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The clinical applications of the cancer genome atlas project for bladder cancer

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Abstract

Introduction: Knowledge of the molecular subtypes of bladder cancer enables powerful generalizations involving the distinctive biology and pathways driving different disease subsets.

Areas Covered: In this review, we summarize the findings of a number of published studies exploring the molecular landscape of bladder cancer by analysis of genomic data from The Cancer Genome Atlas (TCGA). TCGA project has provided a comprehensive data resource of 412 muscle-invasive bladder cancers as characterized by multiple molecular analytical platforms. These data have been and will continue to be utilized in numerous subsequent studies aimed at better understanding the molecular basis of bladder cancer. The catalogue of DNA-level alterations can greatly inform personalized and precision medicine approaches. Molecular subtypes of bladder cancer include distinct “basal/squamous” and “luminal” subtypes, cancers with papillary histology, disease subsets with prominent leukocyte infiltration and immune checkpoint marker expression, and a “neuronal” subtype lacking small cell or neuroendocrine histology. The gene-level alterations and subtypes as revealed by TCGA data are relevant from the standpoint of both basic biology and clinical trial studies.

Expert Commentary: Multiple studies analyzing TCGA muscle-invasive bladder cancer cases point to the existence of five major expression-based molecular subtypes of the disease, with these subtypes having therapeutic implications.

Keywords

The Cancer Genome Atlas; TCGA; Bladder cancer; Genomics; Data integration; Pan-Cancer analysis; Molecular subtypes; Neuroendocrine cancers

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1. The Cancer Genome Atlas (TCGA) bladder cancer project (BLCA)

TCGA was a large scale effort to comprehensively characterize over 10,000 human cancers at the molecular level. These cases were divided among 32 major cancer types (counting colon and rectal as a single colorectal cancer type), including bladder urothelial carcinoma, for which 412 cases were characterized. For nearly all cases in TCGA, multiple types of data have been generated, through the use of various “omic” data platforms: mutations by whole exome sequencing, gene expression by RNA sequencing, microRNA expression by small RNA sequencing, copy number by Single Nucleotide Polymorphism (SNP) arrays, DNA methylation by Illumina arrays, protein expression by Reverse Phase Protein Arrays, and noncoding mutations and structural variants by whole genome sequencing. The associated TCGA-sponsored project for bladder cancer was the TCGA “BLCA” project, which involved two major studies led by TCGA consortium members: an earlier study involving 131 bladder cancer cases [1] (initiated using the set of samples and data that were available at the time), and a subsequent study involving the full cohort of 412 cases for which data are now available [2]. TCGA data are intended to provide an important resource to the research community, which data may be utilized (without any publication restrictions) in any number of subsequent studies aimed at better understanding the molecular basis of cancers such as bladder cancer.

Two of the primary areas of inquiry typically addressed in TCGA cancer type-specific studies involved: 1) the set of “significant” DNA-level alterations, which may involve mutations, copy number alterations, or gene fusions; and 2) the molecular subtypes of the disease, which could be defined in a number of ways and may involve different molecular types including mRNA, microRNA, DNA methylation, protein, and copy number alterations. As most every gene may be found altered at the DNA level in at least one or two cases, algorithms may be used to identify recurrent and non-random patterns of mutation or copy alterations across a set of cancer cases [3, 4, 5]. With a greater number of cancer cases being involved in the study, there is more power to identify genes altered at lower frequencies as statistically significant [3]. Alternatively, one may start with a set of well-known oncogenic pathways of interest and focus on which pathway members are genomically altered [6]. Unsupervised classification of tumors on the basis of molecular profiling data—whereby the tumors are allowed to segregate into groups on the basis of overall similarity or dissimilarity as determined by the data, without any presuppositions—can reveal major subtypes existing within a given cancer type [7, 8]. Molecular subtypes of bladder cancer can point to different biological processes or pathways driving different subsets of the disease, which information could lead to the development of improved therapeutic strategies.

2. Somatic DNA alterations and associated pathways in bladder cancer

A large number of genes have been implicated in bladder cancer through mutation analysis of TCGA data, including multiple genes involved in cell-cycle regulation, chromatin regulation, and kinase signaling pathways [1, 2]. In the initial TCGA BLCA marker paper of 131 cases [1], significant recurrent mutations in 32 genes were identified. In the subsequent

TCGA BLCA marker paper featuring all 412 cases [2], 58 genes were significantly mutated, 34 of which had not been identified as significant in the earlier study. Through DNA alterations, on the order of 70% of bladder cancer cases would harbor potential therapeutic targets, most of these involving either PI3K/AKT/mTOR or RTK/MAPK pathways [1]. Recently, for pathway members in ten selected pathways, gene-level alterations (involving copy-number alterations, mutations, fusions or epigenetic silencing) across the entire TCGA pan-cancer cohort (involving all 32 cancer types) were catalogued [6]. For this review, we have tabulated the pathway-level alteration frequencies for just the TCGA BLCA cases (Figure 1). For each of these pathways, an appreciable number of BLCA cases would be impacted by one or more genes. Genes represented in these pathways and with alterations in over 20% of BLCA cases include *TP53*, *CDKN2A*, *CDKN2B*, *RB1*, and *PIK3CA*. However, even genes with a low frequency of DNA alterations may be critical in individual cancer cases and have a cumulative effect on pathway-level alterations.

In addition to categorizing bladder cancer cases by somatic mutation of specific genes, molecular subtypes of the disease can be defined on the basis of mutational “signatures” [9, 10] and mutational burden. Different mutational processes often generate different combinations of mutation types, termed “signatures,” and analysis of whole exome sequencing data can reveal the presence of these signatures within a particular cancer sample, potentially providing clues as to the specific processes at work in that cancer [9]. In the most recent TCGA BLCA marker paper [2], the 412 bladder cancers cases analyzed could be separated into four major groups, each with a distinct mutational signature, including one associated with mutation of the DNA repair gene *ERCC2* [11] and two others associated with patterns of mutagenesis by APOBEC Cytidine Deaminases [10]. APOBEC-related mutational signature, high mutational burden, and high neoantigen load were each associated with better patient survival [2].

3. Expression-based molecular subtypes of bladder cancer from TCGA-led studies

In contrast to the DNA sequence-based “genotypic” subtyping of bladder cancer as described above (e.g. on the basis of mutations in specific genes or of the presence of mutational signatures), “phenotypic” subtyping may define disease subsets on the basis of gene expression or DNA methylation, which represent molecular states that, in principle, may be changeable or subject to contributions from non-cancer cellular components. A number of previous studies have identified phenotypic molecular subtypes existing within the same set of TCGA BLCA cases [1, 2, 12, 13], which studies represent different analytical approaches and different utilization of the available TCGA data platforms. Molecular subtypes may be called on the basis of a single data platform (e.g. RNA-seq) or from integrated results involving multiple platforms. In the initial TCGA BLCA marker paper of 131 cases [1], RNA expression analysis revealed four expression subtypes, including: a “luminal” subtype enriched for luminal breast and urothelial differentiation factors [14], a “luminal-papillary” subtype with luminal markers and enriched for cases with papillary morphology as well as *FGFR3*-related alterations, and a basal/squamous-like subtype with squamous histology and expression of epithelial lineage genes and stem/

progenitor cytokeratins [14]. In the subsequent TCGA BLCA marker paper featuring all 412 cases [2], RNA expression analysis identified five expression subtypes: luminal-papillary (35%), luminal (6%), basal-squamous (35%), “luminal-infiltrated” (19%), and “neuronal” (5%). The luminal-infiltrated subtype was distinguished from other luminal subtypes by inferred presence of lymphocytic infiltrates, and by strong expression of smooth muscle and myofibroblast gene signatures. The neuronal subtype showed high expression of neuronal differentiation and development genes, as well as typical neuroendocrine markers. In a subsequent integrated analysis involving data from microRNAs and Long Non-coding RNAs, four of the above five subtypes (excepting the neuronal subtype) were identifiable.

Specific gene markers as highlighted in the TCGA BLCA marker studies may be examined in independent bladder cancer cohorts, in order to see what overall molecular patterns may replicate. For this review, we examined an external gene expression profiling dataset from Sjödaahl *et al.* [15] of 308 bladder cancers, classifying each Sjödaahl tumor profile by one of the five TCGA mRNA expression-based subtypes, using previously described methods [12, 13, 16]. As a classifier, the genes originally highlighted in the most recent TCGA BLCA marker study (from Figure 2 of ref[2]) were used. Similar overall trends of in differential expression as originally observed in TCGA cohort could also be observed in the Sjödaahl cohort (Figure 2). For example, in both cohorts, samples in the luminal subtypes showed high expression of uroplakins (*UPK2* and *UPK1A*) and urothelial differentiation markers (*FOXA1*, *GATA3*, *PPARG*). Basal-squamous and luminal-infiltrated subtypes showed increased expression of several immune markers (Figure 2). Basal-squamous tumors also showed strong expression of basal and squamous markers and of specific carcinoma in situ (CIS) signature genes. The Sjödaahl study had originally defined five major urothelial carcinoma subtypes: “urobasal A” (with shorthand “MS1,” subdivided into MS1a and MS1b) corresponding to TCGA luminal-papillary subtype, “genomically unstable” (MS2a subdivided into MS2a1 and MS2a2) corresponding to TCGA luminal and neuronal subtypes, “urobasal B” (MS2b2.1) and “squamous cell carcinoma like” (MS2b2.2) both corresponding to TCGA basal-squamous subtype, and an infiltrated class of tumors (MS2b1) corresponding to TCGA luminal-infiltrated subtype (Figure 2).

4. Molecular subtypes of bladder cancer from independent pan-cancer studies

Independent of the above TCGA consortium-led studies, two pan-cancer studies involved defining molecular subtypes of the TCGA BLCA cases in the context of molecular subtypes as definable in other cancer types. A “pan-urollogic” study [13] involved all 1954 urologic cancers (bladder, kidney, prostate, and testicular) in TCGA, while a subsequent “Pan32” study by the same group [12] involved all 32 cancer types and over 10000 cases in TCGA. Whereas in conventional pan-cancer genomic analyses, most bladder cases (with the exception of squamous-associated cases) will segregate from the other cancer types [12, 17, 18], the above studies utilized an alternate analytical strategy to define molecular subtypes that would not be driven by tumor lineage-specific markers or patterns, namely normalizing or centering the molecular values within each cancer type so that any tissue- or histology-specific markers would not drive the classification. Most of the molecular subtypes of

bladder cancer as defined by these studies were found to span cases from multiple cancer types. In the pan-urolologic study, BLCA cases were primarily subdivided among three subtypes, with a “c1” subtype associating with papillary histology and “luminal” expression-based subtype and with a “c8” subtype associating with squamous-associated features. In the Pan32 study, BLCA cases were primarily subdivided among seven pan-cancer molecular classes (each involving >5% of cases), with one class (c2) lacking distinctive molecular features.

While the existence of different molecular classification schemes of bladder cancer, as represented by all of the above studies, would seem potentially confusing, the subtype classifications as made by various studies can be compared with each other, in order to see what consensus patterns would emerge. For this review, we integrated the various subtype calls on BLCA cases, as defined independently from three different studies: the most recent TCGA BLCA marker study [2], the pan-urolologic study [13], and the Pan32 study [12]. From this exercise (featured in Figure 3), five major BLCA subtypes are found to be reflected across the various studies, representing the same five subtypes—namely luminal, luminal papillary, luminal-infiltrated, basal-squamous, and neuronal—as identified in the marker study by RNA-sequencing platform. The neuronal cases were independently identified in the Pan32 study, with the associated “c4” Pan32 class expressing neuroendocrine and cancer-testis antigen markers and predominantly involving bladder, head and neck, cervical, lung, ovarian, and gastrointestinal cancers. The luminal-infiltrated and basal-squamous cases were associated with lymphocytic infiltrates [2], as also reflected in their associations with the PanUC c8 subtype and the Pan32 c3/c8/c10 classes, each of these involving expression of immune checkpoint pathway members and immune-related gene signatures. Interestingly, the luminal-infiltrated and basal-squamous subtypes did not associate here with immune-related aggregate genomic measures [19] such as neoantigen load and total mutational burden (Figure 3). Neoantigen load and total mutational burden would correlate highly with each other [2, 19], and while these variables are thought to have potential as biomarkers of response [20], not all tumors with a high mutational burden, for example, have been found to positively respond to immunotherapy in other cancer types [21], suggesting that other potential markers, including molecular subtype, should also be considered in the clinical setting.

The observations of the molecular subtypes as defined by the TCGA BLCA marker papers in independent and pan-cancer studies are important. Results of other studies, utilizing different analytical approaches to the same data, reinforce the existence of five major subsets of bladder cancer. In addition, the pan-cancer studies allow us to relate the bladder cancer subtypes with types of cancer other than bladder. For example, based on results from the pan-urolologic study, the PanUC:c2 subtype, which associates here with the luminal and neuronal BLCA subtypes, also associates with a subset of prostate cancer harboring ERG fusions [13]; and the panUC:c1 subtype, which associates here with the luminal papillary BLCA subtype, also associates with kidney chromophobe cancers. It is understood that the immune checkpoint pathway markers underlying the BLCA luminal-infiltrated and basal-squamous subtypes would also involve a wide range of cancer types other than bladder [13, 22]. The Pan32 “c4” pan-cancer class, which expresses neuroendocrine markers, represents ~4% of cancers across the entire TCGA cohort, including the BLCA neuronal cases. A

previous study of TCGA lung cancers had also uncovered a neuroendocrine-related subtype, representing a mix of both lung adenocarcinoma and lung squamous cases and associated with poor differentiation [16]. For both bladder and lung neuroendocrine-related subtypes, there was an observed enrichment for neuroendocrine tumors, but with the majority of cases having no observed histopathologic features suggestive of neuroendocrine origin.

5. Therapeutic implications for bladder cancer from TCGA results

Results of TCGA datasets characterizing bladder cancer would have therapeutic and clinical implications. The mutations and gene fusions detected (including *FGFR3* fusions, most notably *FGFR3-TACC3*) represent targetable pathways such as PI3K/AKT/mTOR pathway or receptor tyrosine kinases, and the relevant alterations as cataloged by TCGA may be incorporated into precision medicine approaches as applied to bladder cancer. The TCGA BLCA 412-case study in Cell also proposed a schema for how bladder cancers may be treated according to expression-based subtype [2]. For example, luminal-infiltrated and basal-squamous cancers could be treated with immunotherapy (including inhibition of PD-L1, PD-1, or CTLA4), luminal-papillary cancers could be treated with *FGFR3* inhibitors, and neuronal cancers (with high cell cycle gene expression) could be treated with Etoposide/Cisplatin neoadjuvant chemotherapy. The association of BLCA neuronal subtype with neuroendocrine tumors would suggest that somatostatin analogues (SSA) may be used to treat this subtype, as these have demonstrated anti-proliferative activity in addition to efficacy for carcinoid symptom control in neuroendocrine tumors [23, 24]. As the BLCA neuronal subtype may in actuality represent a pan-cancer class relevant to multiple cancer types, therapeutic strategies found to be effective in neuroendocrine tumors originating from a specific organ might also be investigated in BLCA neuronal tumors.

While observations regarding therapeutic implications, as put forth in the most recent TCGA BLCA marker paper, were clearly stated as being hypothesis-generating and thus not ready to be used for clinical decision making [2], the findings based on TCGA data could be placed in the context of clinical trials. To our knowledge, no clinical trial has been initiated directly as a result of the BLCA marker studies by TCGA, and data platforms for obtaining expression-based molecular subtyping information have yet to see widespread use in the clinical setting (though such platforms would exist, e.g. ref [20]). At the same time, specific gene correlates of BLCA expression-based subtypes are likely to be considered when interpreting the results of a number of clinical trials. For example, results of currently ongoing, randomized, phase 3 studies evaluating immunotherapeutic approaches in bladder cancer (e.g. NCT02853305, NCT02807636, NCT02516241, NCT03036098) should shed light on candidate biomarkers of response, which might include PD-L1 expression and immune cell infiltration (which may be considered surrogate markers of the luminal-infiltrated and basal-squamous subtypes) as well as mutation burden and neoantigen load [25]. In a recent phase 2 clinical trial of patients with metastatic bladder cancer treated with PD-L1 inhibitor atezolizumab (NCT02108652), increased levels of PD-L1 expression on immune cells and mutation load were each associated with increased response [20]. Regarding *FGFR3* DNA-level alterations (including mutations, fusions with *TACC3*, and/or amplification), enriched within the BLCA luminal-papillary subtype, a number of planned or ongoing phase 1 and phase 2 clinical trials aim to target the FGFR family in bladder cancer

(e.g. NCT03123055, NCT02401542, NCT02365597, NCT02465060) [26]. In addition, several recent studies have linked specific DNA repair pathway defects (e.g. *ERCC2* mutation) with sensitivity to DNA damaging-based therapy such as cisplatin [27], which could also help inform clinical trial design.

6. Expert Commentary

Multiple studies analyzing TCGA muscle-invasive bladder cancer cases point to the existence of five major molecular subtypes of the disease, with these subtypes having therapeutic implications. These molecular subtypes allow us to make powerful generalizations as to the distinctive biology driving different subsets of bladder cancer. While the five subtypes would appear to cover much of the molecular heterogeneity represented in bladder cancer, other analytical approaches or analysis parameters might be used to uncover additional subtypes; however, such additional subtypes would also need to have some unique biology associations for them to be considered relevant. The widespread differential molecular patterns that may be associated with a given subtype can be mined to provide meaningful context to the subtype, in terms of key genes or associated pathways that would help describe the subtype. Genomic studies utilizing larger numbers of bladder cancer cases may also uncover additional subtypes of interest.

Beyond defining the list of frequently altered genes and identifying molecular subtypes, genomics could be used to answer other questions regarding bladder cancer in future studies. TCGA data are rather limited in terms of patient treatment information, including level of response to any targeted therapy. Future studies could, for example, carry out molecular profiles of patient specimens from clinical trials, which could allow us to define molecular signatures and associated pathways of responders versus non-responders. Molecular biology studies of bladder cancer can continue to utilize TCGA data as a resource. Genes shown to be functionally relevant in the laboratory setting could also be examined in TCGA to ascertain their relevance in the setting of human disease; for example, whether expression or alteration of a gene is associated with more aggressive disease or with molecular subtype, or whether two functionally-related genes also appear correlated in expression with each other across human cancer samples.

Large scale genomics initiatives such as TCGA are likely to remain a big team science endeavor, with hundreds of authors involved with the eventual TCGA-led publications. With these types of publications, the range of questions that may be asked of the data may be somewhat limited, in part because of practical limitations in article space, and in part because some team members may not feel as invested in doing a truly “deep dive” into the data as authorship credit may be dispersed among many. Politics also often plays a role in limiting who may contribute to a TCGA-led publication and to what level. In contrast, investigators who are allowed to carry out their own independent research of TCGA data can gain new insight or perspectives that have been missed by TCGA-led efforts. The growing amount of genomic data in the public domain should only further open the range of questions that may be addressed, and integration of results between different datasets and different data platforms offers potentially unlimited possibilities for discovery. To this end, the use of data from large scale genomic initiatives such as TCGA in independent studies

should be actively encouraged, in the interests of the research community collectively utilizing these data to their full potential.

7. Five-year view

The BLCA expression subtypes that may be associated with unique response to therapies could be prospectively tested in clinical trials [2]. In the clinical setting, while it may seem unlikely that patient specimens would be evaluated for multiple molecular data platforms in the same way as was carried out for TCGA, individual molecular markers of bladder cancer molecular subtypes that might seem most relevant from a therapeutic standpoint—including markers explicitly noted in the schema from the TCGA BLCA 412-case study—could potentially be evaluated in the setting of patient care in future studies. In light of the BLCA neuronal subtype, there is also a clear need for additional and better biomarkers of neuroendocrine-like cancers [12, 28]. Future genomics studies and precision medicine approaches may also take into account relevant non-coding mutations [29] and structural rearrangements [30], which can also lead to activation of oncogenes [31, 32, 33, 34, 35] and would be detectable by whole genome sequencing as opposed to whole exome sequencing [36]. In addition to DNA-level somatic alterations, expression of proteins, including phospho-proteins, also should be examined in the context of critical oncogenic pathways, particularly as not all alterations impacting a specific gene may necessarily lead to activation of associated pathways [37, 38]. Lastly, the pathways of tumor evolution of bladder cancer, particularly in response to treatment should become better defined over the next few years, as these may have significant implications for clinical decision making, where recent studies indicate that both the genotype and phenotypic-based (e.g. gene expression-based) subtype have a significant possibility for drift or plasticity across the disease course [39, 40, 41].

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Reference annotations

* Of interest

** Of considerable interest

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The first study of bladder cancer led by TCGA consortium, involving 131 bladder cancer cases for which data were available at the time of the study

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Key issues

- TCGA genomic datasets are a publicly-available resource that enables the comprehensive molecular characterization of over 10,000 human cancers, including 412 bladder cancers.
- TCGA “omic” data platforms provide information on mutations, gene and protein expression, copy number alterations, gene fusions, and DNA methylation.
- The TCGA consortium published two major genomics studies of the TCGA bladder cancer cohort, and subsequent independent studies have also gone through the data and made additional findings.
- The catalogue of DNA-level alterations in bladder cancer involves pathways such as PI3K/AKT/mTOR, receptor tyrosine kinase (RTK) signaling, cell cycle, and p53, and can greatly inform precision medicine approaches.
- Molecular analysis of bladder cancer defines five major gene expression-based subtypes, including luminal, luminal-papillary, luminal-infiltrated, basal-squamous, and neuronal.
- TCGA consortium has put forth a proposed schema for how bladder cancers may be treated according to molecular subtype, as defined by their associated markers.
- Through pan-cancer genomic analyses, the molecular patterns associated with bladder cancer subtypes may be better understood in the context of patterns from other cancer types.
- Therapeutic strategies found to be previously effective in neuroendocrine tumors might also be investigated in bladder cancers of the neuronal subtype.

Figure 1.

Gene-level alterations in muscle-invasive bladder cancer in the context of curated pathways. Figure adapted from [6]. Pathway members and interactions in ten selected pathways are shown, where gene-level alterations (e.g. mutations, copy number alterations, fusions, and epigenetic silencing) across the entire TCGA pan-cancer cohort were previously catalogued by the TCGA PanCanAtlas project, with the alteration frequencies for TCGA BLCA cases being represented here. Genes are altered at different frequencies (shading intensity indicating the average frequency of alteration across the TCGA BLCA cohort) by either

oncogenic activation (for genes with oncogenic roles, alterations including activating mutations, fusions, and gene amplifications) or tumor suppressor inactivation (for genes with tumor suppressive roles, alterations including inactivating mutations, epigenetic silencing, and gene deletions). Italicized genes without boxes denote genes with close to 0% alteration frequencies.

Figure 2.

Observation of patterns associated with TCGA BLCA mRNA expression subtypes in an external bladder cancer expression profiling dataset. For selected gene markers, originally highlighted in the most recent TCGA BLCA marker study (from Figure 2 of Ref. [2]), high differential expression across the 5 mRNA expression-based subtypes (luminal-papillary, luminal-infiltrated, luminal, basal-squamous and neuronal) are represented, in both TCGA BLCA dataset (left) and in an independent dataset of 303 bladder cancers (right, data accession number GSE32894) from Sjödaahl et al. [15]. Black represents relatively high differential expression (>0.4 standard deviations, or SD, from the median across samples). Using the mRNA markers shown, expression profiles in the GSE32894 dataset were classified according to TCGA BLCA subtype. The molecular subtypes originally assigned to the GSE32894 expression profiles in the Sjödaahl study are represented along the bottom of

the GSE32894 data matrix. ECM: extracellular matrix; EMT: epithelial-mesenchymal transition; CIS: carcinoma in situ.

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Figure 3.

Survey of molecular subtypes reported within TCGA BLCA cohort, with emerging consensus patterns. A number of previous studies have identified phenotypic-based molecular subtypes existing within TCGA BLCA cases [1, 2, 12, 13], which studies may have focused exclusively on BLCA or carried pan-cancer analyses, and which subtypes may be defined using a single ‘omic’ data platform (e.g. RNA-sequencing) or an integration of multiple ‘omic’ data platforms. The top panel represents an integration of subtype classifications across the 412 cases in the BLCA cohort from 3 different studies: TCGA

BLCA marker study published in Cell [2]; a ‘pan-urolologic’ cancer study (‘PanUC’) involving TCGA bladder, kidney, prostate, and testicular cases [13]; and a molecular subtyping study of all 32 cancer types in TCGA (‘Pan32,’ counting colon and rectal as a single colorectal cancer type) [12]. The heat map represents the subtypes defined independently from the indicated studies; each row in this heat map denotes membership within a specific subtype as defined by the indicated study. The matrix used for this heat map was clustered by hierarchical clustering [8], whereby five major BLCA subtypes (listed along the top) are found to be reflected across the various studies. The three heat maps below the first heat map respective denote selected pathology features, which cases were included in various studies [1, 2, 6, 12, 13, 17], and data availability by ‘omic’ platform (WXS: whole exome sequencing; WGS: whole genome sequencing; low pass: ~6–8X sequencing coverage; high pass: ~30–60X sequencing coverage; RPPA: reverse phase protein arrays; SNP: single nucleotide polymorphism). Plots for leukocyte fraction (based on DNA methylation analysis), number of neoantigens, and nonsilent mutation rate based on data provided by the TCGA PanCanAtlas project [19].