Molecular subtypes of T1 bladder cancer

Ward, Douglas G; Arnold, Roland; Bryan, Richard T

DOI:
10.1016/j.eururo.2020.07.021

Document Version
Peer reviewed version

Citation for published version (Harvard):
Ward, DG, Arnold, R & Bryan, RT 2020, 'Molecular subtypes of T1 bladder cancer: biomolecular characteristics versus clinical utility', *European urology*, vol. 78, no. 4, pp. 538-539. https://doi.org/10.1016/j.eururo.2020.07.021

MOLECULAR SUBTYPES OF T1 BLADDER CANCER: BIOMOLECULAR CHARACTERISTICS VERSUS CLINICAL UTILITY

Douglas G Ward[1], Roland Arnold[1], Richard T Bryan[1].

[1]Institute of Cancer and Genomic Sciences, University of Birmingham, Birmingham, UK.

INVITED EDITORIAL

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

Keywords:

Bladder cancer; molecular; subtypes; T1; utility.

Correspondence:

Mr Richard T Bryan

The Bladder Cancer Group, Institute of Cancer & Genomic Sciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK.

T +44 121 414 7870, F +44 121 414 3630, E r.t.bryan@bham.ac.uk

Disclosures:

RT Bryan has contributed to advisory boards for Olympus Medical Systems & Janssen, and undertakes research funded by UroGen Pharma.

From low grade Ta to high grade T1, non-muscle-invasive bladder cancers (NMIBCs) are highly heterogeneous both clinically and biologically. As with muscle-invasive bladder cancer (MIBC), high grade NMIBCs have a high mutation burden, multiple copy number changes and loss of tumour suppressors (e.g. *TP53*, *RB1*), whereas low grade NMIBCs exhibit oncogene activation (e.g. *FGFR3*, *RAS*) in a relatively normal genome [1]. Recently, "subtyping" based on gene expression has offered further insights into NMIBC biology [1-3], yet risk stratification (and hence treatment selection) remains entirely based upon clinico-pathological observations, without the inclusion of biomolecular information [4].

The majority of urologists treat high grade T1 patients with induction and maintenance intravescial BCG, with radical cystectomy considered in suitable patients [4]. BCG efficacy varies: over 60% of patients experience durable responses [5;6], up to 20% experience progression to MIBC within 5-years [5;6], and as many as 15% have lymph node metastases at diagnosis [7]. There would be great benefit to patients if such biomolecular insights could be used to predict those more likely to respond to BCG, those who should be treated by other (novel) therapeutics, those who would be optimally managed by early radical cystectomy and, potentially, those who do not require prolonged adjuvant therapies.

In this month's issue of *European Urology*, Robertson et al describe the identification of five subtypes of high grade T1 bladder cancer [8]. The authors utilise a discovery cohort of 73 patients with high grade T1 disease; 84% of these patients had undergone re-TUR, 100% had received induction BCG, and 64% had received maintenance BCG. At 24 months, 32% had recurred and 8% had progressed to MIBC; overall, 9 patients progressed during follow-up. Transurethral resection specimens were subjected to RNA sequencing using standard methodology. State-of-the-art analyses were used to interpret the data at the level of defined pathways and regulons, and to provide insights into the (immune) microenvironment.

In brief, unsupervised consensus clustering based on gene expression was used to define 5 subtypes considered to optimally describe tumour biology and characteristics, with an open-access "single

sample classifier" generated to facilitate subtyping. Regulon analysis identified a dichotomy, with similar transcription factor activity in the T1-Myc and T1-LumGU subtypes and in the T1-TLum and T1-Early subtypes. Although recurrence-free survival curves for the 5 subtypes did not differ significantly, combined T1-Myc/Early versus combined T1-LumGU/Inflam/TLum resulted in a significant difference in recurrence-free survival. As a limited validation, the gene expression patterns were corroborated in a cohort of 26 patients with high grade tumours who underwent radical cystectomy (69% ≤T1, 31% ≥T2), suggesting that the 5 subtypes are intrinsic properties of high grade bladder cancer rather than a result of "overfitting". Subsequent in vitro data highlighted the potential of this classification for the selection of targeted therapies.

Unlike previous studies predominantly focussed on MIBCs and/or NMIBCs of all grades and stages [2;3;9], the advantage of the current study is that it has analysed only high grade T1 tumours from patients treated with at least induction BCG; hence, the influence of intrinsic biology on the outcomes of high grade T1 disease can largely be separated from the confounding effects of grade, stage, and varying treatments (see later). Comparison with existing subtyping methods showed that T1-LumGU shows similarity to LumU (MIBC consensus [9]) and GU (Lund [2]) subtypes, and T1-Inflam shows similarity to cluster 2b (UROMOL [10]) and Basal & Mesenchymal (Lund) subtypes, whilst the remaining proposed T1 subtypes are predominantly similar to LumP (MIBC consensus), cluster 2a (UROMOL) and urothelial-like (Lund). Thus, the subtypes described here provide more detailed characterisation of T1 tumours than existing schema.

The Brief Correspondence format does not do justice to these data, nor permit detailed explanations of methodology and iterative steps. Hence, the analyses are not without the need for clarification; as the authors state, the results require robust independent validation. Although high *MYC* expression is reason to combine T1-Myc and T1-Early (with a significant recurrence-free survival difference versus T1-LumGU/Inflam/TLum), they are derived from opposite regulon clusters, and other shared biology is not evidenced. Also, data relating to immune microenvironment should be interpreted carefully: for

example, MCP-counter does not report absolute measurements of cell-type fractions but individual cell-type scores, which are potentially influenced by normalisation procedures. Furthermore, it remains unclear if the validation readout (expression of two regulons, expression of seven genes, CIS gene-set expression, and MIBC subtype) is not intrinsically modelled in the classifier (which is based on the expression of 300 genes). In addition, the differential outcomes for the subtypes are based on a small number of events in a modest number of patients with no independent validation. Finally, is recurrence-free survival the most clinically-relevant outcome for patients with high grade T1 tumours following induction BCG? Figure 1A illustrates the treatments of the 9 patients who progressed to MIBC: only 1/9 had undergone re-TUR and had received maintenance BCG. Progression in high grade T1 patients after optimal bladder-preserving management (re-TUR, induction and maintenance BCG [4]) is life-threatening, and is the outcome of most interest to clinicians (and their patients). Arguably, this one patient is the only patient who matters in this study – what are the unique biomolecular characteristics of her tumour?

As with interventional clinical trials where one undertakes sample size calculations to adequately "power" the study, it is likely that many hundreds of T1 tumours (accompanied by high quality clinical data and follow-up) need to be analysed to answer the question(s) of most importance for this clinical setting. With RNA sequencing still costly and transcriptome data analysis complex, this remains a challenge. Additionally, subtype is only be one of the biomolecular factors influencing outcomes from high grade T1 disease, with important contributions to pathogenesis from other genomic and epigenomic phenomena, as well as cell and immune biology. There is still a long journey ahead to fully understand high grade T1 disease such that we can optimally stratify patients, and develop new therapies; however, alongside previous analyses [1-3;10], this study represents a promising waypoint.

Reference List

(1) Hurst CD, Platt F, Taylor CF, Knowles MA. Novel tumor subgroups of urothelial carcinoma of the bladder defined by integrated genomic analysis. Clin Cancer Res 2012; 18:5865-5877.

(2) Sjodahl G, Lauss M, Lovgren K, Chebil G, Gudjonsson S, Veerla S, Patschan O, Aine M, Ferno M, Ringner M, Mansson W, Liedberg F, Lindgren D, Hoglund M. A molecular taxonomy for urothelial carcinoma. Clin Cancer Res 2012; 18:3377-3386.

(3) Hedegaard J, Lamy P, Nordentoft I, Algaba F, Hoyer S, Ulhoi BP, Vang S, Reinert T, Hermann GG, Mogensen K, Thomsen MB, Nielsen MM, Marquez M, Segersten U, Aine M, Hoglund M, Birkenkamp-Demtroder K, Fristrup N, Borre M, Hartmann A, Stohr R, Wach S, Keck B, Seitz AK, Nawroth R, Maurer T, Tulic C, Simic T, Junker K, Horstmann M, Harving N, Petersen AC, Calle ML, Steyerberg EW, Beukers W, van Kessel KE, Jensen JB, Pedersen JS, Malmstrom PU, Malats N, Real FX, Zwarthoff EC, Orntoft TF, Dyrskjot L. Comprehensive Transcriptional Analysis of Early-Stage Urothelial Carcinoma. Cancer Cell 2016; 30:27-42.

(4) Babjuk M, Burger M, Comperat EM, Gontero P, Mostafid AH, Palou J, van Rhijn BWG, Roupret M, Shariat SF, Sylvester R, Zigeuner R, Capoun O, Cohen D, Escrig JLD, Hernandez V, Peyronnet B, Seisen T, Soukup V. European Association of Urology Guidelines on Non-muscle-invasive Bladder Cancer (TaT1 and Carcinoma In Situ) - 2019 Update. Eur Urol 2019; 76:639-657.

(5) Thomas F, Noon AP, Rubin N, Goepel JR, Catto JW. Comparative outcomes of primary, recurrent, and progressive high-risk non-muscle-invasive bladder cancer. Eur Urol 2013; 63:145-154.

(6) Martinez-Pineiro L, Portillo JA, Fernandez JM, Zabala JA, Cadierno I, Moyano JL, Solsona E, Unda M, Beardo P, Rodriguez-Molina J, Chantada V, Palou J, Muntanola P, Alonso Dorrego JM, Perez-Garcia FJ, Silva JM, Chesa N, Montesinos M, Ojea A, Madero R, Martinez-Pineiro JA. Maintenance Therapy with 3-monthly Bacillus Calmette-Guerin for 3 Years is Not Superior to Standard Induction Therapy in High-risk Non-muscle-invasive Urothelial Bladder Carcinoma: Final Results of Randomised CUETO Study 98013. Eur Urol 2015; 68:256-262.

(7) Wiesner C, Thomas C, Salzer A, Gillitzer R, Hampel C, Thuroff JW. [Prevalence of lymph node metastases in non-muscle-invasive bladder cancer. Delay of radical cystectomy and upstaging in the cystectomy specimen as risk factors]. Urologe A 2008; 47:1162-1166.

(8) A.G.Robertson et al. Identification of Differential Tumor Subtypes of T1 Bladder Cancer. Eur Urol 2020; in press.

(9) Kamoun A, de RA, Allory Y, Sjodahl G, Robertson AG, Seiler R, Hoadley KA, Groeneveld CS, Al-Ahmadie H, Choi W, Castro MAA, Fontugne J, Eriksson P, Mo Q, Kardos J, Zlotta A, Hartmann A, Dinney CP, Bellmunt J, Powles T, Malats N, Chan KS, Kim WY, McConkey DJ, Black PC, Dyrskjot L, Hoglund M, Lerner SP, Real FX, Radvanyi F. A Consensus Molecular Classification of Muscle-invasive Bladder Cancer. Eur Urol 2020; 77:420-433.

(10) Lindskrog SV, Prip FF, Lamy P, Taber A, Groeneveld CS, Birkenkamp-Demtroder K, Jensen JB, Strandgaard T, Nordentoft I, Christensen E, Sokac M, Birkbak NJ, Maretty L, Hermann GH, Petersen AC, Weyerer V, Grimm MO, Horstmann M, Sjodahl G, Hoglund M, Steiniche T, Mogensen K, de Reynies A, Nawroth R, Jordan B, Lin X, Dragicevic D, Ward DG, Goel A, Hurst

CD, Raman JD, Warrick JI, Segersten U, Sikic D, van Kessel KEM, Maurer T, Meeks JJ, Degraff DJ, Bryan RT, Knowles MA, Simic T, Hartmann A, Zwarthoff EC, Malmstrom PU, Malats N, Real FX, Dyrskjot L. An integrated multi-omics analysis identifies clinically relevant molecular subtypes of non-muscle-invasive bladder cancer. medRxiv 2020;2020.