The Bladder Cancer (BLCA) Analysis Working Group: a Progress Report

John N. Weinstein, M.D., Ph.D.
UT MD Anderson Cancer Center GDAC
American Cancer Society Statistics on BLCA*

- 90% over age 55, > half are over age 73
- Four times more likely in men than women
- Lifetime risk for men 1 in 27; Women 1 in 85
- 4th most common cancer in men

U.S. spends 2.2 billion dollars a year in health care for bladder cancer patients compared with 1.4 billion for prostate cancer**

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**Agency for Health Care Policy & Research, 1995 and 1999, U.S. Public Health Service (HHS)
Low- and High-Grade BLCA

Low-grade: superficial, less likely to invade or metastasize, frequently reappears after resection but amenable to therapy, low mortality

High-grade: propensity to invade and metastasize, high mortality when invasive, but good response to treatment if detected early
Muscle Invasive Bladder Cancer

- 15-20% of patients with Ta, T1 or Tis cancer progress to muscle invasion
- 80% of patients with muscle invasive cancer present de novo
- Distant metastases most common cause of treatment failure
  - Present at the time of cystectomy
  - Occurs in 40-50% within 2 years without additional therapy
Bladder Cancer Treatment

- Cisplatin based multi-agent chemotherapy standard of care for neoadjuvant prior to cystectomy and for measurable metastatic disease
- No new FDA approved drugs for muscle invasive BLCA cancer in over two decades!

Neoadjuvant chemotherapy Meta analysis
(Eur Urol 48:202, 2005)

Metastatic disease
GC: median = 14.0 m (12.3-15.5 m); 13.3% censoring
MVAC: median = 15.2 m (13.2-17.3 m); 15.4% censoring
HR: 1.09 (0.88-1.34)
Log-rank $P = .44$, Wald’s $P = .66$

JCO 23:4602, 2005
BLCA Working Group Chairs and Coordinators

Co-Chairs: Seth Lerner, John Weinstein

Data Coordinator: Chad Creighton

Analysis Coordinator: Rehan Akbani

Admin. Coordinator: Margi Sheth

Manuscript Coordinator: Maggie Morgan

Chromatin Remodeling Chairs: David Kwiatkowsky, Jonathan Rosenberg, Peter Laird
## BLCA Working Group Members

<table>
<thead>
<tr>
<th>Seth Lerner (co-chair)</th>
<th>Semin Lee</th>
<th>Lihua Zou</th>
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<tr>
<td>John Weinstein (co-chair)</td>
<td>Bradley Leibovich</td>
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<td>Rehan Akbani</td>
<td>Monica Liebert</td>
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<td>Bob Penny (BCR)</td>
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Clinical Data on TCGA Samples

- Muscle invasive urothelial cancer
  - Mixed histology allowed up to 9%
- 126 samples in data freeze for marker paper
  - 153 qualified; 138 in pipeline
- Gender: male 72%; female 28%
- Caucasian 85%
- Median age 69 (34-88)
- Follow up (n = 126)
  - Median 209 days (0-131.2 months)
- Event rate
  - Progression – 10 (data available for 31 patients)
  - Deaths 35 – (data available for all 126 patients)
# Staging of TCGA BLCA Cases (N = 124)

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<td>pT4</td>
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<td><strong>Total</strong></td>
<td>73</td>
<td>42</td>
<td>9</td>
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</table>

## AJCC Regional Lymph Nodes (N)

- pNX: 9
- pN0: 73
- pN1: 12
- pN2: 25
- pN3: 5
- Not Available: 2

## AJCC Stage

- Stage I: 1%
- Stage II: 27%
- Stage III: 36%
- Stage IV: 36%
Cigarette Smoking History

N = 126

Smoking History

1-Lifelong Non-Smoker: 31%
2-Current Smoker: 23%
3-Reformed smoker >15 yrs: 19%
4-Reformed smoker <=15 yrs: 21%
5-Reformed smoker NOS: 5%
Not Documented: 1%

N = 74
Status of the BLCA Project

• Accrual a limiting factor but recently accelerated.
• Productive Face-to-Face held in Houston in October 2012
• Data freeze at 126 tumor samples (plus normals) in December 2012
• So data and analyses presented here will be an “interim look”
• Fast progress on Marker Paper
Significantly mutated genes

MLL2

(J. Kim, Meyerson lab)

(Preliminary Analysis)
Unsupervised Clustering of Methylation Data

3,309 most variable probes (s.d. across tumor > 0.275)

18 Normal tissues

126 BLCA Tumors

Batch
DNA methylation cluster
RPMM-based clustering (Houseman et al. 2008)
mRNA-based subtypes

(Preliminary Analysis)
SuperCluster Results in Relation to GO Terms

(Preliminary Analysis) (K Hoadley, B Kim, et al)
Unsupervised NMF Consensus Clustering of miRNA data

304/1216 MIMATs

top 25%

miR-100.MIMAT0000098

N = 121

N0 AJCC disease lymph node stage
N1
N2
N3
N4

T1 AJCC tumor stage
T2
T3
T4

M0 AJCC metastasis stage
M1
MX

I AJCC disease stage
II
III
IV

Unsupervised NMF Consensus Clustering of miRNA data

Preliminary Analysis
(G Robertson, A Chu, A Mungall, M Marra, BCGSC)
Clustering of Proteomic (RPPA) Data

(R Akbani, Z Liu, J Weinstein, G Mills)
(NG-CHM: http://bioinformatics.mdanderson.org/main/TCGA/NGCHM)
SuperCluster Results

- Two to Three clusters found

(Preliminary Analysis)

(R Akbani) (NG-CHM: http://bioinformatics.mdanderson.org/main/TCGA/NGCHM)
Detection of Fusion protein FGFR3-TACC3

A. TCGA-CF-A3MH

1 2 16

FGFR3 1 10 11 16 TACC3

1 2 16 17

1 11 16 Fusion FGFR3-TACC3 pre-mRNA

CCGCGAAAGCTCGTCATGAGGGGCCCACCAAGAACGACGGCACTCGGGCTCACAGCGGGGAAA

AGCTCGTCATGAGGGGCCCACCAAGAACGACGGCACTCGGGCTCACAGCGGGGAAAG

Exon 1-16

CAUGAGGGGCCACCAAGAACGACGGCACU

B.

1 838

TK1 TK2

Ig1 Ig2 Ig3 TM

1 807

TK1 TK2

Ig1 Ig2 Ig3 TM

758 FGFR3

807

838 TACC3

Fusion protein FGFR3-TACC3

Coiled-coil domain

648

(Preliminary Analysis)

(Kucherlapati lab)
CD44 Exon Skip Non-Smoker vs Heavy Smoker
(SpliceSeq Analysis)

(Preliminary Analysis)
Virus integration sites in 7 Samples (VirusSeq Analysis)

- Four samples have integration sites for four different viruses (HPV16, 45, 56, BK) (N = 85).
- The other three samples don’t have any detected integration sites (HPV6, CMV).

### Table: VirusSeq: Software to identify viruses and their integration sites using next-generation sequencing of human cancer tissue

<table>
<thead>
<tr>
<th>SampleIDs</th>
<th>Mate_Number</th>
<th>Virus</th>
<th>Viral_Transcript</th>
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Chromatin Remodeling Theme

(From Peter Laird’s workshop presentation)

 modifications to histone tails

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<th>Modification</th>
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<td>GCN5/PCAF</td>
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<td>SMYD4</td>
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The Chromatin Remodeling Story

Toshinori Hinoue, Jaegil Kim, Moiz Bootwalla, Tim Triche Jr, Hui Shen, Daniel J. Weisenberger, David Kwiatkowski, Gaddy Getz, Stephen B. Baylin, Peter W. Laird
Epigenetic Modifiers Mutated in More than 3 Samples out of 100 Analyzed

(Preliminary Analysis)
1. **KDM6A (27%)** - Histone H3K27 demethylase

2. **ARID1A (26%)** - SWI/SNF complex

3. **MLL2 (26%)** - Histone H3K4 methyltransferase

4. **MLL3 (24%)** - Histone H3K4 methyltransferase

5. **EP300 (17%)** - Histone acetyltransferase
**MLL2 Mutations**

- **Mean DNA methylation difference**
  - **MLL2 Mutant** - wildtype
- **Log_{10}(q value) significance**
  - 33.2%
  - 46.5%
  - 20.3%
- **All probes examined**
  - 17.2%
  - 66.9%
  - 15.9%
- **Significant probes**
  - 20.3% 46.5%
  - 15.9% 17.2%
  - Promoter 66.9%
  - Intergenic
- **Number of probes with DNA methylation changes**
  - 400
  - 200
  - 0
  - 100
- **DNA hypermethylation**
- **DNA hypomethylation**
- **Probe location**
  - Promoter
  - Gene body
  - (Preliminary Analysis)
Draft Pathway Figure

P53/Rb pathway XX% altered
- ATM 10% 41%
  - CDKN2A 4% 57%
  - MDM2 0% 8%
  - TP53 50% 55%
  - Apoptosis
  - CCND1 0% 10%
  - CDKN1A 13% 23%
  - RB1 14% 42%
  - CCNE1 0% 17%
  - FBXW7 10% 41%
  - E2F  Cell Cycle Progression

Histone modification, XX% altered
- KAT2A 4% 10%
  - KAT2B 1% 21%
  - CREBBP 12% 38%
  - EP300 17% 40%
  - MLL 13% 34%
  - MLL2 26% 14%
  - MLL3 24% 12%
  - MLL4 8% 15%
  - SMYD4
  - EZH2 8% 31%
  - SETD2 5% 38%
  - DOT1L me-1-3
  - KDM5A 4% 8%
  - KDM5B 3% 16%
  - KDM1A 13% 15%
  - KDM1B 1% 17%
  - KDM4A 1% 36%
  - KDM4B 1% 36%
  - KDM6A 27% 29%
  - KDM6B 5% 56%
  - KDM6C 1% 36%
  - Demethylases

RTK/Ras/PI3K pathway, XX% altered
- ERBB2 2% 7%
  - FGFR3 7% 8%
  - EGFR 0% 8%
  - HRAS 4% 0%
  - PIK3CA 10% 2%
  - PTEN 3% 42%
  - INPP4B 3% 41%
  - Akt
  - STK11 0% 40%
  - TSC1 6% 37%
  - TSC2 3% 35%
  - mTOR Proliferation, Survival

SWI/SNF complex XX% altered
- ARID1B 5% 45%
  - ARID1A 26% 21%
  - SMARCC2 4% 13%
  - SMARCA2 6% 44%
  - SMARCA4 4% 33%
  - BAF57 7% 33%
  - BAF53 β-Actin
  - BAF60
  - INI

(C Creighton)

(Preliminary Analysis)
### Roles and Word Counts for writing BLCA Marker Paper

**Suggested target lengths -- obviously flexible depending on the story -- for BLCA marker paper:**

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**Figures and Figure Leaders and Participants (Leaders in bold)**

- **Fig. 1.** Mutational spectrum, copy # David, Jaegil
  - David Kwiatkowski, Jaegil Kim

- **Fig. 2.** Pathways
  - Chad Creighton, Niki Schultz, Josh Stuart, Wei Zhang, Yuexin Liu

- **Fig. 3.** Expression (mRNA, miRNA, and/or protein)
  - Chad Creighton, Niki Schultz, Josh Stuart, Wei Zhang, Yuexin Liu

- **Fig. 4.** Chromatin remodeling story (incl. methylation)
  - Rehan Akbani, Billy Kim, Wei Zhang, Yuexin Liu, Andrew

- **Fig. 5.** Smoker/Non-Smoker, viral integration, low-pass NGS for structure, other?
  - Peter Laird, Steve Baylin

- **Figures 1-5**
  - Margaret Morgan, Chad Creighton, Rehan Akbani