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Letter to the Editor

Reply to Pontus Eriksson and Gottfrid Sjödahl's Letter to the Editor re: Tuan Zea Tan, Mathieu Rouanne, Kien Thiam Tan, Ruby Yun-Ju Huang, Jean-Paul Thiery. Molecular Subtypes of Urothelial Bladder Cancer: Results from a Meta-cohort Analysis of 2411 Tumors. Eur Urol 2019;75:423–32

We thank Eriksson and Sjödahl for their critical remarks on our bioinformatic analysis. We believe that our meta-cohort sheds light on bladder cancer (BC) subtypes through a comprehensive analysis of a unique data set of 2411 bladder tumors [1]. Notably, studying non-muscle-invasive BC (NMIBC) and muscle-invasive tumors allowed us to define a molecular portrait of potentially lethal, high-risk NMIBC. Therefore, our study – while imperfect – provides an important resource for the BC community.

Although we have already detailed the limitations of our analysis, Eriksson and Sjödahl pointed out three issues that we would like to address.

First, we agree that batch effects (BEs) cannot be eliminated completely, and BE removal is a nontrivial task [2]. We used principal component analysis and several metrics (Supplementary Fig. 2 [1]) to remove BEs due to major confounding factors. Notably, the same subtypes were found with each platform (Supplementary Fig. 5 [1]), suggesting that intrinsic biological differences were not compromised. No subtype found was exclusive to specific cohorts, platforms, or tissue sources (Supplementary Fig. 3 and Supplementary Table 4 [1]). Results from the Affymetrix and Illumina platforms were in good agreement. A similar strategy was successfully used to build the CSIOVDB ovarian cancer subtype database [3]. Importantly, subtype-specific associations were highly concordant with findings in independent BC gene expression subtype studies (Figs. 1–3 and Supplementary Figs. 5, 6, and 8 [1]). A large, uniformly processed, and well-annotated BC cohort (eg, TCGA [4] and UROMOL [5]) provides practitioners with an invaluable resource; however, BEs still exist even with these cohort types and must be acknowledged [6].

Second, the centroid provided (Supplementary Table 15 [1]) is the median-centered expression of differentially expressed genes in the meta-cohort; it is not a classifier. While application of a classifier to individual cohorts constituting the meta-cohort is valid, centroid-based classifiers require gene standardization with the training data before classification. This is because class imbalance or missing data in a cohort will distort the "high" or "low" expression levels and lead to classification variability [7]. If a reclassification uses the median-centered expression within a cohort, the subtype assignment assumes that all subtypes are present and are balanced in the cohort. This assumption may not be true. The consistency of subtype annotation also depends on tumor heterogeneity. Multiple subtype signatures within a tumor lesion exist, as seen in single-cell and multitumor region studies [8,9] and from our experience in ovarian cancer [10].

Third, we wish to stress that formalin-fixed, paraffin-embedded (FFPE) samples were not limited to the NEURAL subtype. NEURAL and fresh-frozen samples were not mutually exclusive (Supplementary Table 4 [1]); GSE39016 had more samples assigned as non-NEURAL. Importantly, another FFPE cohort, GSE87304, also had multiple subtypes, including NEURAL. Both NEURAL and LUM were enriched in published cancer subtypes identified using microarray and RNA sequencing data (Fig. 1 [1]), indicating that NEURAL and LUM are unlikely to be artifactual.

Importantly, we echo the call of Dyrskjot [11] that perhaps the most critical task now is to establish the clinical relevance of these molecular subtypes. The value of a subtype scheme lies in its biological and clinical relevance. Moving forward, basket trials and multi-omics big-data analyses at multiple centers are needed to determine subtype-specific therapeutic interventions. Consensus on the clinical relevance of these BC subtypes will hopefully become a reality.

Conflicts of interest: Jean-Paul Thiery is a consultant/advisory board member for Aim Biotech Singapore, ACT Genomic, and CSO BioCheetah Singapore. The remaining authors have nothing to disclose.

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Tuan Zea Tan[a]
Mathieu Rouanne[b,c]
Ruby Yun-Ju Huang[a,d,e]
Jean-Paul Thiery[f,g,h,i,*]

[a]*Cancer Science Institute of Singapore, National University of Singapore, Center for Translational Medicine, Singapore*
[b]*Department of Urology, Hôpital Foch, Université Versailles-Saint-Quentin-en-Yvelines, Université Paris-Saclay, Suresnes, France*
[c]*INSERM Unit 1015, Laboratoire de Recherche Translationnelle en Immunothérapie, Gustave Roussy, Université Paris-Saclay, Villejuif, France*
[d]*Department of Obstetrics and Gynecology, National University Health System, Singapore*
[e]*Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*
[f]*Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore*
[g]*Guangzhou Institute of Biomedicine and Health, Chinese Academy of Science, Guangzhou, People's Republic of China*
[h]*CNRS Emeritus CNRS UMR 7057 Matter and Complex Systems, University Paris Denis Diderot, Paris, France*
[i]*INSERM UMR 1186, Integrative Tumor Immunology and Genetic Oncology, Gustave Roussy, Université Paris-Saclay, Villejuif, France*

*Corresponding author. Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, 8 Medical Drive, 117597, Singapore.
E-mail address: bchtjp@nus.edu.sg (J.-P. Thiery).

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