UAB MEDICINE
Knowledge that will change your world

Genomics Advances in Bladder Cancer

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Financial Disclosures

- **Genentech** Pathology Advisory Board
- **UroSEEK** Patent application pending

Overview

- **The Dual Universes of BC**
  NMIBC *vs* MIBC
- **The Impact of Genomics on BC**
  Novel TAXONOMY ?
  Prognostics
  Predictive
  Rx Targets
  ImmunoRx Response Predictors
- **UroSEEK**

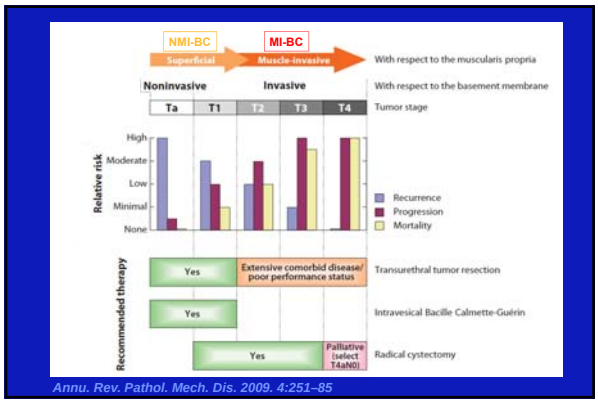
Bladder Cancer (BC)
Disease Costs and Management Opportunities

- Major health care cost burden:
  - Frequent cystoscopy, high rate of recurrence etc..
  - $ 3-4 Billion per year in USA alone
  - HIGHEST COST per patient for any type of cancer
- Unique amenability to applying molecular detection methods to urine sample (e.g. *TERT*)

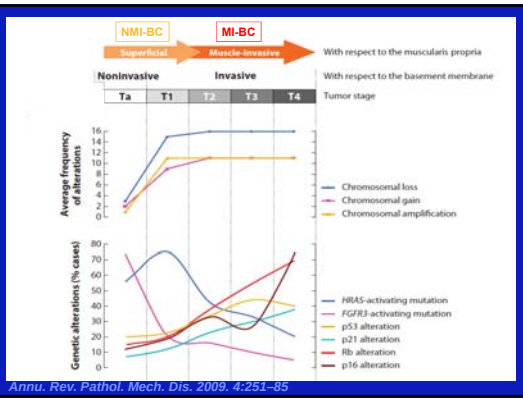
Urothelial Carcinoma
Two Phenotypes?

~~(Superficial)~~ Non-muscle invasive BC **(NMI-BC)**
70-80%

Muscle Invasive BC **(MI-BC)**
20-30%

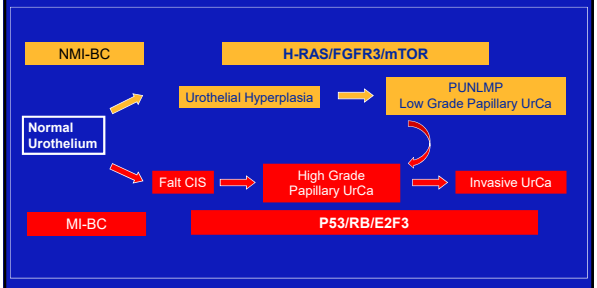


Annu. Rev. Pathol. Mech. Dis. 2009. 4:251–85



Annu. Rev. Pathol. Mech. Dis. 2009. 4:251–85

Bladder Urothelial Carcinoma
Two Distinct Molecular Pathways



The Not So Distant Past ?

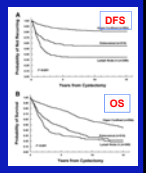
Clinico-Pathologic Prognostic Factors

Radical Cystectomy in the Treatment of Invasive Bladder Cancer: Long-Term Results in 1,054 Patients

By John P. Stein, Gary Lieskovsky, Richard Cote, Susan Groshen, An-Chen Feng, Stuart Boyd, Eila Skinner, Bernard Bochner, Duriayai Thangathurai, Maged Mikhail, Derek Raghavan, and Donald G. Skinner

Stein et al J Clin Oncol 2001

- 1054 pts; 10.2 yrs median F/U
- Rad. Cystectomy ± Adj Chemo radiation
- **DFS 68% at 5yr and 60% at 10 yrs**
- pTNM (OC vs Non-OC) only predictor of DFS/OS
  - OC LN neg group: 85% DFS at 5yr
  - Non OC LN neg group: 58% DFS at 5yr
  - LN positive group: 35% DFS at 5yr



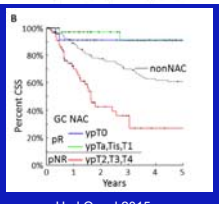
MIBC
Neoadjuvant Chemotherapy (NAC)

Gemcitabine and cisplatin neoadjuvant chemotherapy for muscle-invasive urothelial carcinoma: Predicting response and assessing outcomes

Niloy M. Gandhi, M.D.[a,b,*], Alexander Baras, M.D., Ph.D.[b,c], Enrico Munari, M.D.[d], Sheila Faraj, M.D.[e], Leonardo O. Reis, M.D., Ph.D.[f], Jen-Jane Liu, M.D.[g], Max Kates, M.D.[e], Mohammad Obaidul Hoque, D.S.S., Ph.D.[e], David Berman, M.D., Ph.D.[e], Noah M. Hahn, M.D.[h], Mario Eisenberger, M.D.[i], George J. Netto, M.D.[j], Mark P. Schoenberg, M.D.[k], Trinity J. Bivalacqua, M.D., Ph.D.[a]

NAC Regimens:
GC
MVAC
DD-MVAC

Can we predict Response ????



Urol Oncol 2015

GENOMICS IMPACT on BC

Genomics as Prognostic Tools

PLoS one

Integrated Genomic and Gene Expression Profiling Identifies Two Major Genomic Circuits in Urothelial Carcinoma

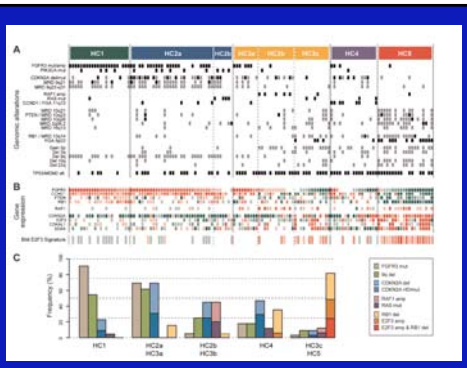
David Lindgren[1], Gottfrid Sjödahl[2], Martin Lauss[2], Johan Staaf[2], Gunilla Chebil[3], Kristina Lövgren[3], Sigurdur Gudjonsson[4], Fredrik Liedberg[4], Oliver Patschan[4], Wiking Månsson[4], Mårten Fernö[3], Mattias Höglund[2]*

Two Genomic Circuits:

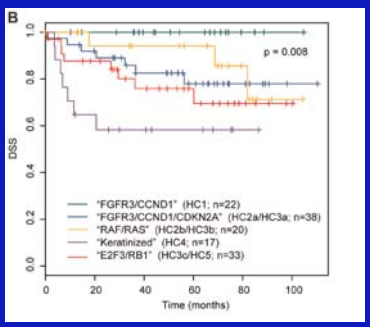
- *FGFR3* mut/Ampl; ⬆ CCND1; PIK3CA mut; 9q (*CDKN2A)* deletions
- *E2F3* ampl; *RB1* del; *PTEN* del; ⬆*CDKN2A*; *CCND1* loss, 5p gain

P53/MDM2 alterations in both circuits at advanced Dz

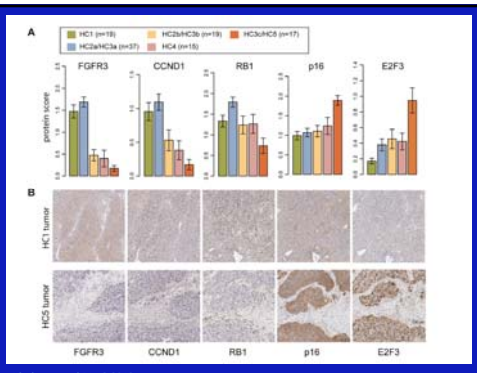
Lindgren et al. PLoS 2012



Lindgren et al PLoS 2012



Lindgren et al PLoS 2012



Lindgren et al PLoS 2012

Comprehensive molecular characterization of urothelial bladder carcinoma

The Cancer Genome Atlas Research Network*

Nature: March 2014

- Integrated genomic Analysis of 131 URCa

Bladder Cancer (MIBC)
TCGA

- Average Genetic Alterations per tumor:
  - 302 mutations
  - 204 segmental CNA
  - 22 rearrangements
- Recurrent mutations in 32 genes:
  - Cell-cycle regulation
  - Chromatin regulation
  - RTK signaling pathways
  - Nine genes not frequently mutated in cancers (MLL2,ERCC2,ELF3,KLF5,RXRA, CDKN1A)
- Rx Targets in 69% of MIBC

NEW GENOMIC TAXONOMY ?
TCGA

INTEGARTED GENE EXPRESSION SUBTYPES

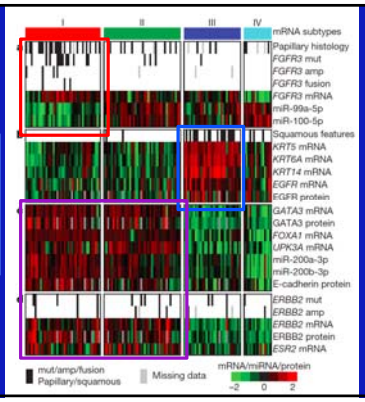
PAPILLARY-LIKE

BASAL / SQUAMOUS-LIKE

LUMINAL / BREAST-LIKE

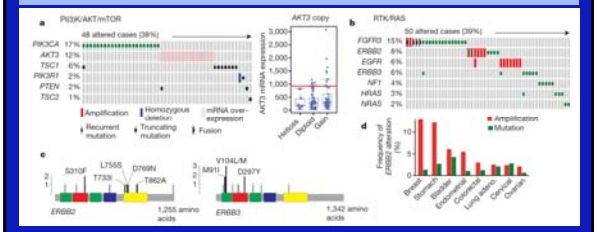
I PAPILLARY-LIKE
III BASAL SQUAMOUS-LIKE
I&II LUMINAL BREAST-LIKE

mRNA/miRNA/Protein



Genomic Based Novel Therapeutic Targets

MIBC TCGA: TARGETS of Rx



Rx Targets in 69% of URCa:
- **mTOR/PIK3CA**
- **RTK/MAPK (ERBB2)**
- **ER**

EGFR as a potential therapeutic target for a subset of muscle-invasive bladder cancers presenting a basal-like phenotype

Sandra Rebouissou,[1,2]* Isabelle Bernard-Pierrot,[1,2]* Aurélien de Reyniès,[3]* May-Linda Lepage,[1,2] Clémentine Krucker,[1,2] Elodie Chapeaublanc,[1,2] Aurélie Hérault,[1,2] Aurélie Kamoun,[1,2] Aurélie Caillault,[1,2] Eric Letouzé,[3] Nabila Elarouci,[3] Yann Neuzillet,[4,5] Yves Denoux,[6] Vincent Molinié,[7] Dimitri Vordos,[8] Agnès Laplanche,[9] Pascale Maillé,[10] Pascale Soyeux,[11,12] Karina Ofualuka,[11,12] Fabien Reyal,[1,2,13] Anne Biton,[1,2,14†] Mathilde Sibony,[15] Xavier Paoletti,[14,16] Jennifer Southgate,[17] Simone Benhamou,[18,19‡] Thierry Lebret,[4,5‡] Yves Allory,[10,11,12,20‡] François Radvanyi[1,2‡§]

www.ScienceTranslationalMedicine.org 9 July 2014

Genomic Based Predictor of Neoadjuvant ChemoRx Response

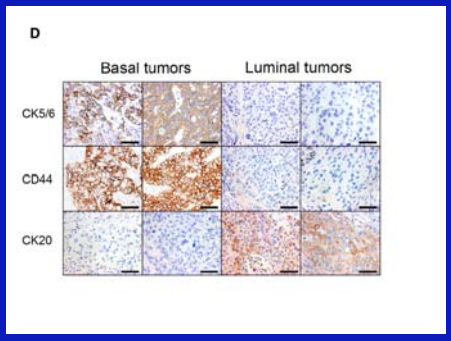
Cell PRESS

Cancer Cell
Article

Identification of Distinct Basal and Luminal Subtypes of Muscle-Invasive Bladder Cancer with Different Sensitivities to Frontline Chemotherapy

Cancer Cell 25, 152–165, February 10, 2014

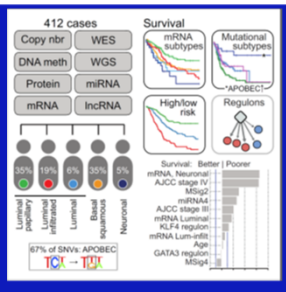
- Whole genome mRNA expression profiling
- Three molecular subtypes of MIBC: **Basal/Luminal/p53-like**
- Shared molecular features with basal and luminal breast cancers
- **Active p53 gene signature "p53-like"** MIBC
  - resistant to neoadjuvant cisplatin-based ChemoRx



Cancer Cell 25, 152–165, February 10, 2014

Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer

Cell 2017



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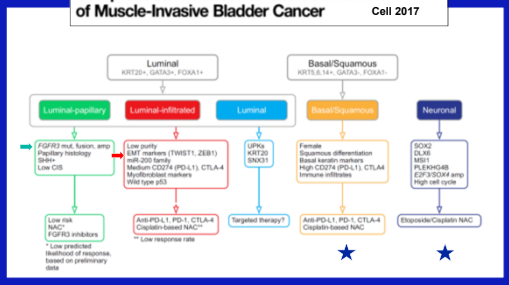


Immune Checkpoint Inhibitors in Bladder Ca.

Immunotherapy in BC
Completed Clinical Trials

Reference	Pembrolizumab: Metastatic second line — Pilmack et al. [15]	Pembrolizumab: Metastatic second line — Bellmunt [16*]	Atezolizumab: Metastatic second line — Powles et al. [12]	Atezolizumab: Metastatic second line — Rosenberg et al. [11**]	Nivolumab: Metastatic second line — Sharma et al. [17**]	Nivolumab: Metastatic second line — Galsky et al. [13]	Durvalumab: Metastatic second line — Massard et al. [18]	Avelumab: Metastatic second line — Apolo et al. [19**]	Atezolizumab: Metastatic first-line cisplatin ineligible — Balar et al. [20**]	Pembrolizumab: Metastatic first-line cisplatin ineligible — Balar et al. [21*]
FDA approval			May 2016				February 2017			
Phase	Ib	III	II	II	II	II	II	II	II	II
n	27	542	100	331	78	245	42	44	119	100
ORR (%)	26 (PD-L1+)	Pembro: 21.1; Chemo: 11.4	21	15	24.4	19.6	31	18.2	23	24
ORR PD-L1+ (%)	26	Pembro: 21.6; Chemo: 6.7	43.3	26	24.0	28.4	46.4	53.8	28	37
AE (%)	15	Pembro: 14.1; Chemo: 8.1	NR	10	18	17.2	NR	6.8	13	18
CR (%)	11	Pembro: 7; Chemo: 3.3	7 (PD-L1+)	5	6.4	2.3	NR	11.4	9	6
PD-L1+ prevalence (%)	53	Pembro: 27.4; Chemo: 23.1	27	32.2	32	30.5	65.6	29.5	27	30
PFS (months)	2	Pembro: 2.1; Chemo: 3.3	6 (PD-L1+); 1 (PD-L1-)	2.1	2.8	2.0	NR	2.9	2.7	NR
OS (months)	13	Pembro: 10.3; Chemo: 7.4	NR (PD-L1+); 8 (PD-L1-)	11.4	9.7	8.7	NR	13.7	15.9	NR
Grade 3/4 AEs (%)	15	Pembro: 15; Chemo: 49.4	4.4	16	22	17.8	4.9	6.8	16	16

Curr Opin Oncol 2017, 29:184–195

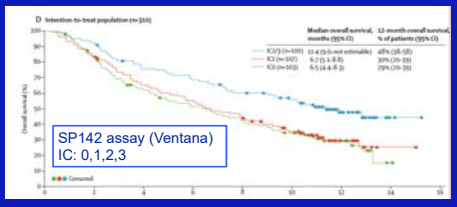
	Grade 1–2	Grade 3	Grade 4
Any event	46 (59%)	17 (22%)	0
Fatigue	26 (33%)	2 (3%)	0
Pruritus	23 (29%)	0	0
Rash, maculopapular	12 (15%)	2 (3%)	0
Lipase elevated	7 (9%)	4 (5%)	0
Nausea	9 (12%)	1 (1%)	0
Arthralgia	9 (12%)	0	0
Anaemia	8 (10%)	0	0
Amylase increased	4 (5%)	3 (4%)	0
Dyspnoea	4 (5%)	1 (1%)	1 (1%)*
Lymphocyte count decreased	3 (4%)	2 (3%)	0
Hyperglycaemia	4 (5%)	1 (1%)	0
Neutrophil count decreased	1 (1%)	2 (3%)	0
White blood cell count decreased	2 (3%)	1 (1%)	0
Hyponatraemia	3 (3%)	1 (1%)	0
Dermatitis acneiform	1 (1%)	1 (1%)	0
Wheezing	3 (3%)	1 (1%)	0
Acute kidney injury	0	1 (1%)	0
Aspartate aminotransferase increased	0	1 (1%)	0
Back pain	0	1 (1%)	0
Colitis	0	1 (1%)	0

Nivolumab monotherapy in recurrent metastatic urothelial carcinoma (CheckMate 032): a multicentre, open-label, two-stage, multi-arm, phase 1/2 trial

Lancet Oncology 2016

2 TREATMENT-RELATED DEATHS:
1 thrombocytopenia /1 pneumonitis

Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial *Rosenberg J et al Lancet 2016*



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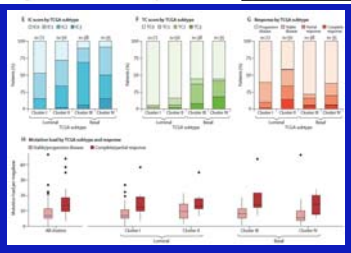
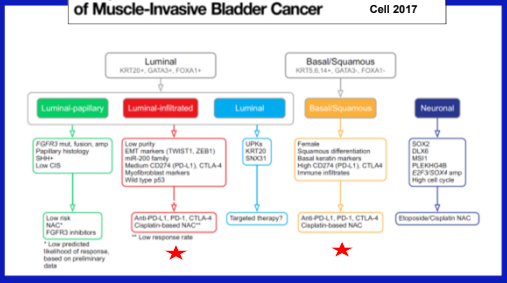


TCGA

TMB

Comprehensive Molecular Characterization of Muscle-Invasive Bladder Cancer **Cell 2017**



Mechanisms Driven Predictive Biomarker for Immune Checkpoint Inhibitors

- **Immunologic Biomarkers IHC/Gene Expression:**
  - PDL-1
  - CD8+
  - CD8+/Ki67
  - TIM3, LAG3, IDO
  - ICOS, GITR
  - Chemokines Signature (Interferon 8)
- **Tumor Mutational Load/Burden:** NSCLC, Melanoma, BC
  - Neoantigens
- **dMMR/MSI: e.g.** CRCa, Endometrial Ca.
- **Viral Oncoproteins:** HTLV-1, HPV, EBV, KSV, MCPyV, HBV, HCV

Molecular Early Detection & Surveillance Markers

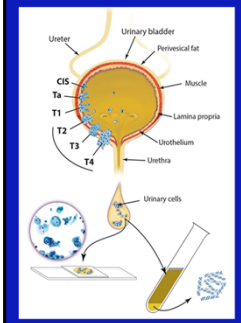
The Power of Genomics

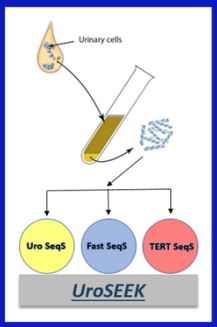
UroSEEK


eLIFE elifesciences.org RESEARCH ARTICLE

Non-invasive detection of urothelial cancer through the analysis of driver gene mutations and aneuploidy

Simeon U Springer[††*], Chung-Hsin Chen[†‡], Maria Del Carmen Rodriguez Pena[§,¶†], Lu Li[§], Christopher Douville[§], Yuxuan Wang[§], Joshua David Cohen[§], Diana Taheri[§,‖], Natalie Silliman[§], Joy Schaefer[§], Janine Ptak[§], Lisa Dobbyn[§], Maria Papoli[§], Isaac Kinde[§], Bahman Afsari[§,**], Aline C Tregnago[§], Stephania M Bezerra[§§], Christopher VandenBussche[§], Kazutoshi Fujita[¶¶], Dilek Ertoy[¶‖], Isabela W Cunha[¶‖], Lijia Yu[¶], Trinity J Bivalacqua[¶#], Arthur P Grollman[¶*,¶#], Luis A Diaz[¶*,¶†], Rachel Karchin[¶,**], Ludmila Danilova[¶,¶‖], Chao-Yuan Huang[‡], Chia-Tung Shun[¶¶], Robert J Turesky[¶§], Byeong Hwa Yun[¶§], Thomas A Rosenquist[¶¶], Yeong-Shiau Pu[‡], Ralph H Hruban[¶], Cristian Tomasetti[¶,¶], Nickolas Papadopoulos[§], Ken W Kinzler[§], Bert Vogelstein[§*], Kathleen G Dickman[¶¶,¶¶*], George J Netto[¶,¶*]

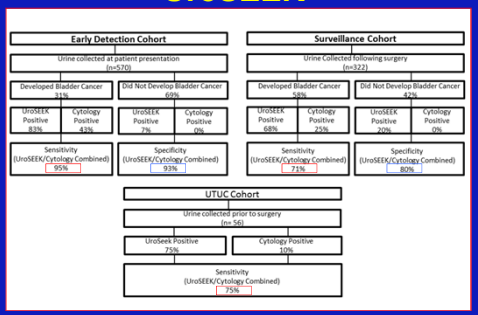




UroSeqs (10 genes)

- CDKN2A
- ERBB2
- FGFR3
- HRAS
- KRAS
- MET
- MLL
- PIK3CA
- TP53
- VHL

UroSEEK



Conclusions

- **Genomics** Impact on Bladder Cancer Management:
  - **PGx**
  - **Predictive**
  - **Rx Targets**
  - **Early Detection and Surveillance**
- New **GENOMIC TAXONOMY** is being refined and awaits TRANSLATION INTO OUR LABS
- Immune Check Point Inhibitors are opening new doors in BC management

Thank You !!!

Conclusions

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- Immune Check Point Inhibitors are opening new doors in BC management

Thank You !!!

TERT **promoter mutations occur frequently in gliomas and a subset of tumors derived from cells with low rates of self-renewal**

Patrick J. Killela[a,1], Zachary J. Reitman[a,1], Yuchen Jiao[b,1], Chetan Bettegowda[b,c,1], Nishant Agrawal[b,d], Luis A. Diaz, Jr.[b], Allan H. Friedman[e], Henry Friedman[e], Gary L. Gallia[c,d], Beppino C. Giovanella[e], Arthur P. Grollman[f], Tong-Chuan He[g], Yiping He[g], Ralph H. Hruban[h], George I. Jallo[c], Nils Mandahl[i], Alan K. Meeker[h,m], Fredrik Mertens[i], George J. Netto[h,j], B. Ahmed Rasheed[e], Gregory J. Riggins[c], Thomas A. Rosenquist[f], Mark Schiffman[j], Ie-Ming Shih[h], Dan Theodorescu[k], Michael S. Torbenson[h], Victor E. Velculescu[h], Tian-Li Wang[h], Nicolas Wentzensen[j], Laura D. Wood[h], Ming Zhang[h], Roger E. McLendon[e], Darell D. Bigner[e], Kenneth W. Kinzler[h], Bert Vogelstein[h,2], Nickolas Papadopoulos[h], and Hai Yan[a,d]

PNAS | April 9, 2013 | vol. 110 | no. 15 | 6021–6026

Priority Report

Cancer Research

TERT Promoter Mutations Occur Early in Urothelial Neoplasia and Are Biomarkers of Early Disease and Disease Recurrence in Urine

Isaac Kinde[1], Enrico Munari[2], Sheila F. Faraj[2], Ralph H. Hruban[2,3], Mark Schoenberg[4], Trinity Bivalacqua[4], Mohamad Allaf[4], Simeon Springer[1], Yuxuan Wang[1], Luis A. Diaz,Jr[1], Kenneth W. Kinzler[1], Bert Vogelstein[1], Nickolas Papadopoulos[1], and George J. Netto[2,3,5]

TERT promoter mutation	pTa LG ($N = 28$)	pTa HG ($N = 31$)	CIS ($N = 17$)	*P*
Present (%)	24/28 (86%)	21/31 (68%)	11/17 (65%)	0.18

Abbreviations: HG, high-grade noninvasive urothelial carcinoma; LG, low-grade noninvasive urothelial carcinoma.

TERT mutation in follow-up urine	Number of patients	Recurred	Did not recur	*P*
Present	8	8/8 (100%)	0/8 (0%)	<0.001
Absent	7	1/7 (11%)	6/7 (89%)	($r = 0.87$)[a]

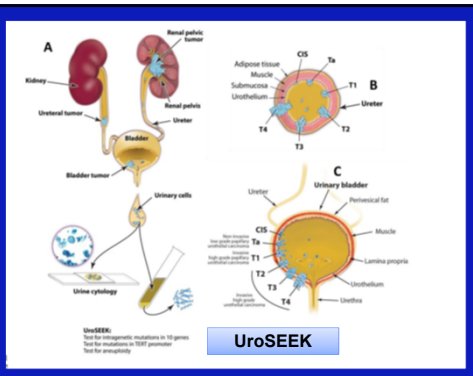
[a]Pearson coefficient of correlation.

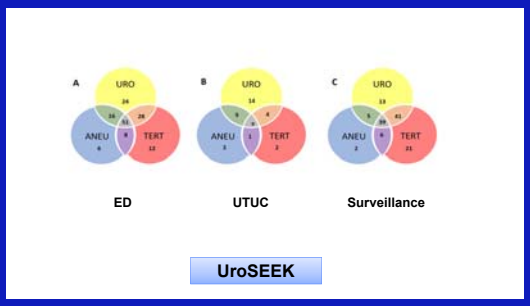
Ludwig Cancer Research
JHU Pathology
JHU Brady Institute

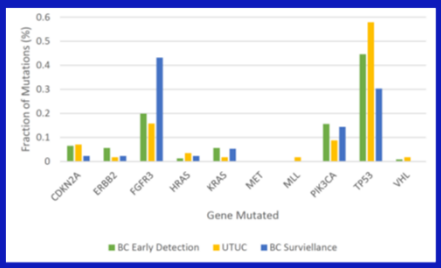
Two Application Settings
- Surveillance
- Primary Screen (Hematuria no prior TCC)

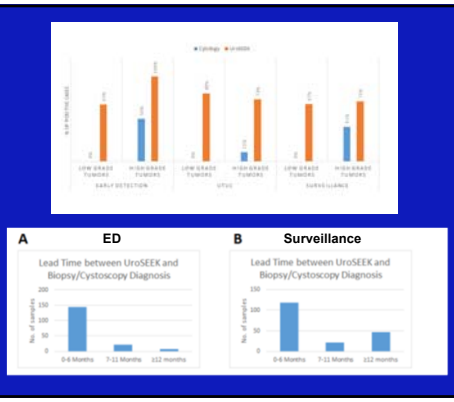
International Collaborators
- Osaka University, Japan
- AC CAMARGO Cancer Ctr, Brasil
- Hacettepe University, Turkey
- Taiwan **UTUC**

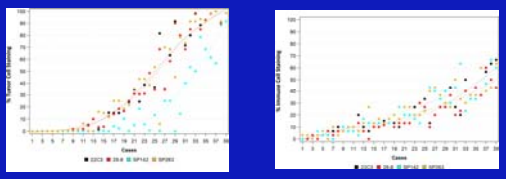
- **>2800 Urine and 400 FFPE Sequenced**











Journal of Thoracic Oncology 2017

Sample	• 39 NSCLC paraffin blocks procured commercially by Dako/Ventana
Assay	• Serial sections were stained by Dako (28-8 & 22C3) and by Ventana (SP142 & SP263)
Slide Readers	• 2 Ventana pathologists (experts for SP142 and SP263, respectively) • 1 Dako pathologist (expert for 28-8 and 22C3)
% score	• Each independently scored the slides for % tumor cells and % immune cell expression of PD-L1
Clinical cut-off	• Each independently scored the slides using only the cut-off associated with each assay.

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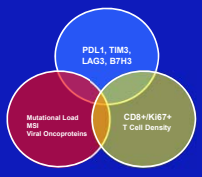
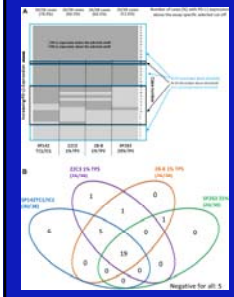
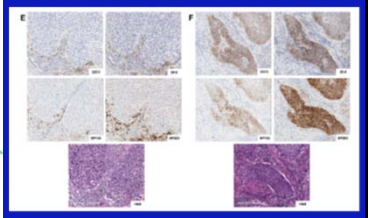
Multifactorial Predictive Biomarkers
of Immune Checkpoint Inhibitors Response







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PD-L1 Immunohistochemistry Assays for Lung Cancer: Results from Phase 1 of the Blueprint PD-L1 IHC Assay Comparison Project

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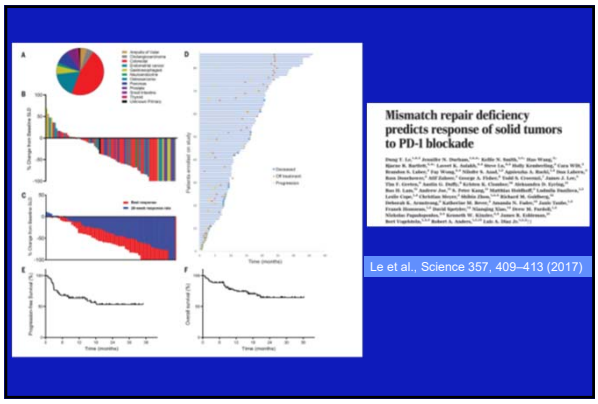
Atezolizumab in patients with locally advanced and metastatic urothelial carcinoma who have progressed following treatment with platinum-based chemotherapy: a single-arm, multicentre, phase 2 trial

Rosenberg J et al Lancet 2016

	Patients, n	Objective response rate, n (% [95% CI])	Complete response	Partial response	Stable disease	Progressive disease
RECIST version 1.1 criteria by independent review						
IC2/3	100	26 (26% [18-36])	11 (11%)	15 (15%)	16 (16%)	44 (44%)
IC1/2/3	207	37 (18% [13-24])	13 (6%)	24 (12%)	34 (16%)	107 (52%)
All patients	310	45 (15% [11-19])	15 (5%)	30 (10%)	59 (19%)	159 (51%)
IC1*	107	11 (10% [5-18])	2 (2%)	9 (8%)	18 (17%)	63 (59%)
IC0*	103	8 (8% [3-15])	2 (2%)	6 (6%)	25 (24%)	52 (50%)
Modified RECIST criteria by investigator review						
IC2/3	100	27 (27% [19-37])	8 (8%)	19 (19%)	31 (31%)	28 (28%)
IC1/2/3	207	45 (22% [16-28])	14 (7%)	31 (15%)	58 (28%)	74 (36%)
All patients	310	58 (19% [15-24])	16 (5%)	42 (14%)	92 (30%)	110 (35%)
IC1*	107	18 (17% [10-25])	6 (6%)	12 (11%)	27 (25%)	46 (43%)
IC0*	103	13 (13% [7-21])	2 (2%)	11 (11%)	34 (33%)	36 (35%)

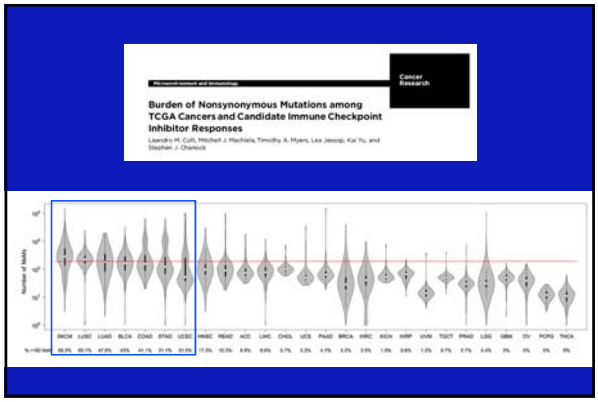


Mismatch repair deficiency predicts response of solid tumors to PD-1 blockade

Le et al., Science 357, 409–413 (2017)

Burden of Nonsynonymous Mutations among TCGA Cancers and Candidate Immune Checkpoint Inhibitor Responses

- ROC curves of checkpoint inhibitor response in **melanoma and NSCLC** studies identified cutoff of **192 NsM**
- In 7,757 samples of **TCGA**: **16.2%** exceeded the 192 NsM threshold
- **> 30%** of **BC, CRCa, Gastric Ca. and Endometrial Ca**. have NsM counts above 192



Burden of Nonsynonymous Mutations among TCGA Cancers and Candidate Immune Checkpoint Inhibitor Responses