Molecular subtypes of T1 bladder cancer

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1 **MOLECULAR SUBTYPES OF T1 BLADDER CANCER: BIOMOLECULAR**

2 **CHARACTERISTICS VERSUS CLINICAL UTILITY**

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6 **INVITED EDITORIAL**

7 **Re: A.G.Robertson et al. Identification of Differential Tumor Subtypes of T1 Bladder Cancer.**

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26   From low grade Ta to high grade T1, non-muscle-invasive bladder cancers (NMIBCs) are highly

27   heterogeneous both clinically and biologically. As with muscle-invasive bladder cancer (MIBC), high

28   grade NMIBCs have a high mutation burden, multiple copy number changes and loss of tumour

29   suppressors (e.g. *TP53*, *RB1*), whereas low grade NMIBCs exhibit oncogene activation (e.g. *FGFR3*, *RAS*)

30   in a relatively normal genome [1]. Recently, "subtyping" based on gene expression has offered further

31   insights into NMIBC biology [1-3], yet risk stratification (and hence treatment selection) remains

32   entirely based upon clinico-pathological observations, without the inclusion of biomolecular

33   information [4].

34   The majority of urologists treat high grade T1 patients with induction and maintenance intravescial

35   BCG, with radical cystectomy considered in suitable patients [4]. BCG efficacy varies: over 60% of

36   patients experience durable responses [5;6], up to 20% experience progression to MIBC within 5-years

37   [5;6], and as many as 15% have lymph node metastases at diagnosis [7]. There would be great benefit

38   to patients if such biomolecular insights could be used to predict those more likely to respond to BCG,

39   those who should be treated by other (novel) therapeutics, those who would be optimally managed

40   by early radical cystectomy and, potentially, those who do not require prolonged adjuvant therapies.

41   In this month's issue of *European Urology*, Robertson et al describe the identification of five subtypes

42   of high grade T1 bladder cancer [8]. The authors utilise a discovery cohort of 73 patients with high

43   grade T1 disease; 84% of these patients had undergone re-TUR, 100% had received induction BCG,

44   and 64% had received maintenance BCG. At 24 months, 32% had recurred and 8% had progressed to

45   MIBC; overall, 9 patients progressed during follow-up. Transurethral resection specimens were

46   subjected to RNA sequencing using standard methodology. State-of-the-art analyses were used to

47   interpret the data at the level of defined pathways and regulons, and to provide insights into the

48   (immune) microenvironment.

49   In brief, unsupervised consensus clustering based on gene expression was used to define 5 subtypes

50   considered to optimally describe tumour biology and characteristics, with an open-access "single

51 sample classifier" generated to facilitate subtyping. Regulon analysis identified a dichotomy, with

52 similar transcription factor activity in the T1-Myc and T1-LumGU subtypes and in the T1-TLum and T1-

53 Early subtypes. Although recurrence-free survival curves for the 5 subtypes did not differ significantly,

54 combined T1-Myc/Early versus combined T1-LumGU/Inflam/TLum resulted in a significant difference

55 in recurrence-free survival. As a limited validation, the gene expression patterns were corroborated

56 in a cohort of 26 patients with high grade tumours who underwent radical cystectomy (69% ≤T1, 31%

57 ≥T2), suggesting that the 5 subtypes are intrinsic properties of high grade bladder cancer rather than

58 a result of "overfitting". Subsequent in vitro data highlighted the potential of this classification for the

59 selection of targeted therapies.

60 Unlike previous studies predominantly focussed on MIBCs and/or NMIBCs of all grades and stages

61 [2;3;9], the advantage of the current study is that it has analysed only high grade T1 tumours from

62 patients treated with at least induction BCG; hence, the influence of intrinsic biology on the outcomes

63 of high grade T1 disease can largely be separated from the confounding effects of grade, stage, and

64 varying treatments (see later). Comparison with existing subtyping methods showed that T1-LumGU

65 shows similarity to LumU (MIBC consensus [9]) and GU (Lund [2]) subtypes, and T1-Inflam shows

66 similarity to cluster 2b (UROMOL [10]) and Basal & Mesenchymal (Lund) subtypes, whilst the

67 remaining proposed T1 subtypes are predominantly similar to LumP (MIBC consensus), cluster 2a

68 (UROMOL) and urothelial-like (Lund). Thus, the subtypes described here provide more detailed

69 characterisation of T1 tumours than existing schema.

70 The Brief Correspondence format does not do justice to these data, nor permit detailed explanations

71 of methodology and iterative steps. Hence, the analyses are not without the need for clarification; as

72 the authors state, the results require robust independent validation. Although high *MYC* expression is

73 reason to combine T1-Myc and T1-Early (with a significant recurrence-free survival difference versus

74 T1-LumGU/Inflam/TLum), they are derived from opposite regulon clusters, and other shared biology

75 is not evidenced. Also, data relating to immune microenvironment should be interpreted carefully: for

76   example, MCP-counter does not report absolute measurements of cell-type fractions but individual

77   cell-type scores, which are potentially influenced by normalisation procedures. Furthermore, it

78   remains unclear if the validation readout (expression of two regulons, expression of seven genes, CIS

79   gene-set expression, and MIBC subtype) is not intrinsically modelled in the classifier (which is based

80   on the expression of 300 genes). In addition, the differential outcomes for the subtypes are based on

81   a small number of events in a modest number of patients with no independent validation. Finally, is

82   recurrence-free survival the most clinically-relevant outcome for patients with high grade T1 tumours

83   following induction BCG? Figure 1A illustrates the treatments of the 9 patients who progressed to

84   MIBC: only 1/9 had undergone re-TUR and had received maintenance BCG. Progression in high grade

85   T1 patients after optimal bladder-preserving management (re-TUR, induction and maintenance BCG

86   [4]) is life-threatening, and is the outcome of most interest to clinicians (and their patients). Arguably,

87   this one patient is the only patient who matters in this study – what are the unique biomolecular

88   characteristics of her tumour?

89   As with interventional clinical trials where one undertakes sample size calculations to adequately

90   "power" the study, it is likely that many hundreds of T1 tumours (accompanied by high quality clinical

91   data and follow-up) need to be analysed to answer the question(s) of most importance for this clinical

92   setting. With RNA sequencing still costly and transcriptome data analysis complex, this remains a

93   challenge. Additionally, subtype is only be one of the biomolecular factors influencing outcomes from

94   high grade T1 disease, with important contributions to pathogenesis from other genomic and

95   epigenomic phenomena, as well as cell and immune biology. There is still a long journey ahead to fully

96   understand high grade T1 disease such that we can optimally stratify patients, and develop new

97   therapies; however, alongside previous analyses [1-3;10], this study represents a promising waypoint.

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