



Reduce bladder cancer recurrence in patients treated for upper urinary tract urothelial carcinoma: The REBACARE-trial



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ABSTRACT

Background: Following radical nephro-ureterectomy for urothelial carcinoma of the upper urinary tract (UUT), the reported bladder recurrence rate of urothelial carcinoma is 22–47%. A single intravesical instillation of chemotherapy within 10 days following nephro-ureterectomy has the potential to decrease the risk of a bladder recurrence significantly. Despite recommendation by the European Association of Urology guideline to administer a single instillation postoperatively, the compliance rate is low because the risk of extravasation of chemotherapy.

Aim: To reduce the risk of bladder cancer recurrence by a single intravesical instillation of Mitomycin immediately (within 3 h) before radical nephro-ureterectomy or partial ureterectomy.

Methods: Adult patients (age ≥ 18 years) with a (suspicion of a) urothelial carcinoma of the UUT undergoing radical nephro-ureterectomy or partial ureterectomy will be eligible and will receive a single intravesical instillation of Mitomycin within 3 h before surgery. In total, 170 patients will be included in this prospective, observational study. Follow-up will be according to current guidelines.

Results: The primary endpoint is the bladder cancer recurrence rate up to two years after surgery. Secondary endpoints are: a) the compliance rate; b) oncological outcome; c) possible side-effects; d) the quality of life; e) the calculation of costs of a single neoadjuvant instillation with Mitomycin and f) molecular characterization of UUT tumors and intravesical recurrences.

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Conclusions: A single intravesical instillation of Mitomycin before radical nephro-ureterectomy or partial ureterectomy may reduce the risk of a bladder recurrence in patients treated for UUT urothelial carcinoma and will circumvent the disadvantages of current therapy.

Abbreviations

CIS	<i>Carcinoma in situ</i>	MMC	Mitomycin
CT	Computer Tomography	RNU	Radical Nephro-Ureterectomy
CTCAE	Common Terminology Criteria for Adverse Events	TNM	Tumor Node Metastasis classification of Malignant Tumors
DNA	Deoxyribonucleic acid	UCB	Urothelial Carcinoma of the Bladder
IRB	Institutional Review Board	UUT	Upper Urinary Tract
METC	Institutional Review Board (IRB); in Dutch: Medisch Ethische Toetsing Commissie (METC)	WHO	World Health Organization

1. Introduction

Urothelial carcinoma of the upper urinary tract (UUT) is a relatively rare disease with an incidence of 2 per 100,000 person/year in Europe [1]. At diagnosis 60% of UUT tumors are invasive versus only 15–25% for urothelial carcinoma of the bladder [2,3]. The outcome of UUT urothelial cancer is rather poor: the 5-year survival rate following radical nephro-ureterectomy (RNU) varies from less than 50% for pathological stage pT2 or pT3 disease versus less than 10% for pT4 disease [3]. The characteristics of high-risk UUT disease are: high-grade tumor at biopsy, multifocality, positive urinary cytology, transmural disease, hydro-nephrosis on imaging and a tumor size ≥ 1 cm [4–6]. For high-risk urothelial carcinoma of the UUT, RNU with excision of the ipsilateral bladder cuff is the treatment of choice, either by open or minimally invasive surgery [2].

Despite this radical surgical procedure, the bladder recurrence rate at two years following RNU for UUT urothelial carcinoma varies from 22 to 47% [4,7–9]. A recent study showed that 70% of these recurrences occurred in the first year following RNU [10]. Risk factors for a bladder recurrence following RNU are previous bladder cancer, tumor multiplicity, tumor location, tumor stage, and the operative modality [7,11]. For the prediction of intravesical recurrences following RNU, two studies designed predictive tools with an accuracy of 62%–69%. This indicates the difficulties in predicting which patients will develop subsequent bladder recurrences [11,12].

Recently, two randomized controlled trials have shown that a postoperative intravesical instillation of chemotherapy reduced the risk of a bladder recurrence following RNU [8,9]. A meta-analysis of these two studies showed that an intravesical instillation with chemotherapy within 10 days following RNU decreased the risk of bladder recurrence with 52%; the absolute risk reduction was 13% [10]. Despite the fact that the European Association of Urology (EAU) guideline recommends a single postoperative intravesical instillation with chemotherapy based on the result of these two studies [2], the compliance rate in clinical practice to this additional treatment is low. A survey among Dutch urologists showed that only 10% actually administers a postoperative instillation [10]. This reluctance is mainly due to the fact that a fresh wound is present in the bladder, which could lead to extravascular leakage of chemotherapy and with that potential life-threatening sequelae [13].

Here, we present the REBACARE study, in which patients receive a single intravesical instillation with chemotherapy just before RNU or partial ureterectomy for an UUT urothelial carcinoma. As subsequent bladder recurrences probably result from intraluminal seeding and the implantation of cancer cells [14,15], a preoperative instillation with chemotherapy could eradicate possible seeding of cancer cells in the

bladder. This neoadjuvant strategy has previously been shown to be effective in the treatment of non-muscle invasive bladder cancer using device-assisted instillations of Mitomycin [16]. The approach of a single instillation with chemotherapy before surgery has the following advantages: i) it will circumvent the possibility of extravascular leakage of chemotherapy; ii) it will spare the patient an invasive diagnostic procedure (cystogram); and iii) it could result in a better compliance of urologists.

2. Study design

2.1. Study management

The REBACARE study is designed as a multicenter, prospective, non-randomized cohort study in a clinical setting. Inclusion of patients will take place from September 2017 till December 2019. The estimated end of the study is December 2021, two years following RNU or partial ureterectomy of the last included patient. The follow-up will be in accordance with the 'EAU guideline for the treatment of upper urinary tract urothelial carcinoma' in which the surveillance regimen consists of cystoscopy, urinary cytology, and CT urography scans [2]. Only bladder recurrences (urothelial carcinoma) within two years following surgery will be counted for study purposes. In case a bladder recurrence is suspected, a diagnostic biopsy is warranted to histologically confirm a urothelial carcinoma of the bladder ([Appendix A](#) for the flow-chart of the trial).

The relapse rate in the study cohort will be compared with the relapse rate of a matched historical cohort. This historical cohort will consist of patients older than 18 years who underwent a RNU or partial ureterectomy for urothelial carcinoma of the UUT, performed between 2001 and 2015 in the participating centers, received no perioperative intravesical instillation of chemotherapy and who had no previous history of a urothelial carcinoma of the bladder.

2.2. Population

Adult patients (age ≥ 18 years) who undergo a RNU or partial ureterectomy (open or laparoscopic) for a primary urothelial carcinoma of the UUT will be eligible. These patients will be selected from participating centers in the Netherlands. Approximately 150 RNU's for urothelial carcinoma of the UUT were performed yearly in the Netherlands between 2006 and 2010.

No exact information is available for the total number of partial ureterectomy procedures performed in the Netherlands for UUT urothelial carcinoma. However, probably, these numbers are increasing due to the growing elderly population who are diagnosed with UUT but

are too frail to undergo a RNU or have impaired renal function. Moreover, evidence is emerging that partial ureterectomy is feasible not only for an imperative indication, such as patients having a solitary kidney [17]. Given this increase in the number of partial ureterectomies performed, it is estimated that at least 90 patients per year can be included in the present study, whereby this number includes both patients undergoing RNU or partial ureterectomy.

See Appendix B for full inclusion and exclusion criteria of the study.

2.3. Study objectives

2.3.1. Primary objective

To demonstrate that a single intravesical instillation of chemotherapy immediately (within 3 h) before RNU or partial ureterectomy for urothelial carcinoma of the UUT reduces the risk of a subsequent urothelial bladder cancer recurrence up to two years after surgery with 40% (from 22–47% to 13.2–28.2%) compared to a matched historical cohort who received no perioperative intravesical instillation.

2.3.1.1. Index objective: risk reduction. A trial by O'Brien et al. randomized 144 patients to receive Mitomycin 40 mg at the time of urethral catheter removal following RNU (median time 7 days) and 140 patients to receive standard of care [9]. In the Mitomycin arm, 105 of 144 patients (73%) and 115 of 140 patients (82%) in the standard of care arm received their allocated treatment. Thirteen of 105 patients who received Mitomycin and 20 of 115 patients allocated to standard of care treatment did not complete follow up. By modified intention-to-treat analysis, 21 of 120 patients (17%) in the Mitomycin arm developed a bladder recurrence in the first year versus 32 of 119 patients (27%) in the standard of care arm ($p = 0.055$). By treatment as per protocol analysis, 17 of 105 patients (16%) in the Mitomycin arm and 31 of 115 patients (27%) in the standard treatment arm developed a bladder recurrence ($p = 0.03$). This resulted in a relative risk reduction in the recurrence rate in the first year following RNU of almost 40%; the absolute risk reduction was 11%. Ito et al. evaluated the efficacy of a single early intravesical instillation of Pirarubicin within 48 h following RNU in the prevention of bladder recurrence [8]. In this smaller study, 36 patients were included in both the intervention and control arm. Significantly fewer patients in the Pirarubicin group compared to the control group had a bladder recurrence at 2 years following surgery (16.9% in the intervention vs. 42.2% in the control group). Consequently, this resulted in a considerable higher relative risk reduction as shown by O'Brien et al. (Ito et al. Odds ratio (OR) 0.280; 95% Confidence Interval (CI): 0.093–0.831, $p = 0.023$ vs. O'Brien et al. OR 0.577; 95% CI 0.310–1.073, $p = 0.82$) [10]. It's possible that they achieved a higher reduction in recurrence due to the administration of chemotherapy within 48 h instead of within 10 days after surgery.

In addition a single instillation of chemotherapy following transurethral resection of bladder tumors (TURBT) for low- and intermediate-risk urothelial carcinoma of the bladder (UCB) induces a relative risk reduction of 40% to prevent a subsequent bladder tumor recurrence [18–20]. To prevent the implantation of tumor cells, the instillation should be given as soon as possible following TURB. In all studies which examined the effectiveness of a single, immediate, postoperative, intravesical instillation of chemotherapy following TURB, the instillation was given within 24 h following surgery [21]. This postoperative instillation following TURBT is most effective when administered within few hours of surgery [22].

2.3.2. Secondary objectives

- To show a $\geq 80\%$ compliance rate and accurate and consistent protocol performance of a single neoadjuvant instillation with MMC 3 h before RNU or partial ureterectomy for a urothelial carcinoma of the UUT.
- To assess the 2-year overall, cancer-specific and recurrence-free survival of a single neoadjuvant instillation with MMC before RNU or partial ureterectomy for UUT urothelial carcinoma compared with no perioperative intravesical instillations.
- The toxicity of the regime as assessed by the CTCAE.
- The impact on the quality of life of the subjects when receiving a neoadjuvant instillation with Mitomycin.
- Costs from a societal perspective using a time horizon of two years and incremental cost-effectiveness ratios.
- A molecular characterization of the UUT urothelial carcinoma and subsequent (recurrent) urothelial carcinoma of the bladder (side-study).

2.3.2.1. Index objective: compliance rate. Despite level I evidence showing that a postoperative instillation with chemotherapy following RNU decreases the risk of a subsequent bladder recurrence, which is also recommended by the EAU guideline (Level B evidence) [2], the compliance rate is low in current clinical practice. A Dutch survey showed a compliance rate of less than 10% [10]. Therefore, by conducting this trial, we aim to show not only that a neoadjuvant instillation of chemotherapy is equally effective as a postoperative instillation in reducing the risk of a subsequent bladder cancer recurrence, but it must also lead to a much higher compliance rate of clinicians to this neoadjuvant strategy because it lacks the potential risk of extravascular extravasation of chemotherapy.

2.3.2.2. Index objective: survival rates. At the time of diagnosis, 60% of all urothelial carcinomas of the UUT are invasive resulting in overall poor survival rates for patients with urothelial carcinoma of the UUT. In a large retrospective study by Adibi et al., the 5-year survival rates among 1462 patients who underwent RNU were less than 50% for stage pT2 or pT3 disease and less than 10% for pT4 disease [23].

Several studies have assessed individual patient risk factors for oncologic outcomes [5,7,12,24,25]. Lughezzani et al. and Mathieu et al. identified tumor stage and grade to be the most significant factors in oncological outcome [5,26]. Moreover, with respect to surgery, cancer-free surgical margins and the method of bladder cuff resection (*trans-* or *extravesical*) had the most significant impact on cancer-specific survival and overall survival. The most significant risk factors for intravesical recurrence of a urothelial cell carcinoma post RNU were a previous history of a urothelial cell carcinoma of the bladder and multifocality of the UUT tumor [25].

In the present study, the 2-years survival rates post RNU and partial ureterectomy will be assessed and stratified by individual patient characteristics. The technique of bladder cuff resection is mandatory, including a *trans-* or *extravesical* approach, and uniformly performed in all study participants. A secondary aim of this study is to develop a novel predictive model for clinical outcome (bladder cancer recurrence and survival) following RNU for urothelial carcinoma of the UUT. Predictive nomograms are used widely in urology to help patients counseling and complex decision-making regarding treatment, but none of these to date have been developed based on prospective data and none have achieved widespread routine use, due to low level of evidence and lack of external validation. In a meta-analysis by Mbeutcha et al. on predictive models for the treatment of urothelial carcinoma of the UUT [24], a positive predictive value of 89% was achieved when

combining hydronephrosis, ureteroscopic grade and urinary cytology for prediction of advanced-stage of UUT urothelial carcinoma [6]. If all three were negative, the negative predictive value was 100%. Xylinas et al. acquired an accuracy of 69% for postoperative prediction of intravesical recurrence risk at 2 years [11]. They combined age, gender, history of bladder cancer, tumor location, clinical stage, concomitant carcinoma *in situ* (CIS), lymph node metastasis, bladder-cuff excision and surgical approach.

2.3.2.3. Index objective: toxicity. Moriarity et al. reported on the safety of an intravesical instillation with MMC or Adriamycin that was administered during RNU in 51 patients. Through a two-way catheter, inserted at the beginning of the procedure, MMC (40 mg) or Adriamycin (40 mg) was instilled. The catheter was clamped for one to 2 h (median time 60 min, range 45–120 min). Just before the bladder was opened for the resection of the ureteric orifice, the chemotherapy was drained passively and the bladder was occasionally irrigated with saline. In total 31 of the 51 RNU's were performed by an extravesical excision of the bladder cuff. The other techniques consisted of intravesical excision of the bladder cuff or intramural ureterectomy. Nine out of 51 patients underwent a distal ureterectomy only. The intra- and postoperative complications were monitored up to 90 days following surgery. No adverse events were reported that were attributable to MMC or Adriamycin instillation [27]. Furthermore, in the studies on the efficacy of a single instillation of post-operative intravesical chemotherapy by O'Brien et al. and Ito et al. only non-serious adverse events were reported [8,9].

Although the reported toxicity is acceptable it is important to recognize and monitor possible side-effects attributable to the MMC instillation. To manifest possible adverse events the toxicity will be assessed in the present study until 3 months following surgery by the National Cancer Institute Common Toxicity Criteria (CTCAE) version 4.0. These toxicity criteria consist of standardized definitions for adverse events that describe the severity of organ toxicity for patients receiving cancer therapy.

2.3.2.4. Index objective: quality of life. It is hypothesized that a neoadjuvant instillation with Mitomycin will not have a negative impact on the quality of life. To address this hypothesis, all patients will have to complete two questionnaires at inclusion (T0), before surgery (with neo-adjuvant treatment), and at two weeks (T2) and three months (T3) following surgery. The EQ5D-5L, a standardized patient-reported instrument to measure general health, and the EORTC QLQ-C30, a questionnaire to assess the quality of life of cancer patients will be used. Both are validated questionnaires for measuring the quality of life within patients suffering from cancer. All time points (T0, T2 and T3) coincide with regular visits to the outpatient department in order to limit the extra burden for participating patients. To be able to adequately address the quality of life end point of the study, the dropout rate for completed questionnaires must be less than 10%.

2.3.2.5. Index objective: costs. The costs consist of direct costs (e.g., single gift of Mitomycin, personnel costs of health professionals involved, disorder related medication, disorder related innervations, time duration of hospital, informal care) and indirect costs (productivity loss) associated with each regimen. The economic evaluation will be a cost-utility analysis and a cost-effectiveness analysis performed from a societal perspective and will only be applicable to the Dutch healthcare system.

2.3.2.6. Index objective: molecular characterization (side-study). Due to the rarity of the disease, little is known about molecular aberrations related to urothelial carcinoma of the UUT and the prognostic profile of

molecular alterations that correspond with or even might predict bladder recurrences. In a time that cancers are increasingly stratified by their molecular alterations and treatment decisions can be based upon these alterations, it is important to investigate the genetic profiles of urothelial carcinoma of the UUT. Sfakianos et al. compared the genetic profile of 59 high-grade urothelial carcinomas of the UUT with another cohort of 102 high-grade UCB by targeted sequencing [28]. The spectrum of genes mutated in tumors of the UUT and UCB was similar, but the frequency of mutations in *FGFR3*, *HRAS*, *TP53* and *RB1* was not. In high-grade urothelial carcinoma of the UUT *FGFR3* and *HRAS* were more frequently mutated, whereas mutations in *TP53* and *RB1* were less prevalent compared to high-grade UCB. Most of the disparity in clinical manifestation between urothelial carcinoma of the UUT and UCB may result from anatomical differences because of the thinner smooth muscle coffering the UUT, but Sfakianos et al. showed that there are also genomic differences that might contribute to this phenomenon [28]. These observations provide evidence that urothelial carcinoma of the UUT and the bladder have distinct biological behaviors despite their histopathological similarities and therefore might require individualized treatment recommendations.

In both retrospective and prospective studies, a high proliferation index as assessed by *Ki-67* expression was associated with disease recurrence and cancer-specific survival in urothelial carcinoma of the UUT [29–33]. Furthermore, alterations in the *mTOR-pathway*, and the genes *HER2*, *BCAT1*, *CDCA5* and *p53* might play a role in the prognosis of high grade urothelial carcinoma of the UUT, but the impact of these biomarkers hasn't been sufficiently validated because of the small portion of samples in single-institution cohorts [24].

Currently two hypotheses for the development of a bladder recurrence following RNU for urothelial carcinoma of the UUT are postulated: a) intraluminal seeding and implantation of cancer cells [14,15]; multifocal tumors are descendants of a single transformed cell, which proliferates and spreads by intraluminal seeding or intraepithelial spread or b) in field cancerization [34], where it is assumed that multiple cells become initiated or partially transformed as a result of carcinogenic hits. In order to address these hypotheses it is important to compare the genomic profile of the primary urothelial carcinoma of the UUT and the subsequent bladder recurrence within the same patients.

Therefore, at inclusion, patients will be asked to provide separate consent for the use of their tumor tissue for molecular analysis. DNA will be isolated from the primary urothelial carcinoma of the UUT, the bladder recurrence and a buccal swap or non-malignant kidney tissue. Genomic sequencing will be performed to investigate tumor-specific somatic mutations and copy number variations to compare the molecular profile of the primary urothelial carcinoma of the UUT and a subsequent carcinoma of the bladder.

2.4. Sample size calculation

The estimated recurrence of urothelial carcinoma of the bladder following RNU for a UUT urothelial carcinoma is based on the literature. It has been shown that in patients not treated with adjuvant intravesical therapy following RNU, the bladder recurrence rate at two years was between 10 and 50% (mean 33.2%, total number of patients reported 995, range 36–223) [10]. We hypothesize a reduction in the risk of a bladder recurrence of 40% after RNU or partial ureterectomy by the neoadjuvant regimen of a single instillation with chemotherapy within 3 h before surgery. Consequently, this translates into a 19.9% estimated bladder recurrence rate for this study. Therefore, it is calculated that a sample size of 170 patients is needed to show a 40% difference two years following surgery with a power of 80% using a two-sided p-value of 0.05.

2.5. Ethical approval

The study abides by the principles of the Declaration of Helsinki. Ethical approval for this study was obtained from the institutional review board of Erasmus University Medical Center Rotterdam (METC 2017–227, NL60919.078.17). Also the board of directors of all participating hospitals have given permission for execution of this particular trial.

3. Statistical analysis

All analyses are based on the intention-to-treat principle, i.e. all eligible patients will be included in the analysis independently of whether they received treatment or not. Data characterized by normal distribution will be expressed as mean \pm standard deviation. Parameters not normally distributed will be expressed as median (range).

3.1. Primary study parameter

The bladder relapse rate at two years following surgery is the primary endpoint of the study. The relapse rate will be compared with the relapse rate of a matched historical cohort on a 1:2 basis (1 intervention cohort:2 historical cohort). The historical cases will be selected by the following criteria: age \geq 18 years, treated by RNU or partial ureterectomy for a histologically proven UUT urothelial carcinoma (cT1-T4 with or without CIS), no lymph node or distant metastasis at the moment of diagnosis as assessed by CT thorax-abdomen (cN0M0), a minimum of two years of follow-up following surgery, no perioperative systemic chemotherapy administered, and no history of urothelial carcinoma of the urinary tract before diagnosis of the UUT urothelial carcinoma. The difference in relapse rate between the intervention cohort and the matched historical cohort will be assessed using a multivariable Cox regression analysis and stratified by the following confounders: age, type of surgery (RNU versus partial ureterectomy), pathological stage, tumor grade, tumor size, tumor location, tumor multiplicity, concomitant CIS, medical center of treatment and surgical techniques (open versus laparoscopic).

3.2. Secondary study parameters

The difference in overall, cancer-specific and recurrence-free survival between the intervention cohort and matched historical cohort will be estimated using a multivariable Cox regression analysis. The toxicity of the treatment at different time points will be tested using a repeated measurements analysis. The quality of life at baseline, at 2 weeks and at 3 months following surgery will be compared using a repeated measurements analysis. Furthermore, potential risk factors will be identified using multivariable Cox proportional hazards. Co-variables included in the analysis are: type of surgery (partial ureterectomy or RNU (laparoscopic or open)), result of pre-operative urine cytology, histological stage and grade of the tumor, tumor location, concomitant CIS and lymph node involvement.

The primary economic analysis will be a cost-utility analysis performed according to the Dutch guideline to determine whether neoadjuvant intravesical instillations with Mitomycin before RNU or organ sparing surgery are a cost-effective alternative to the standard of care (historical cohort) [35]. Additionally, a cost-effectiveness analysis will be conducted to determine the costs per prevented bladder recurrence. The time horizon will be from start of therapy ($t = 0$) till 24 months follow-up to take all relevant costs and effects regarding the MMC and standard of care strategy into account. The costs are defined as direct and indirect costs associated with procedures performed

within each regimen. The costs will be estimated by multiplying resource utilization with the cost per unit of resource (market prices, guideline prices or self-determined prices based on costing methods, i.e. full costing) [35]. The incremental cost-effectiveness ratio (ICER) of MMC will be calculated (i.e., the difference in costs of MMC versus standard of care divided by the average change in QALYs and bladder recurrence rate, respectively). The sensitivity of various costs per unit of resource will be tested in sensitivity analyses.

All statistical analyses will be performed using statistics software (SPSS version 21.0 for Windows, Chicago, IL, USA). A two-tailed p -value of < 0.05 is considered significant.

4. Study procedures

A flow diagram of the REBACARE trial is presented in [Appendix A](#). The following procedures are performed for research purposes at a different time point or in addition to the standardized care.

4.1. Treatment

After consent is obtained for both the primary study and the side-study, patients will be asked to provide a buccal swab for the collection of germline DNA. On the day of surgery, MMC is administered intravesically in all patients within 3 h before surgery. The MMC is given directly into the bladder by an indwelling catheter. The indwelling catheter is inserted through the urethra and after instillation of the MMC the catheter is clamped, which allows the medication to remain in the bladder. The doses will be a suspension of 40 mg MMC in 50 ml sterile saline (NaCl 0.9%) and must remain in the bladder for a period of at least 1 h with a maximum of 2 h, if possible. The patient is then transported to the operating room. Once the bladder is emptied by the indwelling catheter, the bladder will be continuously rinsed with NaCl 0.9% to remove all remains of the MMC and possible floating tumor cells. The indwelling catheter will remain inside the bladder during surgery and the rinsing will be stopped at the moment the treating surgeon is about to incise the bladder wall for excision of the ureteric orifice. The indwelling catheter will remain for some days after surgery until the patient has recovered. The exact number of days the indwelling catheter will remain in the bladder following surgery is at the discretion of the treating physician. From a pilot experiment it is known that neoadjuvantly administered intravesical MMC can no longer be detected on the surgical equipment or inside the operating room once the bladder is appropriately rinsed with 2×50 mL of NaCl 0,9% (*personal communication by Dr. A.G.M. van der Heijden, MD PhD, Radboud UMC*). Therefore, medical personnel who will treat the study participants will not be exposed to MMC.

The surgical procedure is not performed for research purposes, however the participating physicians will be asked to carry out the procedures in a standardized manner; i.e. both the RNU or partial ureterectomy must start with clipping of the ureter distal of the tumor before manipulation of the ureter can take place. For a RNU, the ureteric orifice must be circumcised and resected 'en block' attached to the ureter (bladder cuff). The pathology report must describe the presence of the ureteral clip and, for the latter, the presence of a bladder cuff including the ureteric orifice. The administration of antibiotic prophylaxis is advocated and the antibiotic regimen (orally or intravenously) should be in accordance with the local guidelines of the participating hospitals or based on a urinary culture.

4.2. Follow up

The follow up will be in line with the standardized care and will not include additional investigations. Cystoscopy plus urine cytology will

be performed at 3, 6, 12, 18 and 24 months. The follow-up also includes CT-urography at 6, 12, 18, 24 months. In case of an invasive tumor, follow-up will include a CT-thorax at 6 and 12 months. All patients will complete two questionnaires at three moments during the study (Appendix A) to examine the quality of life following this treatment. To demonstrate side effects, patients will fill in a side-effects form 4 times. In case a bladder recurrence is suspected, it is warranted to take a diagnostic biopsy to histologically confirm and classify urothelial carcinoma of the bladder.

4.3. Side study: molecular analysis

Both a buccal swap and a biopsy of the tumor will be collected from participants who provide separate informed consent. DNA will be isolated from the primary UUT tumor, the subsequent intravesical recurrence and non-malignant kidney tissue or a buccal swab. Genomic sequencing will be performed to investigate tumor-specific somatic mutations and copy number variations to compare the molecular profile of the primary UUT tumor and subsequent bladder tumor.

5. Discussion

5.1. Exposition of protocol

Following RNU for urothelial carcinoma of the UUT, the reported recurrence rate of urothelial carcinoma in the bladder is 22–47% (4–7). Intraluminal seeding [14,15] or in field cancerization [34] are thought to be the two hypotheses of this high recurrence rate. Based on the assumption that intraluminal seeding has most of the impact on this recurrence rate, a single postoperative instillation of chemotherapy following RNU has been introduced, and has shown to decrease the risk of bladder recurrence by 52% (relative risk reduction) [10]. Given the fact that many treating physicians waive the addition of a postoperative instillation with chemotherapy following RNU or partial ureterectomy, despite recommendation by the EAU guideline [2], this could be an indication that another (neo)adjuvant treatment is desirable. To avoid the limitations in current treatment protocols of urothelial carcinoma of the UUT, the REBACARE trial will be the first prospective trial that could change current management. The fact that intravesical instillation will be administered before surgery makes this study unique and plausible positive for the compliance rate.

The REBACARE trial has additional aims besides the reduction of a subsequent bladder recurrence and a safer toxicity profile in comparison with a postoperative instillation, since the study also aims to explore the genetic profile of urothelial carcinoma of the UUT in more detail within a side-study performed during the REBACARE-trial. This is of specific interest, in a time in which the decision of certain treatment modalities of various tumors does not only rely on clinicopathological characteristics, but can possibly also rely on tumor-specific molecular alterations. Furthermore, molecular characterization may reveal tumor-specific alterations that are targets for new anticancer therapies. More important, by performing a molecular comparison of the primary UUT tumor and a subsequent bladder recurrence, the hypothesis that intraluminal seeding is responsible for developing bladder recurrences can be explored. If this is the case, tumors of both the UUT and the subsequent bladder tumor should have identical molecular alterations, i.e. tumor-specific mutations. On the other hand Sfakianos et al. showed differences in the genetic profiles and mutational status between

urothelial carcinoma of the UUT and UCB [28]. Although these alterations were not examined within the same patient, their findings support the hypothesis that subsequent bladder tumors are *de novo* primary tumors with specific molecular alterations. Lastly, a molecular comparison of the primary UUT of the patients who develop a recurrence versus those who do not might reveal important aberrations that could explain the risk of the development of a bladder recurrence or might even be predictive of a bladder cancer recurrence. The REBACARE trial is one of the first studies in which DNA from the primary tumor as well as DNA from the bladder recurrence of the same patient will be compared and this will result in unique information.

5.2. Study limitations

There are several limitations associated with the design of the study. Theoretically, the REBACARE trial could be designed as a prospective randomized controlled trial generating level 1 evidence. However, a randomized controlled trial would not be feasible due to the large number of study participants in relation to the relative low number of patients that will be diagnosed and treated for urothelial carcinoma of the UUT. In addition, due to the low compliance rate for a postoperative instillation with chemotherapy, inclusion of patients in the post-operative instillation arm (standard of care) will take very long. Furthermore, the recommendation in the EAU guideline for a post-operative instillation of chemotherapy following RNU makes it not ethical to conduct a study in which any form of intravesical instillation with chemotherapy is withheld in the control arm.

The exclusion criteria of a previous UCB will limit the inclusion rate of the REBACARE trial as the majority of patients with urothelial carcinoma of the UUT are known to have had one or more episodes of UCB in their history. However, this was necessary, because it is known that these patients are at much higher risk to develop a subsequent bladder recurrence following surgery for urothelial carcinoma of the UUT [11,24,36]. Including these patients will potentially jeopardize the outcome of this trial because it will have an impact on the primary endpoint of this trial.

6. Conclusion

Approximately 40% of patients will develop an intravesical recurrence following a RNU for urothelial carcinoma of the UUT. A single postoperative instillation with chemotherapy reduces the risk of a bladder cancer recurrence significantly. Nevertheless, the compliance rate of clinicians with a single postoperative instillation is low due to the potential risk of extravasation of chemotherapy. The REBACARE trial is the first prospective trial that aims to determine the effect of a single preoperative intravesical instillation with chemotherapy on the risk of a bladder cancer recurrence following RNU for urothelial carcinoma of the UUT. This change in order of treatment may prove to be equally effective as a postoperative instillation but with a safer profile and has the potential to change clinical practice in a definitive way.

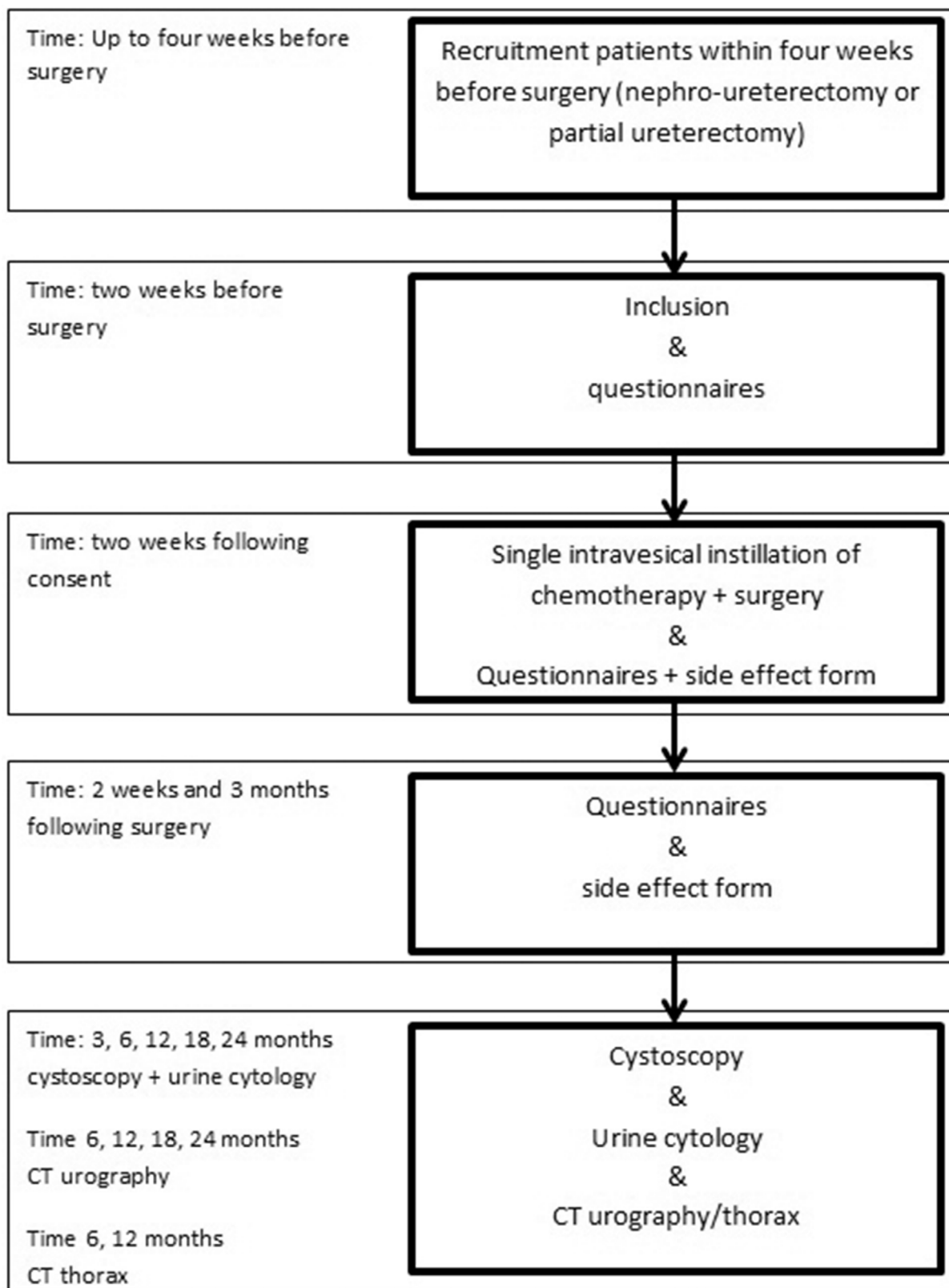
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Appendix C. Supplementary data

Supplementary data related to this article can be found at <http://dx.doi.org/10.1016/j.conctc.2018.01.007>.

Appendix A. Flow-chart of the REBACARE trial.



Appendix B. In- and exclusion criteria of the REBACARE trial.

Inclusion criteria	<ol style="list-style-type: none"> 1. Histologically proven urothelial carcinoma of the UUT with or without concurrent <i>carcinoma in situ</i> (CIS only is also allowed) or patients with a suspicion of a urothelial carcinoma of the UUT on CT-scan plus a urinary cytology sample showing high-grade urothelial carcinoma; 2. Patients planned to be treated either by partial ureterectomy or by a radical nephro-ureterectomy (open or laparoscopic) including a bladder cuff; 3. Age ≥ 18 years; 4. WHO performance status 0, 1 or 2; 5. Negative pregnancy test in woman with childbearing potential; 6. Written informed consent.
Exclusion criteria	<ol style="list-style-type: none"> 1. If pre-operative histology by biopsy: aberrant histology of the UUT tumor of > 50% (adenocarcinoma, small cell carcinoma, squamous cell carcinoma). 2. History or presence of a malignant tumor or <i>carcinoma in situ</i> of the bladder. 3. History of UUT urothelial carcinoma on the contralateral side or presence of bilateral UUT urothelial carcinoma. 4. Known allergy against Mitomycin. 5. Anticipated adjuvant intravesical treatment with chemo- or immunotherapy. 6. Acute urinary tract infection at the time of inclusion as assessed by urinary culturing. 7. Lymphadenopathy or distant metastases as assessed by preoperative CT-scan of thorax and abdomen. 8. Any other concurrent severe or uncontrolled disease preventing the safe administration of intravesical Mitomycin. 9. Breastfeeding women.

References

- [1] J. Ferlay, E. Steliarova-Foucher, J. Lortet-Tieulent, S. Rosso, J.W. Coebergh, H. Comber, et al., Cancer incidence and mortality patterns in Europe: estimates for 40 countries in 2012, *Eur. J. Canc.* 49 (6) (2013) 1374–1403.
- [2] M. Roupert, M. Babjuk, E. Comperat, R. Zigeuner, R.J. Sylvester, M. Burger, et al., European association of urology guidelines on upper urinary tract urothelial cell carcinoma: 2015 update, *Eur. Urol.* 68 (5) (2015) 868–879.
- [3] V. Margulis, S.F. Shariat, S.F. Matin, A.M. Kamat, R. Zigeuner, E. Kikuchi, et al., Outcomes of radical nephroureterectomy: a series from the upper tract urothelial carcinoma collaboration, *Cancer* 115 (6) (2009) 1224–1233.
- [4] Y. Matsui, N. Utsunomiya, K. Ichioka, N. Ueda, K. Yoshimura, A. Terai, et al., Risk factors for subsequent development of bladder cancer after primary transitional cell carcinoma of the upper urinary tract, *Urology* 65 (2) (2005) 279–283.
- [5] G. Lughezzani, M. Burger, V. Margulis, S.F. Matin, G. Novara, M. Roupert, et al., Prognostic factors in upper urinary tract urothelial carcinomas: a comprehensive review of the current literature, *Eur. Urol.* 62 (1) (2012) 100–114.
- [6] J.C. Brien, S.F. Shariat, M.P. Herman, C.K. Ng, D.S. Scherr, B. Scoll, et al., Preoperative hydronephrosis, ureteroscopic biopsy grade and urinary cytology can improve prediction of advanced upper tract urothelial carcinoma, *J. Urol.* 184 (1) (2010) 69–73.
- [7] M.D. Azemar, E. Comperat, F. Richard, O. Cussenot, M. Roupert, Bladder recurrence after surgery for upper urinary tract urothelial cell carcinoma: frequency, risk factors, and surveillance, *Urol. Oncol.* 29 (2) (2011) 130–136.
- [8] A. Ito, I. Shintaku, M. Satoh, N. Ioritani, M. Aizawa, T. Tochigi, 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.* 31 (11) (2013) 1422–1427.
- [9] T. O'Brien, E. Ray, R. Singh, B. Coker, R. Beard, 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.* 60 (4) (2011) 703–710.
- [10] P.J. van Leeuwen JLB, Intravesicale chemotherapie na nefro-ureterectomie verlaagt de kans op urothelcarcinoom van de blaas, *Tijdschrift Urol.* 5 (2015) 52–57.
- [11] E. Xylinas, L. Kluth, N. Passoni, Q.D. Trinh, M. Rieken, R.K. Lee, et al., Prediction of intravesical recurrence after radical nephroureterectomy: development of a clinical decision-making tool, *Eur. Urol.* 65 (3) (2014) 650–658.
- [12] J. Ishioka, K. Saito, T. Kijima, Y. Nakanishi, S. Yoshida, M. Yokoyama, et al., Risk stratification for bladder recurrence of upper urinary tract urothelial carcinoma after radical nephroureterectomy, *BJU Int.* 115 (5) (2015) 705–712.
- [13] J.R. Oddens, A.P. van der Meijden, R. Sylvester, One immediate postoperative instillation of chemotherapy in low risk Ta, T1 bladder cancer patients. Is it always safe? *Eur. Urol.* 46 (3) (2004) 336–338.
- [14] T. Habuchi, Origin of multifocal carcinomas of the bladder and upper urinary tract: molecular analysis and clinical implications, *Int. J. Urol.* 12 (8) (2005) 709–716.
- [15] D.R. Yates, J.W. Catto, Distinct patterns and behaviour of urothelial carcinoma with respect to anatomical location: how molecular biomarkers can augment clinicopathological predictors in upper urinary tract tumours, *World J. Urol.* 31 (1) (2013) 21–29.
- [16] S.M. Di Stasi, M. Valenti, C. Verri, E. Liberati, A. Giurioli, G. Leprini, et al., Electromotive instillation of mitomycin immediately before transurethral resection for patients with primary urothelial non-muscle invasive bladder cancer: a randomised controlled trial, *Lancet Oncol.* 12 (9) (2011) 871–879.
- [17] T. Seisen, B. Peyronnet, J.L. Dominguez-Escrig, H.M. Bruins, C.Y. Yuan, M. Babjuk, et al., Oncologic outcomes of kidney-sparing surgery versus radical nephroureterectomy for upper tract urothelial carcinoma: a systematic review by the EAU non-muscle invasive bladder cancer guidelines panel, *Eur. Urol.* 70 (6) (2016) 1052–1068.
- [18] W. Oosterlinck, K.H. Kurth, F. Schroder, J. Bultinck, B. Hammond, R. Sylvester, A prospective European Organization for Research and Treatment of Cancer Genitourinary Group randomized trial comparing transurethral resection followed by a single intravesical instillation of epirubicin or water in single stage Ta, T1 papillary carcinoma of the bladder, *J. Urol.* 149 (4) (1993) 749–752.
- [19] R.J. Sylvester, W. Oosterlinck, J.A. Witjes, The schedule and duration of intravesical chemotherapy in patients with non-muscle-invasive bladder cancer: a systematic review of the published results of randomized clinical trials, *Eur. Urol.* 53 (4) (2008) 709–719.
- [20] D.A. Tolley, M.K. Parmar, K.M. Grigor, G. Lallemand, L.L. Benyon, J. Fellows, et al., The effect of intravesical mitomycin C on recurrence of newly diagnosed superficial bladder cancer: a further report with 7 years of follow up, *J. Urol.* 155 (4) (1996) 1233–1238.
- [21] [EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder, the 2011 update], M. Babjuk, W. Oosterlinck, R. Sylvester, E. Kaasinen, A. Böhle, J. Palou-Redorta, et al., Guía clínica del carcinoma urotelial de vejiga no musculoinvasivo de la Asociación Europea de Urología. Actualización de 2011, *Actas Urol. Esp.* 36 (7) (2012) 389–402.
- [22] M. Babjuk, A. Böhle, M. Burger, O. Capoun, D. Cohen, E.M. Comperat, et al., EAU guidelines on non-muscle-invasive urothelial carcinoma of the bladder: update 2016, *Eur. Urol.* 71 (3) (2017) 447–461.
- [23] M. Adibi, R. Youssef, S.F. Shariat, Y. Lotan, C.G. Wood, A.I. Sagalowsky, et al., Oncological outcomes after radical nephroureterectomy for upper tract urothelial carcinoma: comparison over the three decades, *Int. J. Urol.* 19 (12) (2012) 1060–1066.
- [24] A. Mbeutcha, R. Mathieu, M. Roupert, K.M. Gust, A. Briganti, P.I. Karakiewicz, et al., Predictive models and prognostic factors for upper tract urothelial carcinoma: a comprehensive review of the literature, *Transl. Androl. Urol.* 5 (5) (2016) 720–734.
- [25] T. Seisen, B. Granger, P. Colin, P. Leon, G. Utard, R. Renard-Penna, 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.* 67 (6) (2015) 1122–1133.
- [26] R. Mathieu, K. Bensalah, I. Lucca, A. Mbeutcha, M. Roupert, S.F. Shariat, Upper urinary tract disease: what we know today and unmet needs, *Transl. Androl. Urol.* 4 (3) (2015) 261–272.
- [27] M.A. Moriarty, M.A. Uhlman, M.T. Bing, M.A. O'Donnell, J.A. Brown, C.R. Tracy, et al., Evaluating the safety of intraoperative instillation of intravesical chemotherapy at the time of nephroureterectomy, *BMC Urol.* 15 (2015) 45.
- [28] J.P. Sfakianos, E.K. Cha, G. Iyer, S.N. Scott, E.C. Zabor, R.H. Shah, et al., Genomic characterization of upper tract urothelial carcinoma, *Eur. Urol.* 68 (6) (2015) 970–977.
- [29] H.G. Jeon, I.G. Jeong, J. Bae, J.W. Lee, J.K. Won, J.H. Paik, et al., Expression of Ki-67 and COX-2 in patients with upper urinary tract urothelial carcinoma, *Urology* 76 (2) (2010) 513 e7–12.
- [30] L.M. Krabbe, A. Bagrodia, A.Q. Haddad, P. Kapur, D. Khalil, L.S. Hynan, et al., Multi-institutional validation of the predictive value of Ki-67 in patients with high

- grade urothelial carcinoma of the upper urinary tract, *J. Urol.* 193 (5) (2015) 1486–1493.
- [31] L.M. Krabbe, A. Bagrodia, Y. Lotan, B.A. Gayed, O.M. Darwish, R.F. Youssef, et al., Prospective analysis of Ki-67 as an independent predictor of oncologic outcomes in patients with high grade upper tract urothelial carcinoma, *J. Urol.* 191 (1) (2014) 28–34.
- [32] Y. Lei, Z. Li, L. Qi, S. Tong, B. Li, W. He, et al., The prognostic role of Ki-67/MIB-1 in upper urinary-tract urothelial carcinomas: a systematic review and meta-analysis, *J. Endourol.* 29 (11) (2015) 1302–1308.
- [33] P. Wu, S. Liu, W. Zhang, Y. Zhang, G. Zhu, D. Wei, et al., Low-level Ki-67 expression as an independent predictor of bladder tumour recurrence in patients with primary upper tract urothelial carcinoma after radical nephroureterectomy, *Jpn. J. Clin. Oncol.* 45 (12) (2015) 1175–1181.
- [34] A.L. Harris, D.E. Neal, Bladder cancer—field versus clonal origin, *N. Engl. J. Med.* 326 (11) (1992) 759–761.
- [35] L.T.S.S. Hakkaart-van Roijen, C.A.M. Bouwmans, Handleiding voor Kostenonderzoek, CVZ, 2010.
- [36] H. Yuan, X. Chen, L. Liu, L. Yang, C. Pu, J. Li, et al., Risk factors for intravesical recurrence after radical nephroureterectomy for upper tract urothelial carcinoma: a meta-analysis, *Urol. Oncol.* 32 (7) (2014) 989–1002.