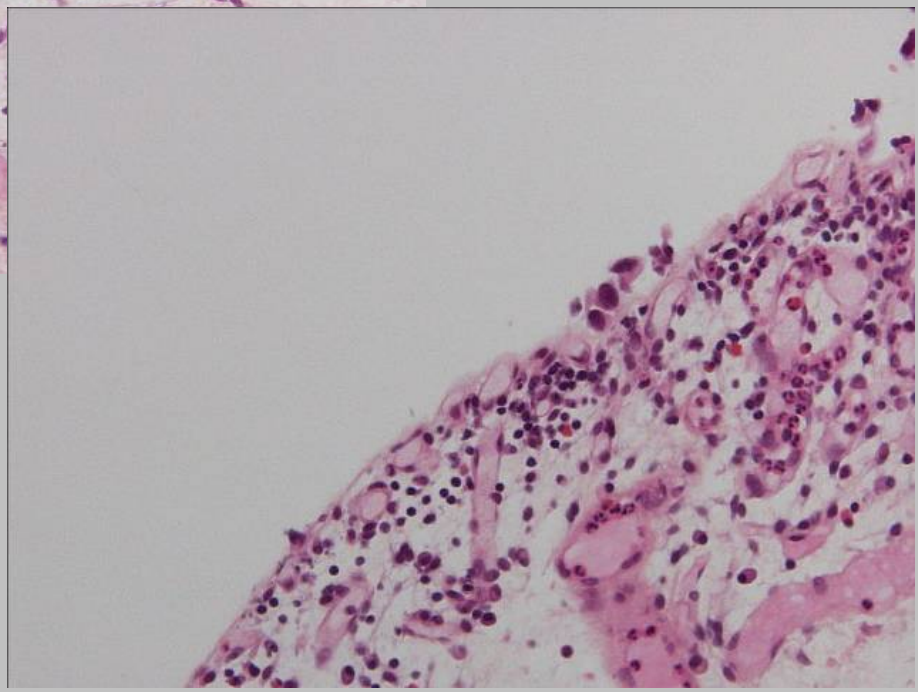

overriding


undermining







Natural history of Cis delvelopment ?

Evolution of aspects in Cis?

- Is it important to recognize different histologic types of CIS
  - Treatment
  - Impact on the patient's prognosis
- Clinical significance of these subtypes remained to be determined
- Recommendation not to subclassify

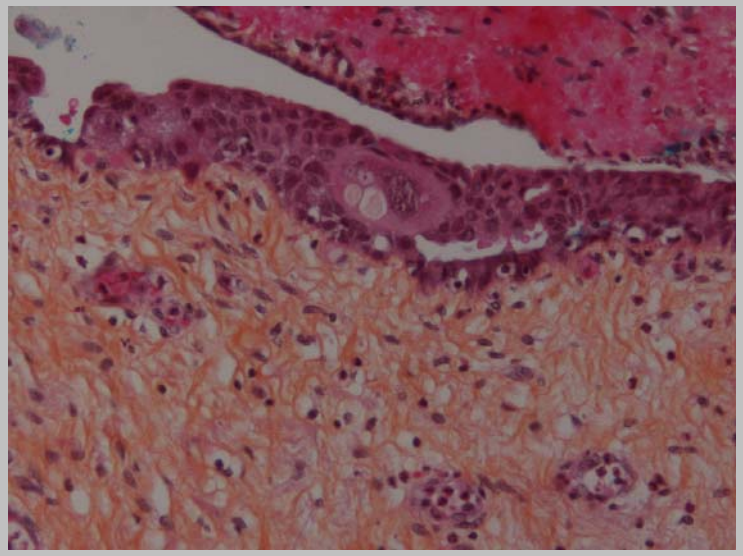
Different aspects, natural history?

- **Aim**
  - Frequency of different subtypes in primary CIS
  - Different morphologic patterns - different outcomes?
  - Identification of these sub- types is related to a change in therapy and/or follow up?

under press Virchow

M&M

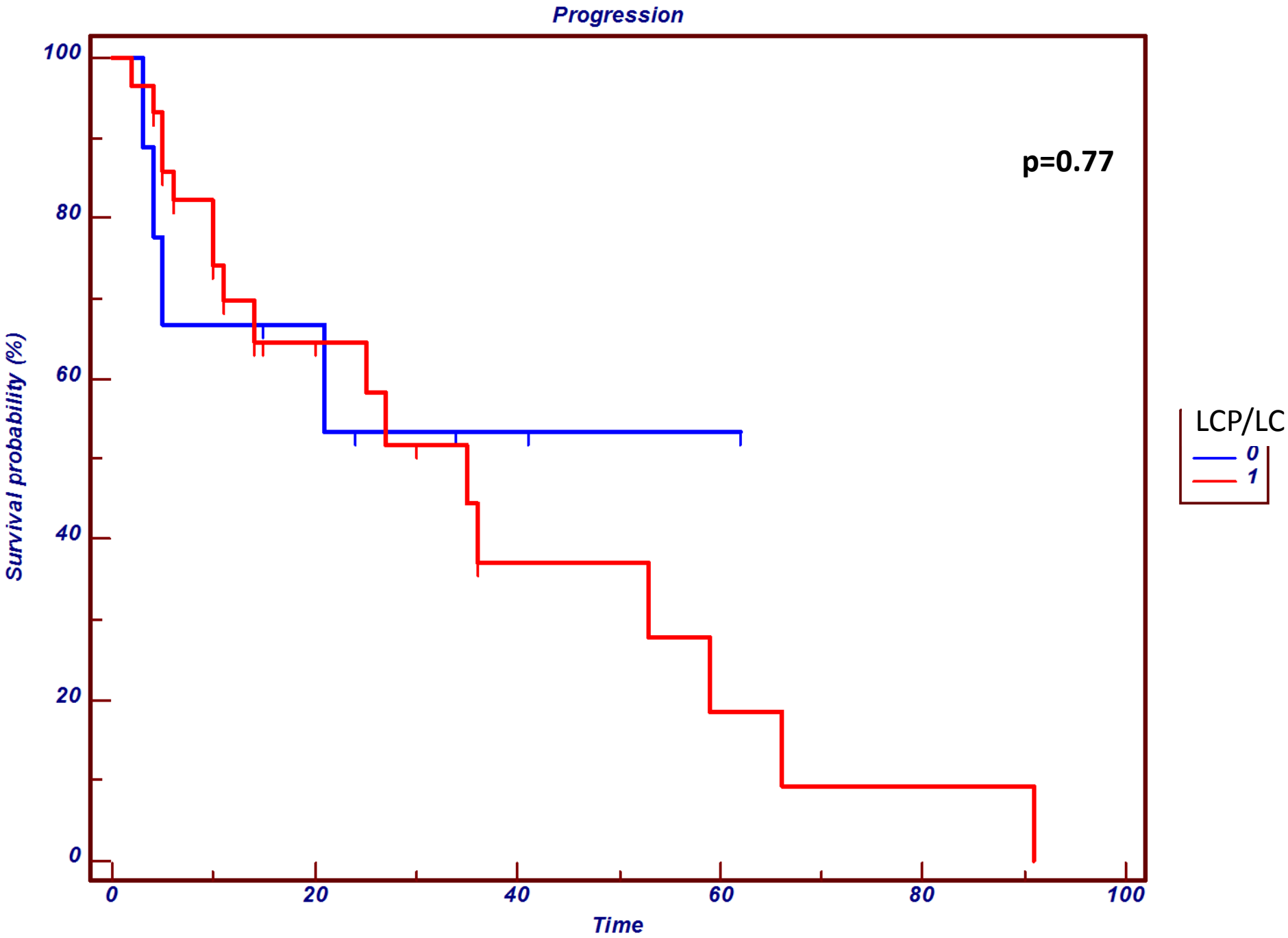
- 39 patients between 2000 and 2010
- First episode of CIS
  - Initial transurethral resections of the bladder or bladder biopsies, n=1325
- 3% of cases

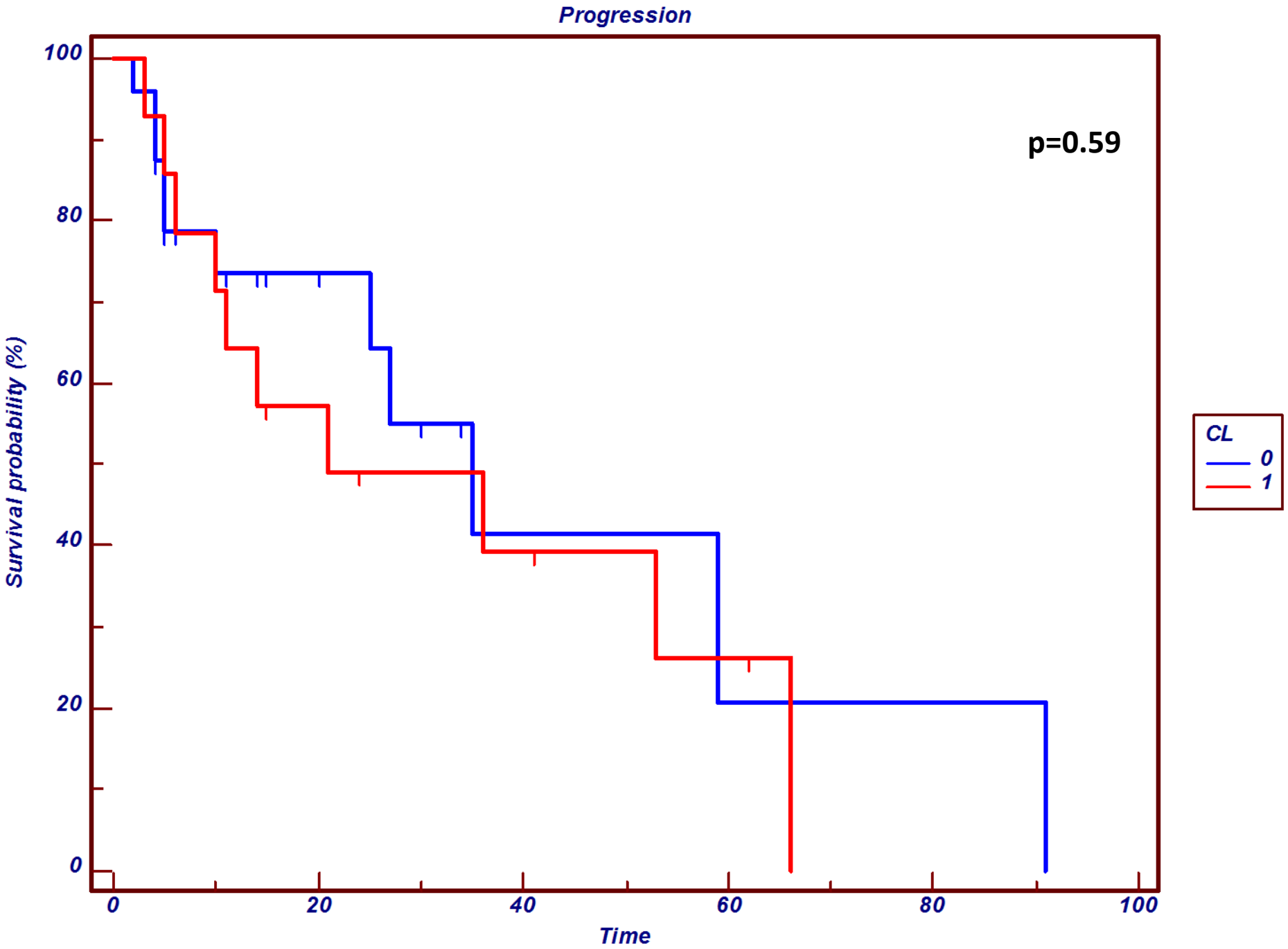


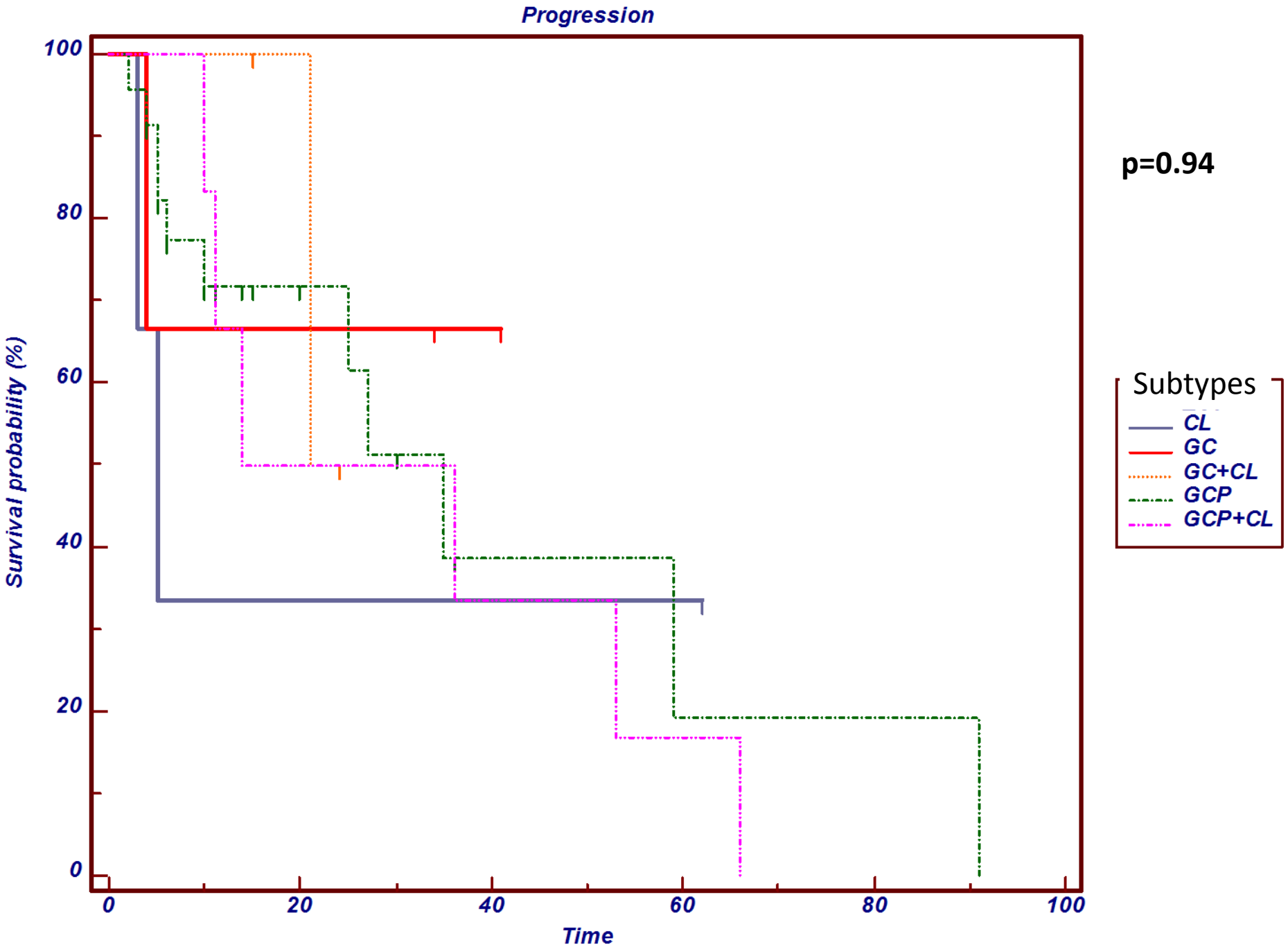
M&M

- 3 groups
  - LCP/LC - CL
    - No pagetoïd, undermining or overriding subtype
    - Two cases of small cell carcinoma → LCP
  - Mixed morphology
    - 51% pure forms of Cis, LCP predominant

- Compare aspects with progression, survival
  - 5-66 month (mean 14.4)


Progression
p=0.77
Survival probability (%)
100
80
60
40
20
0
0
20
40
60
80
100
Time
LCP/LC
0
1


Progression
p=0.59
Survival probability (%)
100
80
60
40
20
0
0
20
40
60
80
100
Time
CL
0
1


Progression
Survival probability (%)
100
80
60
40
20
0
0
20
40
60
80
100
Time
p=0.94
Subtypes
CL
GC
GC+CL
GCP
GCP+CL

Results

- No use in precising different aspects of primary CIS in the report
- No difference in patients' outcomes
- Important to recognize different patterns of CIS

Conclusion

- **Are there different types?**
- Clinically and morphologically different Cis exist
- Genetically too early to confirm differences
- But → Cis specific signature

Witjes, 2007, Nat Clin Pract Urol
Kriegmair, 2002, J Urol
Fradet, 2007, J Urol


Thank you for your attention