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Platinum Priority – Brief Correspondence

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The Cancer Genome Atlas Expression Subtypes Stratify Response to Checkpoint Inhibition in Advanced Urothelial Cancer and Identify a Subset of Patients with High Survival Probability

Jaegil Kim[a], *David Kwiatkowski*[b], *David J. McConkey*[c], *Joshua J. Meeks*[d], *Samuel S. Freeman*[a], *Joaquim Bellmunt*[e], *Gad Getz*[a], *Seth P. Lerner*[f,*]

[a] *The Broad Institute of MIT and Harvard, Cambridge, MA, USA;* [b] *Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA;* [c] *Greenberg Bladder Cancer Institute, Johns Hopkins University, Baltimore, MD, USA;* [d] *Department of Urology and Biochemistry and Molecular Genetics, Northwestern University, Chicago, IL, USA;* [e] *PSMAR-IMIM Laboratory, Bladder Cancer Center, Department of Medicine, Dana-Farber Cancer Institute and Harvard University, Boston, MA, USA;* [f] *Scott Department of Urology and the Dan L. Duncan Cancer Center, Baylor College of Medicine, Houston, TX, USA*

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Abstract

Analysis of the IMvigor 210 trials involving patients with platinum-refractory or cisplatin-ineligible urothelial carcinoma who were treated with the PD-L1 inhibitor atezolizumab identified a resistance signature as an immune biomarker. Transcriptome profiling of 368 tumor samples from this trial revealed that the "genomically unstable" Lund subtype classification was associated with the best response. We developed and applied a novel single-patient subtype classifier based on The Cancer Genome Atlas 2017 expression-based molecular subtypes. We identified 11 patients with a neuronal subtype, with a 100% response rate in eight confirmed cases (2 complete response, 6 partial response), and 72% overall, including 3/11 patients with an unconfirmed response. The survival probability was extraordinarily high for the neuronal subtype, which represents a high-risk cohort with advanced disease, and may be secondary to low levels of TGFβ expression and high mutation/neoantigen burden.

Patient summary: We describe a methodology for genomic classification of an individual patient's bladder cancer tumor and have identified a subtype that is associated with a high response rate to immunotherapy. This is an important step forward in identifying the right treatment for the right patient, which is the goal of personalized precision medicine.

* Corresponding author. Scott Department of Urology, Baylor College of Medicine, 7200 Cambridge Street, Houston, TX 77030, USA. Tel. +1 713 7986841.
E-mail address: slerner@bcm.edu (S.P. Lerner).

Analysis of the IMvigor 210 trials (NCT02951767 and NCT02108652) among patients with platinum refractory or cisplatin-ineligible urothelial carcinoma (UC) [1] who were treated with the PD-L1 inhibitor atezolizumab identified a resistance signature as an immune biomarker [2]. Transcriptome profiling of 368 tumor samples from this trial revealed that the "genomically unstable" Lund subtype classification [3] was associated with the best response to therapy.

We recently reported The Cancer Genome Atlas (TCGA) comprehensive analysis of 408 chemotherapy-naïve muscle-invasive bladder cancers [4]. Using unbiased

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non-negative matrix factorization (NMF) consensus clustering, we identified five RNA-sequencing expression subtypes: luminal-papillary, luminal-infiltrated, luminal, basal-squamous, and neuronal. We reported an association of these subtypes with overall survival (OS), whereby luminal-papillary has the best and neuronal the worst OS. In a LASSO-penalized multivariate Cox regression analysis that included 15 covariates (that were significant in univariate OS calculations), neuronal, luminal-infiltrated, and luminal subtypes retained independent association with worse OS. We proposed a hypothetical model for subtype-directed therapy for testing in prospective clinical trials. In a subsequent analysis, we developed a bladder cancer RNA expression single-patient classifier based on analysis of a reduced set of genes (n = 354; Supplementary Table 1) that faithfully reproduces each of the subtypes derived from unsupervised clustering (based on the 3347 most variable genes). This classifier appears to be robust in discriminating subtypes of bladder cancer with different prognoses in multiple previously published data sets (manuscript in preparation).

We developed a classifier for the TCGA expression subtypes for use on both RNA sequencing and microarray expression data, using NMF. There were four steps in this procedure: (1) determine the sample relevance matrix $\boldsymbol{H}^*_{\mathrm{TCGA}}$ quantifying an association or a "degree of participation" of individual samples to the TCGA subtypes; (2) determine the gene relevance matrix $\boldsymbol{W}^*_{\mathrm{TCGA}}$ strictly conditional on $\boldsymbol{H}^*_{\mathrm{TCGA}}$ in term of expression fold changes, quantifying an association of individual genes to the TCGA subtypes; (3) identify differentially overexpressed subtype markers (n = 354; Supplementary Table 1) for the classifier using $\boldsymbol{W}^*_{\mathrm{TCGA}}$ and mean differences for fold changes; and (4) generate an NMF-based subtype classification scheme explicitly modeling the gene expression vector of a new sample $\boldsymbol{x}_{\mathrm{new}}$ conditioned on $\boldsymbol{W}^*_{\mathrm{TCGA}}$ to best approximate $\boldsymbol{x}_{\mathrm{new}} \sim \boldsymbol{W}^*_{\mathrm{TCGA}}\, \boldsymbol{h}_{\mathrm{new}}$ for the selected 354 markers, determining the association of the new sample with the TCGA subtypes. The methods will be described in detail elsewhere (manuscript in preparation).

Both expression data and relevant clinical data, including the TCGA 2014 and Lund [3] classifications, were downloaded from http://research-pub.gene.com/IMvigor210CoreBiologies/. The expression data were log2-transformed and median-centered, and analyzed using the classifier.

We applied the classifier gene set analysis to the RNA expression data used for the analysis of IMvigor 210 reported by Mariathasan et al. [2]. Fig. 1 shows the distribution of subtype calls for the 348 patients, including comparison to the Lund classification (Fig. 1A), the probability of assignment of patients to the five different TCGA 2017 subtypes (Fig. 1C), and strong correlation with multiple previous expression-based discriminants of bladder cancer subtypes (Fig. 1D). Comparison of our subtype calls with the TCGA 2014 and Lund classifications shows the highest correlation between the TCGA 2017 and Lund classifications, with an adjusted Rand index of 0.38 (Fig. 1B;

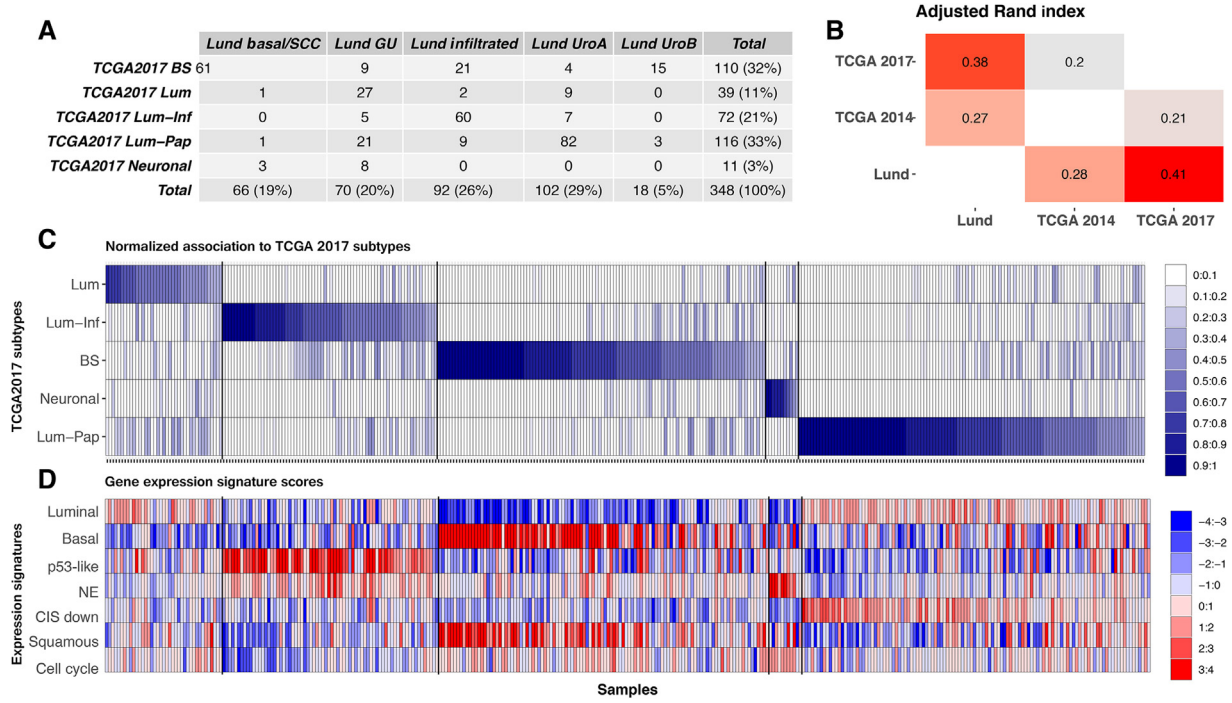


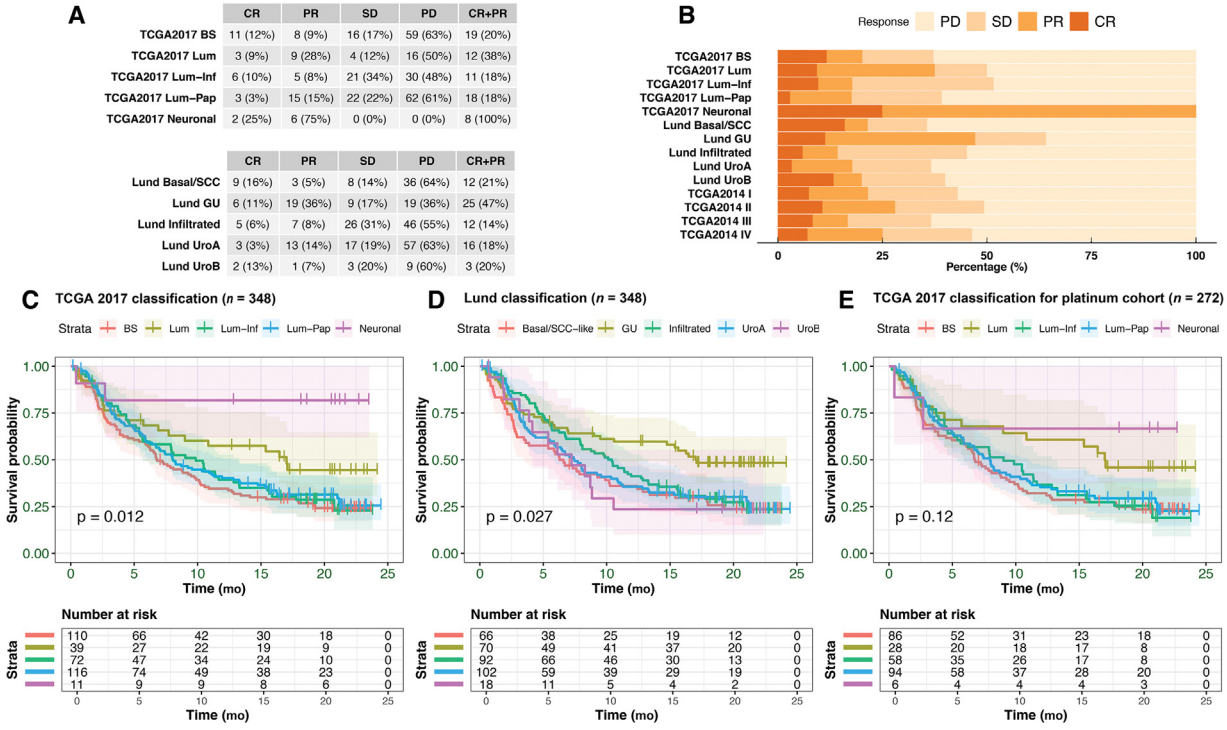
Fig. 1 – The Cancer Genome Atlas (TCGA) single-patient classifier applied to the IMvigor 210 mRNA expression data for 348 tumor samples. (A) Numbers of patients assigned to each expression subtype and comparison to the reported Lund subtypes. (B) Adjusted Rand index (ARI) among TCGA 2017, TCGA 2014, and Lund classifications for 348 patients (upper left) and 298 patients with response data (lower right). (C) Cluster assignment probability for each patient and (D) comparison to common gene expression signatures [3] and their association with each of the subtypes (red denotes high and blue, low expression). Six known NE markers (*CHGA, CHGB, SCG2, ENO2, SYP,* and *NCAM1*) were used to compute the NE signature score. SCC = squamous cell carcinoma; GU = genitourinary; Lum = luminal; Inf = infiltrated; Pap = papillary; CIS = carcinoma in situ; NE = neuroendocrine.

Supplementary Table 2). Interestingly, the TCGA 2017 classifier assigned the Lund genitourinary samples to each of the five TCGA categories.

Both mutation and transcriptome profiles of the TCGA2017 subtypes were highly consistent with the previous analysis (Supplementary Fig. 1A and B); the luminal-papillary subtype was characterized by frequent *FGFR3* mutations (39% vs 8% in others) and the lowest carcinoma in situ score; luminal-infiltrated had the highest *p53*-like and EMT signature expression; basal-squamous was enriched in samples with predominant expression of $CD8^+$ effector T cells (T_{eff}) and immune checkpoint genes; and luminal had the highest level of uroplakins with more frequent *TP53* mutations (64%). Notably, all neuronal samples ($n = 11$) harbored *TP53* mutations ($p = 0.0001$, one-tailed Fisher's exact test), and seven of those (64%) had concomitant *RB1* loss ($p = 0.002$, one-tailed Fisher's exact test) via either mutation ($n = 5$) or downregulation ($n = 2$; log2 fold change < -1.5), consistent with the hallmark loss of wild-type *TP53* and *RB1* in neuroendocrine tumors, and all showed high expression of both neuroendocrine and neuronal markers (Supplementary Fig. 1B).

We examined the association of subtypes defined using the classifier with response to atezolizumab therapy. Remarkably, the neuronal subtype defined by the TCGA 2017 classifier showed a high objective response rate and was associated with the best overall survival ($p = 0.012$; Fig. 2A–C). The luminal subtype was also associated with a better response rate (38%) and better overall survival in comparison to the other three subtypes, but to a much lesser degree (Fig. 2C). Infiltrated and basal subtypes in both the TCGA 2017 and Lund classifications were associated with poor response and survival (Fig. 2C and D). The better survival association of neuronal and luminal subtypes persisted when the analysis was repeated for 298 patients with response data (Supplementary Fig. 2) and 272 patients with prior platinum treatment, although this was not statistically significant (Fig. 2E).

It is striking that the neuronal subtype was associated with the worst survival in the TCGA 2017 cohort [3] and the best survival in this atezolizumab-treated cohort. None of the 11 neuronal subtype tumors were immune-inflamed (as defined by Mariathasan et al. [2]), and 8/11 tumors (77%) were immune excluded, suggesting no major role for $CD8^+$ T_{eff} activity in the response of this subtype (Supplementary Fig. 1A). In addition, the neuronal subtype had an average tumor mutation burden (TMB; median 8 vs 10 per Mb in others; $p = 0.3$, one-tailed Mann-Whitney test) and tumor neoantigen burden (TNB; median 1.02 vs 0.92 per Mb in others; $p = 0.2$, one-tailed Mann-Whitney test), while luminal subtype tumors were highest by those measures (Supplementary Fig. 3A; median TMB 13 vs 7 per Mb in others; $p = 0.004$, one-tailed Mann-Whitney test; median TNB 1.41 vs 0.86 per Mb in others; $p = 0.008$, one-tailed Mann-Whitney test). Remarkably, the neuronal subtype had the lowest level of *TGFB1* and *TGFBR1* expression in comparison to other subtypes (Supplementary Fig. 3B), which was also associated with response in the Mariathasan study [2].



A	CR	PR	SD	PD	CR+PR
TCGA2017 BS	11 (12%)	8 (9%)	16 (17%)	59 (63%)	19 (20%)
TCGA2017 Lum	3 (9%)	9 (28%)	4 (12%)	16 (50%)	12 (38%)
TCGA2017 Lum–Inf	6 (10%)	5 (8%)	21 (34%)	30 (48%)	11 (18%)
TCGA2017 Lum–Pap	3 (3%)	15 (15%)	22 (22%)	62 (61%)	18 (18%)
TCGA2017 Neuronal	2 (25%)	6 (75%)	0 (0%)	0 (0%)	8 (100%)

	CR	PR	SD	PD	CR+PR
Lund Basal/SCC	9 (16%)	3 (5%)	8 (14%)	36 (64%)	12 (21%)
Lund GU	6 (11%)	19 (36%)	9 (17%)	19 (36%)	25 (47%)
Lund Infiltrated	5 (6%)	7 (8%)	26 (31%)	46 (55%)	12 (14%)
Lund UroA	3 (3%)	13 (14%)	17 (19%)	57 (63%)	16 (18%)
Lund UroB	2 (13%)	1 (7%)	3 (20%)	9 (60%)	3 (20%)

Fig. 2 – (A) Stratification of patients in the TCGA 2017 and Lund subtypes into response categories: complete response (CR), partial response (PR), stable disease (SD), progressive disease (PD), and CR + PR. (B) Objective response rate among IMvigor 210 patients to atezolizumab according to TCGA 2017, TCGA 2014, and Lund subtypes. Overall survival probabilities in the IMvigor 210 full cohort ($n = 348$) in (C) the TCGA 2017 and (D) Lund subtypes, and (E) the platinum-treated cohort ($n = 272$) in the TCGA 2017 subtypes. SCC = squamous cell carcinoma; GU = genitourinary; Lum = luminal; Inf = infiltrated; Pap = papillary; CIS = carcinoma in situ; BS = basal-squamous.

The current observations suggest that the TCGA 2017 classifier we have developed has value in the identification of patients most likely to have the best response to immune checkpoint therapy for metastatic UC, namely the neuronal subtype. We recognize that these are preliminary observations that need to be validated in other clinical trials of immune checkpoint inhibitor therapy for bladder cancer, and that the mechanism of this association with response deserves further investigation. We propose that the mechanism of response might be related to the relatively low expression of *TGFB1* and *TGFBR2* by neuronal subtype cancers, and their high expression of neuronal/neuroendocrine proteins that could serve as tissue-restricted antigens and enhance the immune response induced by atezolizumab.

Author contributions: Seth P. Lerner had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Kim, Kwiatkowski, McConkey, Meeks, Bellmunt, Lerner.
Acquisition of data: Kim, Getz.
Analysis and interpretation of data: Kim, Kwiatkowski, McConkey, Meeks, Bellmunt, Freeman, Getz, Lerner.
Drafting of the manuscript: Kim, Kwiatkowski, McConkey, Meeks, Bellmunt, Freeman, Getz, Lerner.
Critical revision of the manuscript for important intellectual content: Kim, Kwiatkowski, McConkey, Meeks, Bellmunt, Freeman, Getz, Lerner.
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Supervision: Getz.
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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.eururo.2019.02.017.

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