Grupo Português
Génito - Urinário

XXVIII Workshop
Urologia Oncológica

Bladder Cancer
Dialogue with Pathologist Today

Carlos Lopes, MD, PhD

ICBAS, IUCS, Unilabs

Bladder Cancer – Dialogue with pathologist today

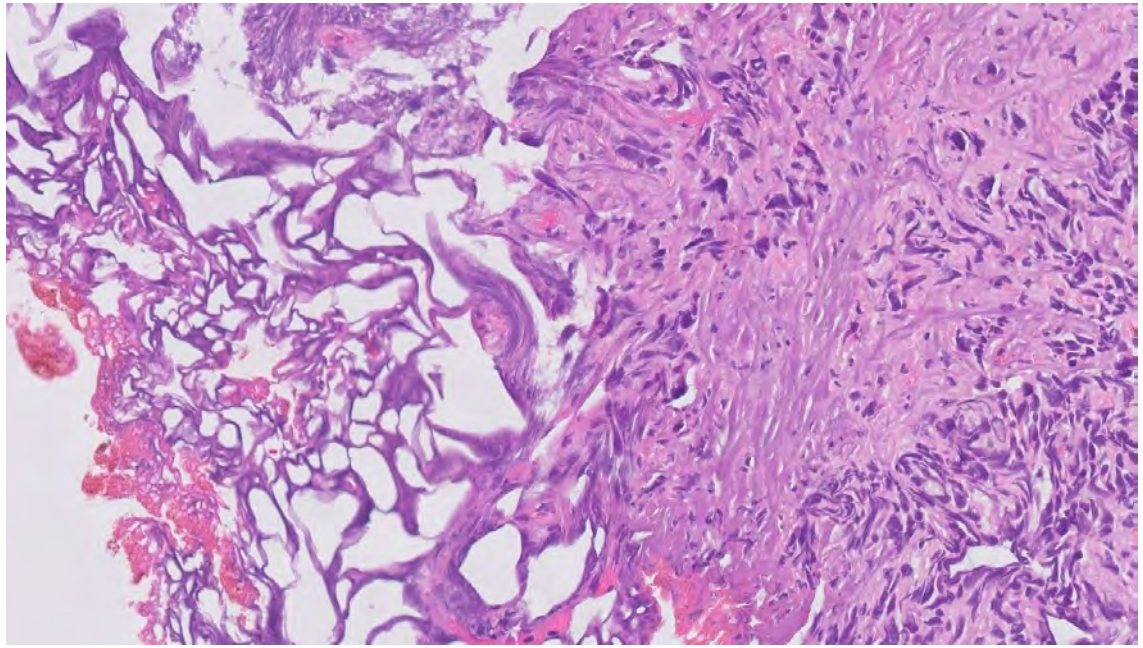
- **Dialog between pathologist and urologist, a necessity and requirement of good practice, frequently repeated…**
- **… but often forgotten and not put into practice, specially when pathologist and urologist do not know each other and never meet or talk.**

So, a first important decision:
to develop a direct contact by Tm or AP …
that goes beyond contacts through administrative level

The urologist should …..

- Take specimens for histopathological diagnosis

- Prevent, if possible, artifacts of fulguration, the worst enemy of histological examination, namely in small specimens.



The urologist should indicate ….

- demographic information and clinical history of the patient, bladder cytology if present, whether it is the first presentation of the tumor and if not, details of previous resection;

- the cystoscopic appearance of bladder mucosa and indicate number, size, location of the tumor/s, the morphological features of the lesion: papillary, solid, or ulcerate;

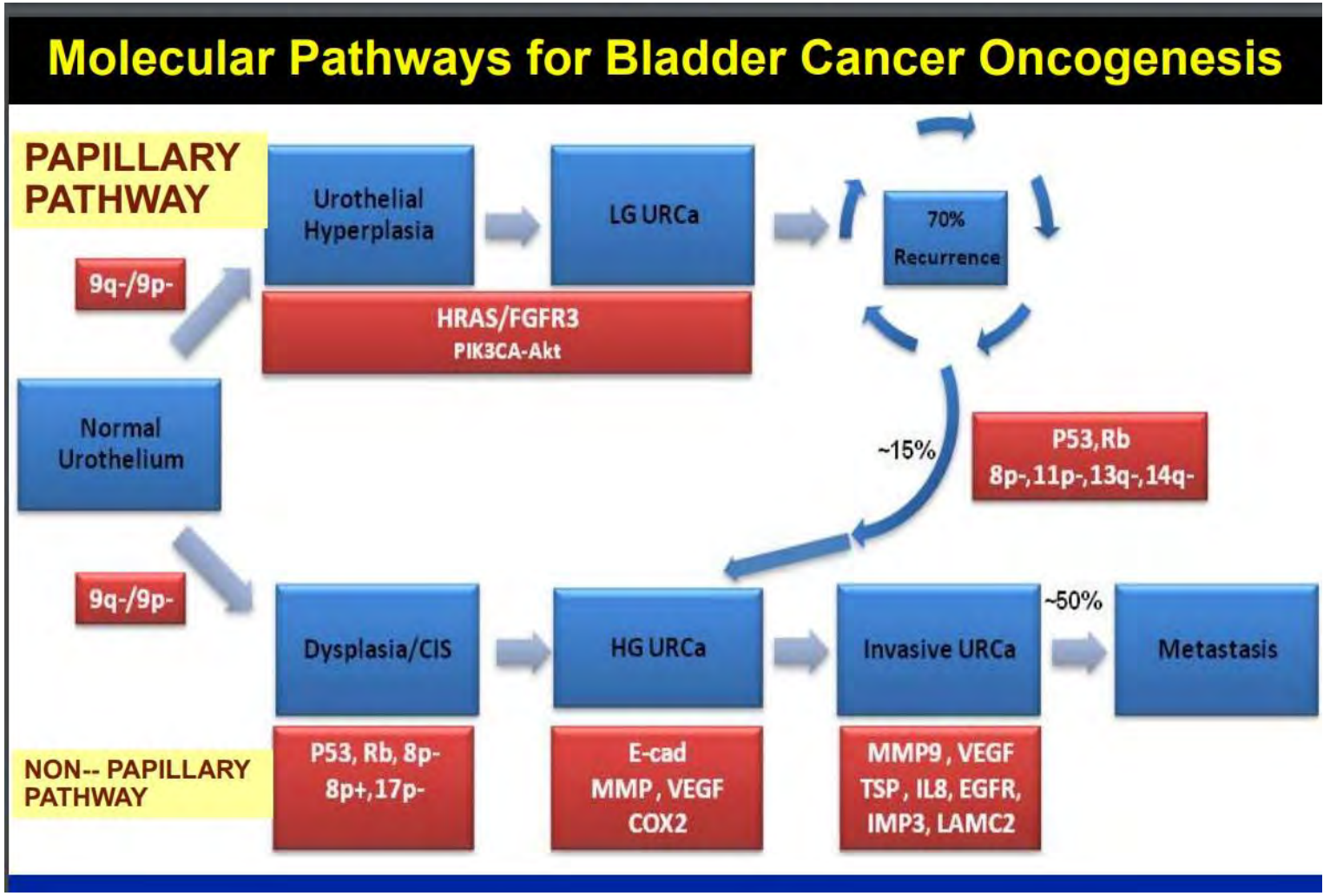
The urologist should indicate ….

- the state of remaining mucosa if further biopsies were performed;
- if previous radiotherapy to the bladder or to adjacent organ were performed;
- if after the first transurethral resection of bladder tumor (TURBT) local treatments, such as bacillus Calmette Guérin (BCG) or Mitomycin C intravesical instillation, were performed.

The histopathological report must contain …

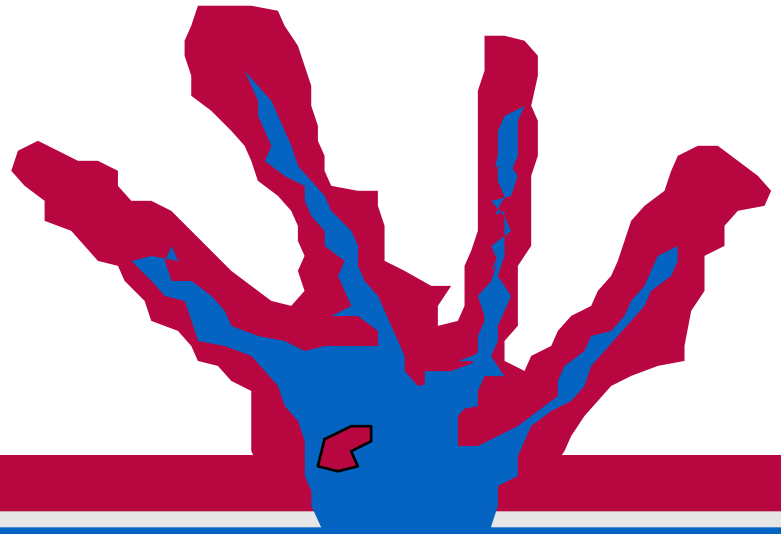
- Specimen representative – muscular propria is present?
- Quality of speciment – artifacts?
- Does exist tumour?
- Histological type of humour (ISUP, WHO)
- Histological tumour GRADE
- Extension of invasion (Pathologic Stage: pTa, pT1, pT2)
- Histological signs of previous therapy
- Markers for therapy
- New molecular and genetic data ???

Bladder Cancer – Dialogue with pathologist today



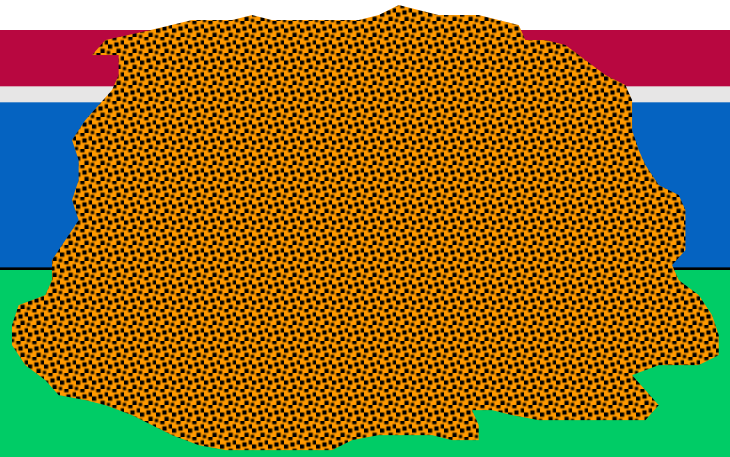
Amin, 2012

SUPERFICIAL PAPILLARY



C.I.S.

NON PAPILLARY INVASIVE



The histopathological report must contain …

- Histological type of humour (ISUP, WHO)
  - Urothelial: PUNLMP

    Papillary non invasive/invasive (low grade/high grade)

    CIS

    Invasive (high grade/low grade)
  - Variants (special types): squamous cells; nested, micropapillary, sarcomatoid, small cells, plasmacytoid, ……

Grading of urothelial lesions

Flat lesions

Normal

Hyperplasia

Reative

Dysplasia

CIS

Atypia of unknown significance

Papillary lesions

Non invasive

Papilloma

PUNLMP

Low grade

High grade

Invasive

High grade

Low grade (rare)

Inverted lesions

Non invasive

Papilloma

PUNLMP

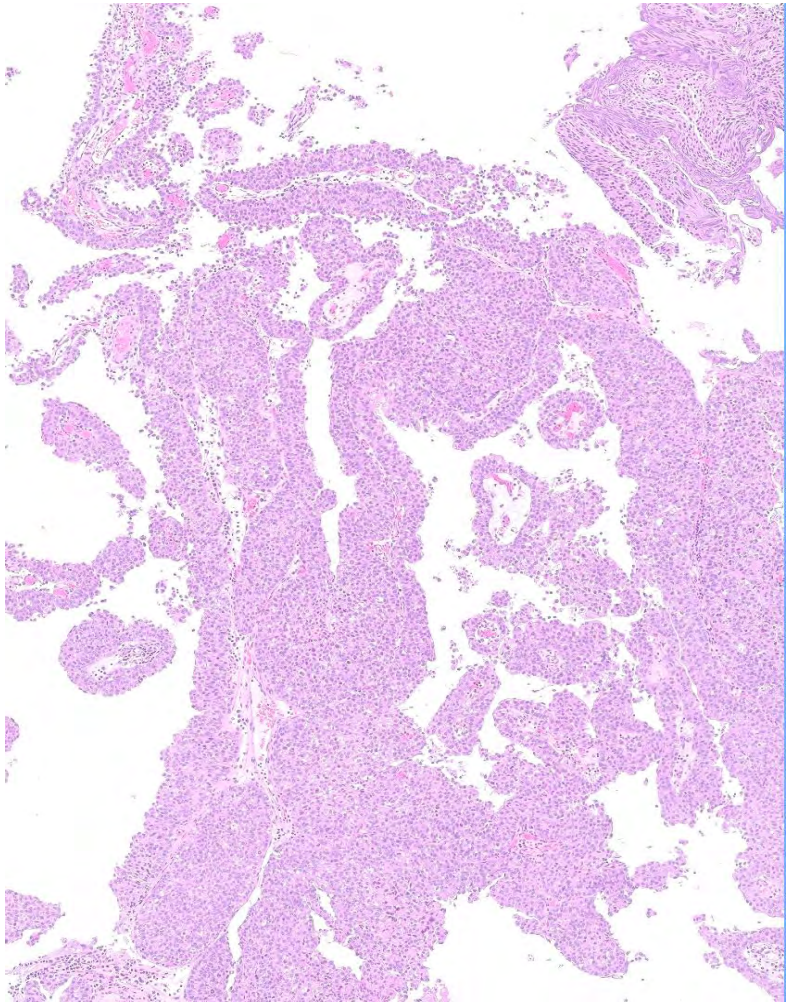
Low grade

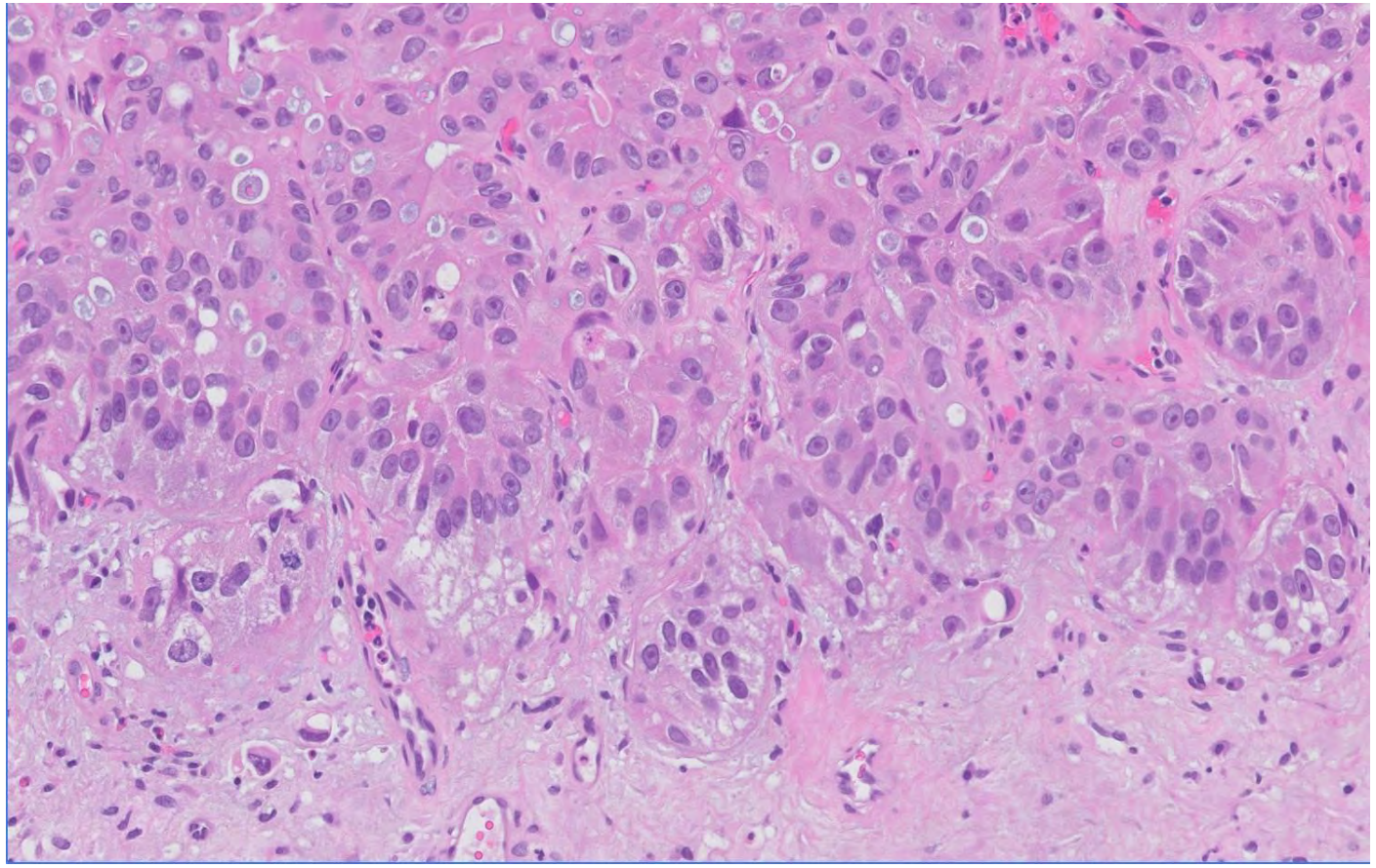
High grade

Invasive

High grade

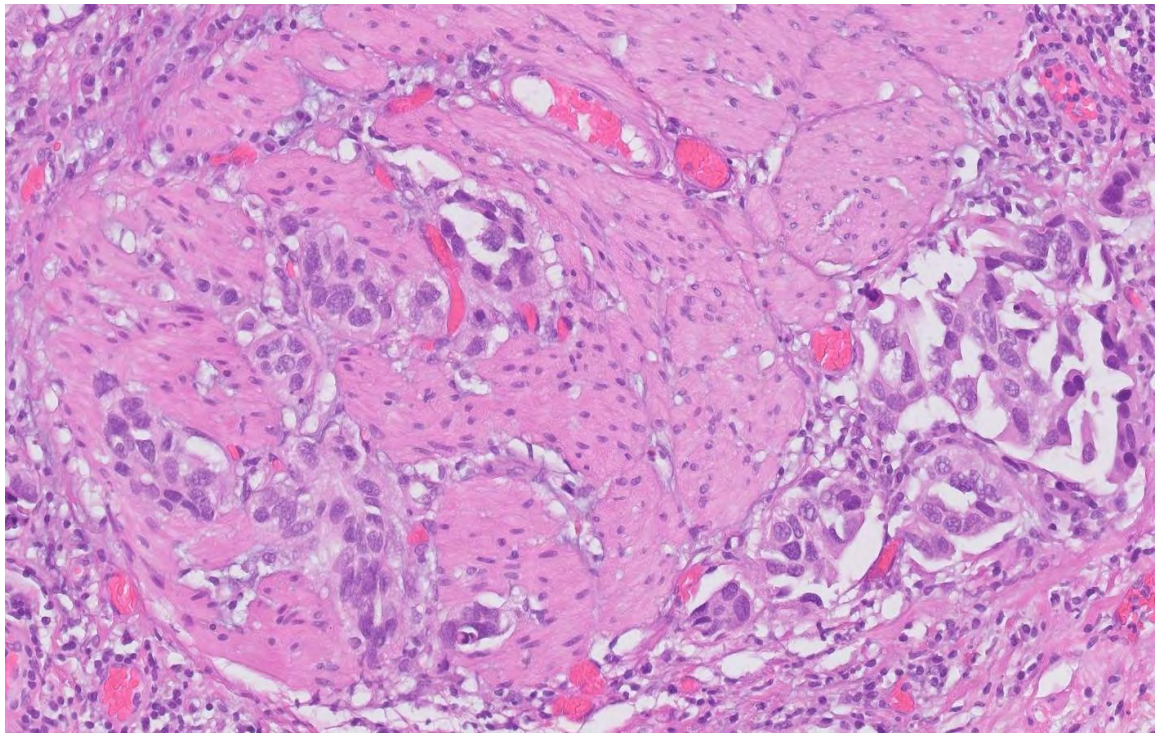
Bladder Cancer – Dialogue with pathologist today



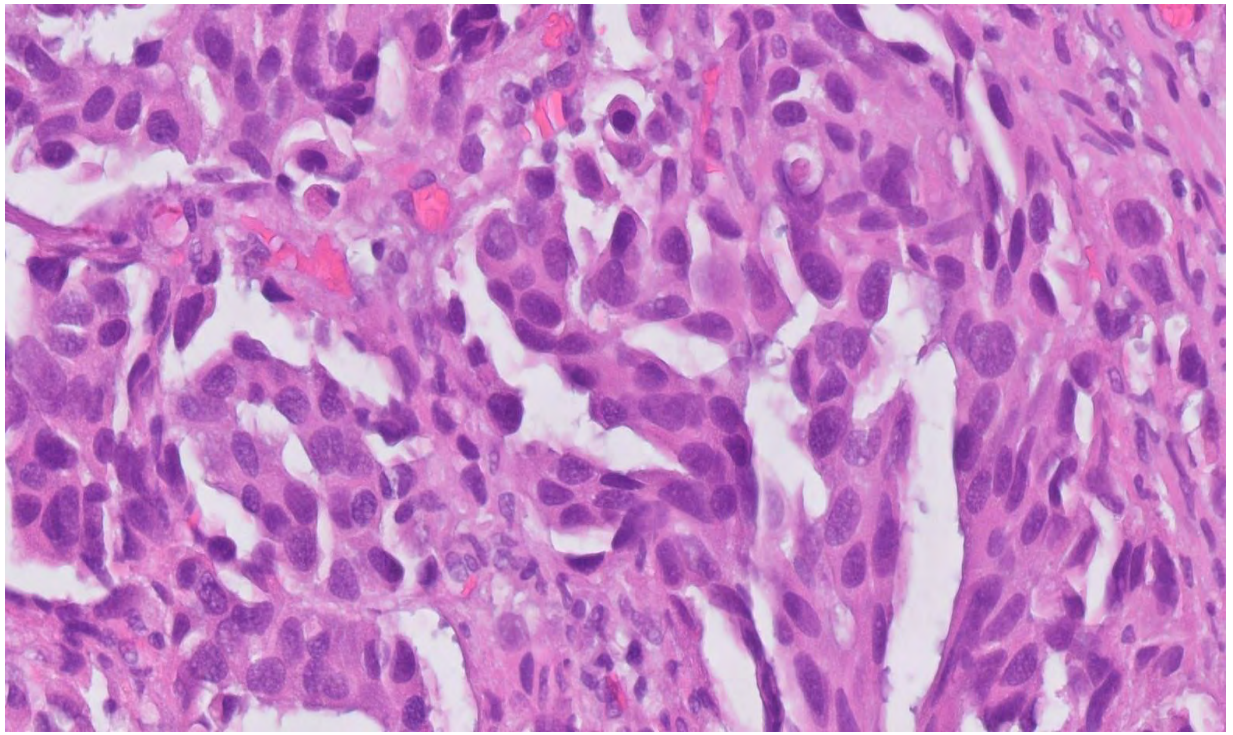


Superficial high grade papillary carcinoma: invasion?

Bladder Cancer – Dialogue with pathologist today



Muscular propria invasion



Micropapillary variant

Bladder Cancer – Dialogue with pathologist today

- New technologies can help?
  - To recognize invasion?
  - In molecular classification?
  - To predict therapy?
  - In metastasis?

Bladder Cancer – Dialogue with pathologist today

Artificial intelligence in evaluation of neoplastic invasion

Yin et al. BMC Medical Informatics and Decision Making (2020) 20:162
https://doi.org/10.1186/s12911-020-01185-z

BMC Medical Informatics and Decision Making

RESEARCH ARTICLE Open Access

Histopathological distinction of non-invasive and invasive bladder cancers using machine learning approaches

Peng-Nien Yin[1], Kishan KC[2], Shishi Wei[2], Qi Yu[2], Rui Li[2], Anne R. Haake[2], Hiroshi Miyamoto[3*] and Feng Cui[1*]

Abstract

Background: One of the most challenging tasks for bladder cancer diagnosis is to histologically differentiate two early stages, non-invasive Ta and superficially invasive T1, the latter of which is associated with a significantly higher risk of disease progression. Indeed, in a considerable number of cases, Ta and T1 tumors look very similar under microscope, making the distinction very difficult even for experienced pathologists. Thus, there is an urgent need for a favoring system based on machine learning (ML) to distinguish between the two stages of bladder cancer.

Methods: A total of 1177 images of bladder tumor tissues stained by hematoxylin and eosin were collected by pathologists at University of Rochester Medical Center, which included 460 non-invasive (stage Ta) and 717 invasive (stage T1) tumors. Automatic pipelines were developed to extract features for three invasive patterns characteristic to the T1 stage bladder cancer (i.e., desmoplastic reaction, retraction artifact, and abundant pinker cytoplasm), using imaging processing software ImageJ and CellProfiler. Features extracted from the images were analyzed by a suite of machine learning approaches.

Results: We extracted nearly 700 features from the Ta and T1 tumor images. Unsupervised clustering analysis failed to distinguish hematoxylin and eosin images of Ta vs. T1 tumors. With a reduced set of features, we successfully distinguished 1177 Ta or T1 images with an accuracy of 91–96% by six supervised learning methods. By contrast, convolutional neural network (CNN) models that automatically extract features from images produced an accuracy of 84%, indicating that feature extraction driven by domain knowledge outperforms CNN-based automatic feature extraction. Further analysis revealed that desmoplastic reaction was more important than the other two patterns, and the number and size of nuclei of tumor cells were the most predictive features.

Conclusions: We provide a ML-empowered, feature-centered, and interpretable diagnostic system to facilitate the accurate staging of Ta and T1 diseases, which has a potential to apply to other types of cancer.

Keywords: Machine learning, Deep learning, Bladder cancer, Histopathology images

Bladder Cancer – Dialogue with pathologist today

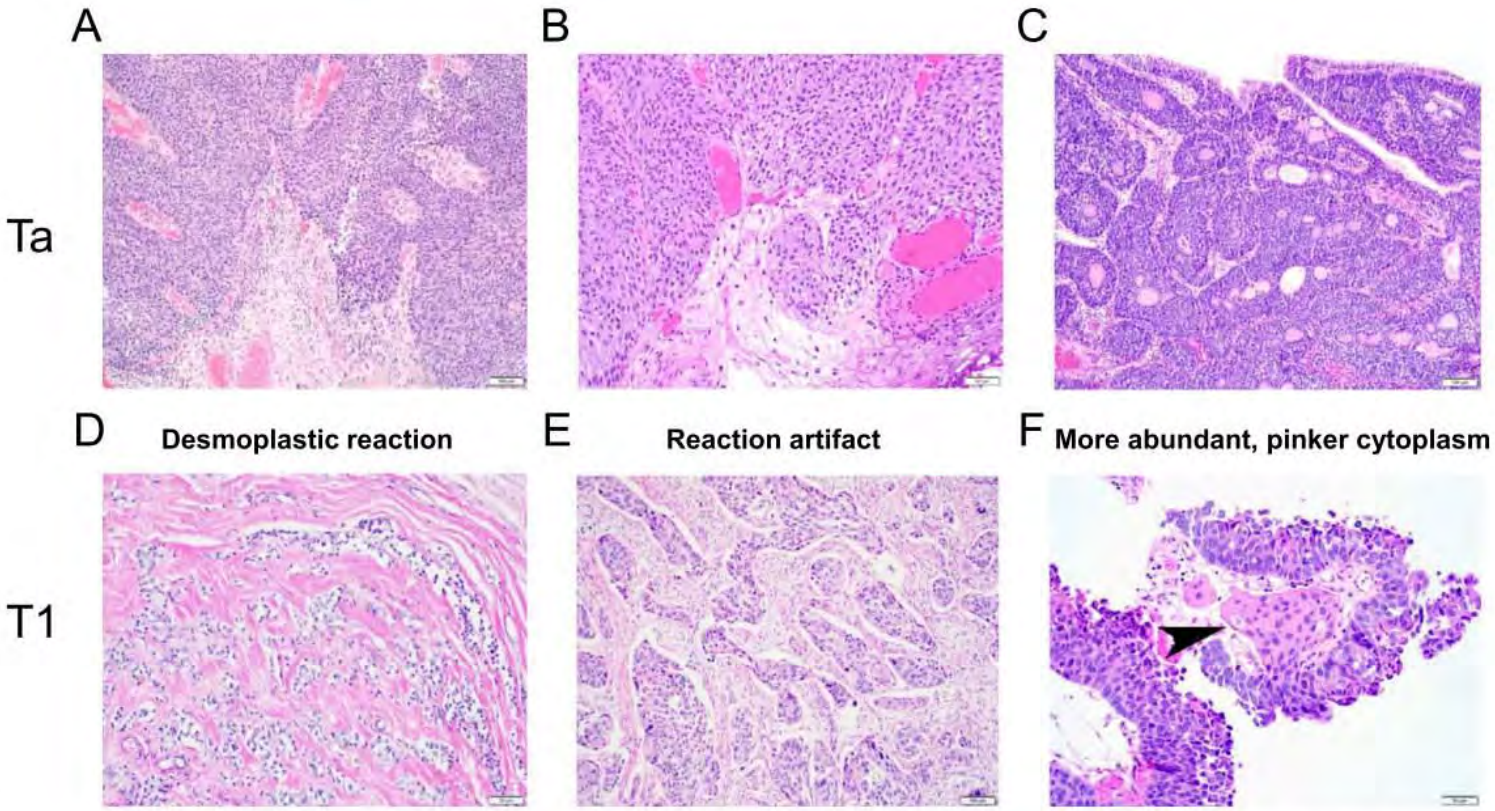


Fig. 1 Histological features of stages Ta (**a-c**) versus T1 (**d-f**) bladder cancers. Three microscopic patterns, including desmoplastic reaction (**d**), retraction artifact (**e**), and more abundant, pinker cytoplasm (**f**, arrowhead), are apparent in invasive components of T1 tumors, but not in Ta tumors without (**a**, **b**) or with (**c**) an inverted growth pattern. Original magnification: **a**, **c**, **e** – 100x; **b**, **d**, **f** – 200x

Bladder Cancer – Dialogue with pathologist today

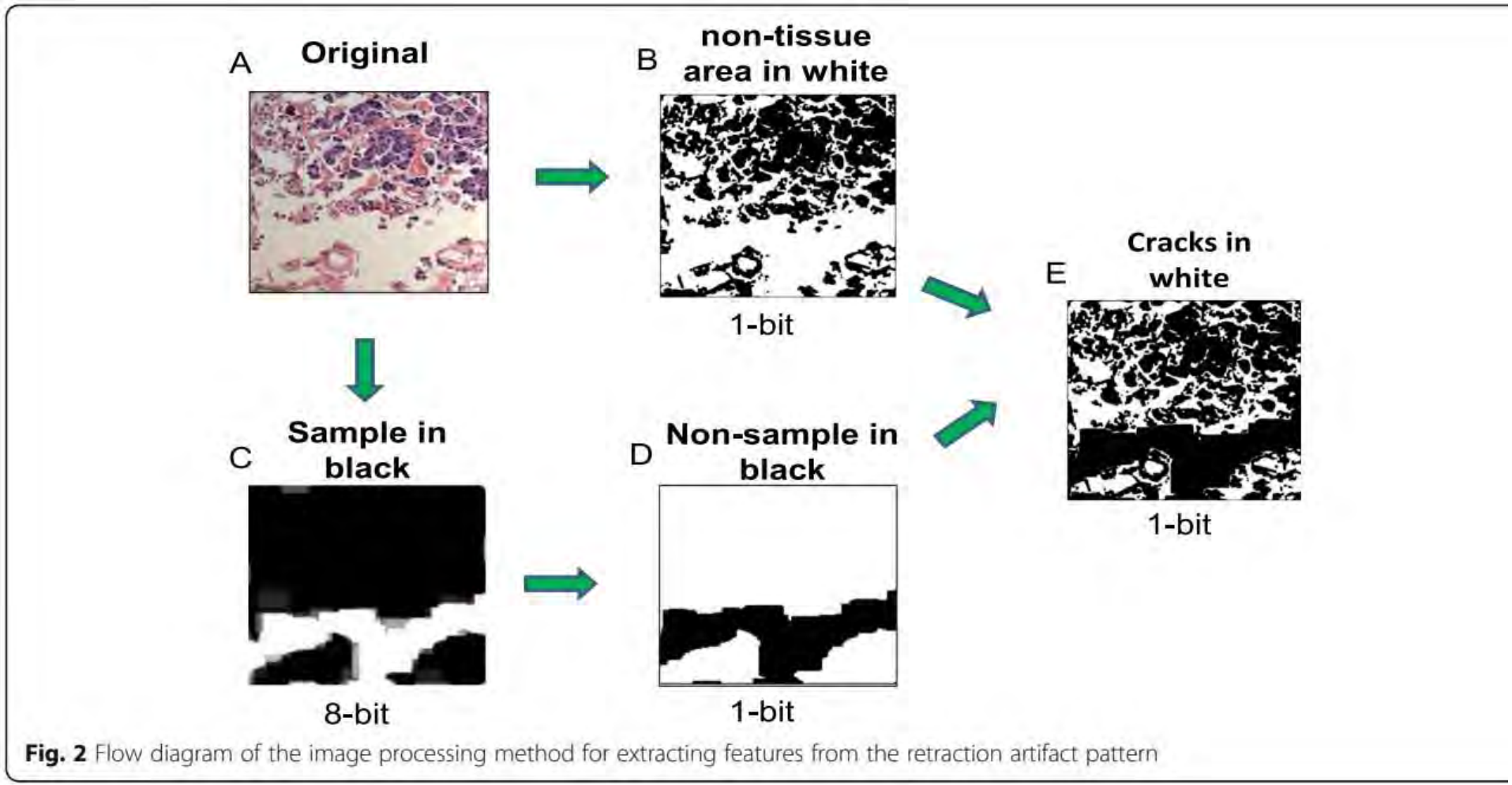


Fig. 2 Flow diagram of the image processing method for extracting features from the retraction artifact pattern

Bladder Cancer – Dialogue with pathologist today

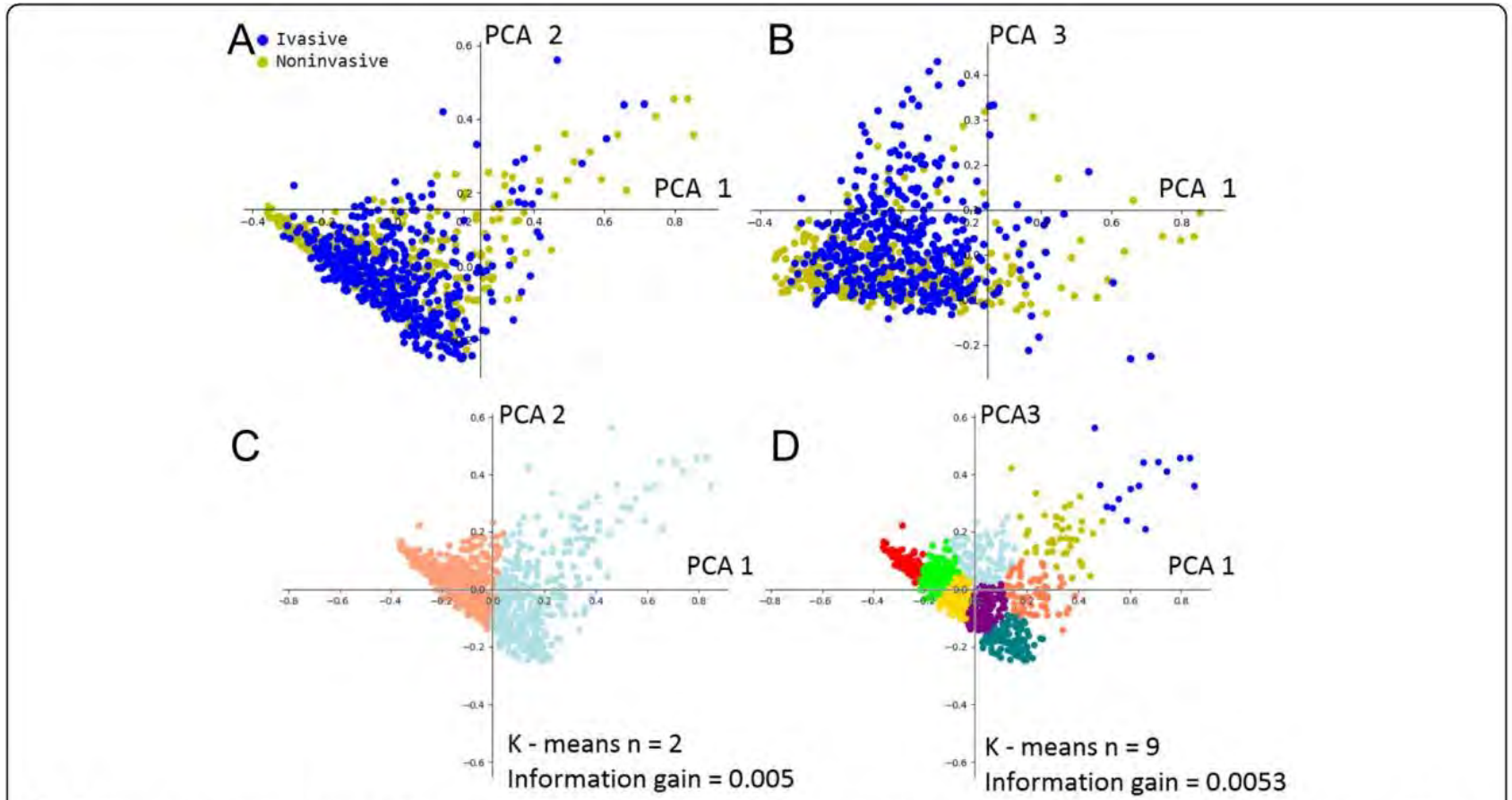


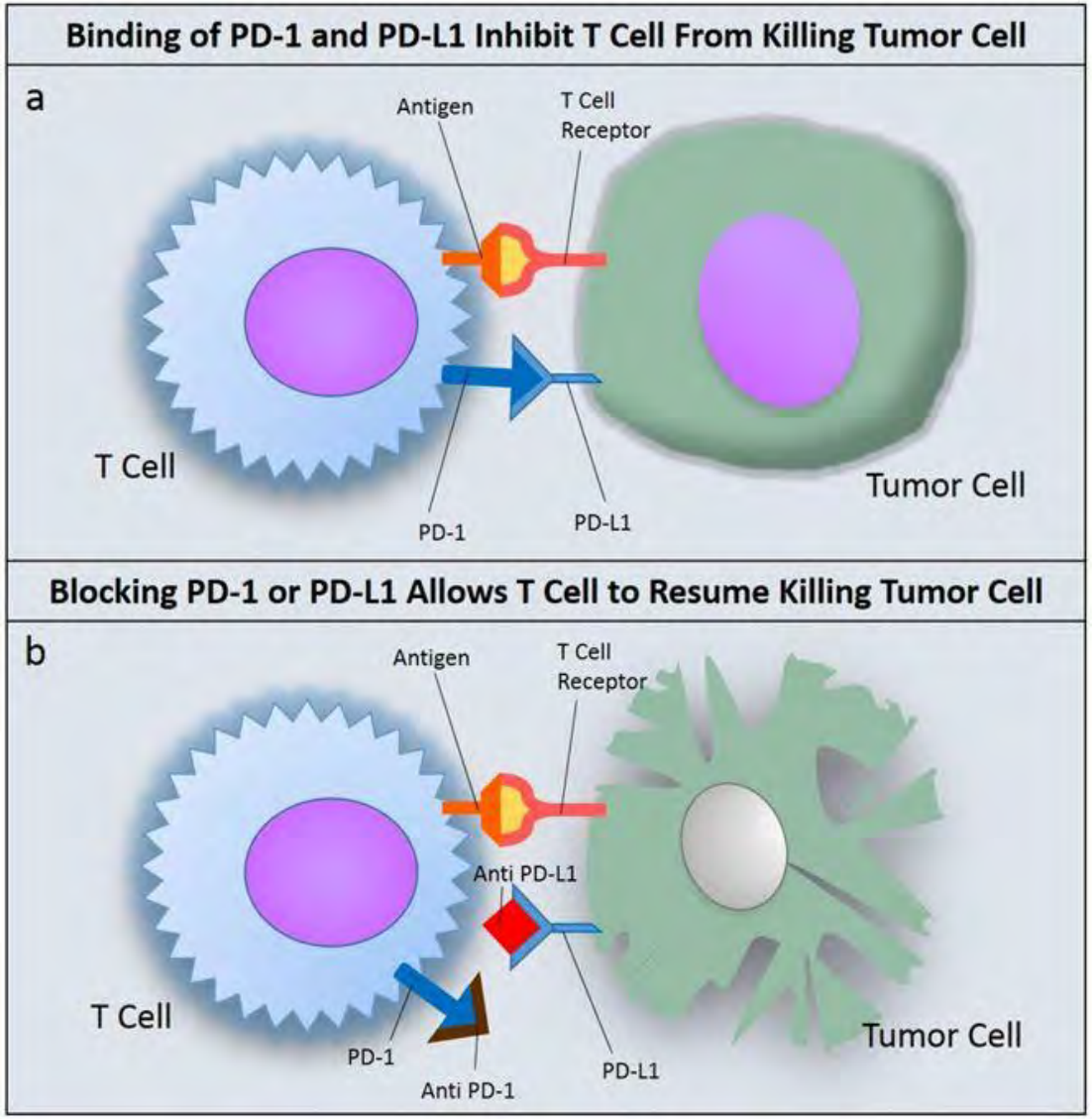
Fig. 3 Clustering analysis of extracted features from Ta (non-invasive) and T1 (invasive) tumor images. The features were first selected by PCA and then clustered using k-means analysis. Plots were made for the first and second components of PCA output (**a**), which were clustered with k = 2 (**c**). Plots were also made for the first and third components of PCA output (**b**), which were clustered with k = 9 (**d**)

PDL-1 in Bladder Urothelial Carcinoma

- **In addition to acting as a prognostic biomarker**, PD-L1 may also be used in future as a predictive biomarker for patients most likely to benefit from adjuvant immunotherapy.

- If cancer cells have high amounts of PD-L1, **they can turn your T cells off so they can't attack the cancer cells**. If high amounts of PD-L1 are found on cancer cells, immunotherapy medicines called "immune checkpoint inhibitors" may be used. These medicines prevent the PD-L1 protein from putting the brakes on T cells.

Bladder Cancer – Dialogue with pathologist today



PDL-1 in cancer

Bladder Cancer – Dialogue with pathologist today

ANTIBODIES AND PLATFORMS STAINING FOR PDL-1

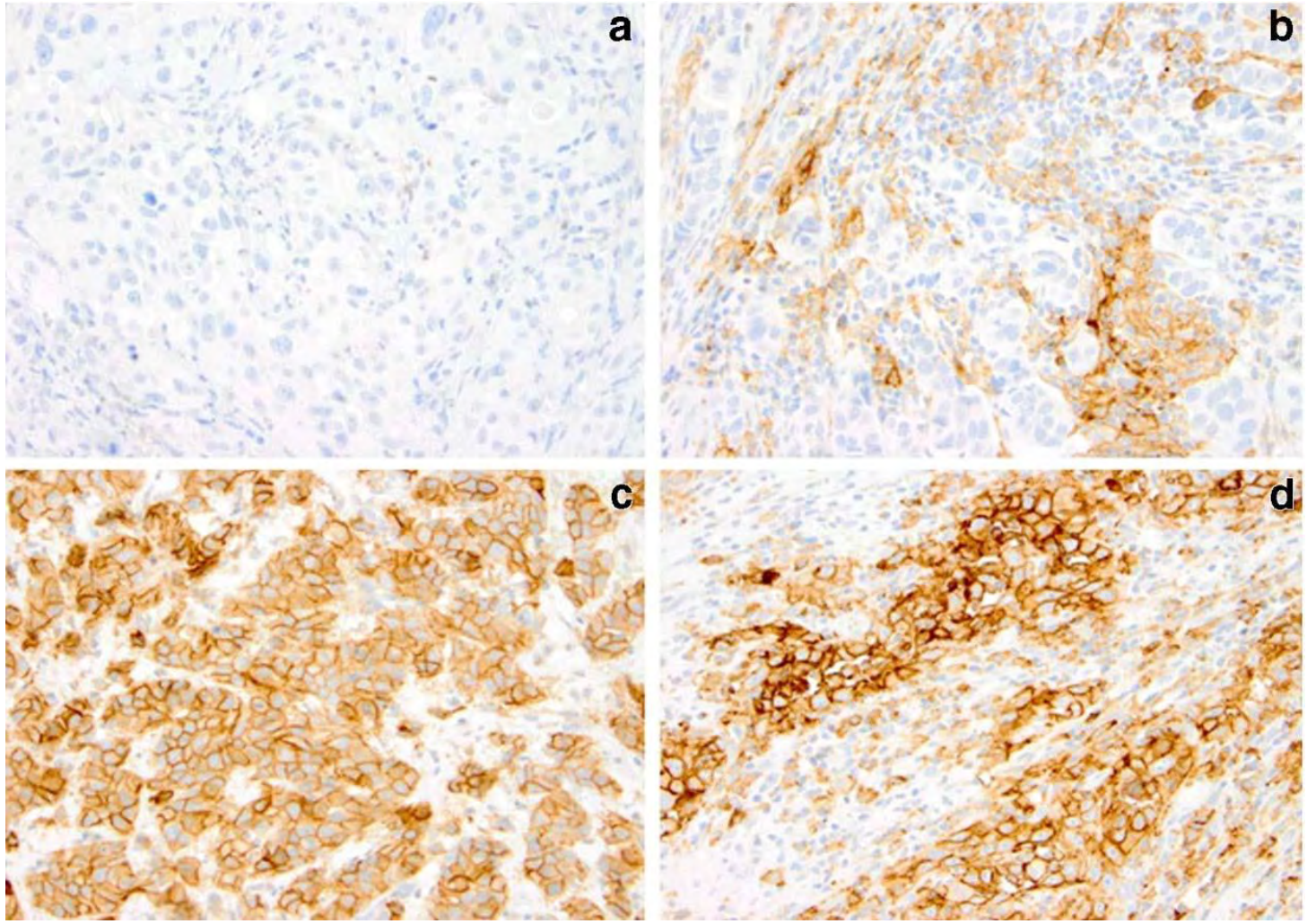
Table 1 US Food and Drug Administration and European Medical Agency approved PD-L1 assays

Diagnostic assay	Staining platform	Staining characteristics	Approved assay for
Ventana SP142	Ventana	• Dot-/ant-like staining pattern • Low tumor cell staining • Developed for immune cell scoring	Atezolizumab [Tecentriq©]
Ventana SP263	Ventana	• Homogenous tumor cell staining • Homogenous tumor cell staining • Mostly strong staining intensity	Durvalumab [Imfinzi©]
Dako 22c3	Dako Link 48*	• Homogenous tumor cell staining • Homogenous tumor cell staining • Mostly weak staining intensity	Pembrolizumab [Keytruda©]
Dako 28-8	Dako Link 48*	• Homogenous tumor cell staining • Homogenous tumor cell staining • Moderate-strong staining intensity	Nivolumab [Opdivo©]

*Currently exclusively approved for the Dako Link 48 platform—approval process for Omnis-platform ongoing.

Bladder Cancer – Dialogue with pathologist today

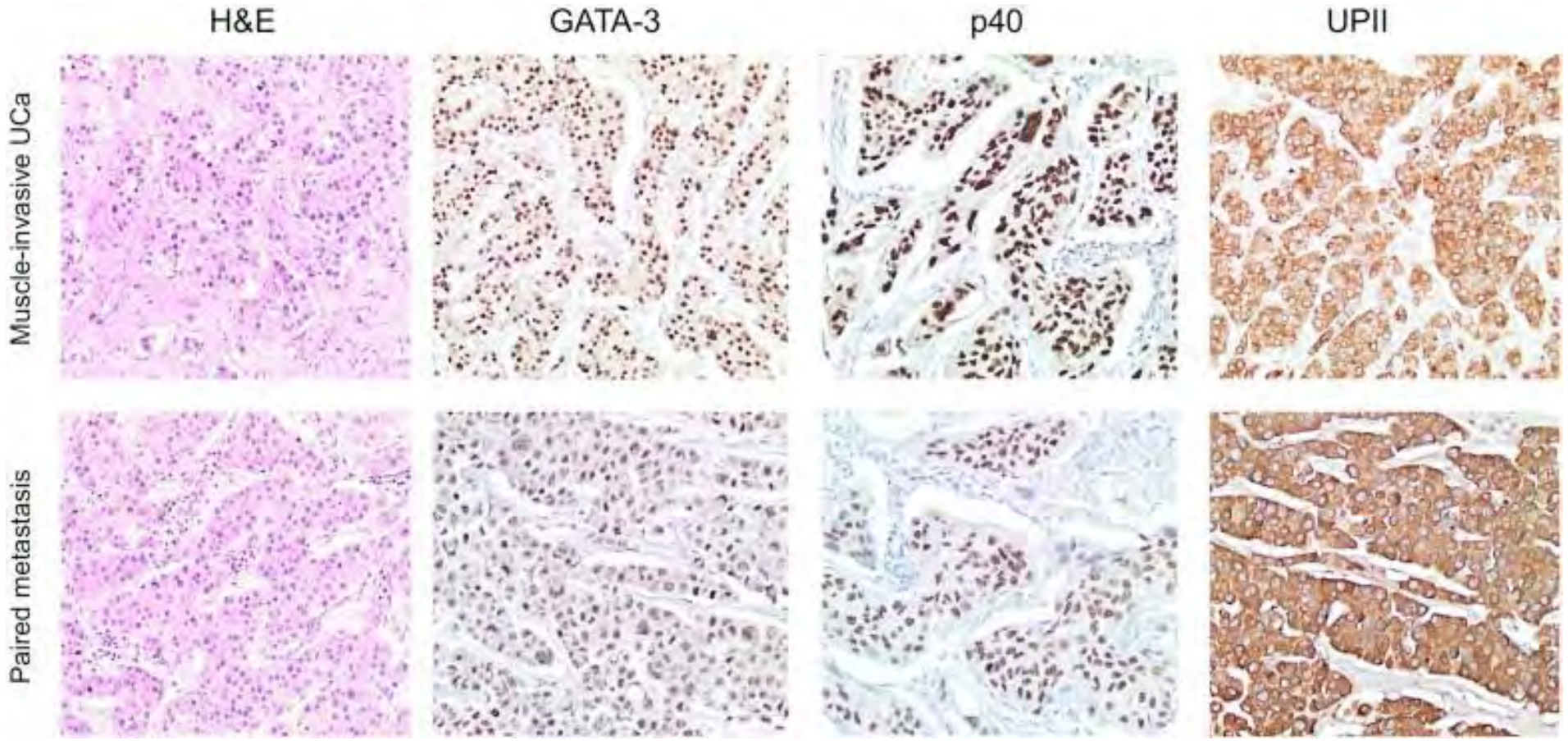
PDL-1 immunorreativity
in tumour cells
and in lymphocytes



Diagnosis of metastatic urothelial carcioma

CK 7 positive	>90%
CK20 positive	40-70%
Uroplakin III positive	50-80%
GATA 3 positive	70-80%
S100P positive	70-80%
Thrombomodulin positive	60-75%
34βE12 (High molecular weight keratin)	60-90%
p63 positive	60-90%
p40 positive	60-90%

Diagnosis of metastatic urothelial carcioma



International Journal of *Molecular Sciences*

MDPI

Review

Are We Ready to Implement Molecular Subtyping of Bladder Cancer in Clinical Practice? Part 1: General Issues and Marker Expression

Francesca Sanguedolce [1,*], Magda Zanelli [2], Andrea Palicelli [2], Stefano Ascani [3], Maurizio Zizzo [4], Giorgia Cocco [5], Lars Björnebo [6], Anna Lantz [6,7], Ugo Giovanni Falagario [8], Luigi Cormio [8,9] and Giuseppe Carrieri [8]

Bladder Cancer – Dialog with pathologist today

International Journal of *Molecular Sciences*

MDPI

Review

Are We Ready to Implement Molecular Subtyping of Bladder Cancer in Clinical Practice? Part 1: General Issues and Marker Expression

Francesca Sanguedolce [1,*], Magda Zanelli [2], Andrea Palicelli [2], Stefano Ascani [3], Maurizio Zizzo [4], Giorgia Cocco [5], Lars Björnebo [6], Anna Lantz [6,7], Ugo Giovanni Falagario [8], Luigi Cormio [8,9] and Giuseppe Carrieri [8]

May be not yet…, but briefly it will come

Molecular classifications systems for bladder cancer

Table 1. Summary of the main molecular classification systems in BC [21–28].

UNC *	MDA **	Lund ***	TCGA ****	Consensus Classification
Basal-like	Basal	SCC-like	Basal–squamous	Basal–Squamous
Luminal	Luminal	UroA	Luminal	Luminal–Papillary (LumP)
	p53-like	UroB	Luminal–papillary	Luminal Non-Specified (LumNS)
		Infiltrated	Luminal-infiltrated	Luminal Unstable (LumU)
		Genomically Unstable	Neuronal	Stroma-rich
				Neuroendocrine-like (NE-like)

* University of North Carolina; ** MD Anderson Cancer Center; *** University of Lund; and **** The Cancer Genome Atlas.

Bladder Cancer – Dialogue with pathologist today

% of MIBC	24%	8%	15%	15%	35%	3%
Class Name	Luminal Papillary (LumP)	Luminal Non-Specified (LumNS)	Luminal Unstable (LumU)	Stroma-rich	Basal/Squamous (Ba/Sq)	Neuroendocrine-like (NE-like)
						
Differentiation	Urothelial / Luminal				Basal	Neuroendocrine
Oncogenic mechanisms	FGFR3 + PPARG + CDKN2A -	PPARG +	PPARG + E2F3 +, ERBB2 + Genomic instability Cell cycle +		EGFR +	TP53 -, RB1 -, Cell cycle +
Mutations	*FGFR3* (40%), *KDM6A* (38%)	*ELF3* (35%)	*TP53* (76%), *ERCC2* (22%) TMB +, APOBEC +		*TP53* (61%), *RB1* (25%)	*TP53* (94%) *RB1* (39%)*
Stromal infiltrate		Fibroblasts		Smooth muscle Fibroblasts Myofibroblasts	Fibroblasts Myofibroblasts	
Immune infiltrate				B cells	CD8 T cells NK cells	
Histology	Papillary morphology (59%)	Micropapillary variant (36%)			Squamous differentiation (42%)	Neuroendocrine differentiation (72%)
Clinical	T2 stage +	Older patients + (80+)			Women + T3/T4 stage +	
Median overall survival (years)	4	1.8	2.9	3.8	1.2	1

* 94% of these tumors present either RB1 mutation or deletion

Molecular classifications systems for bladder cancer

Table I. *Key points regarding consensus molecular classification of muscle-invasive bladder cancer.*

Molecular subtype	Differentiation	Mutation, %	Histology	Clinical features*	Median survival, years
Luminal papillary (LumP)	Luminal	*KDM6A*, 38% *FGFR3*, 40% *CDKN2A*, 33%	Papillary morphology	pT2 and higher	4
Luminal non-specified (LumNS)	Luminal	*ELF3*, 35%	Micropapillary variant	Elderly patients	1.8
Luminal unstable (LumU)	Luminal	*TP53*, 76% *ERCC2*, 26% *APOBEC*$^{+}$			2.9
Stroma-rich	Intermediate				3.8
Basal/squamous (Ba/Sq)	Basal	*TP53*, 61% *RB1*, 25%	Squamous differentiation	Women mostly and pT3/pT4	1.2
Neuroendocrine-like (NE-like)	Neuroendocrine	*TP53*, 94% *RB1*, 39%	Neuroendocrine differentiation		1

APOBEC: Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like; *CDKN2A*: cyclin-dependent kinase inhibitor 2A; *ELF3*: E74-like ETS transcription factor 3; *ERCC2*: excision repair cross-complementing rodent repair deficiency, complementation group 2; *FGFR3*: fibroblast growth factor 3; *KDM6A*: lysine-specific demethylase 6A; *RB1*: retinoblastoma protein; *TP53*: tumor protein p53. *According to (14).

Immunohistochemestry for molecular classification

Table 3. Antibodies and clones used in selected studies (see text).

	Basal Markers												Luminal Markers				
	CD44		CK5 or CK5/6		CK14		P40		P63		CK20		FOXA1		GATA3		
	Antibody	Clone	Antibody	Clone	Antibody	Clone	Antibody	Clone	Antibody	Clone	Antibody	Clone	Antibody	Clone	Antibody	Clone	Ref.
NMIBC			+	XM26											+	L50-823	[78]
NMIBC, MIBC			+	D5/16 B4	+	LL002							+	Q-6	+	L50-823	[100]
MIBC	+	DF1485	+	XM26	+	SP53					+	Ks20.8	+	ab23738	+	L50-823	[44]
NMIBC (CIS)			+	D5/16 B4							+	Ks20.8					[88]
MIBC			+	D5/16 B4	+	LL002					+	Ks20.8			+	L50-823	[107]
MIBC			+	D5/16 B4							+	Ks20.8					[108]
NMIBC	+	DF1485	+	D5/16	+	OIT4A7					+	OTI4A2			+	UMAB218	[81]
NMIBC			+	D5/16 B4			+	BC28	+	4A4	+	SP33			+	L50-823	[83]
NMIBC, MIBC			+	D5/16 B4											+	L50-823	[68]
NMIBC, MIBC			+	D5/16	+	OIT4A7					+	OTI4A			+	UMAB218	[67]
MIBC			+	D5/16 B4	+	LL002					+	Ks20.8			+	HG3-31	[27]

Table 2. Potential responsiveness to different treatments according to the Consensus Classification [25].

Molecular subtypes according to the Consensus Classification	LumP	LumNS	LumU	Stroma-rich	Ba/Sq	NE-like
Potential responsiveness to treatment	FGFR3-targeted therapies	NAC, immunotherapy	Radiotherapy, immunotherapy	-	EGFR-targeted therapies, immunotherapy, NAC	Radiotherapy, immunotherapy

Bladder Cancer – Dialogue with pathologist today

Next-Generation Squencing in Bladder Cancer

Advanced urothelial carcinoma: next-generation sequencing reveals diverse genomic alterations and targets of therapy

Jeffrey S Ross[1,2], Kai Wang[2], Rami N Al-Rohil[1], Tipu Nazeer[1], Christine E Sheehan[1], Geoff A Otto[2], Jie He[2], Gary Palmer[2], Roman Yelensky[2], Doron Lipson[2], Siraj Ali[2], Sohail Balasubramanian[2], John A Curran[2], Lazlo Garcia[2], Kristen Mahoney[2], Sean R Downing[2], Matthew Hawryluk[2], Vincent A Miller[2] and Philip J Stephens[2]

[1]*Department of Pathology and Laboratory Medicine, Albany Medical College, Albany, NY, USA and* [2]*Foundation Medicine, Cambridge, MA, USA*

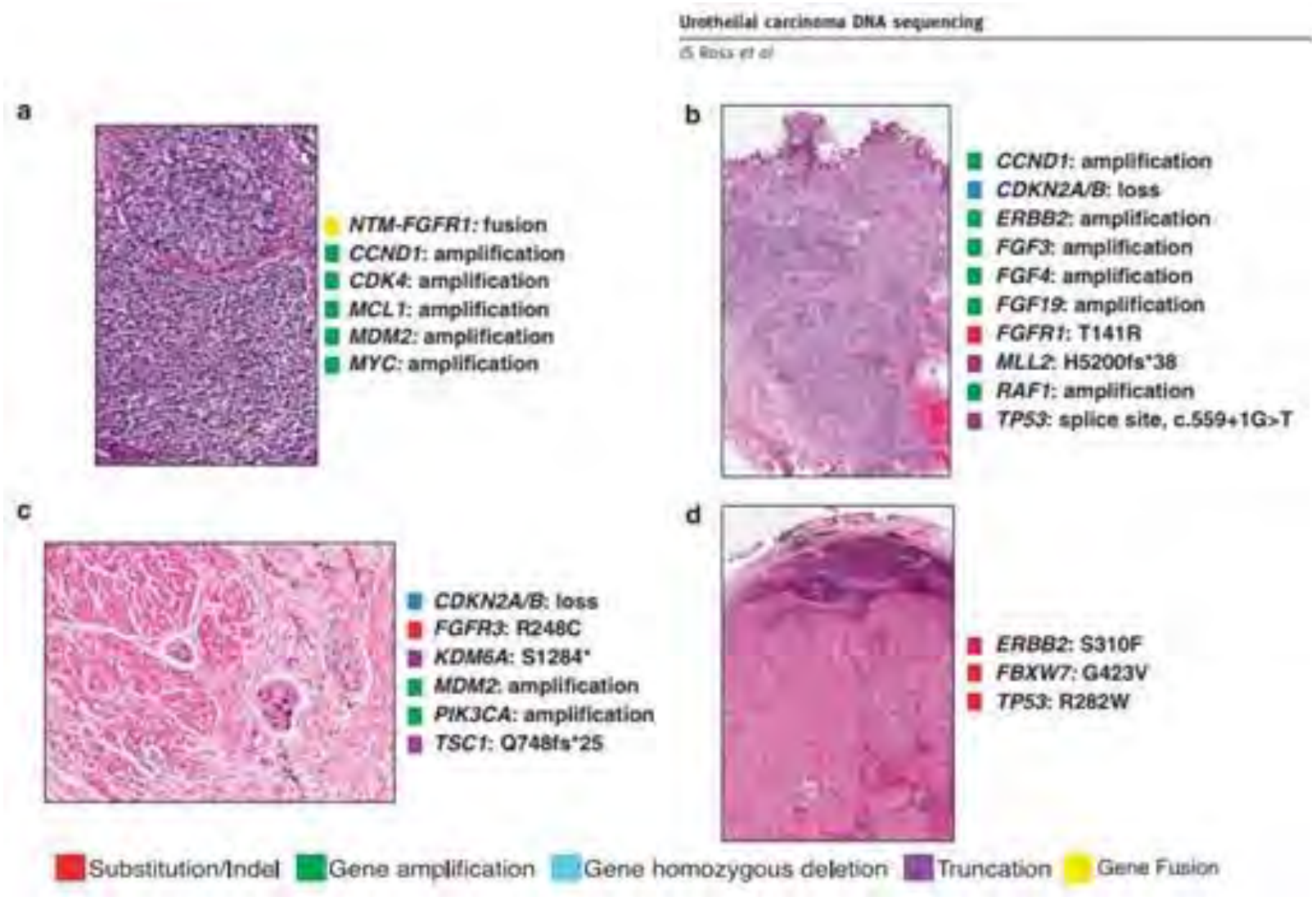
Although urothelial carcinoma (UC) of the urinary bladder generally portends a favorable prognosis, metastatic tumors often follow an aggressive clinical course. DNA was extracted from 40 μm of formalin-fixed, paraffin-embedded (FFPE) sections from 35 stage IV UCs that had relapsed and progressed after primary surgery and conventional chemotherapy. Next-generation sequencing (NGS) was performed on hybridization-captured, adaptor ligation-based libraries for 3320 exons of 182 cancer-related genes plus 37 introns from 14 genes frequently rearranged in cancer to at an average sequencing depth of 1164× and evaluated for all classes of genomic alterations (GAs). Actionable GAs were defined as those impacting the selection of targeted anticancer therapies on the market or in registered clinical trials. A total of 139 GAs were identified, with an average of 4.0 GAs per tumor (range 0–10), of which 78 (56%) were considered actionable, with an average of 2.2 per tumor (range 0–7). Twenty-nine (83%) cases harbored at least one actionable GA including: *PIK3CA* (9 cases; 26%); *CDKN2A/B* (8 cases; 23%); *CCND1* (5 cases; 14%); *FGFR1* (5 cases; 14%); *CCND3* (4 cases; 11%); *FGFR3* (4 cases; 11%); *MCL1* (4 cases; 11%); *MDM2* (4 cases; 11%); *EGFR* (2 cases, 6%); *ERBB2* (*HER2/neu*) (2 cases, 6%); *NF1* (2 cases, 6%) and *TSC1* (2 cases, 6%). Notable additional alterations included *TP53* (19 cases, 54%) and *RB1* (6 cases; 17%). Genes involved in chromatin modification were altered by nonsense mutation, splice site mutation or frameshift indel in a mutually exclusive manner in nearly half of all cases including *KDM6A* (10 cases; 29%) and *ARID1A* (7 cases; 20%). Comprehensive NGS of 35 UCs of the bladder revealed a diverse spectrum of actionable GAs in 83% of cases, which has the potential to inform treatment decisions for patients with relapsed and metastatic disease.

Modern Pathology (2014) **27,** 271–280; doi:10.1038/modpathol.2013.135; published online 26 July 2013

AKT1	ERBB2	NRAS
BRAF	ERBB3	PIK3CA
C3orf70	ERCC2	RHOB
CDKN1A	FBXW7	RXRA
CDKN2A	FGFR3	SF3B1
CREBBP	HRAS	TERT (promoter)
CTNNB1	KDM6A	TP53
ELF3	KRAS	

Table 1. Bladder Cancer Panel gene content.

Bladder Cancer – Dialogue with pathologist today



Recognised mutations in invasive high grade bladder carcinomas:

…. target therapies ??

In addition to neoplastic tissue, tumoral markers can also be found:

- In urine
- In blood (liquid biopsies)

CONCLUSION

Direct, personal, DIALOGUE is mandatory, oral and written,
between pathologist and urologist ….,
from the moment when biopsy is taken,
during the treatment and follow-up.

CONCLUSION

Direct, personal, DIALOGUE is mandatory, oral and written,
between pathologist and urologist ….,
from the moment when biopsy is taken,
during the treatment and follow-up.

But more elements must come:
medical oncology and molecular genetic