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Devil in the Detail: Intratumour Heterogeneity and Personalised Medicine for Bladder Cancer

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Personalised medicine is transforming oncology through the use of molecular markers to guide treatment and inform prognosis [1]. In the field of urothelial carcinoma, patients are selected for immune checkpoint inhibitor therapy on the basis of immunohistochemical expression of PD1 or PDL1. Gene expression signatures can be used to predict response to neoadjuvant chemotherapy in muscle-invasive disease or predict progression in non–muscle-invasive bladder cancer (NMIBC) [2]. Similar to most solid tumours, urothelial carcinoma is spatially heterogeneous. This intratumour heterogeneity (ITH) is the result of temporal acquisition of mutations and corresponding tumour evolution. Histologically, this is reflected in heterogeneity in grade or the presence of variant tumour morphology such as squamous, micropapillary, nested, or plasmacytoid features. Heterogeneity is also seen at the genomic and transcriptomic levels. ITH is beneficial to a tumour; it allows the development of clones that may be more aggressive or better able to withstand chemotherapy. However, this benefit is diminished when too many clones are present within an individual tumour, implying there is a "Goldilocks" level of ITH that balances the benefits and costs of divergent tumour evolution [3].

Any personalised tumour treatment or prognostic indicator requires a test to distinguish patients who will benefit from those who will not. Regardless of the type of test (mutation, gene expression, epigenetic profile, or protein expression), bulk assessment of tumour is currently used. Typically, a pathologist will mark the area of highest tumour percentage on a slide (avoiding areas of background normal tissue and necrosis), and this area is used for nucleic acid extraction and downstream analysis. While this approach has generated useful classifiers, it fails to consider ITH. If a small area of genomically aggressive tumour is not sampled or not detected owing to low variant allele frequency, then a patient could be inappropriately assigned to a lower risk group or not receive a specific therapy from which they might benefit. These cases may comprise a small percentage of a validation cohort and be hidden within the aggregate statistics used to define the accuracy and precision of novel markers. However, for the individual patient, correct subtyping is crucial. To date, few studies have assessed the impact of ITH on the reliability of personalised tumour profiling.

In this issue of *European Urology*, Warrick and colleagues [4] describe ITH in muscle-invasive bladder cancer with multiregion sampling of variant histology and classification according to immunohistochemistry using the Lund approach. The data show significant molecular ITH between variant histologies, with the basal squamous molecular subtype exhibiting the greatest heterogeneity. Furthermore, two cell-cycle markers within the classifier (p16 and RB1) demonstrated mutually exclusive loss in NMIBC, suggesting that these are early genomic events in bladder cancer evolution. These are important findings, highlighting the possibility for misclassification of patients and subsequent suboptimal treatment.

The authors have chosen to only evaluate variant histology, which represents approximately 30% of bladder cancer cases in routine practice, and arguably their findings are only applicable to this patient group. Two previous studies evaluated genomic and transcriptomic ITH in urothelial carcinoma [5,6]. Although the cohorts were small (n = 3 and 4, respectively), these studies showed a mixed

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picture. Multifocal tumours were heterogeneous, whereas unifocal tumours had low levels of ITH. Perhaps more importantly, there was some spatial heterogeneity of clinically actionable mutations and heterogeneity between paired primary and metastatic deposits. A separate study of *FGFR3* mutation status showed genomic heterogeneity between superficial and deep tumour compartments in muscle-invasive cancers [7]. This study also evaluated NMIBC but demonstrated no ITH. Contrary to this, Dyrskjøt et al. [8] showed gene expression heterogeneity in 29% of metachronous tumours in their validation of a 12-gene progression signature in NMIBC.

The present study also highlights the practical issues of incorporating measures of ITH into clinical practice. The supplementary data show that in one-quarter of cases, subtyping could not be undertaken owing to issues with tumour sampling or if any of the 13 immunohistochemical markers was not evaluable. If multiregion sampling is required for valid molecular assessment, then there are further questions outstanding with regard to ITH. How many regions of an individual tumour should we sample? How far apart should these regions be? When is an area of molecular heterogeneity significant? Should metastatic deposits also be subjected to multiregion sampling? These questions need to be addressed in the development of molecular markers and it would seem reasonable that tissue biomarker–stratified studies should include a subgroup or preliminary analysis of the effect of ITH on patient classification. Some groups have tried to address these concerns. Good classification starts with good tumour sampling, and the strategy for sampling multiple tumour regions described by Guarch et al. [9] led to an increase in detection of high-grade renal cell carcinoma. However, multisite sampling generates more specimens per patient for testing and a consequent higher cost. To ameliorate this cost, Joung et al. [10] adopted an approach involving pooled analysis of samples from multiple tumour regions in multiple cancer types and showed this to be a reliable alternative to individually assaying separate tumour regions.

Personalised medicine requires precise subtyping of disease. Currently, the effect of ITH on molecular stratification in bladder cancer is unclear. To properly evaluate this issue may require changes in routine practice on the part of the surgeon and the pathologist to ensure that tumours are adequately sampled at the start of the diagnostic journey. Incorporating an ITH assessment into any molecular analysis would also generate more data on the biology of ITH. This enhanced understanding of ITH will be key in precise and personalised bladder cancer care.

Conflicts of interest: The author has nothing to disclose.

References

[1] Jackson SE, Chester JD. Personalised cancer medicine. Int J Cancer 2015;137:262–6.
[2] Matulay JT, Kamat AM. Advances in risk stratification of bladder cancer to guide personalized medicine. F1000Res 2018;7:1137.
[3] Andor N, Graham TA, Jansen M, et al. Pan-cancer analysis of the extent and consequences of intratumor heterogeneity. Nat Med 2016;22:105–13.
[4] Warrick JI, Sjodahl G, Kaag M, et al. Intratumoural heterogeneity of bladder cancer by molecular subtypes and histologic variants. Eur Urol 2019;75:18–22.
[5] Thomsen MBH, Nordentoft I, Lamy P, et al. Spatial and temporal clonal evolution during development of metastatic urothelial carcinoma. Mol Oncol 2016;10:1450–60.
[6] Thomsen MBH, Nordentoft I, Lamy P, et al. Comprehensive multiregional analysis of molecular heterogeneity in bladder cancer. Sci Rep 2017;7:11702.
[7] Pouessel D, Neuzillet Y, Mertens LS, et al. Tumor heterogeneity of fibroblast growth factor receptor 3 (FGFR3) mutations in invasive bladder cancer: implications for perioperative anti-FGFR3 treatment. Ann Oncol 2016;27:1311–6.
[8] Dyrskjøt L, Reinert T, Novoradovsky A, et al. Analysis of molecular intra-patient variation and delineation of a prognostic 12-gene signature in non-muscle invasive bladder cancer; technology transfer from microarrays to PCR. Br J Cancer 2012;107:1392–8.
[9] Guarch R, Cortés JM, Lawrie CH, López JI. Multi-site tumor sampling (MSTS) improves the performance of histological detection of intratumor heterogeneity in clear cell renal cell carcinoma (CCRCC). F1000Res 2016;5:2020.
[10] Joung J-G, Bae JS, Kim SC, Jung H, Park W-Y, Song S-Y. Genomic characterization and comparison of multi-regional and pooled tumor biopsy specimens. PLoS One 2016;11, e0152574.