Laboratory-Bladder cancer

Synchronous and metachronous urothelial carcinoma of the upper urinary tract and the bladder: Are they clonally related? A systematic review

Thomas van Doeveren, M.D.[a,*], Harmen J.G. van de Werken, Ph.D.[a,b], Job van Riet, Ph.D.[a,b], Katja K.H. Aben, Ph.D.[c,d], Pim J. van Leeuwen, M.D., Ph.D.[e], Ellen C. Zwarthoff, Prof., Dr.[f], Joost L. Boormans, M.D., Ph.D.[a]

[a] Department of Urology, Erasmus MC Cancer Institute, University Medical Center Rotterdam, Rotterdam, The Netherlands
[b] Cancer Computational Biology Center, Erasmus MC Cancer Institute, University Medical Center Rotterdam, Rotterdam, The Netherlands
[c] Netherlands Comprehensive Cancer Organization, Utrecht, The Netherlands
[d] Radboud University Medical Centre, Radboud Institute for Health Sciences, Nijmegen, The Netherlands
[e] Department of Urology, Netherlands Cancer Institute, Amsterdam, The Netherlands
[f] Department of Pathology, Erasmus MC Cancer Institute, University Medical Center Rotterdam, Rotterdam, The Netherlands

Abstract

Purpose: Following radical nephroureterectomy for upper urinary tract urothelial carcinoma (UTUC), intravesical recurrence (IVR) is found in 22% to 47% of patients. Patients with a primary urothelial carcinoma of the bladder (UCB) have an increased risk of a future UTUC (1%−5%). Paired UTUC and UCB might represent clonally related tumors due to intraluminal seeding of tumor cells or might be separate entities of urothelial carcinoma caused by field cancerization. We systematically reviewed all the relevant literature to address the possible clonal relation of UTUC and paired UCB. *Materials and Methods:* MEDLINE, EMBASE, and COCHRANE databases were systematically searched for relevant citations published between January 2000 and July 2019. This study was performed according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses guidelines. Of 5038 citations identified, 86 full papers were screened, and 9 studies met the inclusion criteria. *Results:* The populations studied and the molecular techniques used to assess clonality of UTUC and paired UCB differed largely over time. Eight studies reported on primary UTUC and meta- or synchronous IVR without a history of UCB. A total of 118 tumors (55 UTUC and 63 IVR) from 49 patients were included, of which 94% seemed to be clonally related. Five studies reported on primary UCB and subsequent UTUC with a total of 61 tumors (30 UCB and 31 UTUC) from 14 patients; a possible clonal origin was identified for 85% of the tumors. *Conclusion:* Taking into account the limitations of microsatellite technology in comparison to *Next Generation Sequencing* and currently accepted concepts of tumor heterogeneity and evolution, this systematic review shows that most, if not all, UTUC and paired UCB likely are clonally related. © 2020 The Author(s). Published by Elsevier Inc. This is an open access article under the CC BY-NC-ND license. (http://creativecommons.org/licenses/by-nc-nd/4.0/)

Keywords: Bladder carcinoma; Clonality; Intravesical recurrence; Upper urinary tract; Urothelial carcinoma

1. Introduction

Urothelial carcinomas can arise throughout the entire urinary tract, but the urinary bladder is the predominant side of origin. The incidence of upper urinary tract urothelial carcinoma (UTUC) is 1 to 2 per 100,000 persons/year in Western Europe, and UTUC accounts for 5% to 10% of all urothelial carcinomas [1]. UTUC and urothelial bladder cancer (UCB) are considered similar entities. Accordingly, results of studies on UCB are often extrapolated to UTUC. Although UCB and UTUC share certain histopathological characteristics and have several risk factors in common, with tobacco use as the most imperative one, important clinical and molecular differences exist between the 2 entities [2]. At diagnosis, 60% of UTUC patients have an

*Corresponding author. Tel.: +31 107040704.
E-mail address: t.vandoeveren.1@erasmusmc.nl (T. van Doeveren).

invasive tumor versus 20% to 25% of UCB patients [1,3]. Hence, the prognosis of UTUC is poor with a 5-year overall survival (OS) of approximately 70%; for invasive disease the 5 year OS is less than 40%, which is lower than reported for UCB patients treated with radical cystectomy [4,5]. Recent genomic characterization of UTUC revealed different molecular alterations in comparison to UCB and, in contrast to UCB, UTUC seemed to be associated with Lynch syndrome (LS) [6−8].

Following radical nephroureterectomy (RNU), which is the recommended treatment for nonmetastatic UTUC, intravesical recurrence (IVR) within the first 2 years following surgery is found in 22% to 47% of the patients [1,9,10]. Clinical risk factors for the development of an IVR following RNU are: a history of UCB, tumor multiplicity, tumor location (distal ureter), advanced tumor stage, and the operative modality [11]. Guidelines recommend administration of a single dose of intravesical chemotherapy within 10 days after RNU to reduce the risk of a future IVR [1,12,13]. A neoadjuvant regimen of intravesical Mytomicin C is being evaluated in an ongoing multicenter study [14].

UTUC patients also have an increased risk of developing a tumor in the contralateral upper urinary tract; 2%to 6% develop a recurrence in the contralateral upper urinary tract following RNU [15]. Moreover, the incidence of concomitant UCB at the time of diagnosis of primary UTUC is 17% [16], whereas the risk of developing an UTUC following the diagnosis of a primary UCB is much lower. In a cohort of 1,529 patients with primary nonmuscle invasive UCB, the incidence of a subsequent UTUC was only 2.6%, although the proportion was higher in multifocal and high-risk tumors [17]. In summary, urothelial carcinoma is an important risk factor for developing a subsequent tumor throughout the entire urinary tract; patients with a primary UTUC have the highest risk of developing a recurrence in the bladder.

Two hypotheses have been proposed for the increased risk of recurrence in the urinary tract following a primary diagnosis of urothelial carcinoma. One hypothesis is that the entire urinary tract is affected by carcinogenic hits [18], which results in multifocal tumors that develop independently from one another. These tumors are therefore thought not to share the same progenitor cell. However, this would not explain the difference in incidence of UTUC and UCB in general, nor the difference in incidence of tumors in the contralateral urinary tract vs. the bladder after a primary diagnosis of UTUC. The second hypothesis states that by intraluminal seeding or intraepithelial spread, tumor cells located in the upper urinary tract implant in the bladder and give rise to a recurrence [19,20]. In the latter, IVR will be of monoclonal origin as it arises from the antecedent UTUC. This hypothesis seems plausible taking into account the low incidence of UTUC and hence the chance that a patient would develop 2 or more tumors that derive in different parts of the urinary tract is very low [21,22]. In 2002, a review concluded that the majority of the studies investigating the clonal relationship of multiple urothelial carcinomas of the urinary tract revealed tumors to be of monoclonal origin [23].

We present the results of a systematic review of all the relevant and recent literature addressing whether synchronous and metachronous urothelial carcinoma of the upper urinary tract and bladder are clonally related.

2. Materials and methods

The electronic databases Medline (Ovid) and Embase, the Cochrane Central Register of Controlled Trials, and the Cochrane Database of Systematic Reviews were searched for citations published between January 2000 and July 2019. The review was performed according to the Preferred Reported Items for Systematic Reviews and Meta-analysis statement [24] and the protocol has been published in the PROSPERO database (CRD42018105617).

Original studies that performed a genomic characterization of UTUC and paired IVR (i.e. both tumors diagnosed in the same patient) were included, whereas studies that reported on a molecular analysis of UTUC and UCB samples not derived from the same patient were excluded (see Fig. 1). Keywords arranged in variable combinations included "upper urinary tract urothelial carcinoma," "intravesical recurrences," "ureter," "renal pelvis," bladder urothelial carcinoma," "clonality," and "molecular genetics" (see Supplementary Materials for details of the search strategy). The search was complemented by cross-referencing of the studies included. Two reviewers (T.v.D. and J.L.B.) independently screened all abstracts and full-text articles. Disagreement was resolved by discussion, and if no agreement was reached, a third independent party acted as arbiter (E.C.Z.).

2.1. In- and exclusion criteria

Studies with UTUC patients who developed a subsequent IVR and studies with UCB patients who developed a subsequent UTUC were included. Studies that reported on patients who had recurrences limited to either the upper or lower urinary tract were excluded. At least 1 genomic alteration had to be present in 1 of the 2 paired tumors of a patient in order to be included in the final analysis.

2.2. Definition of a clonal relationship between UTUC and paired UCB

Monoclonal origin: Tumors were considered to be of clonal origin when both the UTUC and paired UCB shared synonymous/non-synonymous or noncoding somatic mutations, microsatellite instability (MSI), methylation and Loss of Heterozygosity (LOH). These molecular alterations had to be identical in expansion or deletion. An interface of 100% between the alterations of the 2 tumors was not considered mandatory since subclones derived from the primary tumor can expand in the number of alterations independently over time. A single concordant alteration, pattern of methylation,
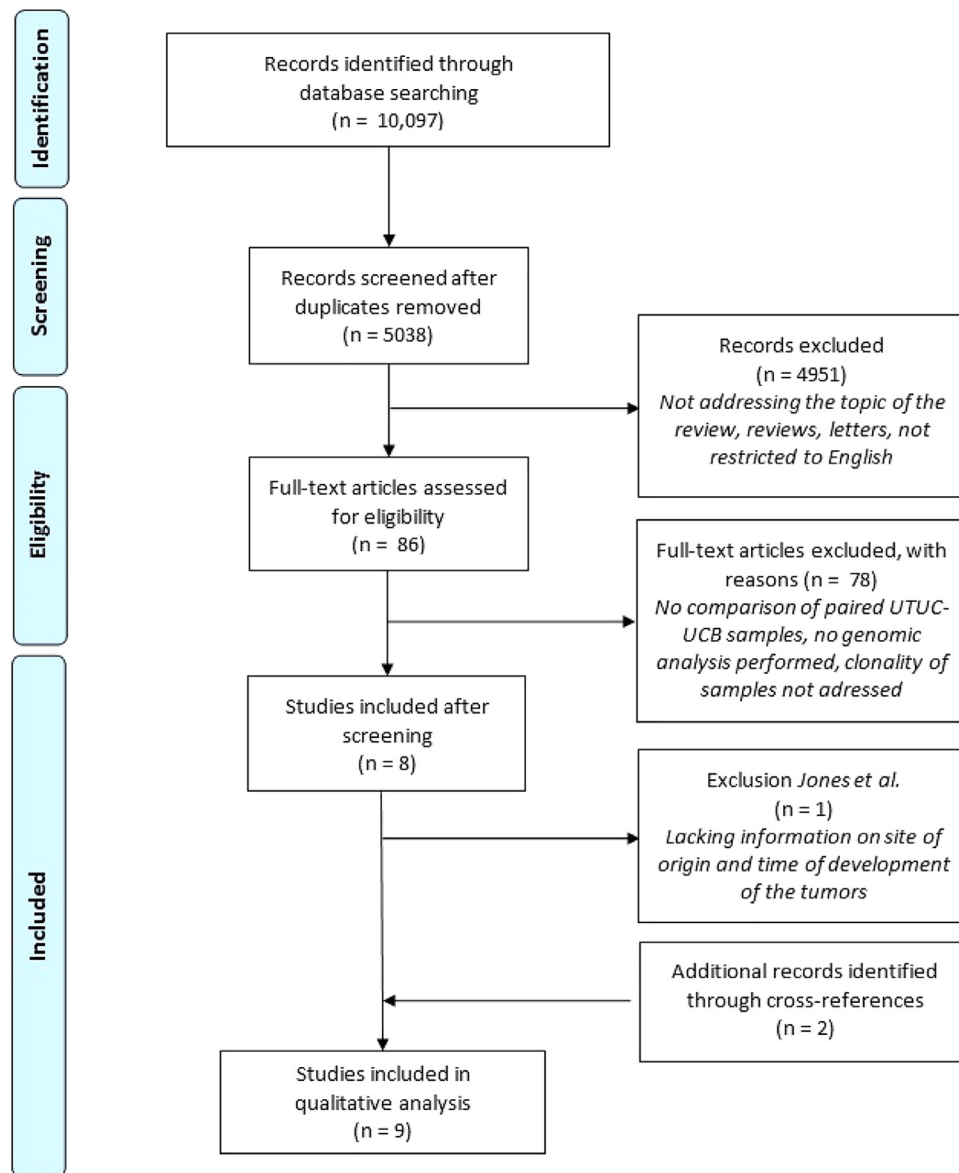
Fig. 1. PRISMA flow diagram of the study.

or LOH between 2 paired tumors, as assessed by *Next Generation Sequencing (NGS), bisulfate sequencing, or Whole Exome Sequencing (WES)*, was considered determinative for a clonal relationship or a shared progenitor cell, as these techniques permit to approach the exact gene position of an alteration. The possibility that a shared molecular alteration alters on the exact same gene position in 2 analyzed tumors of the same patient was considered to be negligible, especially in 'passenger genes' [25].

Undefined clonal origin: In case of absence of concordant molecular alterations, we marked the paired tumors as 'undefined' and not of 'oligoclonal origin'. We chose to do so as for the analysis we were dependent on the (sometimes limited) number of markers/loci analyzed in the studies included. Theoretically, it could be possible that both tumors did share a progenitor cell and were clonally

related but that the specific examined marker(s) did not cover that specific alteration. In those cases, it was not possible to exclude clonality and, as such, tumors were classified as 'undefined'.

It is important to stress that the determination of clonal relatedness by the aforementioned definitions in some cases differed from the original authors' conclusions, which discrepancy might lead to a different assessment of clonally related tumors.

2.3. Definition of synchronous UTUC and UCB

A synchronous recurrence was defined if both tumors, either UTUC or UCB, were diagnosed within 3 months following the diagnosis of the primary tumor.

2.4. Calculation of proportion of clonally related tumors

The large variety of techniques used to analyze clonality of UTUC and paired UCB precluded a formal meta-analysis. All patients were considered to share equal weight in the final analysis, i.e., a patient with multiple recurrences should have the same contribution to the analysis as a patient with only 1 recurrence. To do so, the contribution of a patient for the final analysis was calculated as follows:

$$patients\ contribution = \frac{1}{n} \cdot n_c$$

In which n is the number of recurrences and n_c is the number of clonally related recurrences.

For example: the contribution to the final analysis of a patient who had 5 recurrences ($n = 5$), of which 4 ($N_C = 4$) were clonally related to the primary tumor, was considered 0.8.

$$patients\ contribution = \frac{1}{5}\ x\ 4 = 0.8$$

The final percentage of clonally related tumors per study was calculated with the formula:

$$percentage\ clonally\ related\ tumors = \frac{\sum n_c}{N} \cdot 100\%$$

In which N is the total number of patients from a study and $\sum n_c$ is the sum of all clonal contributions of all included patients of that study.

3. Results

After removal of duplicates, titles and abstracts of 5,038 records identified in the initial search were screened for relevance. In total, 4,951 abstracts were excluded because the inclusion criteria were not met. Eventually, 86 full-text papers were evaluated and 9 studies met the inclusion criteria (see Fig. 1). Forty-six of the 78 studies that were excluded performed a genomic characterization of either UCB or UTUC without a comparison between the 2 entities; 7 studies

focused on prognostic molecular markers; 11 records were reviews; 11 studies did not include any genomic analysis; and 3 studies analyzed unpaired cohorts of UCB and UTUC. Furthermore, a publication by Jones et al. was excluded from the analysis because information on the site of origin in the urinary tract and the timing of tumor development was lacking [26]. See Table 1 for an overview of the 9 studies included in this review.

3.1. Primary UTUC and subsequent UCB

Eight of the 9 studies included patients who had a primary UTUC and a meta- or synchronous IVR without a history of UCB (see Table 2). Since some patients developed more than 1 UTUC and/or IVR, a total of 118 (55 UTUC, 63 IVR) tumors from 49 patients were included in the analysis (Supplementary information Figure S1). The paired tumors had been analyzed for clonality by various techniques, which had changed over time. In total, 93.5% of the patients had concordant patterns of molecular alterations, indicating that a large proportion of IVR and UTUC were of monoclonal origin [27−31,33−35].

Takahashi et al. (2000) analyzed 1 case of primary UTUC and IVR for microsatellite shifts and LOH of chromosomes 2, 4, 8, 9, 11, and 17 using 21 markers [27]. Each tumor showed LOH of chromosomes 9q and 17p, and the IVR had additional LOH of chromosomes 2q, 4p, and 11p (Fig. S1, patient #1). These tumors were considered to be of clonal origin.

Dalbagni et al. assessed mutations of the *TP53* gene in 4 patients who had 16 tumors (5 UTUC, 11 IVR) [28]. All tumors showed identical mutations of *TP53* and thus the paired tumors were considered to be of monoclonal origin (Fig. S1, patient # 2−#5). Hafner et al. assessed mutations in *TP53* exons 5 to 9, LOH of chromosome 9, MSI at 6 loci, and protein expressions of hMLH1 and hMSH2 in 15 patients [29]. This study only reported data on a patient considered not to have clonally related paired tumors; consisting of 1 UTUC followed by 3 IVRs. The UTUC had loss of the short allele of D9S113, whereas the IVRs had

Table 1
Study characteristics of the series that analyzed a possible clonal relationship of UTUC and IVR/UCB ($n = 9$)

	Year	Study design	Number of patients	Paired UTUC-UCB samples	Number of tumors
Takahashi et al. [27]	2000	Case report	1	Yes	1 UTUC, 1 UCB
Dalbagni et al. [28]	2001	Retrospective	13	Yes	11 UTUC, 39 UCB
Hafner et al. [29]	2001	Retrospective	19	Yes	6 UTUC, 16 UCB [a]72 UC
Takahashi et al. [30]	2001	Pro- and retrospective	15	Yes	16 UTUC, 18 UCB
Catto et al. [31]	2006	Prospective	9	Yes	12 UTUC, 20 UCB
Warrick et al. [32]	2010	Retrospective	1	Yes	3 UTUC, 2 UCB
Wang et al. [33]	2013	Retrospective	5	Yes	6 UTUC, 6 UCB
Du et al. [34]	2017	Retrospective	3	Yes	10 UTUC, 4 UCB
Audenet et al. [35]	2018	Prospective	29	Yes	29 UTUC, 29 UCB

Abbreviation: UC = urothelial carcinoma.
[a] *Location in urinary tract not specified.*

Table 2
Overview of the studies that analyzed patients diagnosed with a primary UTUC and who subsequently developed a UCB, the molecular techniques used, and the proportion of clonally related tumors (n = 49 patients)

	Patients (n)	Number of tumors (n)	Median months to recurrence (range)	Target/Technique	Number of patients with clonally related tumors	Percentage clonally related (%)
Takahashi et al., 2000 [27]	1	1 UTUC, 1 IVR	8	LOH (MSI markers): chromosome 9p, 9q, 11p, 17p, 4p, 4q, 2q, 8p;	1/1	100%
Dalbagni et al., 2001 [28]	4	5 UTUC, 11 IVR	14.5 (1−38)	*TP53* (exons 5-8).	4/4	100%
Hafner et al., 2001 [29]	1	1 UTUC, 3 IVR	NA	LOH (chrom 9); p53 (exons 5−9); MSI (six loci); IHC (MLH1, MSH2).	0/1	0%
Takahashi et al., 2001 [30]	13	14 UTUC, 16 IVR	9.0 (0−28)	LOH (MSI markers): chromosome 9p, 9q, 11p, 17p, 4p, 4q, 2q, 8p;	12/13	92.3%
Catto et al., 2006 [31]	5	7 UTUC, 6 IVR	23.4 (0−47)	MSI-H; Methylation promoter regions: *hMLH1, p16, p14, E-cadherin, RARB, RASSF1A, MINT31.*	5/5	100%
Wang et al., 2013 [33]	5	5 UTUC, 5 UCB	NA	LOH (9q21, 9q32, 9q22); *TP53* (17p13).	5/5	100%
Du et al., 2017 [34]	3	5 UTUC, 4 IVR	0	WES; Somatic variants; Copy number; Mutational signature.	1.8[a]/3	60.0%
Audenet et al., 2018 [35]	17	17 UTUC, 17 IVR	22.1 (3-87.8)	MSK-IMPACT (NGS).	17/17	100%

Abbreviations: LOH = loss of heterozygosity; MSI = microsatellite instability (H = high); MSK-IMPACT = Memorial Sloan Kettering Cancer Center integrated mutation profiling of actionable cancer targets (275−468 genes); WES = whole exome sequencing.
[a] 4 of the 5 (80%) tumors from one patient showed a clonal origin.

loss of the longer allele of the same marker. The 3 IVRs also had identical alterations of *TP53*, which were not present in the UTUC. Therefore, we could not consider these tumors as clonally related and were therefore scored as 'undefined' (Fig. S1, patient #6).

Catto et al. combined MSI analysis using 17 markers together with methylation of 7 promoter regions [31]. MSI analysis was performed in 210 patients; only 9 patients had a UTUC and an IVR showing MSI. Five of these 9 patients had a primary UTUC followed by 1 or multiple IVR(s). All paired tumors of these 5 patients shared at least 1 identical alteration of the methylation markers or had a similar pattern of MSI indicating a clonal relationship (Fig. S1, patient #20−#24).

In a second study by Takahashi et al. (2001), which used identical markers as in the 2000 study, a total of 14 UTUC patients who developed 16 IVRs were analyzed [30] (Fig. S1, patient #7−#19). Only 1 patient seemed to have discordant molecular alterations; the primary UTUC showed no alterations in the analyzed markers, while the 2 IVRs had LOH of a marker on chromosome 11p (Fig. S1, patient #17). Therefore, we considered the clonal relationship of the paired tumors as 'undefined'.

Wang et al. analyzed paired tumors of 5 patients by 3 markers for LOH of chromosome 9 and exons 5 to 8 of the *TP53* gene [33] (Fig. S1, patient #25−#29). All 5 paired samples showed identical patterns of chromosomal loss or *TP53* mutations. One patient, however, had an identical

pattern of *TP53* and D9S303, with a complete loss of D9S171 in the UTUC. Conversely, the IVR only had a loss of the shorter allele (Fig. S1, patient #26). It is possible that tumor cells of the primary tumor had seeded or migrated to the bladder in the possession of LOH of the shorter allele of D9S171. Due to evolution, the UTUC might have lost the other allele, contributing to a discordant pattern of this marker between the 2 tumors. However, an identical LOH pattern is considered an indication of clonality [23], whereas identical point mutations in the *TP53* gene are considered even a stronger indication because many possible point mutations exist that lead to inactivation of *TP53*. Hence, we concluded that all tumors were clonally related (Fig. S1, patient #25−#29).

Du et al. analyzed by *whole exome sequencing* (WES) 3 cases: 1 female patient had 3 synchronous UTUCs and 2 IVRs; the other 2 patients each had 1 UTUC and 1 IVR [34] (Fig. S1, patient #30−#32). The 3 UTUCs and 1 IVR shared the same alterations in *TP53, BRAF*, and *APC* genes. The other IVR had a mutation in *MTOR* and shared no alterations with the other tumors, so there was no proof of clonality (Fig. S1, patient #31). One of the 2 other patients showed a clonal relationship of both tumors (Fig. S1, patient #32). The other patient showed no shared alterations and, since Du et al. used WES, this is a strong indication that these tumors were not clonally related (Fig. S1, patient #30).

Audenet et al. applied NGS with the targeted 230 to 468-gene MSK-IMPACT oncopanel to analyze primary UTUCs

of 17 patients who subsequently developed an IVR [35,36]. All paired tumors had identical point mutations, which is a strong indicator of monoclonal origin as the chance that identical point mutations develop independently is highly unlikely. Comparing the somatic mutations between the initial UTUC and the subsequent IVR revealed that 86% of the mutations were present in both tumors. Hence, the additional mutations of the IVR were presumably caused by ongoing tumor evolution (Fig. S1, patient #33−#49).

3.2. Primary UCB and subsequent UTUC

Five of the studies included evaluated the possible clonal relationship in patients diagnosed with primary UCB who subsequently developed a recurrence in the upper urinary tract. Since some patients developed more than 1 UCB and/or UTUC following the primary diagnosis of UCB, a total of 14 patients having 30 UCBs followed by 31 UTUCs (see Table 3) were included. A total of 85.1% of the tumors were considered to be of monoclonal origin [29−32,35].

In 4 of the studies, which included 11 patients with 19 UCBs and 15 UTUC recurrences, all tumors originating from 1 patient had identical alterations, indicating a monoclonal origin [30−32,35]. The studies by Catto et al., Takahashi (2001) et al. and Audenet et al. are discussed in Section 3.1 above [30,31,35]. The techniques used to analyze a clonal relationship did not differ for patients having a primary UCB and a subsequent UTUC. These 3 studies showed all paired tumors to be of monoclonal origin (Fig. S2, patient #4−#6 and #8−#14).

Warrick et al. included 1 patient having 1 UCB and 3 UTUC and found with the use of NGS identical mutations in the genes *HRAS, FLT4, MLL2, NTRK3,* and *PIK3CA* [32]. Copy number analysis and LOH revealed a compatible pattern of gain and loss between the paired tumors (Fig. S2, patient #7).

Hafner et al. included patients having 1 or multiple UCB(s) followed by 1 or more UTUC [29]. Two patients had 1 UCB with 1 subsequent UTUC and both tumors could not be defined as clonally related (Fig. S2, patient #1 and #2). The other patient had multiple urothelial carcinomas, i.e., 9 UCBs with 3 subsequent UTUCs (Fig. S2, patient #3). Clustering, based on the reported molecular markers, showed that multiple UTUC and UCB shared common alterations and these were therefore marked as clonally related (Fig. S3). One UCB had a distinct pattern of alterations, however, and was marked as 'undefined'.

4. Discussion

We conducted a systematic review of the relevant literature on the possible clonal relation of synchronous and metachronous urothelial carcinoma of the upper urinary tract and bladder. Based on the available literature, we concluded that the majority of UTUC and paired UCB had a clonal relation. Literature on this matter, however, was scarce and the techniques used differed significantly between series and over time. Some of the techniques used are nowadays considered less accurate to address a possible clonal relation of 2 tumor entities. Conversely, currently available large-scale sequencing techniques such as NGS or *Whole Genome Sequencing* can much better provide profound evidence whether paired UTUC and UCB samples are of monoclonal origin, as the probability that point mutations occur multiple times independently from

Table 3
Overview of the studies that analyzed patients diagnosed with a primary UCB and who subsequently developed a UTUC, the molecular techniques used, and the proportion of clonally related tumors (n = 14 patients)

	Patients (n)	Number of tumors (n)	Median months to recurrence (range)	Target/Technique	Number of patients with clonally related tumors	Percentage clonally related (%)
Hafner et al., 2001 [29]	3	11 UCB, 5 UTUC	24 (0−43.0)	LOH (chrom 9) TP53 (exons 5-9) MSI (six loci) IHC (MLH1, MSH2)	0.92[a]/3	30%
Takahashi et al., 2001 [30]	1	5 UCB, 3 UTUC	*NI*	LOH (MSI markers): chromosome 9p, 9q, 11p, 17p, 4p, 4q, 2q, 8p; Subchromosomal breakpoints.	1/1	100%
Catto et al., 2006 [31]	2	6 UCB, 2 UTUC	17.0 (0−31.0)	MSI-H; Methylation: *hMLH1, p16, p14, E-cadherin, RARB, RASSF1A, MINT31.*	2/2	100%
Warrick et al., 2015 [32]	1	1 UCB, 4 UTUC	0	NGS (409 genes); LOH; CNV.	1/1	100%
Audenet et al., 2018 [35]	7	7 UCB, 7 UTUC	7.3 (3.9−21.7)	MSK-IMPACT (NGS).	7/7	100%

Abbreviations: CNV = copy number variations, IHC = immunohistochemistry, NGS = next generation sequencing, NI = not informative.
[a] 12 of the 13 (92%) tumors from 1 patient showed a clonal origin.

another is negligible. Hence, the more recent studies included in this review provide more conclusive evidence on clonally related UTUC and paired UCB.

The order of clinical detection of multiple tumors in visceral organs is not always in line with the molecular development of the tumors. This characteristic has previously been proposed for multiple metachronous UCB by van Tilborg et al. [37]. Moreover, clones that derive from a primary tumor of the upper urinary tract could evolve over time and develop additional genomic alterations. An IVR derived from such a clone, however, could be diagnosed prior to the primary UTUC and molecular analysis of both entities will, in such cases, reveal more genomic alterations of the IVR in addition to overlapping mutations. This 'tumor evolution' could also apply to the primary UTUC. Therefore, not all alterations will necessarily be shared by 2 paired tumors due to evolution of tumors, although a large proportion will. Consequently, a 100% overlap of alterations is rarely present in clonally related UTUC and paired UCB, as Audenet et al. demonstrated with an 86% overlap [35]. Therefore, when analyzing recurrences in the urinary tract and when interpreting a clonal or a nonclonal relationship of both entities, one should be aware that the clinical order is not necessarily the molecular order of tumor development [31,37].

The proportion of patients diagnosed with a primary UCB who later developed a UTUC recurrence might be overestimated in the studies included. Twelve of the 29 (41.3%) patients analyzed by Audenet et al. had a primary UCB followed by a diagnosis of UTUC, which is a higher proportion than that reported in the literature (1%−5%) [1,17]. Four of these 12 patients, however, showed a clonal relationship compatible with a previously developed UTUC instead of a primary UCB, as the UCBs showed a surplus of alterations compared to the UTUCs. Therefore, it is possible that the UTUCs originated first and the UCBs were clones or subclones of the UTUC with an accumulation of molecular alterations, and had developed later than the UTUCs.

Whether IVR are formed by seeding/migration of tumor cells originating from the upper urinary tract or by field cancerization remains subject of debate [18−20]. The majority of patients develop an IVR within 2 years following RNU, possibly due to manipulation of the tumor during surgery [11]. This hypothesis of distributing tumor cells by manipulation is further supported by the fact that a diagnostic ureterorenoscopy prior to RNU increases the risk of an IVR [38]. In addition, a systematic review showed that instability of the UTUC, defined by presence of necrosis and positive preoperative urinary cytology, correlated with the risk of IVR [11]. As we found in the present review that 94% of the primary UTUC and IVRs were clonally related, we assume that in primary UTUC patients the most important mechanism of developing an IVR is seeding or migration of tumor cells. However, it is not excluded that field cancerization could contribute to the development of separate entities of urothelial carcinoma in the upper and lower urinary tract.

Analyzing a cohort of 512 UTUCs, Xylinas et al. showed that smoking was significantly associated with the risk of an IVR [39]. Du et al. addressed exposure to the Aristolochic Acid (AA) [34], a widely used herb in Chinese medicine, in a Chinese patient cohort and found that all tumors had predominant T to A transversions in the 5'-CpTpG-3'motif, which is a mutational signature caused by AA [40]. The mutagenic aspect of this herb might contribute to field cancerization in patients and hence to the development of non-clonally related urothelial tumors. Patients with Lynch syndrome (LS) have a higher risk of developing urothelial carcinoma, mainly UTUC [8]. LS is a hereditary cancer syndrome characterized by mutations in mismatch repair genes leading to mismatch repair deficiency and MSI. Possibly, LS could lead to the independent development of UTUC and UCB, but literature is lacking on this matter. One LS patient analyzed by Audenet et al. showed a clonal relation of paired UTUC and IVR *(personal communication F. Audenet)*.

Clonality of primary tumors and metachronous or synchronous intracaval recurrences have been analyzed in malignancies originating from other hollow visceral organs than the urinary tract, such as the lung, colon, and oral cavity. LOH analysis and mutational status of *EGFR, TP53,* and *KRAS* in multifocal lung cancer ($n = 115$) revealed that 64%−79% of multiple synchronous intrapulmonary, mostly nonsmall cell carcinomas (NSCLCs), were clonally related [41−43]. For tumors of the oral cavity, however, it was not clear whether multiple tumors resulted from field cancerization or intraluminal spread [44]. With the use of microarray-based SNP and copy-number genotyping of 104 paired synchronous colorectal cancers, a clonal relationship was found in 36% [45]. Patients with oligoclonal NSCLCs seemed to have a better outcome than patients with NSCLCs of monoclonal origin, which has also been reported for patients with oligoclonal colorectal tumors [43,45]. These data show that clonality of paired tumors originating from the same hollow visceral organ might correspond with clinical outcome. Therefore, it is of importance to investigate this phenomenon in the urinary tract by larger, prospective studies.

In case UTUC and IVR are clonally related, the way is paved for the identification of patient-specific genomic alterations that can be used to develop noninvasive urine-based assays for the diagnostic surveillance following RNU. Cystoscopy, which is invasive and causes discomfort to the patient, might be replaced by this alternative urine-based strategy [46]. Large-scale genomic characterization of UTUC and paired bladder recurrences could also identify new biomarkers that correlate with the risk of a future urinary tract recurrence or clinical outcome and possibly new actionable molecular alterations. With an accuracy of only 62% to 69% of 2 previous designed predictive tools for the risk of IVR development after RNU, addition of biomarkers might provide a better prediction of recurrences [47,48].

5. Conclusion

Patients diagnosed with an urothelial carcinoma of the urinary tract are at increased risk of developing a subsequent tumor throughout the entire urinary tract. Patients with a primary UTUC have the highest risk of developing a future UCB. We systematically reviewed all the relevant literature to address whether UTUC and paired UCB derive from the same progenitor cell or whether they develop independently as a result of field cancerization. The populations studied and the molecular techniques used to assess clonality differed largely between the studies and over time. Taking into account the limitations of microsatellite instability technology versus NGS and the currently accepted concepts of tumor heterogeneity and evolution, we conclude that it is highly likely that UTUC and paired UCB of one patient are clonally related and most likely are formed by seeding of tumor cells.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Supplementary materials

Supplementary material associated with this article can be found in the online version at https://doi.org/10.1016/j.urolonc.2020.01.008.

References

[1] Roupret M, Babjuk M, Comperat E, et al. European Association of Urology Guidelines on Upper Urinary Tract Urothelial Carcinoma: 2017 Update. Eur Urol 2018;73(1):111–22.
[2] Colin P, Koenig P, Ouzzane A, et al. Environmental factors involved in carcinogenesis of urothelial cell carcinomas of the upper urinary tract. BJU Int 2009;104(10):1436–40.
[3] Margulis V, Shariat SF, Matin SF, et al. Outcomes of radical nephroureterectomy: a series from the Upper Tract Urothelial Carcinoma Collaboration. Cancer 2009;115(6):1224–33.
[4] Adibi M, Youssef R, Shariat SF, et al. Oncological outcomes after radical nephroureterectomy for upper tract urothelial carcinoma: comparison over the three decades. Int J Urol 2012;19(12):1060–6.
[5] Hautmann RE, de Petriconi RC, Pfeiffer C, Volkmer BG. Radical cystectomy for urothelial carcinoma of the bladder without neoadjuvant or adjuvant therapy: long-term results in 1100 patients. Eur Urol 2012;61(5):1039–47.
[6] Moss TJ, Qi Y, Xi L, et al. Comprehensive genomic characterization of upper tract urothelial carcinoma. Eur Urol 2017;72(4):641–9.
[7] Sfakianos JP, Cha EK, Iyer G, et al. Genomic characterization of upper tract urothelial carcinoma. Eur Urol 2015;68(6):970–7.
[8] Roupret M, Yates DR, Comperat E, Cussenot O. Upper urinary tract urothelial cell carcinomas and other urological malignancies involved in the hereditary nonpolyposis colorectal cancer (lynch syndrome) tumor spectrum. Eur Urol 2008;54(6):1226–36.
[9] Azemar MD, Comperat E, Richard F, Cussenot O, Roupret M. Bladder recurrence after surgery for upper urinary tract urothelial cell carcinoma: frequency, risk factors, and surveillance. Urol Oncol 2011;29(2):130–6.
[10] Matsui Y, Utsunomiya N, Ichioka K, et al. Risk factors for subsequent development of bladder cancer after primary transitional cell carcinoma of the upper urinary tract. Urology 2005;65(2):279–83.
[11] Seisen T, Granger B, Colin P, et al. A systematic review and meta-analysis of clinicopathologic factors linked to intravesical recurrence after radical nephroureterectomy to treat upper tract urothelial carcinoma. Eur Urol 2015;67(6):1122–33.
[12] Ito A, Shintaku I, Satoh M, et al. Prospective randomized phase II trial of a single early intravesical instillation of pirarubicin (THP) in the prevention of bladder recurrence after nephroureterectomy for upper urinary tract urothelial carcinoma: the THP Monotherapy Study Group Trial. J Clin Oncol 2013;31(11):1422–7.
[13] O'Brien T, Ray E, Singh R, Coker B, Beard R. British Association of Urological Surgeons Section of O. Prevention of bladder tumours after nephroureterectomy for primary upper urinary tract urothelial carcinoma: a prospective, multicentre, randomised clinical trial of a single postoperative intravesical dose of mitomycin C (the ODMIT-C Trial). Eur Urol 2011;60(4):703–10.
[14] van Doeveren T, van Leeuwen PJ, Aben KKH, et al. Reduce bladder cancer recurrence in patients treated for upper urinary tract urothelial carcinoma: the REBACARE-trial. Contemp Clin Trials Commun 2018;9:121–9.
[15] Li WM, Shen JT, Li CC, et al. Oncologic outcomes following three different approaches to the distal ureter and bladder cuff in nephroureterectomy for primary upper urinary tract urothelial carcinoma. Eur Urol 2010;57(6):963–9.
[16] Cosentino M, Palou J, Gaya JM, Breda A, Rodriguez-Faba O, Villavicencio-Mavrich H. Upper urinary tract urothelial cell carcinoma: location as a predictive factor for concomitant bladder carcinoma. World J Urol 2013;31(1):141–5.
[17] Millan-Rodriguez F, Chechile-Toniolo G, Salvador-Bayarri J, Huguet-Perez J, Vicente-Rodriguez J. Upper urinary tract tumors after primary superficial bladder tumors: prognostic factors and risk groups. J Urol 2000;164(4):1183–7.
[18] Harris AL, Neal DE. Bladder cancer−field versus clonal origin. N Engl J Med 1992;326(11):759–61.
[19] Habuchi T. Origin of multifocal carcinomas of the bladder and upper urinary tract: molecular analysis and clinical implications. Int J Urol 2005;12(8):709–16.
[20] Yates DR, Catto JW. Distinct patterns and behaviour of urothelial carcinoma with respect to anatomical location: how molecular biomarkers can augment clinico-pathological predictors in upper urinary tract tumours. World J Urol 2013;31(1):21–9.
[21] Antoni S, Ferlay J, Soerjomataram I, Znaor A, Jemal A, Bray F. Bladder cancer incidence and mortality: a global overview and recent trends. Eur Urol 2017;71(1):96–108.
[22] Raman JD, Messer J, Sielatycki JA, Hollenbeak CS. Incidence and survival of patients with carcinoma of the ureter and renal pelvis in the USA, 1973-2005. BJU Int 2011;107(7):1059–64.
[23] Hafner C, Knuechel R, Stoehr R, Hartmann A. Clonality of multifocal urothelial carcinomas: 10 years of molecular genetic studies. Int J Cancer 2002;101(1):1–6.
[24] Moher D, Liberati A, Tetzlaff J, Altman DG, Group P. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. BMJ 2009;339:b2535.
[25] Ostrovnaya I, Seshan VE, Begg CB. Using somatic mutation data to test tumors for clonal relatedness. Ann Appl Stat 2015;9(3):1533–48.
[26] Jones TD, Wang M, Eble JN, et al. Molecular evidence supporting field effect in urothelial carcinogenesis. Clin Cancer Res 2005;11(18):6512–9.
[27] Takahashi T, Habuchi T, Kakehi Y, et al. Molecular diagnosis of metastatic origin in a patient with metachronous multiple cancers of the renal pelvis and bladder. Urology 2000;56(2):331.

[28] Dalbagni G, Ren ZP, Herr H, Cordon-Cardo C, Reuter V. Genetic alterations in tp53 in recurrent urothelial cancer: a longitudinal study. Clin Cancer Res 2001;7(9):2797–801.

[29] Hafner C, Knuechel R, Zanardo L, et al. Evidence for oligoclonality and tumor spread by intraluminal seeding in multifocal urothelial carcinomas of the upper and lower urinary tract. Oncogene 2001;20 (35):4910–5.

[30] Takahashi T, Kakehi Y, Mitsumori K, et al. Distinct microsatellite alterations in upper urinary tract tumors and subsequent bladder tumors. J Urol 2001;165(2):672–7.

[31] Catto JW, Hartmann A, Stoehr R, et al. Multifocal urothelial cancers with the mutator phenotype are of monoclonal origin and require panurothelial treatment for tumor clearance. J Urol 2006;175(6): 2323–30.

[32] Warrick JI, Hovelson DH, Amin A, et al. Tumor evolution and progression in multifocal and paired non-invasive/invasive urothelial carcinoma. Virchows Arch 2015;466(3):297–311.

[33] Wang Y, Lang MR, Pin CL, Izawa JI. Comparison of the clonality of urothelial carcinoma developing in the upper urinary tract and those developing in the bladder. Springerplus 2013;2:412.

[34] Du Y, Li R, Chen Z, Wang X, Xu T, Bai F. Mutagenic factors and complex clonal relationship of multifocal urothelial cell carcinoma. Eur Urol 2017;71(5):841–3.

[35] Audenet F, Isharwal S, Cha EK, et al. Clonal relatedness and mutational differences between upper tract and bladder urothelial carcinoma. Clin Cancer Res 2019;25(3):967–76.

[36] Cheng DT, Mitchell TN, Zehir A, et al. Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT): a hybridization capture-based next-generation sequencing clinical assay for solid tumor molecular oncology. J Mol Diagn 2015;17(3):251–64.

[37] van Tilborg AA, de Vries A, de Bont M, Groenfeld LE, van der Kwast TH, Zwarthoff EC. Molecular evolution of multiple recurrent cancers of the bladder. Hum Mol Genet 2000;9(20): 2973–80.

[38] Marchioni M, Primiceri G, Cindolo L, et al. Impact of diagnostic ureteroscopy on intravesical recurrence in patients undergoing radical nephroureterectomy for upper tract urothelial cancer: a systematic review and meta-analysis. BJU Int 2017;120(3):313–9.

[39] Xylinas E, Kluth LA, Rieken M, et al. Impact of smoking status and cumulative exposure on intravesical recurrence of upper tract urothelial carcinoma after radical nephroureterectomy. BJU Int 2014;114 (1):56–61.

[40] Hoang ML, Chen CH, Sidorenko VS, et al. Mutational signature of aristolochic acid exposure as revealed by whole-exome sequencing. Sci Transl Med 2013;5(197):197ra102.

[41] Shimizu S, Yatabe Y, Koshikawa T, et al. High frequency of clonally related tumors in cases of multiple synchronous lung cancers as revealed by molecular diagnosis. Clin Cancer Res 2000;6(10):3994–9.

[42] Wang X, Wang M, MacLennan GT, et al. Evidence for common clonal origin of multifocal lung cancers. J Natl Cancer Inst 2009;101 (8):560–70.

[43] Warth A, Macher-Goeppinger S, Muley T, et al. Clonality of multifocal nonsmall cell lung cancer: implications for staging and therapy. Eur Respir J 2012;39(6):1437–42.

[44] Angadi PV, Savitha JK, Rao SS, Sivaranjini Y. Oral field cancerization: current evidence and future perspectives. Oral Maxillofac Surg 2012;16(2):171–80.

[45] Perea J, Garcia JL, Corchete L, et al. Redefining synchronous colorectal cancers based on tumor clonality. Int J Cancer 2018.

[46] van Kessel KE, Kompier LC, de Bekker-Grob EW, et al. FGFR3 mutation analysis in voided urine samples to decrease cystoscopies and cost in nonmuscle invasive bladder cancer surveillance: a comparison of 3 strategies. J Urol 2013;189(5):1676–81.

[47] Ishioka J, Saito K, Kijima T, et al. Risk stratification for bladder recurrence of upper urinary tract urothelial carcinoma after radical nephroureterectomy. BJU Int 2015;115(5):705–12.

[48] Xylinas E, Kluth L, Passoni N, et al. Prediction of intravesical recurrence after radical nephroureterectomy: development of a clinical decision-making tool. Eur Urol 2014;65(3):650–8.