Letter to the Editor

Reply to Anirban P. Mitra, Tanner Miest, and Colin P.N. Dinney's Words of Wisdom re: Genomic Predictors of Good Outcome, Recurrence, or Progression in High-Grade T1 Non-Muscle-Invasive Bladder Cancer. Eur Urol. In press. https://doi.org/10.1016/j.eururo.2020.10.020

We read with interest the comments provided by Mitra et al [1] on our recently published paper on the genomics of high-grade T1 (HGT1) bladder cancer [2]. We appreciate the experts' comments on the relevance of our genomic characterization of the alterations in HGT1 disease. However, the authors highlight a series of limitations of the methodology used in our study that we would like to address in greater detail.

Regarding the unavailability of surgical management details to determine the implications regarding recurrence and progression, in our paper we reference the clinical details for this series of patients that were included in a previous protocol (NCT02113501). A careful description of their urological management has been published elsewhere [3].

Concerning germline samples, Mitra et al correctly state that adjacent normal bladder tissue may not be an appropriate control because of the urothelial "field effect". Indeed, we provide a specific analysis of this aspect in the Supplementary material to our paper [2]. In brief, we quantified the "tumor in normal" contamination computationally using deTiN software. We found that higher contamination of normal samples decreased the variant calling precision, while the sensitivity remained unaffected. We also used normal samples with very low contamination to assess the performance of somatic variant calling without matched germline tissue. The overall sensitivity and precision were 80% and 73%, respectively, and we obtained comparable median mutation rates in the matched (284/Mb) and tumor-only (319/Mb) analyses.

We share the authors' concerns regarding the difficulty in calling somatic variants when no control sample is available; for this reason, we applied very hard filters, namely: removal of non-coding or silent variants; coverage and allele frequency thresholds; and ExAC and COSMIC databases for germline filtering. These filters are extensively used and it has been shown that they yield high performance, especially when used in a selected region of interest [4]. Accordingly, to reduce false positives, we further restricted the analysis to 95 genes known to be somatically mutated in bladder cancer, which increased the sensitivity and precision to 83% and 90%, respectively.

We want to clarify the authors' statement that our study lacks external validation. On reviewer request, we searched for publicly available data sets comprising HGT1 patients for external validation. Although no truly comparable data set exists, we identified the Nassar cohort [5], the HG NMIBC Memorial series, and the UROMOL group. We validated the correlations between DNA damage response (DDR) and ERCC2 mutations with tumor mutational burden (TMB), and ERCC2 mutations with COSMIC5. Notably, the prognostic associations of TMB and ERCC2 and DDR mutations with good outcome were also reproduced. It should be noted that whereas all purely genomic findings were statistically significant, some of the prognostic associations were not. We attribute this lack of significance mostly to technical differences and major limitations of these data sets, such as smaller sample size, shorter follow-up, and a limited number of events.

Although broader validation is still needed, most of our findings were validated in several—technically very different—independent data sets. The validations also confirm that our methodology successfully removed germline variants as far as the main findings of the study are concerned.

Conflicts of interest: The authors have nothing to disclose.

CRediT authorship contribution statement

Júlia Perera-Bel: Conceptualization, Writing - original draft. **Joaquim Bellmunt:** Conceptualization, Writing - review & editing.

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