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Clinical-Bladder cancer

Toward noninvasive follow-up of low-risk bladder cancer – Rationale and concept of the Uro*Follow* trial*

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Abstract

Background: Follow-up recommendations for patients with nonmuscle invasive bladder cancer (NMIBC) are largely based upon expert opinion. A growing body of evidence suggests that current follow-up strategies for bladder cancer patients with low and intermediate risk represent overdiagnosis and may lead to overtreatment. The goal of this study is to explore the options of a noninvasive follow-up in patients with pTa G1-2/low-grade NMIBC.

Methods: The risks and options for a urine marker-guided, noninvasive follow-up of patients with pTa G1-2/low-grade NMIBC were defined and the study design for a prospective randomized trial (Uro*Follow*) was developed based upon the current literature.

Results: The investigators postulated that follow-up of patients with pTa G1-2/low-grade NMIBC requires a high sensitivity of urinary tumor markers. However, data from prospective studies with prediagnostic urine samples are scarce, even for approved markers, and cross-sectional studies with symptomatic patients overestimate the sensitivity. So far, cell-based markers (e.g., uCyt+ and UroVysion) in urine appeared to have higher sensitivities and specificities in low-grade NMIBC than urine cytology and markers analyzing soluble tumor-associated antigens. Marker panels are more sensitive than single-marker approaches at the expense of a lower specificity. Given a prospective randomized comparison with a marker sensitivity of 80% compared to usual care with cystoscopy, the sample size calculation yielded that 62 to 185 patients under study per arm are needed depending on different recurrence rates.

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Conclusions: Based upon these findings the Uro*Follow* trial has been designed as a prospective randomized study comparing a noninvasive marker-based (UroVysion, NMP22, urine cytology, and ultrasound) follow-up with the current standard of care over a period of 3 years. © 2020 Published by Elsevier Inc.

Keywords: Urinary tumor markers; Bladder cancer; Low grade; Follow-up; Surveillance; Disease management

Introduction

Current guidelines recommend urethrocystoscopy as the primary tool for the follow-up of patients with nonmuscle invasive bladder cancer (NMIBC) [1−3]. Exemplified by looking at the EAU guidelines, frequency of cystoscopy and imaging as well as duration of follow-up should be based on the patient's individual risk. Whereas recommendations for high-risk patients (pTa/T1 G3/high-grade tumors, carcinoma in situ [pTis], and NMIBC > 3 cm) are more detailed, the suggestions become less stringent for low-risk patients (primary, unifocal pTa G1, and <3 cm) and are even unclear for the follow-up of patients with intermediate risk (others). So far, patients with low-risk disease should undergo cystoscopy 3 and 12 months after primary transurethral resection of the bladder (TURB) followed by annual cystoscopies until year 5. For the patients with intermediate-risk lesions an unspecified "individualized" follow-up scheme using cystoscopy is recommended.

It is recognized that recurrence in patients with low-risk disease has a very high likelihood of being of low stage and low grade (low grade/G1) and that recurrent small, pTa/G1 low-grade papillary lesions neither represent an immediate threat nor is the early detection essential for the fate of the disease [4,5]. For these patients, office-based fulguration of recurrent small papillary lesions [6] or surveillance in selected cases [5,7,8] is even discussed. Thus, especially in this latter group of patients, strategies to avoid unnecessary invasive procedures are highly warranted to decrease disease burden (increase quality of life [QoL]) and to reduce costs [9].

The statement that "*recommendations for follow-up are mainly based on retrospective data and there is a lack of randomized studies investigating the possibility of safely reducing the frequency of follow-up cystoscopy*" [1] highlights the current dilemma: It is a general rule in oncology to diagnose and treat tumor recurrence as early as possible. The rationale behind this concept is to prevent potential tumor progression and tumor-specific mortality. For bladder cancer, this concept appears reasonable in patients with high-grade NMIBC being at risk of developing fatal tumor progression and metastasis. However, due to the low risk of tumor progression in patients with low/intermediate risk the relevance of intense and invasive routine follow-up must be questioned.

Despite many efforts over the last decades to develop experimental and commercial assays for urinary tumor markers and the fact that the U.S. Food and Drug Administration has approved urine-based testing for bladder cancer, there is common agreement that markers cannot replace cystoscopy [1−3,9−11]. However, noninvasive diagnosis using urine cytology or markers might be helpful in tumor surveillance and guiding the use of invasive cystoscopy. Urine is in contact with the tumor lesion and very simple to obtain for investigation. Furthermore, cell adherence is decreased in tumor cells resulting in preferential shedding of malignant cells into urine. In general, this concept of shedding tumor cells thus markers into urine looks attractive, sparing at least a significant part of patients from potentially unnecessary invasive cystoscopy. However, it remains doubtful if the performance of urine cytology and currently available urine markers is sufficient in this situation with mostly very small tumors exerting low grades of anaplasia [3,10,12,13].

The Uro*Follow* trial is the first prospective randomized study comparing noninvasive follow-up using commercially available urine markers along with abdominal ultrasound (US) vs. standard of care (SOC) based on routine cystoscopy. The primary aim of Uro*Follow* is to investigate whether commercially available marker assays can guide a noninvasive follow-up regimen of patients with recurrent pTa G1-2 NMIBC. This manuscript summarizes the considerations and rationale behind the study design.

Trial design

Defining the patient population to safely implement follow-up using urine-based markers

Although the target population harboring low-risk lesions may have the lowest probability of tumor progression even if a recurrent tumor is missed at the first re-investigation, a residual risk of tumor progression cannot entirely be ruled out. Therefore, it was mandatory for the investigators to calculate this residual risk as precisely as possible. Best data available so far can be obtained from risk tables developed by the European Organization for Research and Treatment of Cancer (EORTC) [14]. The EORTC XP calculator is based upon data obtained from approximately 2,600 patients from 7 prospective randomized trials and thus should provide the best estimate of progression rates even though the underlying trials have been performed some 25 years ago. Nearly 80% of patients received intravesical treatment, mostly chemotherapy. Notably, patients did not undergo Re-TURB or received Bacillus Calmette-Guerin treatment. This calculator

predicts for intermediate-risk tumors (defined as multifocal, recurrent pTa G2 disease with <3 cm diameter, and without concomitant pTis) a progression risk of 4% in the first 3 years. In low-risk tumors (primary, unifocal pTa G1 with <3 cm diameter, and without concomitant pTis) the corresponding risk is less than 1%. As this calculator has not been validated for the 2004 WHO tumor grade classification, risk group definition in the Uro*Follow* trial followed the 1973/1998 classification [15].

Study endpoints

The primary endpoint of Uro*Follow* is to investigate, if marker-guided (noninvasive) follow-up of patients with low- and intermediate-risk NMIBC is equally effective to detect tumor recurrence and progression compared to the SOC based on cystoscopy and urine cytology as optional adjunct. Equal effectiveness was defined as detecting not less than 80% of recurrences in the control arm with a delay not exceeding 6 months. Additional (secondary) study endpoints are validation of candidate markers with a prospective study design, the number of cystoscopies saved, the QoL using the EORTC QLQ-NMIBC questionnaire module to assess patient-reported overall QoL in both arms, and patient compliance in the marker arm.

As patient safety appeared of utmost importance a stopping rule in case of the occurrence of 2 patients with undetected progression to muscle invasive bladder cancer in the marker arm was implemented.

Study population and randomization

Patients with pTa G1/G2 NMIBC, a tumor size less than 3 cm, and with no accompanying pTis are eligible for participation in Uro*Follow*. Patients after Bacillus Calmette-Guerin treatment or persisting bladder cancer 3 months after TURB or Re-TURB (as assessed by cystoscopy) are not considered. Eligible patients are randomized (1:1) to either usual-care or the marker arm and further stratified according to primary or recurrent tumor and tumor grade (G1/G2) within each center (Fig. 1).

*The Uro*Follow *test battery*

Selection of markers to guide follow-up

A noninvasive surveillance strategy in low-grade tumors must meet specific requirements. As the general risk of tumor progression is low in this study population, markers should detect recurrence of low-risk NMIBC of moderate and clinically relevant size. In addition, it is of importance not to miss high-grade tumor recurrence or progression to muscle invasive tumors.

The sensitivity of almost all urine markers correlates with tumor grade [12,16–18]. Therefore, for urine cytology and the majority of all commercially available assays, reported marker sensitivity in G1 urothelial cancer is moderate to poor but increases with higher tumor grade. Selection of suitable markers with sufficient sensitivity in G1-2 NMIBC

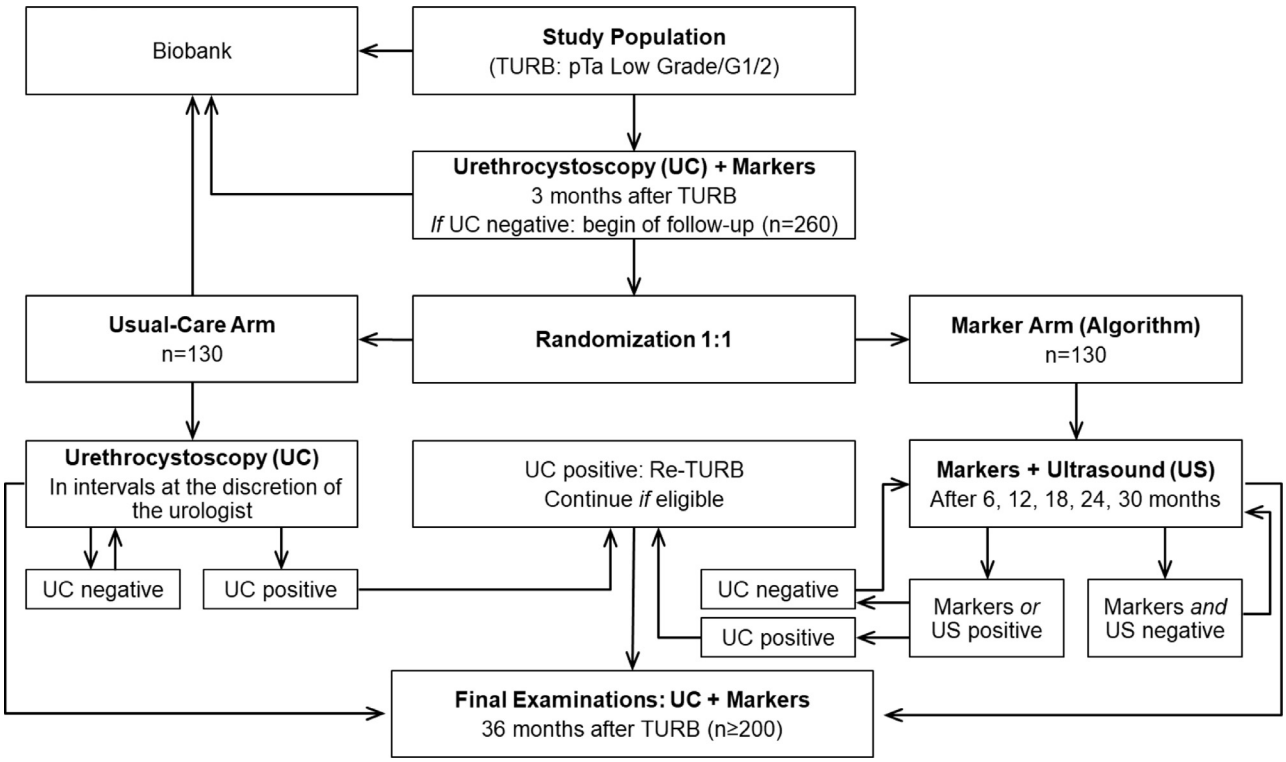

Fig. 1. The Uro*Follow* study design.

obviously represents a specific challenge of noninvasive surveillance.

There is some evidence that smaller tumors (i.e., very early recurrent lesions) might be more difficult to detect prior to the occurrence of symptoms by urine markers [12,19]. A retrospective longitudinal study on 36 patients undergoing follow-up for pTa G1-2 disease using immunocytology (uCyt+) found a positive uCyt+ assay in 12 out of 13 primary tumors (92.3%) but a decreased sensitivity (13 out of 20, 65%) in recurrent tumors [20]. The authors hypothesize that the smaller size of recurrent lesions is responsible for this difference. Prospective studies analyzing prediagnostic urine samples are scarce. The study UroScreen demonstrated in chemical workers that cytology and UroVysion achieved sufficient sensitivity within the year before diagnosis of bladder cancer [13].

The identification of markers with sufficiently high sensitivity appears even more demanding given the estimates of their performance from cross-sectional studies, including those of poorer epidemiological quality [12,13,18]. Larger tumors in symptomatic patients overestimate the sensitivity of markers as compared with the detection of tumors prior to the occurrence of symptoms [18]. Characterized by heterogeneous study populations, inadequate cross-sectional designs, and sometimes poor reporting of methodology, reference standards, and procedures, published sensitivities can hardly be used as reliable estimates for the performance in prediagnostic urine samples or even for systematic reviews and meta-analyses [13]. For cell-based assays, such as urine cytology, UroVysion, and uCyt+, the results may be further biased by varying expertise of the reporting centers [11,12]. In addition, comparative marker analyses are rare.

Table 1 summarizes information on the sensitivity of urine cytology and selected commercially available urine markers from previously published reviews, mostly on cross-sectional studies [12,16,17]. As information from high-quality prospective trials is missing, current reviews represent the only source for selecting appropriate markers although they just permit a raw estimate of marker performance. After narrative review of the literature the authors concluded that cell-based assays might be more sensitive to detect G1/G2 tumors if compared with assays targeting tumor-associated antigens in urine. This aspect along with the required expertise of participating centers conducting cell-based assays triggered the decision for including the UroVysion and the uCyt+ assay in the diagnostic algorithm.

As the sensitivity of a single assay was assumed to be insufficient, multi-marker testing was implemented to improve sensitivity. The hazard of multi-marker testing is a decreased overall specificity because each single positive test triggers an additional cystoscopy. However, this hazard was accepted because an unnecessary cystoscopy would be the only consequence of a false-positive marker result. Nevertheless, the specificity when testing multiple markers should still be sufficiently high in order to reduce the number of cystoscopies in cancer-free patients in the marker arm.

Information on multi-marker testing is rare. In the prospective UroScreen study, combinations of cytology, UroVysion, and NMP22 showed an increased sensitivity in the surveillance of chemical workers, particularly in urine samples collected within 12 months prior to the occurrence of symptoms [13]. In a cross-sectional study comprising 483 patients with a history of NMIBC who received testing for cytology, UroVysion, NMP22, and uCyt+, the combined use of these markers led to an increased detection of recurrent tumors and, particularly, high-grade tumors [21]. Combinations of NMP22 with either cytology, UroVysion, or uCyt+ yielded sensitivities of 87%, 88%, and 90%; whereas the sensitivities of cytology, UroVysion, NMP22, and uCyt+ as stand-alone markers were only 69%, 74%, 66%, and 70%, respectively. It is of note that the high number of false-positive NMP22 tests in the 2-marker combinations decreased the specificity to 41%, 39%, and

Table 1
Sensitivity and tumor grade for selected urine markers as reported by different reviews (modified after [12])

Marker (reference)	Number of studies considered	Grade 1 (range)	Grade 2 (range)	Grade 3 (range)
Cytology				
Lotan [17]	8	12 (4–31)	26 (17–37)	64 (38–84)
Van Rhijn [16]	9	17	34	58
BTA stat				
Lotan [17]	8	47 (38–56)	73 (58–83)	94 (55–99)
Van Rhijn [16]	7	45	60	75
NMP22 (ELISA/bladder check)				
Lotan [17]	7	61 (35–81)	71 (41–90)	79 (63–89)
Van Rhijn [16]	3	41	53	80
uCyt+/Immunocyt				
Van Rhijn [16]	1	78	90	100
Schmitz-Dräger [12]	19	75	84	84
FISH (Urovysion)				
Van Rhijn [16]	2	56	78	95
Schmitz-Dräger [12]	21	53	81	79

38%, respectively. Furthermore, in the context of Uro*Follow* with a need not to miss a G3/high-grade tumor recurrence a high negative predictive value for high-grade disease is mandatory. Using a combination of cytology, UroVysion, and NMP22, a negative predictive value of 99% for G3 disease was observed in this study [21].

A retrospective analysis of urine marker combinations in Uro*Follow* may allow the calculation of optimal cut-offs for different combinations. This can be done using receiver operating characteristics curve analysis defining an optimal threshold in a test model. In a study with 808 patients without a history of bladder cancer, cytology, UroVysion, uCyt+, and NMP22 [22] showed a better performance of using marker combinations rather than single markers. However, specific 2-, 3- and 4-test combinations with only 1 marker positive (mostly NMP22) had to be considered as a negative test result. In summary, based upon the expectation that marker sensitivity may be overestimated in cross-sectional studies, considering the reduction of specificity due to false-positive NMP22 tests, and high requirements concerning the detection of progression, Uro*Follow* has been designed as a multi-marker study.

Based upon the reported sensitivities and the necessary expertise of participating centers for G1/G2 NMIBC, UroVysion, and uCyt+ were chosen as cell-based assays (Table 1). NMP22 has been added to include a widely used assay which is not restricted to specialized laboratories. Due to the high fraction of false-positive tests [13,23], particularly in patients with urinary tract infections, positivity of NMP22 was considered for diagnostic work-up in leukocyte-free urines only. The ELISA version was included as a quantitative adjunct because it has provided more robust performance in previous investigations [24] and to avoid that the point-of-care assay performed at the study centers might affect cystoscopy findings [25]. Urine cytology was added to detect potential G3/high-grade tumor recurrence. Markers were complemented by US examination of the bladder to exclude the presence of larger lesions.

Prospective studies last over several years and are prone to changes in the availability and the production of biomarker kits [18]. After the production of the uCyt+ assay has been discontinued by the manufacturer in 2016 the study investigators and the data safety and monitoring board agreed to continue the study without this assay. The rationale for this decision was that a diagnostic overlap between uCyt+ and the UroVysion assay, both cell-based markers, could be assumed [21,22].

"New markers"

Facing the general lack of prospective studies in marker research, the Uro*Follow* trial was also considered a platform for validation of new innovative markers targeting different tumor-related changes at different molecular levels also including morphological technologies (Table 2). For consideration of experimental assays certain requirements had to be fulfilled which include good sensitivity in G1/G2 NMIBC, an innovative approach, and the fact that markers should be directed toward targets not covered by the existing panel.

We rephrased: Performance of the new markers will be calculated a posteriori. The findings will be related to the next cystoscopy (indicated by positive marker results or end of study cystoscopy) and considered as positive or negative in an anticipatory way. Direct comparison between the new markers will identify optimal markers or marker combinations. It is obvious that these simulations represent ex posteriori analyses and will require further validation.

Morphology: CellDetect is a unique histochemical stain enabling color and morphological discrimination between malignant and benign cells based on differences in metabolic signature [26]. Raman and coherent anti-Stokes Raman scattering microscopic imaging techniques are label-free approaches that characterize cellular samples with high spatial and lateral resolution. The pixel spectra of these microscopic images reflect the biochemical composition

Table 2
Summary of new or experimental urine markers included in the Uro*Follow* trial

Assay [reference]	Target/technique	Manufacturer/distributor
Morphology		
CellDetect [26]	Cytologic special staining	Zetiq, Tel Aviv, Israel
Raman and coherent anti-Stokes Raman scattering [27]	Microscopic imaging	Department of Biophysics, Bochum
Protein level		
UBC rapid [28]	Cytokeratine 18/20 expression	Concile, Freiburg, Germany
Survivin [29]	ImmunoPCR	Experimental, IPA*, Bochum, Germany
RNA level		
Xpert bladder cancer monitor [30]	mRNA signature	Cepheid, Europe, Maurens-Scopont, France
DNA level		
Automated image analysis (FISH technique) [31]	Polyploidy, aneuploidy, copy number variations	Experimental, IPA*, Bochum, Germany
FGFR3 [32]	Somatic DNA mutations	Experimental, IPA*, Bochum, Germany
hTERT [33]	Somatic DNA mutations	Experimental, IPA*, Bochum, Germany

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of the specimen at the corresponding pixel position. These methods are very suitable to monitor molecular changes in urothelial cells, enabling the differentiation between cancerous and noncancerous cells [27].

Protein level: The UBC Rapid Test measures soluble fragments of cytokeratins 8 and 18 in urine [28]. Survivin plays a crucial role in cell division particularly during the development of the fetus and in the onset and progression of most tumors. Antibodies generated from recombinant survivin were utilized to develop a new sandwich ELISA [29].

mRNA level: A newly developed urine assay, Xpert Bladder Cancer Monitor (Xpert), measures 5 mRNA targets (ABL1, CRH, IGF2, UPK1B, and ANXA10) that are frequently overexpressed in BC [30].

DNA level: A modified UroVysion assay that detects DNA gain at chromosomes 3, 7, and 17 and loss at the 9p21 locus in urothelial cells permitting automated analysis of large cell numbers was developed and added to the trial [31]. *FGFR3* and *hTERT* alterations represent frequent genetic changes in bladder cancer and have been suggested to be suitable for surveillance in patients with tumors harboring the respective alterations [32,33].

Overall, comprising more than 10 different urine markers ("decision-triggering" and experimental) and the resources for future marker research using supernatant and DNA from the biobank (Fig. 2), the Uro*Follow* trial may be considered the currently largest platform investigating urine markers in the follow-up of patients with previous pTa G1/G2 NMIBC.

Follow-up intervals

Eligible patients were investigated with cystoscopy and markers 3 months after TURB. Cancer-free patients were randomized and patients in the usual-care arm were investigated in intervals chosen at the discretion of the respective urologist. Urologists were informed about the current guidelines for follow-up of bladder cancers [2]. Based upon these recommendations patients have to undergo up to 10 cystoscopies.

The screening intervals for marker testing over the planned 3-year follow-up period also had to be defined. Similar to the SOC, prospective randomized trials aimed at determining optimum intervals are missing and guideline recommendations are based on expert opinion. Therefore, the definition of screening intervals in the marker arm had to be arbitrary. In general, screening intervals should be based on the growth rate of the disease [18]. Nevertheless, again, data about the tumor volume doubling time are scarce. Assuming that the current 3-month interval in this tumor entity results in overdiagnosis and overtreatment and

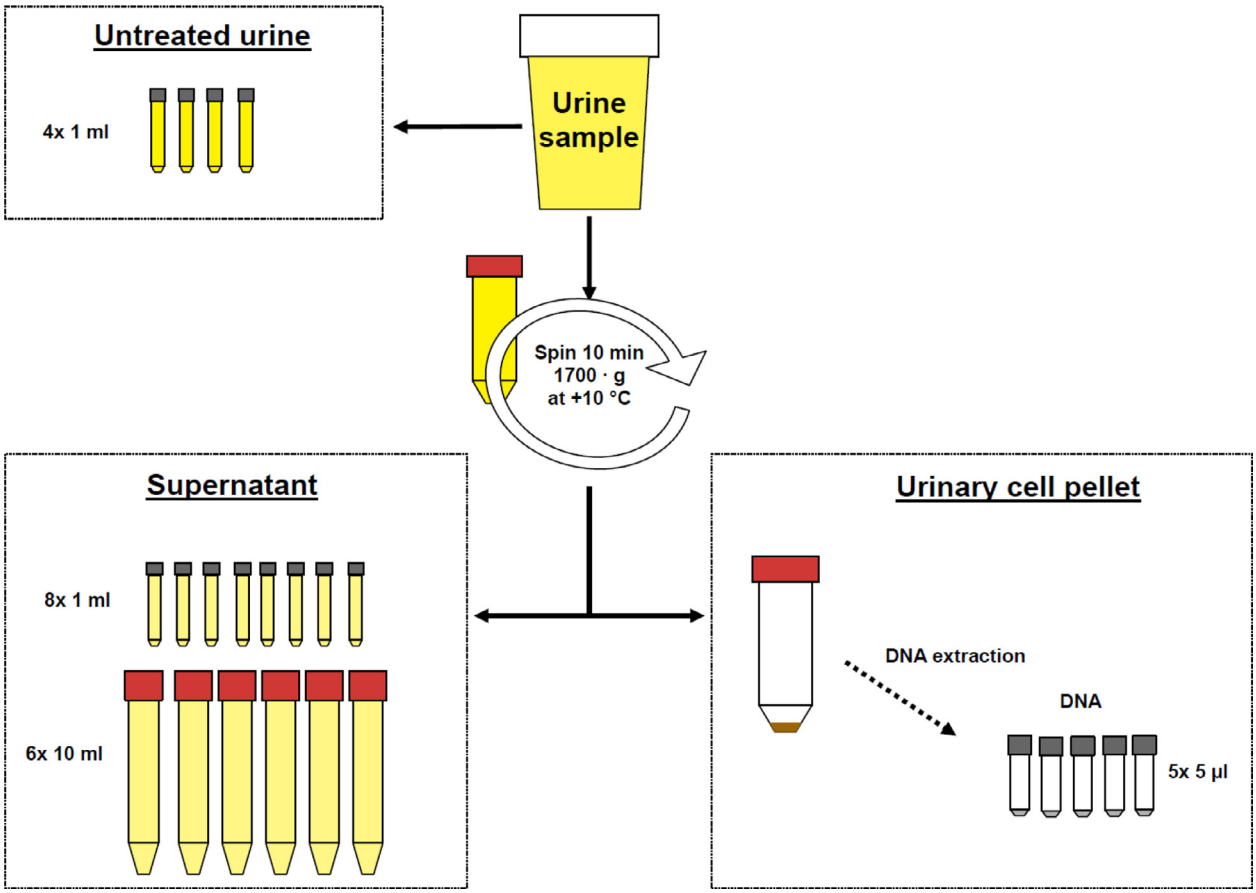

Fig. 2. The Uro*Follow* biorepository.

considering the low tumor progression rate, it was decided to perform marker determinations every 6 months. This screening interval could result in up to 6 possible cystoscopies in case of positivity of the decision algorithm for diagnostic work-up. To decrease the risk of a delayed detection, 3-monthly visits were permitted in both arms with urine analysis (dip stick, sediment, cytology according to the physicians' decision) and US of the bladder in the marker arm.

Power calculation

The power calculation (sample size calculation) based upon a marker sensitivity of 80% compared to cystoscopy yielded numbers between of 62 and 185 evaluable patients per arm depending on different recurrence rates (Table 3). The EORTC risk tables suggest 3-year recurrence rates between 25% (primary, unifocal, and small pTa G1 tumor without concomitant pTis) and up to 75% (recurrent, multifocal, and small pTa G2 tumor without concomitant pTis). These numbers include a persister rate of about 10% at 3 months after TURB, when randomization is performed in cancer-free patients into the 2 arms. Assuming an average recurrence rate of 30% between months 3 and 36, we considered recruitment of 124 patients per arm sufficient to answer the primary objective of the trial.

Comparison between marker arm and control arm will include equivalence and noninferiority testing. Marker performance over time and an imbalance between the number of cystoscopies and the number of marker analyses are challenges of the prospective design with serial measurements.

Urine and tumor biorepository

As prospective randomized trials are laborious, expensive and long lasting and therefore the bottleneck in marker research, it is reasonable to collect and store samples for future investigations [18]. In addition, collections of consecutive prediagnostic samples within the framework of a trial are extremely rare and provide a valuable source for the validation of novel markers. Therefore, storage of urine samples has been added the Uro*Follow* trial.

Fig. 2 provides an overview on biobanking in Uro*Follow*. Voided urine is collected, cooled down to 4 to 10°C and immediately shipped to the central laboratory. Aliquots of the samples are either stored untreated or centrifuged (800 *g*/10 min). Supernatants are stored without fixation, while the urinary cell pellets are used for DNA isolation. All samples are stored at −80°C. The accompanying information of each sample is documented in the laboratory information management system. A consortium comprising the principal investigators of the Uro*Follow* trial will decide on project proposals.

Standard of care

Considering the low levels of evidence and therefore the resulting low recommendation grade the Uro*Follow* coordinators did not implement a guideline as a SOC comparator to the marker arm. Uro*Follow* trialists were recommended performing follow-up in the SOC arm along the best available evidence and existing guideline recommendations [2]. The procedure in daily practice is recorded and will be analyzed. As an imbalance between the number of cystoscopies and the number of marker analyses will necessarily result from the trial design, correction for this effect will be performed using mixed effect models.

Discussion

With the high costs of surveillance on recurrent tumors and the recent advent of immunotherapy, bladder cancer research has regained large interest. Through the last years, multiple high-quality trials focusing on new therapeutic approaches including checkpoint inhibition or other targeted approaches have been published [34]. Despite this enormous development, comparable research addressing early diagnosis and noninvasive surveillance of bladder cancer is still largely underrepresented.

From a patient's perspective, reducing the frequency of invasive cystoscopies during surveillance of low-grade disease would be highly desirable as more than one third of patients undergoing cystoscopy report pain and discomfort [35]. However, the use of molecular markers in urine of follow-up patients with NMIBC lacks sufficient evidence from prospective studies and is therefore not recommended by current guidelines so far [1−3]. Reported performance of markers suggests that replacement of cystoscopy may be possible in low-risk NMIBC. However, these estimates are mainly derived from cross-sectional studies, and the concept has not been proven by prospective randomized comparison using cystoscopy as current usual care.

Although the risk of tumor progression appears to be very low in patients with low-grade bladder cancer it must be taken into account that the EORTC risk calculator might underestimate this risk as the underlying data originate

Table 3
Power and sample size calculation for the Uro*Follow* study

Difference between both study arms	No of patients required (per arm)			
	Recurrence rate 20%	Recurrence rate 30%	Recurrence rate 45%	Recurrence rate 60%
20%	185	*124*	83	62

from patient cohorts being diagnosed over 3 decades ago. While some validation studies confirm the prognostic accuracy [36,37], a report by an Australian group suggests higher progression rates in pTa G1/G2 patients [38]. To address this problem, a trial termination criterium has been included in the protocol if two patients in the marker arm develop progression to muscle invasive tumors that are not detected during noninvasive follow-up by test battery and US.

The underlying power calculation is highly dependent on the estimation of the tumor recurrence rates, which widely vary by tumor grade, size, focality, and other factors. Therefore, an overestimation of tumor recurrence would pose a risk for achieving the primary study endpoint because the study would be, consequently, underpowered, if the observed recurrence is lower than expected. The underlying power calculation for Uro*Follow* was based on previously published estimates of historic recurrence rates and on marker sensitivity reports from mostly cross-sectional studies (EORTC risk tables). Although a recent publication [36] suggests concordance of tumor recurrence rates in a contemporary German cohort with those of the EORTC risk tables, it must be taken into account that more recent changes in tumor management (e.g., blue-light TURB, routine re-TURB, and immediate postoperative chemotherapy) might also affect recurrent tumor rates [39,40]. Nevertheless, even in the case of overestimation, with obvious consequences for the statistical power of the trial, the prospectively collected data in Uro*Follow* will be a valuable source of information on other facets of follow-up of patients with low- and intermediate-risk NMIBC, in particular on those of the secondary study endpoints and of marker research within the framework of a prospective study design by using serial prediagnostic samples.

There is evidence that patients with positive markers and negative cystoscopy will eventually develop tumor recurrence during follow-up. This might lead to an underestimation of marker performance, primarily in cross-sectional designs [41]. This event has been reported to occur within the following 6 to 24 months [13,20,42]. As Uro*Follow* is a longitudinal study with serial marker measurements over 3 years, visible tumor recurrence in anticipatory positive cases should be detected in most patients.

A G3/high-grade recurrence of a G1-2 NMIBC picked up by cytology is rare. Indication for upper urinary tract assessment and bladder biopsy (systematic, blue light-guided) in patients with positive cytology follows current guidelines in both arms. As cytology is mandatory in the marker arm and optional in the control arm, there is a possibility that cytology-based assessment of the upper urinary tract and/or bladder biopsy might occur more frequently in the marker arm.

Doubt has been expressed concerning previous reports on the sensitivity and specificity of urine markers [3,10,12,13] as marker performance may be biased by a long list of potentially relevant factors. Until today, cross-sectional study designs, ill-defined patient cohorts, and study endpoints are methodological shortcomings in marker research [18]. This list is complemented by a small sample size in many studies, ex-post cut-off definition, and unspecified expertise for cell-based assays. The deficits of reporting quality raise further doubts on the validity of marker studies in general, particularly in older publications [43]. Despite this, even today good laboratory practice is largely neglected in many studies and quality criteria as postulated by the "Quality Assessment of Diagnostic Accuracy Studies included in Systematic Reviews" group are heavily underused [44]. These considerations underline the urgent need of prospective randomized trials.

So far, only 1 randomized study investigating the role of urine markers (microsatellite analysis) of patients under surveillance for intermediate-/high-risk NMIBC has been published: the Cost-Effectiveness of Follow-Up of Urinary Bladder Cancer (CEFUB) trial has convincingly demonstrated that the knowledge of a positive test result significantly improves sensitivity of office cystoscopy [25]. Based upon the CEFUB results, it appears reasonable to conclude that, particularly in high-risk tumors, upfront urine cytology and/or a molecular marker assay will improve early detection of tumor recurrence.

The Uro*Follow* trial represents another prospective marker study with important differences when compared to the CEFUB trial: (1) In contrast to the CEFUB trial, the primary goal of Uro*Follow* is to provide evidence if markers may safely replace unnecessary cystoscopies. (2) In order to permit translation of the results into daily practice, Uro*Follow* focuses on commercially available approved markers. Although not triggering decision making, the validation of candidate markers will generate important information and are valuable adjuncts to the trial. (3) Examination of the current SOC and the idea of optimizing current surveillance strategies for low-risk bladder cancer represent another focus of the Uro*Follow* trial. The trial is currently in progress with a total of 168 eligible patients included per October 31st, 2019.

Authors Contribution

Natalya Benderska-Söder: study coordination, manuscript drafting; Jan Hovanec: study coordination, manuscript drafting; Beate Pesch: study concept, supervision, manuscript drafting; Peter Goebell: study concept, manuscript drafting; Florian Roghmann: patient recruitment, manuscript drafting; Joachim Noldus: study concept, manuscript drafting; Juri Rabinovich: patient recruitment, manuscript drafting; Katharina Wichert: data management and statistics, manuscript drafting; Jan Gleichenhagen: development of the Survivin assay and biobank, manuscript drafting; Heiko U. Käfferlein: study concept, manuscript drafting; Christina Köhler: automated analysis of the UroVysion results, manuscript drafting; Georg Johnen: survivin assay, manuscript drafting; Karoline Kernig: patient recruitment, manuscript drafting;

Oliver Hakenberg: study concept, manuscript drafting; Daniela Jahn: NMP22 analysis, manuscript drafting; Tilman Todenhöfer: patient recruitment, manuscript drafting; Arnulf Stenzl: study concept, manuscript drafting; Jochen Gleissner: patient recruitment, manuscript drafting; Klaus Gerwert: study concept, manuscript drafting; Samir El-Mashtoly: Raman and coherent anti-Stokes Raman scattering studies, manuscript drafting; Thomas Behrens: study concept, supervision, manuscript drafting; Thomas Brüning: study concept, supervision, manuscript drafting; Bernd J. Schmitz-Dräger: study concept, principal investigator, patient recruitment, manuscript drafting.

Disclosures

BJSD is consultant and trialist for Cepheid Europe, speaker and trialist for Concile, Freiburg, and speaker and trialist for Zetiq, Tel Aviv, AS is trialist for Cepheid Europe and for Concile, Freiburg, Germany.

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