Developments in intravesical therapy for non-muscle invasive bladder cancer

Kees Hendricksen

Developments in intravesical therapy for non-muscle invasive bladder cancer

een wetenschappelijke proeve op het gebied van de Medische Wetenschappen

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Contents

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Nativ O, Witjes JA, Hendricksen K, Cohen M, Keidar D, Sidi A, Colombo R, Leibovich I. Combined thermochemotherapy for the management of patients with non-muscle invasive bladder cancer who failed intravesical BCG treatment. Accepted for Journal of Urology

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Chapter 1

General introduction and outline of the thesis

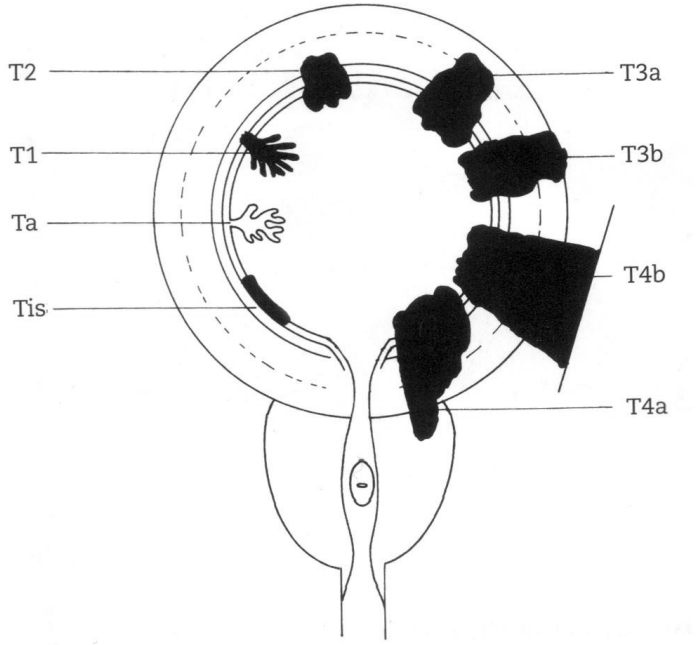
Algemene inleiding en overzicht van het proefschrift

Bladder cancer is the fourth most common malignancy among men in the Western world, following prostate, lung and colon cancers. In Europe and the United States, bladder cancer accounts for 5-10% of all malignancies among men. The risk of developing bladder cancer at < 75 years of age is 2-4% for men and 0.5-1% for women. The median age at diagnosis is 65-70 years. The two most well-known risk factors for bladder cancer are cigarette smoking and occupational exposure. Familial bladder cancer is a fairly rare phenomenon.

More than 90-95% of all bladder tumours are urothelial cell carcinoma (UCC). The second most prevalent is squamous cell carcinoma (SCC), accounting for 3-5% of bladder tumours in Western countries. In countries where the schistosoma haematobium parasite is endemic (Egypt, Middle East), urinary bladder SCC is the most prevalent form of cancer in men overall. The third most common type of bladder cancer is adenocarcinoma, comprising 0.5-2% of bladder tumours. Other types of malignant, primary non-urothelial tumours are rare in Western countries. This thesis is focused on the most common type of bladder cancer, UCC.

Approximately 70-85% (in the Netherlands 72.5%) of patients with bladder UCC present with non-muscle invasive bladder cancer (NMIBC), which is disease confined to the mucosa (stage Ta, carcinoma in situ (CIS or Tis)) or the submucosa (stage T1) (figure 1). The remainder of patients present with bladder cancer initially invading the detrusor muscle (stage T2), followed by the perivesical fat (stage T3) and the organs surrounding the bladder (stage T4). Of all patients with NMIBC, about two thirds of NMIBC patients present with Ta disease, 30% with T1 disease and 5% with CIS. Currently, two classifications are used for grading of NMIBC (table 1). Stage and grade are important determinants of bladder cancer treatment.

Figure 1 Staging of bladder cancer.



Adapted from J. Blandy, Lecture notes on Urology, 5th edition.

Table 1 World Health Organization (WHO) grading in 1973 and 2004

1973 WHO grading	**2004 WHO grading**
Urothelial papilloma	Urothelial papilloma
Grade 1: well differentiated	Papillary urothelial neoplasm of low malignant potential (PUNLMP)
Grade 2: moderately differentiated	Low-grade papillary urothelial carcinoma
Grade 3: poorly differentiated	High-grade papillary urothelial carcinoma

The classic way to categorize patients with Ta-T1 tumours is to divide them into risk groups, based on prognostic factors derived from multivariate analyses. These risk groups were used to choose adjuvant intravesical therapy after transurethral resection of the bladder tumour(s) (TURBT), based on an estimation of the risk of NMIBC recurrence and progression to muscle invasive disease. The low-risk group classifies single TaG1 tumours ≤ 3 cm in diameter, that exhibit a low recurrence rate with nearly no progression. The high-risk group contains an aggressive form of NMIBC, with T1G3, multifocal or highly recurrent (≥ 3 recurrences in 24 months) Ta-T1 tumours, and CIS. Up to 75% of these patients will develop disease recurrence, and 30-50% muscle invasive disease. The intermediate-risk group

contains all other tumours between the low- and high-risk groups, namely Ta-T1, G1-G2, multifocal, > 3 cm in diameter. These patients often develop NMIBC recurrence but progression is uncommon. Recently, a scoring system and risk tables have been developed by the European Organization for Research and Treatment of Cancer (EORTC) to separately predict the short-term and long-term risks of both recurrence and progression in individual patients. The scoring system is based on the six most significant clinical and pathological prognostic factors (table 2). Their weights are used to calculate recurrence and progression scores, which are subsequently used to predict probabilities of recurrence and progression (table 3). The last column of table 3 shows an improved alternative of dividing patients into low-, intermediate- and high-risk groups.

For patients with muscle invasive bladder cancer, cystectomy is the treatment of choice. Despite this radical therapy, patients with primary muscle invasive bladder cancer have a 5-year tumour-specific survival of 55%. In comparison, patients with NMIBC have a 5-year tumour-specific survival of 88-90%. However, for patients with muscle invasive bladder cancer who have a history of NMIBC, the 5-year tumour-specific survival drops to only 28%. These percentages clearly emphasize the need for more effective treatment options against bladder cancer, and more in particular, the need for improved treatment of patients with a high risk of recurrence and/or high risk of progression of NMIBC. At the same time, treatment should give as little side effects as possible, with preservation of the normal function of the urinary bladder.

In this thesis, developments in intravesical therapy for patients with low-, intermediate-, and high-risk NMIBC are discussed. First of all, a review of adjuvant intravesical pharmacotherapy for all risk groups of NMIBC is provided in **chapter 2**, discussing conventional treatment, emerging drugs, and device-assisted therapies.

Table 2 Weights used to calculate recurrence and progression scores

Factor	Recurrence	Progression
Number of tumours		
Single	0	0
2 to 7	3	3
≥8	6	3
Tumour diameter		
< 3 cm	0	0
≥ 3 cm	3	3
Prior recurrence rate		
Primary	0	0
≤1 rec/yr	2	2
>1 rec/yr	4	2
Category		
Ta	0	0
T1	1	4
Concomitant CIS		
No	0	0
Yes	1	6
Grade (1973 WHO)		
G1	0	0
G2	1	0
G3	2	5

Table 3 Probability of recurrence and progression according to total score

Recurrence score	Probability of recurrence at 1 yr		Probability of recurrence at 5 yr		Recurrence risk group
	%	(95% CI)	%	(95% CI)	
0	15	(10-19)	31	(24-37)	Low-risk
1-4	24	(21-26)	46	(42-49)	Intermediate-risk
5-9	38	(35-41)	62	(58-65)	Intermediate-risk
10-17	61	(55-67)	78	(73-84)	High-risk

Progression score	Probability of progression at 1 yr		Probability of progression at 5 yr		Progression risk group
	%	(95% CI)	%	(95% CI)	
0	0.2	(0-0.7)	0.8	(0-1.7)	Low-risk
2-6	1	(0.4-1.6)	6	(5-8)	Intermediate-risk
7-13	5	(4-7)	17	(14-20)	High-risk
14-23	17	(10-24)	45	(35-55)	High-risk

CI: confidence interval

The results of the preclinical animal studies are discussed in chapters 3 and 4. In **chapter 3**, an orthotopic rat bladder UCC model is studied. It is highly desirable to have such a clinically relevant model, to be able to test potential new drugs against NMIBC already in a preclinical setting. In the present study growth of UCC is assessed in the orthotopic rat bladder model over time, to optimize the future starting point of experimental intravesical treatment. In addition, the model is augmented by the use of serial cystoscopy for in vivo tumour assessment and follow-up (FU).

The results of a preclinical study in pigs are discussed in **chapter 4**. Pemetrexed (Alimta®) has shown broad antitumour activity in a variety of solid tumours, and antitumour activity was observed in a trial with advanced bladder cancer patients. To test the potential of pemetrexed for intravesical use, a pig model is used to study pharmacokinetics and toxicity of pemetrexed. The study contains a dose escalation phase and 6-week instillation phase in which animal well-being, potential myelosuppression, drug absorption, and bladder wall histology are studied.

The results of the clinical studies concerning new and enhanced treatment options for NMIBC are discussed in chapters 5, 6, 7 and 8. In chapters 5 and 6 the relatively new drug apaziquone (EO9 or EOquin®) is discussed. This drug generates cytotoxic species under aerobic as well as hypoxic conditions, and has proven to be effective (i.e. much more effective than mitomycin C (MMC)) against UCC of the bladder in vitro. In a marker lesion study a promising histological complete response of 67% was demonstrated in patients with NMIBC after apaziquone instillations. These promising results have led to the following clinical studies. In **chapter 5**, the results of a phase I/II study are described. The safety, tolerability and pharmacokinetics of a single immediate post-TURBT instillation of apaziquone are evaluated for patients with NMIBC, by means of adverse event and safety parameter assessment, and blood sample evaluation. In **chapter 6**, the results of 2-year FU of

the phase II marker lesion study are described. Initially, 46 patients underwent TURBT with the exception of an untouched marker lesion, and were treated with six intravesical instillations of apaziquone. Of these patients, 31 had a complete response (67%), i.e. complete destruction of the marker lesion two to four weeks after TURBT. In continuation of this study, both the complete responders as well as the non-responders are followed for a period of two years, with assessment of long-term adverse events and recurrence-free survival.

Another drug used for intravesical chemotherapy, epirubicin, is studied in three schedules and compared in a phase III study in **chapter 7**. Epirubicin has a good tolerability profile and efficacy in the prevention of recurrences of NMIBC. Epirubicin, and intravesical chemotherapy in general, have been studied in randomized clinical trials, but the optimal schedule and duration of treatment are unknown. To investigate the hypothesis whether there is a more efficient instillation scheme of epirubicin, we studied the additive effect of either an early instillation or maintenance instillations of adjuvant intravesical epirubicin, as compared to the epirubicin "standard" treatment schedule only, in patients with NMIBC.

The results of chemotherapy combined with intravesical hyperthermia in patients with CIS are discussed in **chapter 8**. Bladder wall hyperthermia is induced via an energy-delivering unit in the tip of a special catheter, equipped with internal thermocouples to monitor the temperatures, to be around 42-43°C (the Synergo®-system). This system is currently used in combination with intravesical instillations of MMC (thermochemotherapy), and several randomized trials have shown its superiority over intravesical treatment with MMC alone. We analysed 51 patients with CIS that were treated with thermochemotherapy, and focused on eradication of CIS and tumour recurrence.

In all, several new drugs and strategies are emerging in the field of NMIBC treatment, and this thesis focuses on several of these new therapies, including their preclinical testing.

Algemene inleiding en overzicht van het proefschrift

Na de prostaat, long en dikke darm is de blaas het orgaan waarin kanker het meest voorkomt bij mannen in de Westerse wereld. In Europa en de Verenigde Staten betreft blaaskanker 5-10% van alle kankersoorten bij mannen. De kans op het ontwikkelen van blaaskanker op een leeftijd < 75 jaar is 2-4% voor mannen en 0.5-1% voor vrouwen, waarbij de diagnose gemiddeld op 65- tot 70-jarige leeftijd wordt gesteld. De twee meest bekende risicofactoren voor het ontwikkelen van blaaskanker zijn roken en blootstelling aan kankerverwekkende (werkgerelateerde) stoffen. Erfelijke blaaskanker is zeldzaam.

Van alle maligne (kwaadaardige) blaastumoren gaat het in 90-95% om een urotheelcelcarcinoom (UCC). In 3-5% van de maligne blaastumoren gaat het om een plaveiselcelcarcinoom (PCC). In gebieden (Egypte, het Midden-Oosten) waar de Schistosoma haematobium parasiet endemisch is, de verwekker van schistosomiasis (bilharzia), komt PCC van de blaas uitzonderlijk veel voor, en is daar zelfs de meest voorkomende vorm van kanker. De diagnose adenocarcinoom wordt in 0.5-2% van de maligne blaastumoren gesteld. Andere vormen van maligne blaastumoren zijn zeldzaam in de westerse landen. Dit proefschrift is gericht op de meest voorkomende vorm van blaaskanker: UCC.

Van alle patiënten met UCC van de blaas presenteert 70-85% (in Nederland 72.5%) zich met een niet-spierinvasieve blaaskanker. Niet-spierinvasief wil zeggen dat de groei van de tumor beperkt blijft tot de mucosa (tumorstadium Ta, carcinoma in situ), de eerste laag van de blaas, of tot de submucosa (tumorstadium T1), de tweede laag van de blaas (figuur 1). De overige patiënten presenteren zich met een spierinvasieve blaaskanker, wat betekent dat de tumor door de submucosa de blaasspier in groeit (tumorstadium T2), waarna het omliggende vet (tumorstadium T3) en de omliggende organen (tumorstadium T4) volgen. Van de patiënten met een niet-spierinvasieve blaaskanker heeft ongeveer tweederde een Ta tumor,

30% een T1 tumor en 5% carcinoma in situ (CIS of Tis). Momenteel worden twee classificatiesystemen gebruikt om niet-spierinvasieve blaaskanker te graderen (tabel 1). De stadiëring (Ta, CIS, T1-4) en gradering (G1-3) van een tumor zijn in belangrijke mate bepalend voor de keuze van behandeling van blaaskanker.

Patiënten met een niet-spierinvasief carcinoom van de blaas worden eerst behandeld met een transurethrale (via de plasbuis) resectie van de blaastumor (TURT). Na deze chirurgische behandeling kunnen patiënten met een Ta of T1 blaastumor aan de hand van prognostische factoren worden ingedeeld in risicogroepen, welke het risico weergeven op het terugkomen van de blaaskanker (het ontwikkelen van een recidief) en de ontwikkeling (progressie) tot een spierinvasieve blaaskanker. Ook worden de risicogroepen gebruikt om een vorm van adjuvante (aanvullende) intravesicale (in de blaas) therapie te kiezen. Meestal zijn dit blaasspoelingen met mitomycine C of epirubicine (chemotherapie), of BCG (immunotherapie). De laagrisico groep wordt gevormd door patiënten met één TaG1 tumor van ≤ 3 cm; deze patiënten hebben een lage kans op recidivering van de tumor en progressie is zeldzaam. De hoogrisico groep bevat patiënten met een agressieve vorm van het niet-spierinvasief blaascarcinoom: T1G3, multifocale (op meerdere plekken) of snel recidiverende Ta-T1 tumoren (≥ 3 recidieven in 24 maanden), en CIS. Naar schatting zal 75% van deze patiënten een recidief ontwikkelen en ontwikkelt 30-50% een blaastumor die spierinvasief wordt. De intermediairrisico groep bevat alle tumoren tussen de laag- en hoogrisico groep in: Ta-T1, G1-2, multifocaal en > 3 cm in diameter; veel van deze patiënten ontwikkelen een niet-spierinvasief recidief, waarbij progressie naar een spierinvasieve blaaskanker weinig voorkomt. Recent heeft de Europese Organisatie voor Onderzoek en Behandeling van Kanker (EORTC) een systeem bedacht waarbij de zes belangrijkste (klinische en pathologische) prognostische factoren elk een specifieke waarde krijgen toegekend, om zodoende per patiënt een individuele risicoscore te kunnen berekenen (tabel 2). De risicoscore laat zich aflezen in

de risicotabel (tabel 3) en voorspelt het risico op het ontwikkelen van een recidief of progressie naar spierinvasieve ziekte, op de korte en de lange termijn. In tabel 3 is te zien dat het scoringssysteem van de EORTC een verbetering geeft van de begrippen laag-, intermediair- en hoogrisico op recidivering en progressie van blaaskanker.

Patiënten met een spierinvasieve blaaskanker worden behandeld met een cystectomie, een operatie waarbij de blaas volledig wordt verwijderd. Ondanks deze radicale behandeling is de 5-jaars overleving van patiënten met een primair spierinvasieve blaaskanker slechts 55%. Voor patiënten met een niet-spierinvasieve blaaskanker is de 5-jaars overleving 88-90%. Echter, voor patiënten met een spierinvasieve blaaskanker die in het verleden zijn behandeld voor een niet-spierinvasieve blaaskanker, zakt de 5-jaarsoverleving naar 28%. Deze getallen benadrukken de vraag naar effectievere behandelopties tegen blaaskanker, en meer specifiek, betere behandelopties voor patiënten met een hoog risico op een recidief en/of progressie van een niet-spierinvasieve blaaskanker. Tegelijkertijd zou een conservatieve behandeling zo min mogelijk bijwerkingen moeten geven, waarbij de normale functie van de blaas behouden blijft.

In dit proefschrift worden nieuwe ontwikkelingen gepresenteerd op het gebied van intravesicale therapie voor patiënten met een laag-, intermediair- en hoogrisico niet-spierinvasief blaascarcinoom. Allereerst wordt in **hoofdstuk 2** een overzicht gegeven van de huidige adjuvante intravesicale farmacotherapieën voor de verschillende risicogroepen van het niet-spierinvasief blaascarcinoom. Daarbij worden de meest gangbare behandelingen, de op relatief korte termijn te verwachten nieuwe medicamenten en de zogenaamde apparaatgeassisteerde behandelingen besproken.

In het preklinische deel van dit proefschrift worden de resultaten van twee dierexperimentele studies gepresenteerd. In **hoofdstuk 3** wordt het orthotope blaas tumormodel in de rat besproken. Bij ratten worden via een dunne katheter UCC cellen in een

voorbehandelde blaas gebracht, met als doel deze uit te laten groeien tot een niet-spierinvasieve blaaskanker. Dit is een voor de kliniek zeer relevant model, omdat bij tumorgroei in de rattenblaas de effectiviteit van potentiële nieuwe medicijnen tegen het niet-spierinvasief blaascarcinoom reeds in een preklinische setting kan worden onderzocht. In deze studie wordt gekeken hoe de opgewekte blaastumoren zich bij de rat ontwikkelen in de tijd, om zodoende te bepalen op welk moment een toekomstige experimentele intravesicale behandeling zou moeten plaatsvinden. Daarnaast wordt er op verschillende momenten met een cystoscoop, via de plasbuis, in de blaas gekeken om tumorgroei in vivo (binnen het levende organisme) te kunnen observeren.

In **hoofdstuk 4** wordt een varkensmodel gebruikt om de veiligheid van pemetrexed (Alimta®) te onderzoeken. Pemetrexed is een middel waarvan de werkzaamheid reeds is aangetoond tegen een aantal vormen van kanker en daarbij bleek in één studie pemetrexed ook effectief tegen het spierinvasief blaascarcinoom (na toediening via een infuus). Dit middel zou dus als blaasspoeling ook werkzaam kunnen zijn tegen het niet-spierinvasief blaascarcinoom. Alvorens de effectiviteit van dit middel op patiënten te onderzoeken, is het wenselijk om met een varkensmodel (de urinewegen van het varken gelijken sterk op die van de mens) meer te weten te komen over de farmacokinetische eigenschappen en toxiciteit van pemetrexed, wanneer het intravesicaal wordt toegediend. Daartoe worden eerst oplopende doses pemetrexed als blaasspoeling toegediend aan varkens, waarbij het welzijn van de dieren, potentiële beenmergonderdrukking, geneesmiddelopname door het bloed en schade aan de blaaswand worden bestudeerd. Vervolgens wordt de hoogst tolereerbare dosis gedurende 6 weken wekelijks toegediend.

In het klinische deel van dit proefschrift worden nieuwe en verbeterde behandelopties tegen het niet-spierinvasieve blaascarcinoom gepresenteerd. In hoofdstuk 5 en 6 wordt het relatief nieuwe middel apaziquone (EOquin®) besproken. Apaziquone is een

chemotherapeuticum dat is afgeleid van het veel gebruikte middel mitomycine C (MMC). Bij celkweekonderzoek bleek apaziquone effectief te zijn tegen verschillende UCC cellijnen, en zelfs 27 keer effectiever dan MMC. Vervolgens lukte het in een fase II markerlaesie studie (bij patiënten met multipele blaastumoren wordt tijdens de TURT bewust één blaastumor van 5-10 mm niet weggehaald, de zogenaamde markerlaesie) om in 67% van de patiënten de markerlaesie compleet te laten verdwijnen met 6 wekelijkse blaasspoelingen apaziquone. Dit waren goede eerste onderzoeksresultaten voor dit middel en deze hebben de basis gevormd voor verschillende klinische vervolgstudies. In **hoofdstuk 5** worden de resultaten van een fase I/II studie weergegeven, waarin patiënten met een niet-spierinvasief blaascarcinoom direct na de TURT een eenmalige blaasspoeling met apaziquone krijgen toegediend. Om de veiligheid en farmacokinetiek van apaziquone direct na TURT te onderzoeken, wordt geobserveerd of patiënten bijwerkingen ondervinden van het middel en vindt bloedonderzoek plaats. **Hoofdstuk 6** is het vervolg van de bovengenoemde fase II markerlaesie studie. In het eerste deel van de studie werd bij 46 patiënten een markerlaesie achtergelaten, welke bij 31 patiënten (67%) 2-4 weken na de laatste blaasspoeling met apaziquone was verdwenen. Zowel de complete responders (markerlaesie verdwenen) als de non-responders (markerlaesie niet verdwenen, hiervoor werd alsnog een TURT verricht) werden gedurende 2 jaar gecontroleerd, waarbij werd gekeken naar de bijwerkingen van apaziquone op de lange termijn, en de recidiefvrije overleving.

In **hoofdstuk 7** worden drie verschillende behandelschema's van het veel gebruikte chemotherapeuticum epirubicine met elkaar vergeleken in een fase III studie. Blaasspoelingen met epirubicine worden door patiënten relatief goed getolereerd, en het middel is effectief in het voorkomen van niet-spierinvasieve recidieven van het blaascarcinoom. Ondanks verschillende gerandomiseerde klinische studies naar epirubicine, en intravesicale chemotherapie in het algemeen, is nog steeds niet bekend wat het optimale behandelschema

is, of hoe lang de therapie zou moeten worden gegeven. Om te onderzoeken of er een beter werkzaam behandelschema bestaat, hebben we de toegevoegde waarde onderzocht van 1) een blaasspoeling direct na TURT, of 2) twee onderhoudsblaasspoelingen in aanvulling op 3) het standaard behandelschema.

De resultaten van thermochemotherapie (Synergo® behandeling) voor patiënten met CIS van de blaas worden gepresenteerd in **hoofdstuk 8**. De gedachte achter thermochemotherapie is dat het toevoegen van warmte (hyperthermie) aan een intravesicaal toegediend chemotherapeuticum een synergistisch effect bewerkstelligt. Verschillende gerandomiseerde studies hebben reeds aangetoond dat MMC gecombineerd met hyperthermie inderdaad significant effectiever is tegen het niet-spierinvasief blaascarcinoom, dan behandeling met MMC alleen. In deze studie worden 51 hoogrisico patiënten met CIS van de blaas behandeld met thermochemotherapie, waarna het complete respons percentage (verdwijnen van CIS na 3 maanden) en de recidiefvrije overleving worden bepaald.

Samenvattend zijn er op het gebied van behandeling van het niet-spierinvasief blaascarcinoom verschillende nieuwe geneesmiddelen en behandelstrategieën in opkomst, welke in dit proefschrift zowel in een preklinische als klinische setting zullen worden onderzocht.

Chapter 2

Adjuvant intravesical pharmacotherapy for non-muscle invasive bladder cancer

K Hendricksen

JA Witjes

European Urology 2008;53(1):45-52

Current Opinion in Urology 2007;17(5):352-357

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Abstract

Objective: Review adjuvant intravesical pharmacotherapy for non-muscle invasive bladder cancer (NMIBC).

Methods: Search of published literature on conventional treatment of NMIBC, emerging drugs, and device-assisted therapies.

Results: In low-risk NMIBC patients an immediate instillation with chemotherapy is sufficient. For patients with intermediate- or high-risk tumours, additional adjuvant instillations are needed. For intermediate-risk patients chemotherapeutic instillations, usually with mitomycin-C or epirubicin, are safe and effective in reducing the risk of recurrence in the short term, but efficacy is only marginal in the long term. Newer drugs have promising results, but long term follow-up is limited or lacking. In these patients BCG does not seem to be more effective, only more toxic. In high-risk NMIBC, or patients in whom chemotherapy fails, BCG is the best choice with lower rates of recurrence and progression. For BCG failures cystectomy is therapy of choice, although the combination of BCG and interferon-alpha can be considered, just as device-assisted therapies such as thermochemotherapy and electromotive drug administration.

Conclusions: Risk-adapted first-line adjuvant therapy for NMIBC after TURBT is well established but has its limitations because recurrences are still numerous. Some new drugs and second-line therapies are promising, but efficacy should be confirmed.

Introduction

Bladder cancer is the fourth most common malignancy among men in the Western world, following prostate, lung and colon cancers, but the high recurrence rates makes it probably the most prevalent malignancy of these four, and certainly the most expensive per patient treated [1]. More than 90% of bladder cancers are urothelial cell carcinoma (UCC), and on average 70% of bladder UCCs present as non-muscle invasive bladder cancer (NMIBC). The initial treatment of NMIBC is transurethral resection of the bladder tumour (TURBT). After TURBT, patients receive adjuvant instillations of chemotherapy or immunotherapy to lower the (high) recurrence rate and to prevent or delay progression to muscle-invasive disease. As indicated, the progression rate and high recurrence rate are problems for the patient, the urologists and the community. The probability of recurrence or progression can be calculated with risk tables as provided by the European Organization for Research and Treatment of Cancer (EORTC), based on six clinical and pathological parameters [2]. Similar prognostic factors have been used by the European Association of Urology (EAU) to divide NMIBC into low-, intermediate- and high-risk groups, with subsequent therapeutic advisories. Risks of recurrence at 5 yr vary from 31% in low-risk patients to 78% in high-risk patients, the risk of progression from <1% to 45%, respectively.

In this review, we discuss adjuvant intravesical treatment strategies for NMIBC, with emphasis on currently available drugs and long-term results of those drugs used for longer periods.

Chemotherapy

Single immediate instillation

An immediate instillation after TURBT is advised for all patients with NMIBC because it reduces the risk of recurrence by about 50% at 2 yr, and ≥15% at 5 yr [3-6]. Several surveys and personal communications, however, suggest that approximately half of the European urologists, and a minority of the American urologists do, indeed, give an immediate instillation**.** Although an instillation within 6 h after TURBT seems to be most effective, Sylvester et al, in a meta-analysis of seven trials comparing TURBT alone to TURBT plus one immediate instillation, could not find significant differences in efficacy as long as the instillation is given within 24 h [6]. This meta-analysis also found that for one immediate instillation all chemotherapeutic drugs studied appear to have similar efficacy. In two EORTC studies, 30 mg mitomycin-C (MMC) or 50 mg doxorubicin was given and early (≤24 h) versus delayed (days 7-15) therapy regimens were compared [5]. For both agents early treatment with or without maintenance therapy was slightly superior to delayed therapy with maintenance. Although in general one immediate instillation is safe, as long as there is no evident bladder perforation, bladder complications have been reported anecdotally.

Data for one instillation with a new drug are very limited. An early single-instillation pharmacokinetic study has also been done with gemcitabine, a novel deoxycitidine analogue with a broad spectrum of antitumor activity, and considered standard in systemic therapy for advanced UCC of the bladder [7]. In nine patients, 2000 mg gemcitabine in 50 mL was instilled in the bladder during 1 h, immediately after TURBT. Grade 2 leukopenia and vomiting were seen, and the highest peak concentration of 4.26 μg/mL was found after extended bladder resection. Although the authors concluded that this approach is feasible with

acceptable toxicity, these results appear somewhat less favourable than the results we reported earlier [8], so very clearly more studies are needed.

In all, an immediate instillation can be considered sufficient for patients with low-risk tumours. For patients with intermediate- or high-risk tumours, additional adjuvant instillations are needed.

Multiple delayed instillations

A cycle of instillations with a chemotherapeutic drug is treatment of choice for patients with intermediate-risk NMIBC because it is able to reduce the short-term risk of recurrence. For this, the most used drugs are MMC and epirubicin. MMC is used in dosages of 20-60 mg and is administered once a week for 4-8 wk, eventually followed by some kind of maintenance schedule [9]. The same applies for epirubicin in dosages of 30-80 mg [10]. Both agents have relatively few side-effects. The results with intravesical chemotherapy, however, are relatively limited with long-term follow-up. In an analysis by Pawinski et al, the long-term efficacy of TURBT alone was compared to TURBT plus adjuvant treatment in 2535 patients with Ta-T1 NMIBC [11]. With a median follow-up of 4.6 yr for disease-free survival, 5.5 yr for muscle invasion, and 7.8 yr for survival, adjuvant treatment resulted in an only 6% decrease in the risk of recurrence (47%) as compared to the no treatment group (52.6%). Although there was a significantly favourable impact on the disease-free interval, there was no difference in terms of time to muscle invasion, duration of survival or progression-free survival. In a study by Hendricksen et al, 1000 patients (the majority multiple Ta G1-2 UCC) were randomised among three different treatment schedules of epirubicin [12]. The results at 5-yr follow-up were even lower, as in the above-mentioned analysis; 44.0% of the patients were recurrence-free and 88.6% of the patients progression-free, with no significant differences between the three schedules. Obviously, the results with these drugs, although they have been studied

extensively and are considered standard of care, have limitations. One of the possibilities to improve these somewhat disappointing results was reported by Au et al [13]. In the "optimized treatment arm" they instructed the patients to decrease urine output and used urine alkalinisation and a double dose of MMC. These changes led to an approximately 17-mo longer median time to recurrence and an approximately 15% higher recurrence-free rate at 5 yr. However, how much of this improvement is attributable to the double dose remains unclear.

Results with newer drugs are emerging, but long term follow-up data are still lacking, and as demonstrated above, long-term data are extremely important. One of these newer drugs, gemcitabine, appears to have minimal toxicity when used intravesically in doses up to 2000 mg/50 mL for 2 h [8;14]. Ablation of a marker lesion is seen in up to 56% [15-17]. Dalbagni et al even obtained a complete response in 15 of 30 patients (50%) refractory to Bacillus Calmette-Guérin (BCG) refusing cystectomy, although with a 1-yr recurrence-free survival rate of only 21% [18]. A study with prophylactic intent reported a 1-yr recurrence rate of 25.9% (21 of 81 patients) with intermediate-risk tumours, of which again 6 of 24 patients (25%) were refractory to BCG therapy [19]. However, patients with high-risk tumours had a recurrence rate of 77.1% (27 of 35 patients). Of even more recent date are the studies with apaziquone or EO9, a novel indolequinone derivative of MMC. Both drugs are inactive prodrugs which require activation by cellular reductase enzymes to become cytotoxic [20]. The enzyme that has a crucial role in the activation of EO9, deoxythymidine-diaphorase (DTD), has a high activity in about 40% of bladder tumours as compared to normal bladder tissue. This suggests that selective efficacy against tumour cells may be achieved [21]. In preclinical research the concentration of EO9 needed to achieve 50% cell kill at 37°C was 6-78 times lower than that of MMC depending on the cancer cell line used [22]. In a marker lesion study on patients with low- to intermediate-risk NMIBC, 30 of 45 patients (67%)

achieved a histologically proven complete response 2-4 wk after the last of six instillations of 4 mg/40 mL EO9 [23]. The side-effects of EO9 were comparable to other chemotherapeutic agents used against NMIBC.

Recently, also a phase 1 trial with intravesical docetaxel was reported in patients with recurring NMIBC [24]. In a dose-escalation study 18 patients were treated. No grade 3 or 4 dose-limiting toxicities, nor systemic absorption of docetaxel were reported. Eight (44%) of 18 patients experienced grade 1 or 2 toxicities, predominantly dysuria. Ten (56%) of 18 patients had no evidence of disease at their posttreatment cystoscopy and biopsy; there was no progression in relapsing patients. Intravesical docetaxel appears safe, and further studies are needed.

In all, these studies show that chemotherapeutic instillations are safe and effective in reducing the risk of recurrence in the short term. Drugs that are currently used as standard are MMC and epirubicin. Unfortunately, there is only marginal long-term efficacy, which is not good for the patients, a burden for the urological practice, and expensive for the community. Newer drugs are interesting and have promising results in the patient groups studied, but long term follow-up is limited or completely lacking.

Immunotherapy

Because intravesical chemotherapy clearly has its limitation, intravesical immunotherapy becomes interesting. Intravesical immunotherapy is predominantly done with BCG, and comparisons clearly show that intravesical chemotherapy is less effective than intravesical BCG. Krege et al compared TURBT alone to TURBT plus adjuvant MMC or BCG in patients with Ta-T1, G1-3 NMIBC [25]. Patients receiving TURBT alone had a significant increase in risk of recurrence as compared to the adjuvant treatment groups, but there was no significant

difference between the MMC or BCG groups. Witjes et al found similar results in a study comparing nine instillations with 30 mg MMC versus six instillations with either BCG-Tice or BCG-RIVM [26]. Recurrence rates, even in patients with carcinoma in situ (CIS), where numbers were low, were not significantly different.

In these older studies, however, predominantly low- and intermediate-risk patients were included, where a difference in efficacy is more difficult to prove. Moreover, in both studies no maintenance BCG was used. Only recently was the superior efficacy of BCG over chemotherapy clearly proven by several large meta-analyses. Sylvester et al performed a meta-analysis of 24 clinical trials with 4863 patients comparing TURBT plus intravesical BCG to either resection alone or resection plus another treatment than BCG [27]. They found that adjuvant BCG is superior to TURBT alone and more effective than adjuvant chemotherapeutic drugs with regard to progression-free survival; after a median follow-up of 2.5 yr, progression was seen in 9.8% in the BCG-treated group versus 13.8% in the non-BCG group (odds ratio [OR] = 0.73, p = 0.001). This difference was even larger when only maintenance trials were used: OR 0.63, p = 0.00004. However, the follow-up is relatively short, resulting in a low absolute number of patients with progression: 6.4% in patients with papillary tumours and 13.9% in patients with CIS. Moreover, several small trials were included and finally no difference in tumour-related survival.

Boehle et al came to similar conclusions after their meta-analysis, comparing nine trials with 1328 NMIBC patients treated with adjuvant MMC, to 1421 patients treated with BCG [28]. Without a clear separation of results for patients with intermediate- or high risk-NMIBC, the overall recurrence rate was 46.4% for MMC and 38.6% for BCG, after a mean follow-up of 26 mo. BCG also had a statistically significant superiority in reducing the risk of progression; after a median follow-up of 26 mo the rate of progression using all trials was 7.7% for BCG-treated patients versus 9.4% for MMC-treated patients (OR= 0.77, p = 0.08).

Including only the five trials that used BCG maintenance increased the OR to 0.66 (p = 0.02). Also in this meta-analysis follow-up was limited. Whether with longer follow-up the difference between BCG and MMC increases or decreases remains a question. One of the very few long-term studies comparing BCG and MMC was recently published. Gardmark et al reported the 10-yr follow-up of a randomised study comparing 2 yr of maintenance BCG (Danish strain) or MMC (40 mg) in 261 patients with intermediate- and high-risk NMIBC [29]. After a median follow-up for survivors of 123 mo, disease progression was found in 58 of the 250 evaluable patients; 34 in the MMC group and 24 in the BCG group (p = 0.26). Overall survival was also similar (log-rank test, p = 0.98), with 32% dying due to bladder cancer. Whether the use of this specific BCG strain explains this lack of difference, or the use of this maintenance MMC schedule remains unclear. Arguments in favour of an extensive MMC schedule can also be found in a recent German study [30]. They studied 495 intermediate- and high-risk NMIBC patients. Patients received 6 weekly instillations with BCG RIVM 2 x 10^8 colony-forming units (CFUs) or 20 mg of MMC, or 20 mg of MMC for 6 wk followed by monthly instillations for 3 yr. They found that 3 yr of MMC significantly increased the recurrence-free rates compared to BCG or 6 wk of MMC (86.1% vs. 65.5% vs. 68.6%, log-rank test, p = 0.00), without increasing the toxicity in the maintenance MMC group.

Clearly the reduction in the risk of progression is only achieved with maintenance BCG, which, in its turn, leads to more frequent and more severe local and systemic side-effects than with intravesical chemotherapy. Lamm et al also showed significantly improved recurrence-free survival time in high-risk NMIBC with BCG maintenance therapy [31], but also found increased side effects; 5% of patients had to stop during induction therapy and 20% of patients during maintenance therapy [32]. In an attempt to control these side-effects the EORTC randomised 957 patients with intermediate- and high-risk NMIBC for adjuvant

treatment with BCG, BCG and isoniazid, or epirubicin [33]. The superiority of BCG was again proven; at 3 yr, 49% of patients receiving epirubicin were recurrence-free and about 65% of patients treated with BCG (with or without isoniazid). Progression to muscle-invasive disease was infrequent (5%) and similar in the three groups. As also expected, in the epirubicin group drug-induced cystitis was less and there were no systemic side-effects. Isoniazid, unfortunately, did not reduce BCG toxicity, nor, by the way, did it influence BCG efficacy.

Surprisingly, a recent study in 115 BCG-naïve patients, treated with BCG and 200 mg ofloxacin or placebo, showed that prophylactic ofloxacin decreased the incidence of moderate to severe side effects and improved compliance to BCG therapy [34].

Obviously, another way of trying to overcome BCG toxicity is lowering the BCG dose. Ojea et al recently reported a trial comparing MMC (30 mg) to a one-third (27 mg) and one-sixth (13.5 mg) dose of BCG in 430 intermediate-risk patients [35]. Instillations were weekly during 6 wk and once every 2 wk during again 6 wk. The 27-mg BCG dose had the lowest recurrence rate, but only significantly better than MMC. Toxicity was similar in both BCG groups, less in the MMC group. This study suggests that the optimal BCG dose lies around 1/3 of the full dose.

BCG is apparently also more cost effective than intravesical chemotherapy. Uchida et al recently calculated costs of BCG therapy during a 86-mo observation period in 138 tumours [36]. Because BCG was the most significant factor preventing recurrence, improving the 5-yr recurrence-free survival rate from 28% to 78%, the cost-effectiveness ratio of BCG therapy was approximately 3900 dollars/5-yr recurrence-free period.

In all, for patients with intermediate-risk NMIBC, BCG does not seem to be superior to chemotherapy, and has significantly more side-effects. However, in studies with more high-risk NMIBC patients included, the superiority of BCG is evident with regard to the reduction

in recurrence rate, progression, and costs. BCG should, therefore, be reserved for intermediate-risk patients in whom intravesical chemotherapy fails, but it is the treatment of first choice for patients with high risk NMIBC.

Patients in whom adequate BCG treatment fails, especially high-risk patients, are confronted with the threat of radical therapy, such as radical cystectomy as treatment of choice [37]. Alternative intravesical therapy is an option, but imposes an oncological risk. Tumour-specific survival in case of cystectomy for BCG failures is between 80% and 90% in 5 yr, approaching the tumour-specific survival of 88%-90% of the whole group of patients with NMIBC [38;39]. In comparison, a small study conducted by Schrier et al showed a 5-yr tumour-specific survival of 55% for patients with primary muscle-invasive tumour, and only 28% 5-yr tumour-specific survival for patients with progressive invasive tumour [40]. Still, patients may refuse or be unfit for major surgery.

The combination of interferon-α (IFN-α) and BCG for BCG failures has been the subject of a large multicenter phase 2 trial [41]. In all, 467 patients in whom BCG failed were treated with low-dose BCG plus IFN-α. Twenty-seven percent of these patients had isolated or concomitant CIS. With a median follow-up of 24 mo 45% remained tumour free, compared to 59% in the BCG-naïve group (n = 536). The authors concluded that this combination could be effective for BCG-failing patients but also stress that certain characteristics influence durable response. So again, also this approach needs confirmation.

Device-assisted therapy

Other possibilities are new conservative approaches such as device-assisted therapies. Of course, one should realise that currently available device-assisted therapies are not used on a

large scale (yet), and mainly in small groups of high-risk patients or as second-line therapy. This means that these results also clearly have to be interpreted with caution.

Thermochemotherapy

The Synergo® system induces bladder wall hyperthermia around 42-43°C with a special catheter, also equipped with internal thermocouples to monitor the temperature. It is currently used in combination with intravesical MMC (thermochemotherapy), and several trials have shown its superiority over MMC alone [42;43]. It has significantly more side-effects, although these are moderate and transient. Van der Heijden et al reported the use of thermochemotherapy with prophylactic intent in 90 patients with intermediate- and high-risk NMIBC [44]. After 1 yr and 2 yr follow-up. respectively, 14.3% and 24.6% of all patients experienced a recurrence. In 41 patients in whom BCG failed the recurrence rates, respectively, were 23% and 41%. Witjes et al recently presented a multicentre study in which 57 patients (40 BCG failures, 29 with concomitant papillary tumours) with CIS were treated with 6-8 weekly and 4-6 monthly sessions of thermochemotherapy [45]. Forty-five of 48 patients (94%) evaluable for response, had a biopsy- and cytology-proven complete response. Despite promising initial results, obviously long-term follow-up is awaited.

Electromotive drug administration

Electromotive drug administration (EMDA) is based on the concept of temporarily enhancing penetration of drugs through the urothelial barrier of the bladder with an electrical gradient between the bladder wall and the bladder contents. Colombo et al compared four weekly ablative sessions prior to TURBT in low-intermediate risk patients undergoing either thermochemotherapy (n = 29), or EMDA (n = 15) or MMC only (n = 36), obtaining complete responses in, respectively, 66%, 40%, and 27.7% [46]. Di Stasi et al compared MMC only,

MMC combined with EMDA, and BCG in 108 high-risk patients, obtaining complete responses in, respectively, 31%, 58%, and 64%, after 6 mo of follow-up [47]. Side-effects with EMDA were more than with MMC alone, but still significantly less than with BCG. In a study by the same authors, 212 patients with stage T1 UCC were randomised for BCG alone versus sequential BCG and MMC/EMDA, with maintenance therapy in both arms [48]. With a mean follow-up of 88 mo, sequential BCG and MMC/EMDA had a higher disease-free interval of 69 mo versus 21 mo for BCG only, a lower recurrence rate of 41.9% versus 57.9%, a lower progression rate of 9.3% versus 21.9%, and a lower disease-specific mortality of 5.6% versus 16.2%. Even if this is only one smaller study, especially the significant difference in the progression rate after sequential use of MMC/EMDA is remarkable.

Photodynamic therapy

Photodynamic therapy (PDT) is just emerging as a potential new treatment option. It combines photo sensitizers that selectively bind to tumour and a powerful intravesical light source to destroy the complex of tumour cell and photo sensitizer. The first studies on PDT were performed after oral administration of 5-aminolevulinic acid (5-ALA), causing hemodynamic side-effects (hypotension, tachycardia) in the majority of patients [49]. These could be avoided by the use of intravesical 5-ALA [50]. Berger et al showed that 16 of 31 PDT-treated NMIBC patients were recurrence free after a median follow-up of 23.7 mo (4 of 10 BCG failures). Local side-effects were minimal and included dysuria and haematuria. PDT was proposed as a second-line treatment for patients with multiple comorbidities, who are not surgical candidates. With the newer generation of photo sensitisers, which at least have improved diagnostic potential, these results might even be better. Clinical data, however, are not available as yet.

Conclusions

An immediate intravesical instillation after TURBT with a chemotherapeutic drug is considered first-line therapy for all patients with NMIBC and is sufficient for patients with low-risk tumours. For intermediate-risk patients an additional course of chemotherapy is indicated because BCG offers little advantage in this group and is more toxic. Still, with long-term follow-up the effect is limited, and the risk of progression is not reduced at all. New drugs are under investigation, but follow-up is limited. Patients in whom chemotherapy fails and those with high-risk NMIBC should be treated with maintenance BCG. BCG is superior in reducing recurrences in these patients, and additionally it reduces the risk of progression although BCG has more and more severe side-effects.

For patients in whom BCG therapy fails, cystectomy should be considered. If patients are unwilling or unfit, BCG plus IFN-α offers some potential, as does novel device-assisted therapies such as thermochemotherapy and EMDA. Results are promising but should be confirmed.

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Chapter 3

Evaluation of an orthotopic rat bladder urothelial cell carcinoma model by cystoscopy

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Abstract

Objectives: To enable preclinical testing of intravesical therapies against non-muscle invasive bladder cancer (NMIBC) in an orthotopic rat bladder tumour model, augmented by the use of serial cystoscopy for in vivo tumour assessment and follow-up.

Materials and methods: Fischer F344 rats had a 16-G transurethral cannula placed. The bladder mucosa was conditioned with an acid rinse, followed by a 1-hour instillation of 1.5 x 10^6 AY-27 rat bladder urothelial cell carcinoma (UCC) cells (day 0). Cystoscopy (1 mm) was done on day 0 (control) and at 3, 4, 5, 6, 7, 10, 13 and 17 days. At the scheduled times the rats were killed after cystectomy (four at each time) for histopathological examination of the bladder.

Results: Overall, tumour establishment was >80%, with predominantly carcinoma in situ preceding or concomitant with invasive tumour growth. All tumours were formed at 3-5 days, and remained non-muscle invasive up to 5 days. From 6 days, tumours progressed to muscle invasive disease in 40% of the rats. Visibility at cystoscopy was excellent and tumours were apparent in >90% of rats from 5 days on, with a specificity and sensitivity >90%. Cystoscopy could not distinguish NMIBC from muscle invasive disease.

Conclusions: This is a reliable model of orthotopic rat bladder UCC, with early high grade NMIBC growth, immediately followed by muscle invasive growth, i.e. the recommended time to start intravesical therapy would be 5 days after tumour cell inoculation. Tumour growth can easily be monitored by cystoscopy, but cannot be used to distinguish NMIBC from muscle invasive bladder cancer.

Introduction

On average, 70% of bladder tumours present as non-muscle invasive bladder cancer (NMIBC) [1]. Transurethral resection and adjuvant intravesical instillations with chemotherapy or immunotherapy are considered the standard treatment for NMIBC, to lower the recurrence rate and the risk of progression to muscle invasive bladder cancer. The European Organisation for Research and Treatment of Cancer calculated that the probabilities for recurrence and progression of NMIBC are 31-78% and <1-45% at 5 years respectively [2]. These rates illustrate the modest success of currently available treatments and underline the need for improved adjuvant treatment.

To test potential new drugs against NMIBC in a preclinical setting, a clinically relevant orthotopic bladder tumour model is highly desirable. Xiao et al. described an orthotopic rat model resembling human urothelial cell carcinoma (UCC), with reproducible tumour growth [3]. The use of rats was advantageous, as these are of sufficient size to test experimental chemotherapy, immunotherapy and device-assisted therapies. Until recently, four separate research groups adapted this model and published the results of various experimental therapies. A potential demerit in these studies is the timing of intravesical therapy after tumour cell inoculation; in some rats tumours had not formed by this time, while other rats had already developed muscle invasive tumours. Moreover, tumour behaviour could only be determined at autopsy, not in vivo.

Therefore, the primary objective of the present study was to assess the growth of UCC in the orthotopic bladder rat model over time, aiming to have a maximum number of rats with NMIBC at a particular time and thus optimize the future starting point of experimental intravesical treatment. In addition, our secondary objective was to improve in vivo tumour assessment by using serial fine-needle cystoscopy.

Material and methods

AY-27 transitional cell carcinoma cells (kindly provided by Dr Ronald Moore, University of Alberta and Cross Cancer Institute, Edmonton, Alberta, Canada), derived as primary bladder tumour from Fischer F344 rats fed with FANFT (N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide), were grown as a monolayer culture in RPMI-1640 medium with L-glutamine, supplemented with 10% fetal bovine serum, 100 U/mL penicillin G and 100 µg/mL streptomycin, in a humidified 95% air/5% carbon dioxide atmosphere.

Animal procedures were carried out in compliance with national and European regulations, and approved by the Committee for Animal Experiments (Radboud University Nijmegen Medical Centre, The Netherlands). Female Fischer F344 rats (150-175 g; Charles River, L'Arbresle Cedex, France) were housed in type 3 cages (Techniplast, Milan, Italy) with 'gold-flakes' bedding (SPPS, Frasne, France) and environmental enrichment.

The tumour establishment procedures were done under inhalation anaesthesia, using isoflurane 2-5% (induction), followed by isoflurane 2%, nitric oxide 0.5 L/min and oxygen 1 L/min. The rat bladder was catheterized via the urethra with a 16-G (1.4 mm) plastic i.v. cannula (BD Biosystems, Erembodegem-Aalst, Belgium) and drained. To facilitate tumour seeding, the bladder mucosa was damaged by a 15-s instillation of 0.4 mL 0.1 M hydrochloride (HCl) and neutralized by adding 0.4 mL 0.1 M potassium hydroxide (KOH) for 15 s. The bladder was then drained and flushed three times with 0.8 mL 0.01 M phosphate-buffered saline (PBS). Immediately after bladder conditioning, freshly harvested AY-27 cells (1.5×10^6 in 0.5 mL medium; time between cell harvesting and bladder inoculation <1 h) were instilled via the catheter and left indwelling for 1 h. The position of the rats was changed every 15 min by 90° to facilitate full bladder wall exposure, and were observed for leakage of the cell suspension around the catheter. The catheter was removed after 1 h and the rats were

allowed to void spontaneously. The well-being of the animals was monitored daily, with special emphasis on haematuria and weight loss.

Macroscopic tumour growth was assessed by cystoscopy before bladder conditioning (day 0) and at 4, 7, 10, 13 and 17 days after. A fibre-optic needle arthroscope (Karl Storz, Tuttlingen, Germany) with 1.0 mm diameter, 6.5 cm long and with a miniature straight forward 0° telescope was used. The catheter functioned as sheath with an airtight rubber plug connected to it. The cystoscope was inserted and the bladder distended with 0.8 mL of air. The bladder surface was inspected systemically and images were recorded with the AIDA^{TM} DVD (Karl Storz).

On each day that cystoscopy was done, four pre-scheduled rats were killed by carbon dioxide inhalation, for histopathological examination. After cystectomy the bladders were fixed in 10% buffered formalin, laminated, embedded in paraffin, and tissue sections of 5 μm were cut and stained with haematoxylin and eosin (HE). Two observers (C.A.H.K. and K.H.) evaluated the number of tumours, tumour stage (2002 TNM staging system) and tumour grade (1998 WHO classification). Inflammation in the mucosa and submucosa was quantified as no reaction, mild, moderate or severe reaction.

Results

In our initial experiments the first rats were scheduled to be killed at 4 days; evaluation earlier seemed to be unwarranted, based on observations by Xiao et al. who reported two of three tumours were NMIBC at 16 days [3], and by Asanuma et al., who found various stages of tumour formation in all 22 rats assessed at 7-10 days [4]. However, based on the results presented later, we did additional experiments in which four groups of four rats had cystoscopy, and were killed on day 3, 4, 5 and 6 days, respectively.

Serial catheterization and anaesthesia were in general well tolerated, and only one rat died during anaesthesia on day 0. Some rats had mild temporary haematuria directly after bladder conditioning or catheterization. One rat had leakage of the cell suspension after 17 min, but it did not hamper later tumour formation. On day 0 the mean (range) weight was 162.8 (141.9-183.1) g, and remained unchanged until 10 days for the rats in the initial experiments. At 13 and 17 days, respectively, two of eight and three of four rats lost weight, with a mean loss of 13.1 (7.6-26.1) g, accompanied by lethargy, inactivity, hunched back, muffled skin and a prominent red neck (Harder's gland secretion). One rat scheduled for assessment at 17 days was killed at 13 days. At 17 days, one rat had bladder (tumour/clot) retention, which was palpable and relieved after catheterization. Four rats in the short-period experiments lost weight, by 17.7 (11.3-29.7) g, at respectively 2, 3, 4 and 5 days, and two rats were killed earlier than planned (at 2 and 3 days).

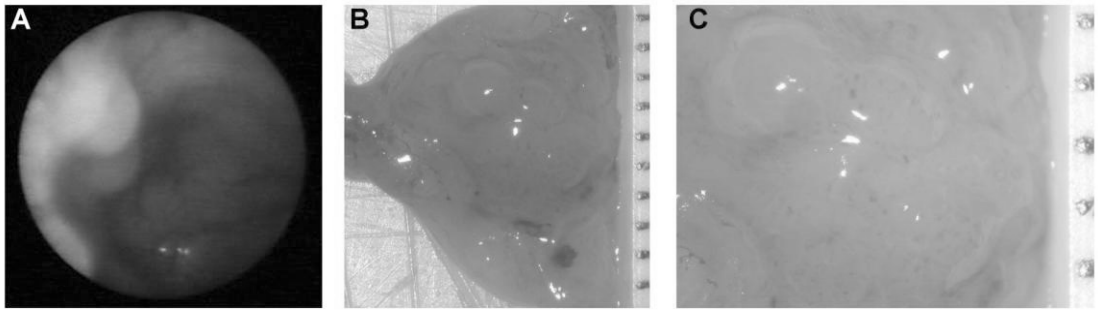
The first cystoscopies were done after distending the bladder with PBS, but urine and blood mixed with PBS resulted in poor visibility. Therefore we changed to pneumo-cystoscopy, which resulted in excellent bladder visualization. No abnormalities were seen on day 0. The first tumour formation was seen at 3 days (Table 1). Until 4 days, tumours were predominantly solitary, and most formed tumour plaques later (Figure 1). We could not discriminate bladder wall irritation, carcinoma in situ (CIS) or early tumour growth. Bladder stones were often multiple (one to six) and present in the same rats throughout time. A 2-mm stone was isolated on bladder dissection and subsequently analyzed by Fourier transform-infrared spectroscopy to assess the chemical composition. It contained magnesium ammonium phosphate (struvite), which is produced by the combination of a urinary tract infection and bacteria producing a urease [5].

Table 1 Tumour growth assessment and bladder wall inspection with cystoscopy

		Macroscopy*				
Day	No. of rats	Solitary tumours	Tumour plaques	Redness	Stone formation	No. of rats with tumour†
4	20	15	3	4	0	8
7	16	25	14	9	1	15
10	12	23	19	2	3	12
13	8	6	9	3	4	7
17	4	3	4	2	3	4
3	4	0	1	1	0	1
4	2	1	1	2	1	1
5	4	2	3	0	1	4
6	4	0	4	1	1	4

*Solitary tumours, respectively, tumour plaques, are counted in absolute numbers per rat and taken together. Redness could be irritation, CIS or early tumour growth. Stone formation is scored as the number of rats with stone formation. †Scored as the number of rats with solitary tumour(s) and/or tumour plaques.

Figure 1 Tumour macroscopy



A, tumour plaque formation on the right lateral wall, visualized with cystoscopy (at 10 days). **B**, x6, and **C**, x10, a dissected bladder with central tumour plaque formation (at 10 days). The scale is in millimetres.

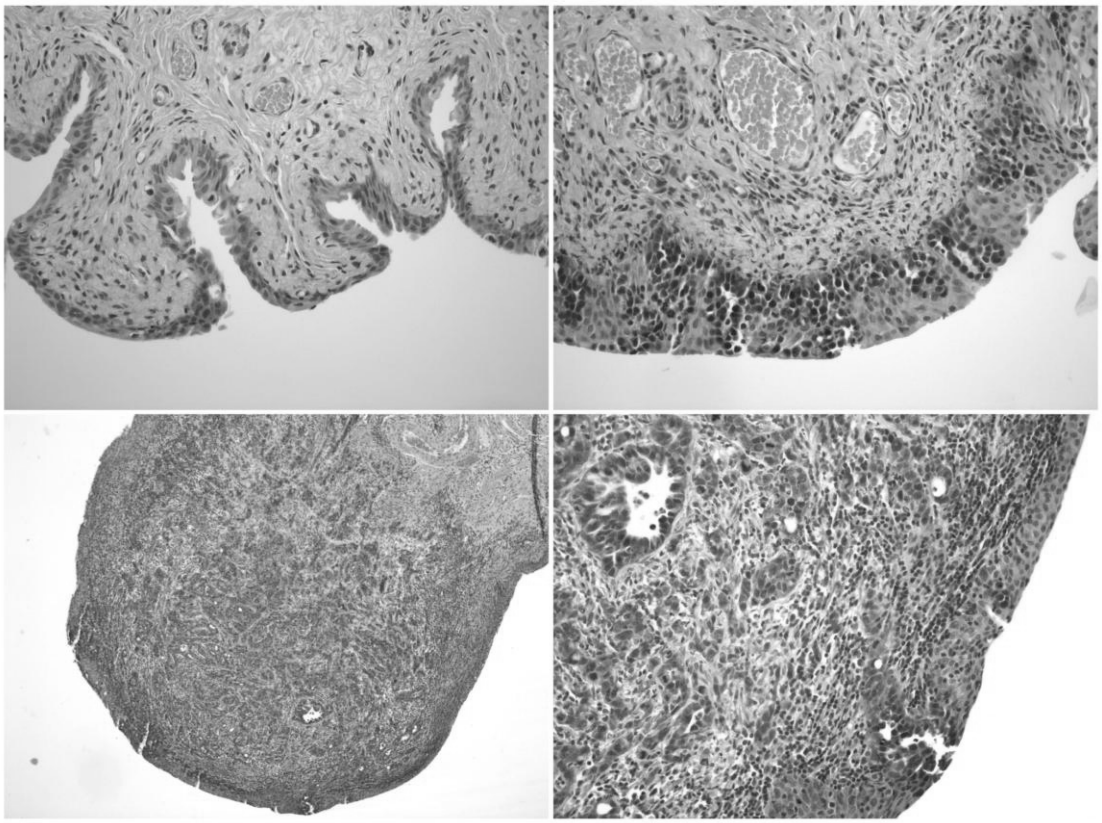
Tumour stage was scored for the highest category per rat (Table 2). All tumours were solid UCCs of grade 3, with a slight tendency towards adenomatous differentiation. The cystectomy samples showed no extravesical abnormalities. No progression to T3-4 tumours was seen. CIS was present alone or concomitant with tumour formation in 25 of 35 cases and appeared to precede invasive growth in the submucosa. Furthermore, CIS appeared as the Pagetoid subtype in 24 of 25 rats, showing dispersed aberrant nests in the pre-existing urothelium (Figure 2). Only one rat (at 3 days) had the diffuse CIS type. At 17 days, one rat

Table 2 Microscopic tumour evaluation of four rats scheduled per time point, compared with macroscopic tumour assessment

Day	No. of rats	Tumour stage						No. of rats with tumour	
		None	CIS only	T1	T1/CIS	T2	T2/CIS	On microscopy	On cystoscopy
2*	1	1	0	0	0	0	0	0	not done
3	4	1	2	0	1	0	0	3	1
4†	6	1	3	0	2	0	0	5	1
5	4	0	1	0	3	0	0	4	4
6	4	0	0	0	3	0	1	4	4
7	4	0	0	0	2	0	2	4	3
10	4	0	0	0	2	0	2	4	4
13	4	1	0	0	1	2	0	3	3
17‡	4	2	0	1	0	1	0	2	4

*One rat of the additional experiments was killed earlier than planned; †The two rats that died in the additional experiments were deducted from this group; ‡One rat had reactive papillary hyperplasia with stone formation.

Figure 2 Bladder histology



A, a normal-appearing urothelium of a pilot rat, after bladder conditioning with no tumour inoculation, at 4 days (x200). **B**, CIS extending in a Pagetoid manner along the basement membrane, at 4 days (x200). **C**, T2 tumour with CIS, at 10 days (x50), and **D** (x200), respectively.

with massive stone formation on cystoscopy had acute inflammation with reactive papillary hyperplasia on histology, with no concomitant CIS or tumour.

The pre-existing urothelium consisted of three cell layers in the absence of tumour formation, and reactively thickened to nine cell layers at sites of tumour formation or of moderate to severe inflammation. Mild (nine of 35 rats) and moderate (17 of 35 rats) inflammation was seen in mucosa and submucosa, mainly around tumour formation and invasion. Eight rats had erosion of the mucosa, of which four had severe inflammation of the submucosa, and one had abscess formation with signs of bladder perforation, but tumour formation was not hampered. The rat that had to be killed at 2 days had a purulent bladder inflammation with wall necrosis, and intravascular bacteria.

Discussion

To enable preclinical testing of intravesical therapies against NMIBC, we validated an orthotopic rat-bladder tumour model [3], and attempted to augment the model by the use of serial cystoscopy for in vivo tumour assessment. Using 1.5 x 10^6 AY-27 cells, our overall tumour establishment was >80% in rats killed at the scheduled times, at 3 to 17 days. Histology confirmed tumour growth from 3 days on, and, in the case of tumour growth, solely NMIBC (CIS, T1/CIS) up till 5 days.

Several research groups have adapted the orthotopic rat bladder UCC model as initially reported by Xiao et al [3], but the implementation of the model differs [4,6-8], particularly the number of tumour cells inoculated after bladder conditioning, and the times chosen for the start of treatment vary. In addition, most groups do not report the use of control rats, raising uncertainty about tumour existence, or the existence of muscle invasive tumour at the start of treatment. Xiao et al. administered 1.0-3.0 x 10^6 AY-27 cells and recommended

14-16 days as the most suitable time to start intravesical treatment [3], but changed to 13 days [9], and later to 10 days [10]. In their initial report, tumour was found in five of eight rats at 12-13 days, in 80 of 82 rats at 16-17 days, and in all 12 rats at 22-50 days, with NMIBC (CIS, T1/CIS) in, respectively, all five, 52 (65%) and three of twelve of these rats. Kamuhabwa et al. used 1.0 x 10^6 AY-27 cells and started treatment at 6-8 days [7]. Gronlund-Pakkanen et al. used 0.45 x 10^6 AY-27 cells and only found NMIBC grade 2-3, in 33 of 40 (83%) control rats at 15 days [8].

Our findings differ from the initial report by Xiao et al, who described three stages of tumour growth, i.e. early tumour establishment (1-13 days), mid-stage intravesical progression (14-21 days), and advanced intravesical progression and extravesical spread (22-50 days) [3]. In the present experiments, there was tumour cell growth and extension along the basement membrane only at 3 and 4 days (Figure 2B) and it appeared as a patchy or Pagetoid subtype of CIS, followed by direct invasion of the stroma as solid grade 3 UCC, with no preceding or concomitant differentiation towards papillary tumours. From 6 days on, 40% of rats had early progression to muscle invasive disease. A plump papillary aspect of the mucosa, which had a reactive rather than neoplastic aspect, accompanied by severe inflammation and massive stone formation, was found in only one rat (at 17 days). The observed increase of tumour aggressiveness might be explained by more passages in vitro (personal communication, Z. Xiao, Edmonton, Alberta, Canada). We received the AY-27 cells in passage 3, and rats were challenged with passage 11-13.

Accurate in vivo tumour assessment could improve the starting point of treatment in an orthotopic rat bladder model of UCC. Ideally, all rats should have formed tumours, all with detectable NMIBC. The latter is a difficult issue; gross haematuria and weight loss, or palpation of an abdominal mass, are easily monitored, but fairly indicative for advanced disease. Cytology was tried, but the correlation with actual bladder cancer formation was poor

[8]. We did not use cytology in the present rats, but it is predictable that there would be a poor correlation with bladder cancer formation due to the patchy growth pattern of CIS in our model. With imaging techniques like MRI it is feasible to construct tumour growth curves [11], but the technique is difficult for small early lesions, and is relatively complicated [12]. Xiao et al. reported little benefit from MRI, due to the tumour detection limit of ≥2 mm, and the high reproducibility of their model [3]. Rooks et al. found intra-abdominal tumours as small as 1.5 mm with ultrasonography in mice, and that were already muscle invasive [13]. Techniques like fluorescent imaging and bioluminescence imaging have the potential to become valuable tools for the early detection of tumour growth in the rat bladder, but still need further exploration.

With serial cystoscopy to 17 days we were able to compare in vivo macroscopy with the histology of four rats per sample time. Although there was tumour in eight of 20 rats at 4 days in the initial experiments, none of the eight rats were scheduled for death. In our additional experiments, one of two rats at 4 days had tumour on cystoscopy, confirmed by histology. From 5 days on, tumour formation was seen by cystoscopy in 22 of 24 rats, while 21 of 24 rats had actual tumour formation, as confirmed by histology. In two rats we reached a false-positive diagnosis with cystoscopy (both at 17 days), of which one lesion appeared to be papillary hyperplasia on histology. In one rat our diagnosis was false-negative (at 7 days), accounting for a sensitivity and specificity of >90%. A Japanese group used serial cystoscopy in rats inoculated with 4.0 x 10^6 AY-27 cells, and found NMIBC in 20 of 22 rats (91%) at 5-14 days. However, the rats were killed whenever tumour was detected [4].

A potential pit fall of cystoscopy in the rat model might be the different appearance of tumour growth than present in humans. The mucosa is mainly intact, with submucosal tumour growth leading to the ‘solid bulgy appearance’ of the surface (Figure 1A). This might have led to fewer tumours being detected on cystoscopy for the earliest study, while we could see

all four tumours on cystoscopy at 5 and 6 days in the additional experiments. However, the tumour we misinterpreted at 7 days was so massive that the entire bladder wall had the same, normal appearance. Redness of the bladder wall could have been CIS, but we did not score this as tumour. In this particular model the misdiagnosis of CIS was allowed, as these lesions were immediately followed by invasive tumour growth, which was visible by cystoscopy. Most invasive bladder tumours were detected with cystoscopy. However, non-muscle invasive growth (T1) could not be distinguished from muscle invasive growth (≥T2).

In conclusion, the orthotopic AY-27 rat bladder tumour model is reliable, with early high grade non-muscle invasive tumour establishment (CIS and/or pT1), that is immediately followed by muscle invasive growth. Invasive tumour growth and further tumour development can be easily monitored by cystoscopy, but cystoscopy cannot differentiate NMIBC from muscle invasive bladder cancer. The fast progression toward muscle invasive growth leaves a narrow window for the start of experimental intravesical treatment against NMIBC, preferably at 5 days after tumour cell inoculation. However, it is anticipated that therapeutic effects can be assessed rapidly.

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Chapter 4

Potential and toxicity of intravesical pemetrexed: a preclinical study in pigs

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Abstract

Purpose: In search for a new drug for intravesical use in superficial urothelial cell carcinoma of the bladder, a pig model is used for pharmacokinetics and toxicity measurements after intravesically administered pemetrexed.

Experimental Design: In the dose escalation phase, two groups of two pigs received 5 and 10 mg/kg pemetrexed intravesically; four groups of three pigs received 15, 20, 25 and 30 mg/kg, respectively. The well-being of the animals was monitored. Blood was studied for pharmacokinetic analysis and signs of myelosuppression. Posttreatment urine samples were collected to measure the concentration of pemetrexed after instillation. Twenty-four hours posttreatment, the animals were cystectomized and sacrificed. Histopathologic examination of the bladder wall was done. In the second study phase, five pigs were instilled weekly with the maximum tested dose for 6 weeks. The same methods were applied.

Results: All doses (5-30 mg/kg) in the first study phase were well tolerated, enabling the use of 30 mg/kg in the second study phase. In both study phases, the pigs' well-being was not influenced. Full blood counts showed no sign of myelosuppression. Systemic absorption was not observed. Urine pemetrexed concentrations remained almost unchanged. Histopathological examination of the bladder wall did not reveal significant abnormalities. Bladder mucosa remained intact at any time, without hemorrhage.

Conclusions: Intravesically administered pemetrexed in pigs is well tolerated, not absorbed systemically, and causes no bladder wall toxicity.

Introduction

Superficial urothelial cell carcinoma of the bladder has a high incidence and even higher prevalence due to a high recurrence rate after primary transurethral resection. Adjuvant intravesical instillations are used to lower the recurrence rate and the chance of progression to muscle invasive disease. For intravesical instillations, the options are in principal twofold: chemotherapy or immunotherapy. Intravesical chemotherapy has modest effect on recurrence rate and, moreover, has repeatedly shown to have no influence on tumor progression. Intravesical immunotherapy, mostly bacillus Calmette-Guerin (BCG), clearly reduces recurrence rate significantly more compared with intravesical chemotherapy, although at a cost of inducing more systemic side effects [1]. In addition, BCG is able to delay bladder tumor progression [2]. Ultimately, in the treatment of patients with superficial bladder cancer, a new drug should be able to combine the benefits of existing intravesical therapies, with higher efficacy and less toxicity.

Pemetrexed (Alimta, LY231514, MTA; Eli Lilly and Company, Indianapolis, IN) is a multi-targeted antifolate with structural similarity to methotrexate, the most commonly used antifolate today. It inhibits at least three enzymes involved in folate metabolism, and purine and pyrimidine synthesis. Used systemically, pemetrexed has shown broad antitumor activity in human phase II [3-5] and III [6, 7] trials in a variety of solid tumors. Moreover, in advanced bladder cancer patients, the safety and efficacy of pemetrexed was explored in a phase II study [8]. The antitumor activity observed in the trial was encouraging and identified pemetrexed as a potent drug against urothelial cancer.

We carried out a pig study consisting of a dose escalation and 6-week instillation phase. We studied animal well-being, potential myelosuppression, drug absorption and bladder wall histology.

Materials and Methods

Approval was granted from the Committee for Animal Experiments (Radboud University Nijmegen Medical Centre, The Netherlands) before the study was undertaken. The animals used for this study were 3-month-old sows (crossbred York x York/Dutch pig). Rationale for the female pig was its close resemblance to humans with regard to the urogenital tract and the ease of transurethral catheterization [9]. The sows were housed in special swine stainless steel battery cages and fed with universal swine food (Hendrix UTD B.V., Boxmeer, The Netherlands). The first part of the study contained the dose escalation phase. Procedures were done under general anesthesia. Premedication contained a mixture of 10 mg/kg ketamine, 0.5 to 1.0 mg/kg midazolam, and 1 ml atropine i.m. in one shot. Sedation maintenance was done by the same mixture in half the dosage, without atropine. Sixteen pigs were split in six smaller groups and received in increasing dose a single intravesical instillation of pemetrexed (Eli Lilly and Company) dissolved in 50 ml 0.9% NaCl. First, a French Foley 10ch catheter with luer lock system was inserted, and the bladder was emptied. Pretreatment and posttreatment urine was tested by pH indicator sticks. Respectively, 5 and 10 mg pemetrexed per kg body mass was instilled in two groups of pigs and 15, 20, 25 and 30 mg/kg in four groups of three pigs. The solution remained in the bladder for 1 hour, after which the bladder was emptied, and the urine was collected; 1.8 ml was directly frozen in cryovials at -80°C for analysis of pemetrexed concentration (Taylor Technology, Inc., Princeton, NJ). This was done by a liquid chromatography tandem mass spectroscopy method. The analyte was extracted from pig urine by precipitation of proteins with 7% perchloric acid. The supernatant was then chromatographed under reverse-phase conditions on an YMC Basic (100 Å 3 µm, 2.0 x 50 mm/50°C) column that used a gradient system with water and acetonitrile containing 0.2% formic acid. [$^{2}H_{4}$]pemetrexed was used as the internal standard. The compounds were detected

and quantified by tandem mass spectrometry with electrospray ionization. The liquid chromatography tandem mass spectroscopy method for determination of pemetrexed in both urine and plasma are validated for the concentration range of 1,000 to 200,000 ng/ml using 0.5 ml of pig urine/plasma, respectively. A 12.5-fold dilution was validated to show the ability of the assay to analyze samples at higher concentrations.

Blood samples were taken for pharmacokinetic analysis and full blood count. In the right jugular groove, 2 cm above the manubrium, the cephalic vein or internal, external or communal jugular vein was punctured, depending on puncture angle and depth of needle penetration. The samples for pharmacokinetic analysis were taken before instillation of pemetrexed, 30 and 60 minutes after instillation and 30, 60 and 120 minutes after emptying the bladder. Blood was collected in 3 ml lithium heparin tubes with gel divider and transferred on ice to the laboratory for plasma processing within 30 minutes. At 4°C, the blood was centrifuged for 12 minutes at 3000 rpm. Plasma was then transferred with a pipette into a 1.8 ml cryovial, stored at -80°C, and shipped on dry ice for analysis (Taylor Technology). This was done in a similar way as described above.

Samples for full blood count (i.e., signs of myelosuppression) were collected in a 5-ml potassium EDTA tube 60 minutes and 24 hours (just before cystectomy) after instillation. The samples were analyzed within 2 to 4 hours in the laboratory, using Advia 120 Hematology System (Bayer, Munich, Germany) and ADVIA multi species software version 3.1.8. Posttreatment, an experienced staff (veterinarian, animal keepers and K.H.) examined the pigs' well-being by a meticulously selected protocollary list of possible signs and symptoms of toxicity (Table 1). Twenty-four hours after the instillation, the pigs were once more anaesthetized as described above. Mouth and vulva were inspected for signs of mucosal toxicity. Subsequently, the pigs received a mixture of O_2, N_2O, and isoflurane by inhalation and were cystectomized. After cystectomy, sacrifice was executed using an overdose of

pentobarbital. Bladder biopsies of 1 cm^2 were taken from dome, trigone, right lateral wall, and left lateral wall and transferred into 10% formalin in PBS for 24 hours. Then the biopsies were embedded in paraffin, sectioned, and stained with H&E. The fresh slides were consistently evaluated by two observers (C.A.H.K. and K.H.) for signs of inflammation and allergic reaction in submucosa and mucosa. Quantification on a 0 to 3 scale corresponded with, respectively, no reaction to extreme reaction. Two unaffected bladders were gathered from a trauma course that used comparable pigs, and used as histopathologic controls.

Table 1 One of the items from the observation list

Skin color	**Time point**		
	1	**2**	**3**
Pink	O	O	O
Pale pink	O	O	O
Pale	O	O	O
Icteric	O	O	O
Cyanotic	O	O	O

To be scored at the day of housing, after pemetrexed instillation and just before cystectomy

The second part of the study contained the 6-week instillation and observation phase. Five pigs received weekly instillations of pemetrexed, with the maximum tested treatment dose of the first phase of the study. Plasma for pharmacokinetic analysis was taken weekly 60 minutes after pemetrexed instillation (i.e., just before emptying the bladder). The puncture for full blood count at 24 hours was the only act for which the animals did not receive anesthesia. Again, the well-being of the animals was observed accurately. Posttreatment urine was collected and tested for pH. Twenty-four hours after the sixth instillation the animals were cystectomized and sacrificed.

Results

Sixteen pigs were studied the first part of the study with a mean weight of 38.4 kg (range, 36.0-41.7 kg). After an average of five housing days, the pig's weight lessened to 35.7 kg (range, 32.3-39.6 kg) just before instillation of pemetrexed, with hardly any weight difference 24 hours later at cystectomy. This weight loss is what can be expected by pig distress due to moving.

The five pigs in the second phase all lost on average 2 kg up till the third week but then gained 3.8 kg in the last weeks. The mean instillation time for both phases of the study was 62 minutes (range, 59 – 72 minutes). Throughout both study phases, the animals showed no deterioration of their well-being. Inspection of the vulva revealed a hyperemic urethral orifice in one pig treated with 30mg/kg. In the second study phase, this was seen in two pigs at the fifth instillation and in the other three pigs at the sixth instillation. No additional signs of mucosal toxicity were observed. Pig 13 (25 mg/kg) accidentally had a traumatic catheterization and did not receive pemetrexed. Its bladder was used for reference histopathology.

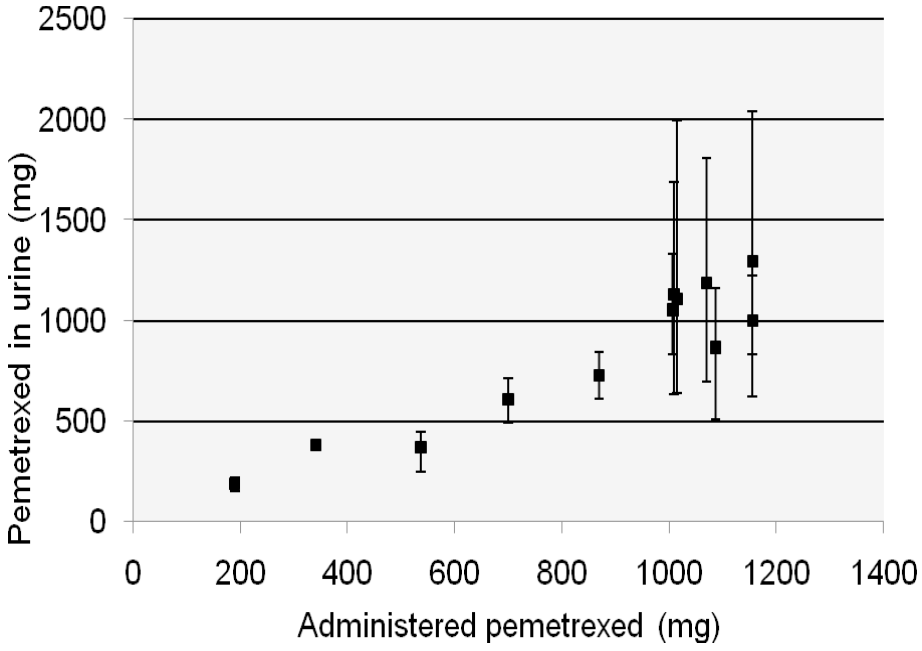
Posttreatment urinary pH (mean, 6.3; range, 5.3-8.0) was not influenced by the instillation of pemetrexed and comparable to pretreatment urinary pH (mean, 6.5; range, 5.0-8.5). Posttreatment urinalysis showed high amounts of pemetrexed (Table 2). This is more obvious when the urine concentration of pemetrexed is multiplied by end-treatment urine volume (Figure 1). No difference was seen between a single dose of 30 mg/kg and six weekly instillations with 30 mg/kg.

Table 2 Per treatment group, the administered and measured end-treatment amount of pemetrexed

Treatment dose (mg/kg)	Administered				End treatment					
	Concentration (mg/ml)		Total amount (mg)		Concentration (mg/ml)		Urine volume (ml)		Total amount (mg)	
5	3.8	(3.6-3.9)	190	(182-198)	3.2	(2.8-3.6)	59	(56-61)	188	(154-221)
10	6.8	(6.7-7.0)	341	(334-348)	5.8	(5.5-6.0)	66	(64-68)	380	(373-386)
15	10.7	(9.7-11.6)	537	(485-578)	7.0	(4.7-8.8)	53	(46-61)	367	(248-449)
20	14.0	(13.8-14.2)	699	(688-710)	10.2	(9.6-10.9)	59	(49-65)	607	(492-617)
25	17.4	(17.0-17.8)	869	(850-888)	8.9	(7.9-9.9)	82	(78-85)	727	(613-841)
30	21.7	(21.5-22.0)	1,086	(1,074-1,098)	10.4	(7.1-16.6)	89	(70-126)	866	(507-1,163)
1 x 30	20.1	(19.5-20-8)	1,006	(975-1,026)	12.8	(6.2-27.1)	100	(49-167)	1,051	(830-1,327)
2 x 30	20.3	(19.7-21.1)	1,015	(984-1,053)	14.8	(7.2-25.9)	81	(58-143)	1,105	(640-1,994)
3 x 30	20.2	(19.3-20.7)	1,009	(966-1,035)	15.8	(14.6-18.0)	72	(42-116)	1,128	(635-1,693)
4 x 30	21.4	(21.1-21.8)	1,069	(1,056-1,089)	18.3	(10.6-24.4)	65	(51-86)	1,184	(699-1,806)
5 x 30	23.1	(22.6-23.6)	1,155	(1,128-1,182)	16.3	(13.3-19.7)	62	(42-78)	997	(621-1,221)
6 x 30	23.1	(22.1-23.9)	1,155	(1,104-1,194)	19.2	(15.1-24.0)	67	(56-85)	1,292	(912-2,040)

Mean concentration and total amount (lowest and highest values). The values were not rounded for the calculations that are made.

Figure 1 Total amounts of pemetrexed. Intravesically administered pemetrexed (dose multiplied by pig weight) compared with end-treatment amount of pemetrexed (measured end-treatment concentration multiplied by urine volume).



Signs of myelosuppression were not observed (Figures 2 and 3). Full blood count remained within the reference range. Only pig 1 in the second study phase had a reversible major platelet drop towards the second instillation (Figure 3), without additional changes in its peripheral blood count. In all plasma samples, the concentration of pemetrexed was below the detection limit of 1,000 ng/ml.

Figure 2 WBC count in five pigs during six weekly instillations

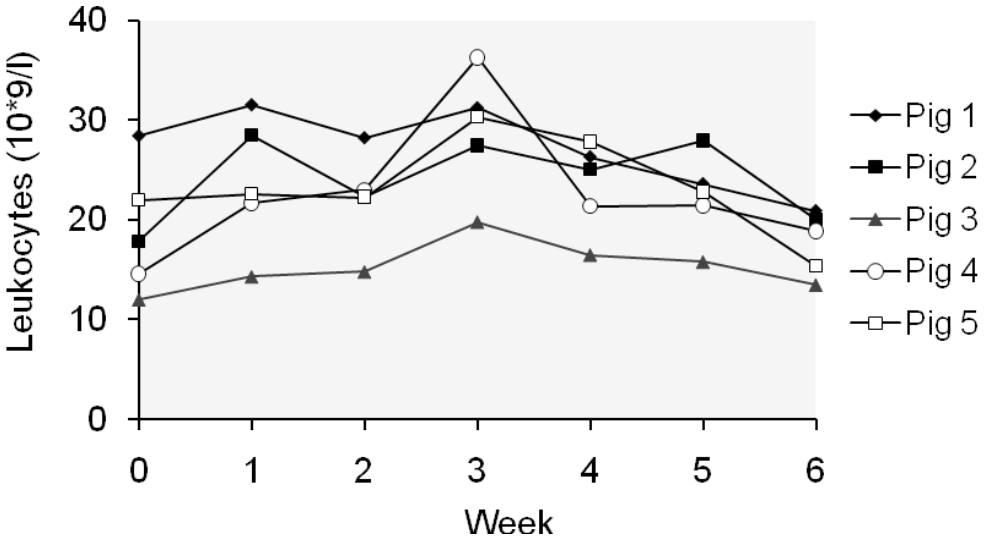
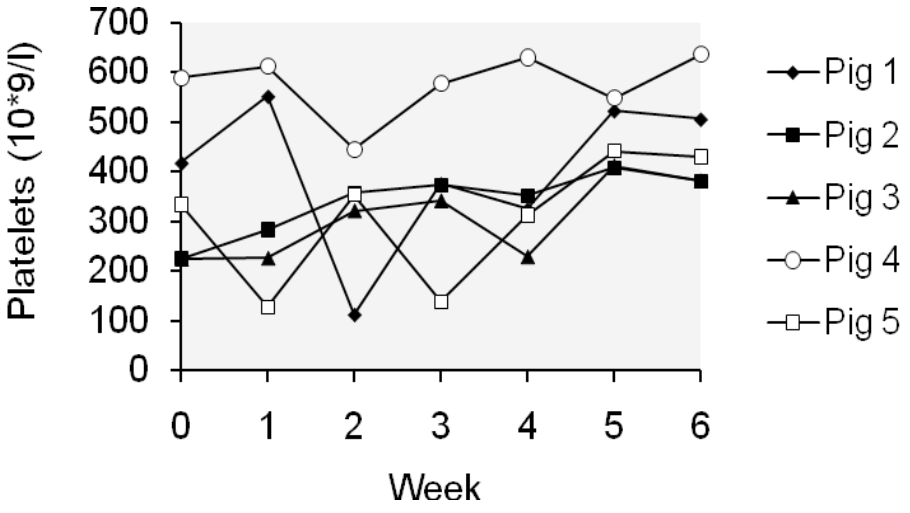


Figure 3 Platelet count in five pigs during six weekly instillations



The resected bladders showed no macroscopic abnormalities. For quantification of microscopic abnormalities, a mean score was calculated per group (the 0, 1, 2 and 3 scores counted and divided by the number of biopsies; Table 3). The mucosa remained intact without signs of erosion or hemorrhage, with the exception of three pre-erosive lesions attributed to mechanical damage by the catheter. Mucosal atypia or detrusor muscle changes were not observed. Neutrophil and lymphocyte infiltrations were mild and not related to dosage. Submucosal edema, hyperemia, and hemorrhage were mild.

Table 3 Per dose of pemetrexed, the mean histopathologic item score (maximum score per group), based on a 0 to 3 score per biopsy

Treatment dose (mg/kg)	Cases	Total no. biopsies	Submucosa					Mucosa	
			Edema	Hyperaemia	Erythrocyte extravasation	Neutrophil infiltration	Lymphocyte infiltration	Neutrophil infiltration	Lymphocyte infiltration
0	3	12	0.08 (1)	0.08 (1)	0 (0)	0.17 (1)	0.33 (2)	0 (0)	0 (0)
5	2	8	0 (0)	0 (0)	0 (0)	0 (0)	0.88 (1)	0 (0)	0 (0)
10	2	8	0.63 (1)	0.75 (1)	0.75 (1)	0.88 (2)	1.13 (2)	0.88 (2)	1 (2)
15	3	12	0.25 (1)	0.42 (1)	0.25 (1)	0.25 (1)	0.83 (2)	0.17 (2)	0.17 (2)
20	3	12	0.08 (1)	0.33 (1)	0.42 (2)	0.17 (1)	0.50 (1)	0.17 (2)	0.17 (1)
25	2	8	0.38 (1)	0.63 (1)	0 (0)	0.63 (1)	1.00 (2)	0.50 (1)	0.75 (2)
30	3	12	0.75 (1)	1.08 (2)	0.75 (1)	1.00 (1)	0.25 (1)	0 (0)	0 (0)
6 x 30	5	20	0.80 (2)	1.15 (2)	0 (0)	0.60 (1)	1.30 (2)	0.75 (2)	0.70 (2)

Discussion

To lower superficial bladder cancer recurrence and progression rates and reduce the side effects of intravesical therapy as seen with the currently used intravesical agents, the search for new intravesical drugs remains legitimate.

Pemetrexed is a pyrrolo-pyrimidine analog of folic acid that inhibits multiple folate-dependant enzyme targets involved in both purine and pyrimidine synthesis. Polyglutamated

to the active pentaglutamide, which is the predominant intracellular form, it is a potent direct inhibitor of thymidylate synthase [10]. The polyglutamation process of pemetrexed is more efficient than, for example, methotrexate, resulting in increased cellular retention of pentaglutamate and a 60-fold more potent inhibition of thymidylate synthase than its parent compound [11]. Inhibition of the enzymes dihydrofolate reductase and glycinamide ribonucleotide formyltransferase seems to be of lesser importance.

Pemetrexed in its systemic application has shown a broad spectrum of clinical activity in multiple tumor types in phase II and III studies, including mesothelioma [7, 12], colorectal [13-16], breast [17], non-small cell lung [5, 6], pancreatic, head and neck [18], and cervical cancers [19]. Several dosing schedules have been tested systemically in phase I studies [3]. In an every-21-day schedule, 600 mg/m^2 seemed to be the maximum tolerated dose. Treating 20 patients with 600 mg/m^2, a mean maximum plasma concentration of 137 μg/ml was attained with a harmonic mean half-life of 3.1 hours (range, 2.2-7.2 hours). The mean clearance value was similar to that of creatinine clearance in the age range of the patients, and the volume of distribution reflects limited distribution outside the bloodstream [20]. The disposition of pemetrexed did not change after multiple doses, and no accumulation seemed to occur with multiple courses. Various phase II studies copied this every-21-day schedule with 600 mg/m^2 , but often required dose reductions to 500 mg/m^2 due to toxicity [3], particularly when used in combination with other cytotoxic agents [13]. Among the mostly seen side effects were grade 4 neutropenia and grade 4 thrombocytopenia, respectively, in 12% to 39% and 8% to 55% of the cases. These percentages were higher when also grade 3 toxicities were included. Other side effects included rash, mucositis, nausea, vomiting, fatigue, anorexia, elevation of liver transaminases, and febrile neutropenia. Patients at risk for these toxicities were found to have elevated pretreatment levels of either total plasma homocysteine concentrations, methylmalonic acid, or both [21, 22]. To minimize the risk of severe toxicity, it was decided

in 1999 to add folic acid and vitamin B_{12} supplementation to all patients receiving pemetrexed. Because of this intervention, death rates and incidence of (non)hematologic toxicities decreased across all studies, with also significantly reduced mean baseline total plasma homocysteine concentration levels. There was no evidence for loss of efficacy of pemetrexed.

In this animal experiment, we hypothesized systemic absorption of pemetrexed through bladder wall passage to be unlikely, due to the antifolate's three charged groups and molecular weight of 597.46 g/mol. Furthermore, we partially aimed at intravesical dosages of pemetrexed that would give toxicity when administered systemically. We locally instilled pemetrexed in a one-time dosage range of 182-1,098 mg and serial dosages with the highest amount. In none of the dosages, the pig's well-being deteriorated. Loss of body weight was already present before instillation with the drug and even improved at the end of the second study phase. Myelosuppression did not occur. Plasma analysis revealed little or no absorption of pemetrexed by the bladder. The remaining high amount of pemetrexed measured in post-instillation urine is in accordance with these findings, although, some of the analyzed urine samples contained even higher amounts of pemetrexed than actually given (Table 2, Figure 1). This can be explained by a minimal spread in validation accuracy and precision of the analysis method. The spread becomes more obvious when multiplying end-treatment urine volumes with urine concentrations of pemetrexed, to enable comparison of total amounts of pemetrexed. Histopathologically, there was a mild degree of inflammation in part of the cases, unrelated to drug dosage. The mucosa remained intact at all times without signs of erosion or substantial hemorrhage.

Due to a lack of formula for a pig's distribution volume, it is almost impossible to translate a systemic human maximum tolerated dose of mg/m^2 to a precise intravesical dose of mg/kg in pigs. Studies with other intravesical drugs have indicated that much higher dosages

could be given and tolerated, than when given systemically. In those cases, the systemic dose in mg/m^2 corresponded with the intravesical dose in mg/50 ml (cisplatin, doxorubicin, epirubicin, and gemcitabin). In our study, we have certainly treated with high dosages of pemetrexed but were not able to establish a toxic dose.

Many studies have analyzed the efficacy of pemetrexed when administered i.v. It has shown efficacy against advanced urothelial bladder cancer when administered systemically. To our knowledge, this is the first study in which pemetrexed is used intravesically. Throughout our animal experiment, the drug was safe in all aspects studied, and there was no systemic absorption. These features indicate that pemetrexed could be an attractive chemotherapeutical drug for intravesical instillation in the treatment of urothelial cell carcinoma.

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Chapter 5

Safety and side effects of an immediate instillation apaziquone following transurethral resection in patients with non-muscle invasive bladder cancer

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Abstract

Purpose: We studied the safety, tolerability and pharmacokinetics of a single immediate post-transurethral resection intravesical instillation of apaziquone, for patients with non-muscle invasive bladder cancer.

Materials and Methods: Patients with cTa-T1, G1-G2 urothelial cell carcinoma of the bladder underwent a transurethral resection of bladder tumor(s) (TURBT) followed by a single intravesical instillation of apaziquone 4 mg/40 ml for 1 hour within 6 hours of transurethral bladder tumor resection. Adverse events and safety parameters were assessed on days 8 and 15 after transurethral bladder tumor resection. Blood samples were drawn before and during the instillation for pharmacokinetic analyses. The first 10 patients with pTa-T1, G1-G2 non-muscle invasive bladder cancer were also evaluated by cystoscopy 3 months after treatment to determine mucosal healing.

Results: Of 20 patients receiving apaziquone 13 (65%) reported 35 adverse events, mostly grade 1 to 2. Eight patients (40%) reported 13 adverse events related to treatment, in particular dysuria, hematuria, bladder spasm, abdominal pain, asthenia and postoperative urinary retention. Three grade 3 and one grade 4 event(s) occurred, but these were considered unrelated to treatment. No other significant clinical changes were observed. Apaziquone (EO9) and the active metabolite EO5a were not detected with pharmacokinetic analyses at any point of time. After 3 months, no evidence of impaired mucosal healing was observed.

Conclusions: A single immediate post-transurethral bladder tumor resection instillation of apaziquone was well tolerated with an expected good safety profile. Apaziquone and its metabolite EO5a were not detected systemically with pharmacokinetic analyses. These results have lead to further study of a single immediate instillation of apaziquone.

Introduction

On average, 70% of patients with bladder tumors present as non-muscle invasive bladder cancer (NMIBC), of which approximately 70% present as Ta lesions, 20% as T1 lesions and 10% as carcinoma in situ (CIS) [1]. Standard treatment for NMIBC is transurethral resection of the bladder tumor (TURBT), followed by adjuvant intravesical instillation(s) of chemotherapy and/or immunotherapy (mainly Bacille Calmette-Guérin, BCG). Which type and schedule of adjuvant treatment to choose, depends on the risk of recurrence and progression of NMIBC [2]. One immediate instillation of chemotherapy decreases the risk of recurrence after TURBT in patients with stage TaT1 single and multiple bladder lesions approximately 12% [3] to 17% [4], and is recommended as the initial treatment in all patients with NMIBC [4;5]. This treatment is considered sufficient for patients with a single low risk papillary tumor, whereas for patients with intermediate risk tumors an additional cycle of chemo-instillations is recommended, and for patients with high risk tumors a cycle of BCG with maintenance instillations. Currently mitomycin-C (United States and Europe) and epirubicin (Europe) are the mostly used chemotherapeutic drugs for NMIBC, with comparable efficacy.

Apaziquone (EO9 or EOquin®, which is apaziquone formulated for intravesical administration) is a novel fully synthetic bioreductive alkylating indoloquinone, that as a pro-drug generates cytotoxic species after enzymatic activation. The enzyme deoxythymidine-diaphorase (DTD) has a prominent role in the activation of apaziquone under aerobic conditions, but apaziquone is also cytotoxic under hypoxic conditions, as well as in cells with low DTD activity. In a marker lesion study by Van der Heijden et al patients with pTa-T1 NMIBC were treated with apaziquone (4 mg/40 ml) instillations for 6 consecutive weeks [6]. A histological complete response was achieved in 30 of 45 (67%) patients and local side

effects were comparable to other chemotherapeutic instillations. The objectives of this study were to investigate the safety, tolerability and pharmacokinetics of a single immediate post-TURBT intravesical instillation of the new drug apaziquone for patients with NMIBC.

Materials and Methods

Six hospitals participated in this prospective phase 1 trial. After ethical committee approval in each of the participating hospitals (local institutional review board for DG, JY, AG and SL, central institutional review board for DS, local and central institutional board for KH and JAW), the study was conducted in accordance with the ethical standards laid down in the Declaration of Helsinki amended version in 1989.

Patient selection

Patients that were judged to have a Ta or T1, grade 1 or grade 2 urothelial cell carcinoma (UCC) of the bladder on preoperative cystoscopic examination before TURBT, were eligible for the study. Tumors were classified according to the recommendations of the World Health Organization (WHO) [7] and the International Union Against Cancer [8]. Patients were fully informed of the investigational nature of the study, a signed written informed consent was obtained, and patients had to agree on the use of an effective method of contraception while on the study and for 4 weeks following the end of treatment. Patients with the likelihood of muscle invasive disease (T2 or greater) or CIS were excluded. The Appendix provides further inclusion and exclusion criteria.

Appendix Inclusion and exclusion criteria

Inclusion	Exclusion
Patient ≥18 years old	Recurrence ≤3 months from last previous manifestation of NMIBC
ECOG performance status 0-2	>4 lesions or largest diameter ≥3.5 cm
Absolute neutrophil count ≥1.5x10^9/L	≥3 prior single dose instillations of chemotherapy
Platelets ≥100x10^9/L	One prior 6-week course of chemotherapy
Serum creatinine and bilirubin ≤1.5x ULN	Any prior BCG treatment or any apaziquone treatment
Serum AST and ALT ≤3x ULN	(Suspected) bladder perforation or deep resection exposing perivesical fat
	Localization of UCC in prostatic urethra or upper urinary tract
	Concurrent malignancy (except BCC or SCC of the skin)
	Uncontrollable urinary tract infection
	Any significant genitourinary disease
	Congenital or acquired immunodeficiency
	Positive pregnancy test
	Expected poor compliance with the protocol

Treatment

TURBT was performed and included resection of all visible bladder tumors, with muscle in the specimen, and biopsies of all suspect sites. The tissue samples were examined by the local pathologist. A catheter was left to drain the bladder. A single adjuvant instillation of 4 mg apaziquone in 40 ml of instillate (Spectrum Pharmaceuticals, Inc., Irvine, California) was administered transurethrally within 6 hours of completion of TURBT, and retained for 1 hour. In the event of serious side effects or toxicity in the first 6 patients of the study, the dose for subsequent patients could be modified.

Patient evaluation and follow-up

Presence, severity and frequency of adverse events were assessed in the weeks following apaziquone administration, and defined according to the National Cancer Institute (NCI) Common Toxicity Criteria (CTC) Version 3.0. Monitoring included examination of the medical history and concomitant medication, physical examination, vital sign measurements (blood pressure, pulse, temperature), hematology (hemoglobin, platelet count, white blood cell and differential count), blood chemistry (creatinine, urea, sodium, potassium, calcium,

albumin, aspartate transaminase, alanine aminotransferase, blood sugar) and urinalysis (macroscopic and microscopic examination) within 2 weeks before TURBT, on the day of TURBT, on day 8 (day 6 to 12) and day 15 (day 13 to 17) after TURBT, and 3 months (±2 weeks) after TURBT.

For the first 10 patients with pTa-T1, G1-G2 histology, cystoscopic evaluation of the bladder urothelium was performed to document mucosal healing at the TURBT site as suggested by regulatory authorities. Urine cytology was also performed. Patients in whom additional instillation therapy was indicated because of higher tumor stage or grade were taken off the study after day 15.

Pharmacokinetics

Pharmacokinetic investigations were performed at selected sites, and required additional informed consent by the patient. Three ml blood samples were drawn before the start of the apaziquone instillation, and 5, 15, 30, 45 and 60 minutes (the time of apaziquone drainage) after instillation. Measurements of apaziquone and its metabolite EO5a were performed by a validated liquid chromatography/mass spectrometry method (Slotervaart Hospital, Amsterdam, The Netherlands), described in detail previously [9].

Statistical considerations

Since there is no formal statistical hypothesis being tested in this study, no sample size calculations were performed. The number of patients included was requested by regulatory authorities. Statistical analyses are descriptive, with frequencies and gradings, and appropriate spread and central values used.

Results

Patient characteristics

A total of 23 patients were enrolled, of whom 20 patients received the apaziquone instillation within 6 hours of TURBT. Three patients did not receive apaziquone, because of, respectively, a bladder perforation during TURBT, absence of tumor at TURBT and hospitalization before TURBT for an unrelated medical problem. Fifteen patients were male and 5 patients female. Mean patient age was 72.5 years (range 54 to 86), and 18 (90%) patients were white.

Tumor characteristics

The mean number of tumors was 1.3 (range 1 to 4). The majority of patients had 1 lesion (85%). Fourteen patients had a primary tumor and 6 patients had recurrence (1 to 3 previous occurrences). Histology confirmed TaG1 in 6 patients, and in respectively 5 patients TaG2, 2 TaG3, 1 T1G1, 2 T1G3, 1 T2G3 and 1 CIS. Two patients had no malignancy.

Safety and tolerability

All 20 patients tolerated the apaziquone instillation, which was retained for a mean of 60.6 minutes (57.0 to 66.0). Thirteen patients (65%) reported 35 adverse events. The most common adverse events are summarized in Table 1, mostly grade 1 to 2. Less common events were grade 1 lymphadenopathy, diabetes mellitus and dizziness, and grade 2 dyspnea. Eight patients (40%) reported 13 adverse events that were classified by the investigator as (possibly, probably or definitely) related to the study drug (Table 2).

Three grade 3 and one grade 4 event(s) were reported: one patient with a single Ta G2 lesion had post-procedural haemorrhage, urinary retention (both grade 3) and bacterial cystitis

(grade 4). One patient with a single Ta G1 lesion had flank pain (grade 3), but also had a ureteral stent in situ. These events were considered by the investigator to be unrelated to the study drug, and related to TURBT. The adverse events of the first of two patients above were reported as serious adverse event, because of hospitalization. In two more patients serious adverse events were reported as hospitalization was prolonged due to (possibly related to study drug) post operative urinary retention (grade 2) and haematuria (grade 2).

No clinically significant changes were found by physical examination, vital sign measurements, hematology, blood chemistry studies or urinalysis, during the apaziquone instillation or over the course of the study. On day 85 cystoscopy showed re-epitheliazation of the bladder mucosa in all 10 patients without evidence of impaired wound healing. No recurrences were observed, nor was malignant cytology found in these 10 patients (6 TaG1, 3 TaG2 and 1 T1G1 at TURBT).

Pharmacokinetics

Blood samples were collected from 6 patients. With a lower limit of quantification less than 0.5 ng/ml, apaziquone and EO5a were not detected at any point of time in any of the patients.

Discussion

To our knowledge this is the first study investigating safety, tolerability and pharmacokinetics of a single immediate post-TURBT intravesical instillation of apaziquone for patients with low and intermediate risk NMIBC. One immediate post-TURBT instillation of chemotherapy used to be the advice in patients with solitary low grade tumors only, but currently it is recommended as the initial treatment for all patients with NMIBC [4;5]. In a meta-analysis of seven randomized trials (1,476 patients with Ta-T1 NMIBC, median follow-up 3.4 years),

Table 1 Adverse events

	No. reports	No. grade*			
		1	2	3	4
Renal + urinary disorders:					
Dysuria	7	5	2		
Hematuria	3	2	1		
Urinary retention	2		1	1	
Bladder spasm	1		1		
Flank pain	1			1	
Urgency	1	1			
Gastrointestinal disorders:					
Lower abdominal pain	5	2	3		
Abdominal pain	1	1			
Nausea	1	1			
Infections + infestations:					
Urinary tract infection	2	1	1		
Cystitis	1				1
Pneumonia	1		1		
Upper respiratory tract infection	1	1			
General disorders:					
Asthenia	1	1			
Pyrexia	1	1			
Procedural complications:					
Postop haemorrhage	1			1	
Postop urinary retention	1		1		

*Maximum severity graded according to NCI-CTC, version 3.0.

Table 2 Possibly, probably or definitely related adverse events

	No. reports related to study drug	Grade*	
		1	2
Renal + urinary disorders:			
Dysuria	4	2	2
Haematuria	2	1	1
Bladder spasm	1		1
Gastrointestinal disorders:			
Lower abdominal pain	3		3
General disorders:			
Asthenia	1	1	
Procedural complications:			
Postop urinary retention	1		1
Nervous system disorders:			
Dizziness	1	1	

*Maximum severity graded according to NCI-CTC, version 3.0.

patients receiving a single post-TURBT chemo-instillation (epirubicin, mitomycin-C, thiotepa and pirarubicin) had a 39% reduction in the relative risk of recurrence (12% reduction in absolute risk) compared to TURBT alone [3]. Both patients with a single tumor and multiple tumors benefited from a single instillation, but the recurrence rate for patients with multiple tumors remained high, resulting in the conclusion that one immediate post-TURBT instillation in itself was not sufficient for these patients. In a meta-analysis from the American Urological Association 2 of the 7 randomized trials from the Sylvester meta-analysis were included (only regarding mitomycin-C, 427 patients), finding a 17% decrease in median recurrence rate [4]. The effect of a single immediate instillation may be explained either by destroying circulating tumor cells that could implant at the site of resection, or by chemo-resection of tumor left after an incomplete TURBT [10]. Timing of the instillation appears crucial. Kaasinen et al found a doubling in the risk of recurrence when the instillation was not given on the same day as the TURBT [11]. Pan et al studied inhibition of murine bladder tumor cell implantation by the use of thiotepa immediately, less than 1 hour and less than 24 hours after tumor cell inoculation, and found that the control and less than 24 hours group had significantly higher tumor implantation rates than the immediate and less than 1 hour group [12]. In other studies the instillation was administered at least within 24 hours, and mostly within 6 hours, to which we confirmed our study design despite the fact that superiority of an instillation within 6 hours over an instillation within 24 hours was not proven in the earlier mentioned meta-analysis [3].

In the majority of clinical trials studying a single immediate instillation post-TURBT, the emphasis is on drug-efficacy. However, little is written about local adverse events and systemic toxicity. This may be due to the low incidence of side effects [13], or the often doubtful relationship between adverse events and study drug, as patients also undergo general or spinal anesthesia and TURBT. The meta-analysis of Sylvester et al described mild transient

irritative bladder symptoms including dysuria, urinary frequency and macroscopic hematuria as the most frequent side effects occurring in approximately 10% of patients [3]. Systemic toxicity was extremely rare. Allergic skin reactions were noted in 1% to 3% of the patients. Bouffioux et al compared early (the day of TURBT) versus delayed (7 to 15 days post-TURBT) instillations of mitomycin-C and doxorubicin, and reported chemical cystitis (nondefined) in respectively 3% and 2.2% of the patients receiving early instillations, vs 0% and 0.5 % of the patients receiving delayed instillations [14]. Systemic toxicity included mainly cutaneous pruritus, dizziness and malaise.

In our pilot study, the number of adverse events is by approximation comparable to the percentages previously mentioned for other chemotherapeutic drugs. The main complaints were as expected of the lower urinary tract and lower abdomen, and in two patients we observed systemic toxicity, being dizziness and asthenia. The risk of side effects to other tissues than the bladder was estimated low beforehand, as any drug reaching the bloodstream would be rapidly metabolized [15;16]. Three grade 3 events and one grade 4 event were not expected, but they occurred in two patients and were not considered drug related, but most likely related to the TURBT. For one of these patients it was possible that post-procedural hemorrhage (grade 3) triggered urinary retention (grade 3) and bacterial cystitis (grade 4), or vice versa. The other patient had flank pain (grade 3), while having a ureter stent in situ. In a study of 2,821 patients, postoperative hemorrhage (clinical signs of hypotension, decreased hemoglobin or repeat intervention) was reported as the most common complication of TURBT, occurring in 5.1% of the patients [17]. Bladder perforation was the second most frequent complication, occurring in 1.3% of the patients. One of our patients indeed was excluded because of a bladder perforation since an immediate postoperative instillation should be avoided in the case of a clear or even suspicion of bladder wall perforation and drug leakage may lead to serious complications [18].

A potential limitation was the patients selected. Of the 20 patients 8 did not meet the inclusion criteria as 2 patients had no malignancy, 4 had grade 3 tumors, 1 had CIS and 1 had a pT2 tumor. In a study from the EORTC for example, where patients with solitary tumours suspected to be Ta or T1 were randomized for an immediate instillation with epirubicin or water, only 81 out of 512 (16%) patients were ineligible with no tumor in 19, CIS in 22, pT2 or greater in 25 and other conditions in 15 [19]. This appears less, but since grade is difficult to estimate, this leaves only 4 in 20 (20%) patients with unexpected histological findings, which is in line with the EORTC study. Moreover, in a study population also including multiple tumors, more higher grades and stages are to be expected. Finally safety, tolerability and pharmacokinetics, the primary objective of this study, is not expected to be better or worse in patients with these higher grades and stages. On the contrary, one might hypothesize that side effects would have been more in patients with worse (for example higher grade and deeper infiltrating) tumors due to a more extensive TURBT wound, but this was not the case. The patients with grade 3 and 4 toxicity both had a single lesion, Ta G1 and Ta G2. A second limitation might be the absence of review pathology. We did not choose to review pathology in this study since it would not have changed the planned treatment (one immediate instillation) and since the value of review pathology is documented to be limited [20;21]. A third limitation is the small number of patients, which is inherent to a pilot study, and finally, pharmacokinetic investigations were performed in only 6 patients. Currently a multicenter, randomized, placebo-controlled, double blind phase 3 study has started in the United States and Canada, comparing the efficacy of a single immediate post-TURBT instillation of 4 mg/40 ml apaziquone with sterile water.

Conclusions

One instillation of apaziquone administered intravesically within 6 hours of TURBT was well tolerated with a safety profile that is comparable to other chemotherapeutic drugs used for this indication. The most common adverse events observed were also known complications of TURBT. Apaziquone nor its active metabolite EO5a were absorbed in any detectable amounts through the bladder. These results of safety, tolerability and pharmacokinetic analyses encourage further study of the efficacy of the drug apaziquone administered as a single immediate instillation after TURBT.

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Chapter 6

Two-year follow-up of the phase II marker lesion study of intravesical apaziquone for patients with non-muscle invasive bladder cancer

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Abstract

Objectives: To study the time to recurrence and duration of response in non-muscle invasive bladder cancer (NMIBC) patients, with a complete ablative response after intravesical apaziquone instillations.

Methods: Transurethral resection of bladder tumour(s) (TURBT) was performed in patients with multiple pTa-T1 G1-2 urothelial cell carcinoma (UCC) of the bladder, with the exception of one marker lesion of 0.5-1.0 cm. Intravesical apaziquone was administered at weekly intervals for 6 consecutive weeks, without maintenance instillations. A histological confirmed response was obtained 2-4 weeks after the last instillation. Routine follow-up (FU) was carried out at 6, 9, 12, 18 and 24 months from the first apaziquone instillation.

Results: At 3 months FU 31 of 46 patients (67.4%) had a complete response (CR) to ablative treatment. Side-effects on the long-term were only mild. Two CR patients dropped out during FU. On intention-to-treat (ITT) analysis 49.5% of the CR patients were recurrence free at 24 months FU, with a median duration of response of 18 months. Of 15 no response (NR) patients, only two received additional prophylactic instillations after TURBT. On ITT-analysis 26.7% of the NR patients were recurrence free (log rank test, $p = 0.155$). The overall recurrence-free survival was 39% (18 of 46 patients) at 24 months FU.

Conclusions: The CR of the marker lesion in 67% of patients was followed by a recurrence-free rate of 56.5% at 1-year FU, and 49.5% at 2-year FU. These long-term results are good in comparison with the results of other ablative studies.

Introduction

The initial treatment of non-muscle invasive bladder cancer (NMIBC) consists of complete transurethral resection of the bladder tumour(s) (TURBT), followed by adjuvant intravesical instillations of chemotherapy or immunotherapy [1;2]. Choice of type and schedule of adjuvant treatment depends on prognostic factors that are used to determine patient's risk of NMIBC recurrence, and progression to muscle invasive disease, as for example the factors used in the European Organisation for Research and Treatment of Cancer (EORTC) risk tables [3]. According to these tables, the calculated probabilities for recurrence of disease range from 15-61% at 1 year to 31-78% at 5 years, and for progression from <1-17% at 1 year to <1-45% at 5 years. These probabilities of recurrence and progression of disease indicate that current treatment regimens are suboptimal and demonstrate a clear need for more effective treatment of NMIBC.

Apaziquone is a derivative of the clinically used mitomycin C (MMC). It is a novel fully synthetic bioreductive alkylating indoloquinone pro-drug, that is converted to cytotoxic species after enzymatic activation [4]. The enzyme deoxythymidine-diaphorase (DTD) plays a prominent role in the activation of apaziquone under aerobic conditions, but apaziquone is also cytotoxic under hypoxic conditions, also in cells with low DTD activity [5]. In preclinical research, the concentration of apaziquone needed to achieve 50% cell death, was 6-78 times lower than the concentration of MMC, depending on the urothelial cell carcinoma (UCC) cell line used [6].

In the initial efficacy analysis of this phase II study with EO9/EOquin (apaziquone formulated for intravesical administration) for NMIBC a histological complete response (CR) of the marker lesion in 67% of the patients was demonstrated after 6 weekly apaziquone instillations, with local side effects comparable to side effects due to other chemo-instillations

[7]. However, the follow-up (FU) period was relatively short. Recently, Jain et al reported long-term FU of 8 phase I study patients treated with apaziquone [8], with a median time to first recurrence of 21.5 months [9] (R. Puri, personal communication).

In the present study, we report the 2-year efficacy results of intravesical apaziquone after a CR for patients with intermediate- and high-risk NMIBC.

Methods

Seven hospitals participated in this prospective phase II trial. The study was conducted in accordance with the ethical standards of the Declaration of Helsinki amended version 1989. The study was approved by the individual Institutional Review Board/ethical committee of each participating hospital.

Patient selection

Patients with multiple (≤10) histologically confirmed Ta-T1 G1-G2 UCC of the bladder were included. All visible lesions were completely resected with TURBT as evidenced by the inclusion of muscle tissue in the resected specimens, confirmed by pathology. Additionally, random biopsies were taken, and in case of areas suspect for carcinoma in situ (CIS) also targeted biopsies. One marker lesion measuring between 0.5 and 1.0 cm in greatest diameter was left intact. The resection loop (0.6 cm) was used as reference for tumour size estimation. Images of the site of the marker lesion were recorded.

Patients gave written informed consent before being enrolled in the study. The patients with a likelihood of muscle invasive disease (≥T2), G3 tumours or CIS were excluded. Further inclusion and exclusion criteria have been described in detail before [7].

Treatment

Intravesical instillations were started 14 ± 3 days after TURBT. Apaziquone 4 mg in 40 ml of instillate (Spectrum Pharmaceuticals, Inc., Irvine, California) was administered at weekly intervals for 6 consecutive weeks. Apaziquone was retained in the bladder for 1 h. When local side effects occurred, treatment was delayed or the dose of apaziquone adjusted to 2 mg/40 ml.

Patient evaluation and follow-up

Two to 4 weeks after the last instillation, the marker lesion response was evaluated under anaesthesia. Complete disappearance of the marker lesion as judged by visual inspection was confirmed by biopsy (CR). When tumour persisted, the marker lesion was resected [no response (NR)]. Partial responses were not evaluated in view of the limitations in tumour measurements. Tumours were classified according to standard recommendations [10;11].

The presence, severity and frequency of adverse events were assessed in the weeks following apaziquone administration, and defined according to the National Cancer Institute (NCI) Common Toxicity Criteria (CTC) Version 3.0. Further details of FU during treatment have been described in detail before [7].

The patients with NR remained in the study, and for these patients the decision to give additional treatment was left to the discretion of the investigator. A routine FU was carried out at 6, 9, 12, 18 and 24 months from the first apaziquone instillation. Evaluations consisted of urea and creatinine assessment, urinalysis, urine cytology and cystoscopy. The presence of recurrent UCC had to be confirmed with biopsies.

Statistical considerations

For the primary analysis we assumed a CR rate of approximately 50%. With a power of 80%, α error of 5% and β error of 20%, and to reject with the same power a response rate of <30%, 44 patients needed to be enrolled.

Patients were followed for 24 months or until disease recurrence or progression was observed. Intention-to-treat analysis (ITT) was performed on all patients with a CR at 3 months FU. The recurrence-free rate was defined as the percentage of CR patients with no recurrence in FU at a certain point of time. The duration of disease-free interval was estimated according to the Kaplan-Meier method. The progression of disease was defined as occurrence of UCC stage ≥T2.

At the beginning of the study the individual risk of recurrence was calculated according to EORTC risk tables [3], utilizing the pathology results of TURBT. Further statistical analyses were descriptive, with frequencies and gradings, and appropriate spread and central values used. Statistical analyses were performed in SPSS version 16.0.

Results

Patient and tumour characteristics

A total of 46 patients were enrolled in the study; 42 patients were male and 4 were female. The age of patients ranged from 37 to 93 years (median 67.5). The patient characteristics including six prognostic factors and calculated recurrence scores according to the EORTC risk tables and prior adjuvant intravesical therapy are shown in Table 1 [3].

Table 1 Six prognostic factors in accordance with the EORTC risk tables, calculated recurrence scores [3] and prior adjuvant intravesical therapy categorized for patients with CR and NR.

Calculation of EORTC recurrence score		**CR**		**NR**	
Variable	**Weight**	**Number of patients**	**Mean (spread)**	**Number of patients**	**Mean (spread)**
Number of tumors					
Single	0	NA		NA	
2 to 7	3	29	3.5 (2-10)	14	4.2 (2-8)
≥8	6	2		1	
Tumor size					
<3 cm	0	26	NA[a]	12	NA[a]
≥3 cm	3	5		3	
Prior recurrence rate					
Primary	0	8		1	
≤1 rec/year	2	16	3.1 (0-13)[b]	9	3.7 (0-11)[b]
>1 rec/year	4	7		5	
T category					
Ta	0	26	NA	12	NA
T1	1	5		3	
Grade					
G1	0	7		4	
G2	1	23	NA	11	NA
G3	2	1[c]		NA	
CIS					
No	0	31	NA	15	NA
Yes	1	NA		NA	
Recurrence score					
	0	NA		NA	
	1-4	5	6.6 (4-10)	0	7.4 (5-11)
	5-9	25		13	
	10-17	1		2	
Prior adjuvant intravesical therapy					
No therapy		13	0	4	0
Chemotherapy		11	1.5 (1-3)[d]	6	1.3 (1-2)[d]
BCG		1	1‡	0	0‡
BCG and chemotherapy		6	4.8 (2-5)[d]	5	3.6 (2-5)[d]

CR: complete response, NR: no response, NA: not applicable, CIS: carcinoma in situ
[a]It was possible to estimate the maximum tumour size < or ≥ 3 cm, but not to measure every single tumour
[b]Number of previous occurrences
[c]Formally ineligible, approved by principal investigator
[d]Number of courses of adjuvant intravesical therapy

Safety

Side effects have been described in detail before [7]. In short, side effects did not exceed NCI-CTC grade 3. In 15 patients apaziquone treatment was postponed for 1 week due to local side effects, but there was no need to lower the dose of apaziquone to 2 mg/40 ml in any patient. During the 6-24 months FU period one patient had grade 1-2 (probably related) lower abdominal pain up to 16 months FU, and one patient had grade 1 (possibly related) dyspnoea/pulmonary oedema up to 12 months FU. Both patients recovered without further therapy. No clinical meaningful changes were found by blood chemistry or urinalysis.

Efficacy

Our analysis 2-4 weeks post treatment showed a histological CR in 30 of 45 patients (66.7%), or CR in 31 of 46 patients (67.4%, one patient refused biopsy of a 'CR' on cystoscopy) [7].

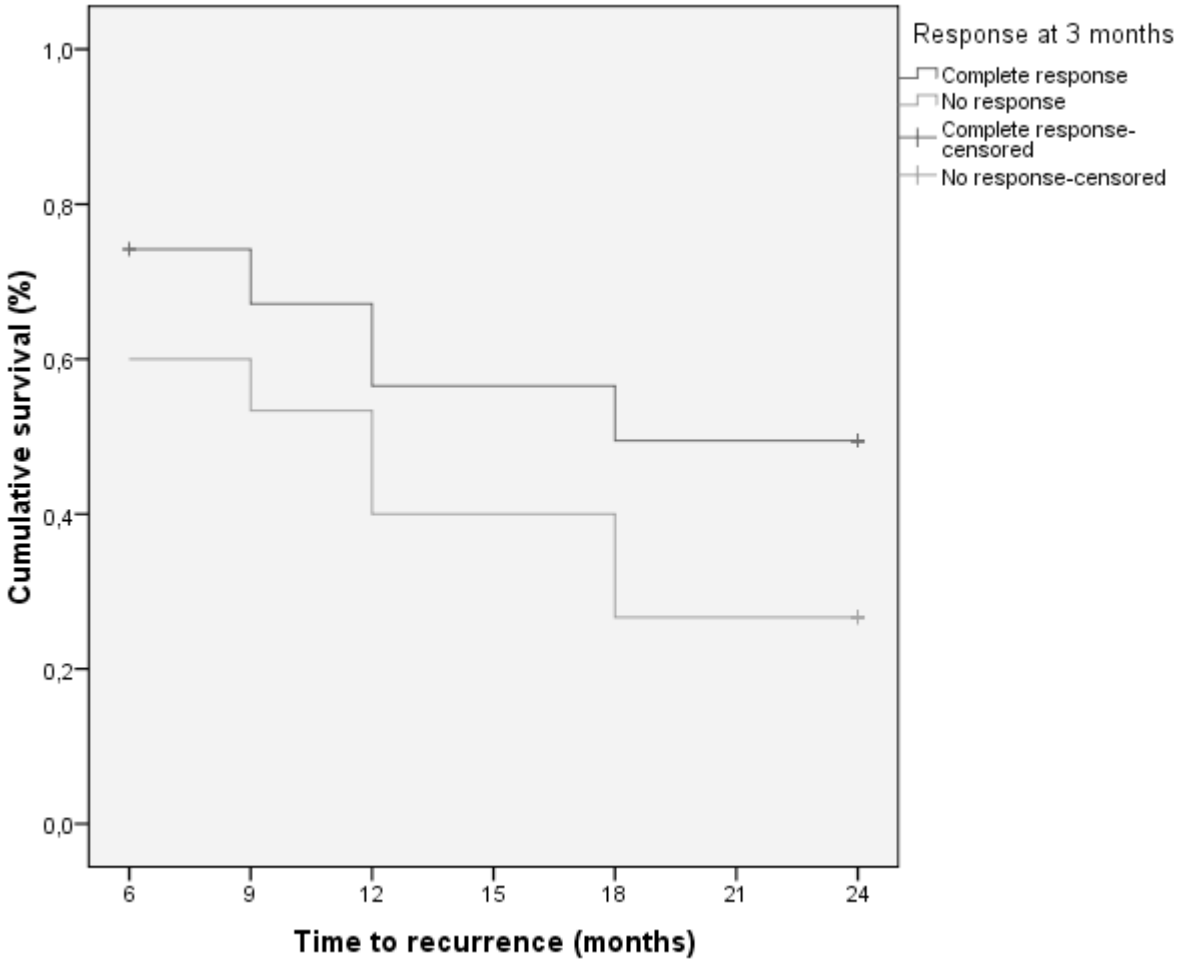
For the current analysis, the FU period concerns 31 CR patients. Two patients dropped out at 6 months FU; one patient refused further cystoscopies, and one patient underwent major surgery for a rectum carcinoma. At 24 months FU, 49.5% of the CR patients were recurrence free without further instillations (Table 2). The median duration of response was 18 months. None of the CR patients showed progression to muscle invasive disease. Of 15 NR patients, only two patients received BCG instillations after TURBT; both patients had a recurrence at 6 and 9 months FU, respectively. Because most patients in the NR group did not receive further treatment, we also performed ITT-analysis for these patients. At 24 months FU, 26.7% of the NR patients were recurrence free (Table 2). One NR patient had progression to T2 disease at 18 months FU. Kaplan-Meier curves for time to recurrence are presented in Figure 1 (log rank test, $p = 0.155$). When the CR and NR patients are combined, the overall recurrence-free survival of patients with NMIBC treated with intravesical installations of apaziquone is 18/46 (39%) after two years of FU.

Table 2 Per FU-visit the number of patients and the recurrence-free survival for the CR and NR group.

FU month	Marker lesion response			
	CR		NR	
	No. of patients	Recurrence-free survival (%)	No. of patients	Recurrence-free survival (%)[a]
3	31	100	15	100
6	21	74.2	9	60
9	19	67.1	8	53.3
12	16	56.5	6	40
18	14	49.5	4	26.7
24	14	49.5	4	26.7

FU: follow-up, CR: complete response, NR: no response
[a]After TURBT, recurrence-free survival is set to 100% for the NR patients at 3 months FU

Figure 1 Time to recurrence by response group.



Discussion

This study describes the efficacy of six weekly instillations of apaziquone after a complete ablative response for patients with intermediate- and high-risk NMIBC, with 2 years of FU.

In this study, the CR rate of 67%, 2-4 weeks after the last apaziquone instillation was superior to most marker lesion studies performed with other (neo-) adjuvant drugs (discussed in [7]). A marker lesion study represents an excellent, safe method to test the ablative efficacy of a given treatment, with rates of progression to muscle invasive disease of <1% [12]. Marker lesion studies are recommended before embarking on any prophylactic study to avoid treatment with an ineffective agent [13;14]. Only three studies also report about 'long-term' drug efficacy in a small number of CR patients [15-17]. Brausi et al [15] randomised patients with intermediate-risk NMIBC for either 40 mg of MMC or electromotive drug administration (EMDA)/MMC for 8 weeks, and obtained a CR in 41.6% and 40% of patients, respectively. Only two of five MMC-treated patients with a CR were recurrence free after 11 months, and five of seven EMDA/MMC-treated patients with a CR after a mean FU of little more than a year. Newling et al [16] treated patients with refractory UCC with or without CIS of the bladder with six courses of 800 mg valrubicin, and obtained a histological CR in 18/39 (46%) patients. Their estimate of the mean time to recurrence was 248 days for CR and NR together, and 5 of 39 (12.8%) patients remained recurrence free after the FU period of 2 years. Maffezzini et al [17] treated patients with low- and intermediate-risk NMIBC with 2000 mg of gemcitabine for 4 weeks, and obtained a CR in 13 of 28 (46.4%) patients. Of the 28 (67.8%) patients, 19 experienced a recurrence within the first year. In comparison to other drugs used in marker lesion studies where also longer term FU was reported, the 2-year recurrence-free rate in our study (49.5% in the CR group and 39% in the whole group) represents a good result. The low percentage of recurrence-free patients from the reported

marker lesion studies might be explained by the recruitment of patients with a high risk of recurrence: these patients obviously had multiple tumours, and often failed previous intravesical therapy.

We also calculated probabilities of recurrence according to the EORTC risk tables [3], which were based on an analysis of 2596 NMIBC patients that were treated between 1979 and 1989, but treatment of NMIBC at that time obviously differed from the current NMIBC guidelines [1;2]. Twenty percent of patients from the analysis initially received no treatment, <10% received an immediate instillation of chemotherapy, a second look TURBT was not performed in high-risk patients, and BCG was given without maintenance instillations. Therefore, the predicted recurrence and progression rates of the risk tables might be higher compared to current clinical practice. Nevertheless, the reported restrictions are by approximation also applicable to the patients in our study. For patients with a risk score of 1-4, 5-9 and 10-17, the corresponding probabilities of recurrence after 2 years are 34, 51, and 71%, respectively. In our study, the majority of patients (38 of 46 patients, 82.6%) had a risk score of 5-9 (Table 1) with a 51% probability of recurrence at 2 years FU. The overall recurrence-free survival at 2 years FU after apaziquone treatment was 18/46 (39%). This seems to be worse than the predicted probabilities of recurrence by the EORTC. However, it is important to realize that most of our patients with NR underwent TURBT without further prophylactic treatment, which otherwise would have been given as part of current routine treatment and would have influenced clinical outcome. Furthermore, the failure of the NR patients to respond to at least one kind of intravesical therapy (in this case apaziquone) may increase the risk of recurrence for these patients. Therefore, the recurrence-free survival with prophylactic treatment is expected to be higher than 39%. Sub-analyses were performed between the EORTC recurrence score groups, but the 1-4 and 10-17 groups were too small to

note any significant differences with regard to treatment response. Of note: 'all' five patients with a score of 1-4 had a CR to apaziquone treatment.

As discussed, it is difficult to compare our results with results from literature and the EORTC tables. It is difficult to predict whether the good CR-rate of 67% at 3 months FU also is an indication of a long-term effect in preventing recurrences. Apparently, the difference in recurrence-free survival between the CR and NR group (Figure 1) is already present from FU month 6, which does not support an ongoing protective effect in CRs.

Conclusions

After six apaziquone treatments, the CR of the marker lesion in 67% of patients was followed by a recurrence-free rate of 56.5% at 1-year FU, and 49.5% at 2-year FU. These long-term results are good in comparison with long-term results of other ablative studies, and appear to approach the probabilities of recurrence according to the EORTC risk tables, which are derived from studies with prophylactic intent.

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Chapter 7

Comparison of three schedules of intravesical epirubicin in patients with non-muscle invasive bladder cancer

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Abstract

Objectives: To study the additive effect of either an early instillation or maintenance instillations of adjuvant intravesical epirubicin, as compared to the epirubicin "standard" treatment schedule only, in patients with non-muscle invasive bladder cancer.

Methods: Patients with intermediate and high risk urothelial cell carcinoma of the bladder, except carcinoma in situ, were randomised for adjuvant intravesical instillations with 50 mg epirubicin/50 ml NaCl for 1 h. Group 1 received 4 weekly and 5 monthly instillations (standard schedule), group 2 received the same schedule as group 1, but with an additional instillation <48 h after transurethral resection of bladder tumour (TURBT) and group 3 received the same scheme as group 1, but with additional instillations at 9 and 12 mo (maintenance schedule). Standard follow-up was 5 yr and consisted of cystoscopy, cytology and registration of adverse events.

Results: A total of 731 patients were eligible for quasi intention-to-treat analysis. Side effects were minimal for all treatment groups. After 5-yr follow-up, respectively, 44.4%, 42.7% and 45.0% (log-rank test, p = 0.712) of the patients in groups 1, 2 and 3 were recurrence free, and respectively 90.0%, 87.7% and 88.2% (log-rank test, p = 0.593) of the patients were progression free.

Conclusions: In the quasi intention-to-treat analysis there is no difference in the 5-yr recurrence-free period between the treatment groups, despite one instillation within 48 h of TURBT or two maintenance instillations up to 1 yr, in addition to the "standard" schedule.

Introduction

Standard therapy for non-muscle invasive bladder cancer (NMIBC) is transurethral resection (TURBT) and adjuvant intravesical instillations to reduce the risk of recurrence and/or progression. The choice of adjuvant treatment is based on prognostic factors [1]. In patients with low risk tumours an immediate (early) post-TURBT instillation with a chemotherapeutic agent significantly lowers the recurrence rate in the first few years, and is considered sufficient as adjuvant treatment for this risk group. Patients with intermediate risk tumours benefit from a single immediate instillation of chemotherapy, but need a further course of adjuvant chemotherapy instillations for 4-8 wk, or immunotherapy with bacillus Calmette-Guérin (BCG) [2]. BCG is indicated for patients with high risk tumours.

Epirubicin (4′ epi-doxorubicin) is a chemotherapeutic drug with a good tolerability profile, and efficacy in the prevention of recurrences of NMIBC. In a multicentre randomised trial with 431 NMIBC (Ta-T1) patients, a single immediate instillation of epirubicin was compared to sterile water as placebo and showed a significantly lower recurrence rate in patients treated with epirubicin [3]. The European Organization for Research and Treatment of Cancer (EORTC) randomised 957 patients with intermediate and high risk NMIBC for either adjuvant treatment with BCG, BCG and isoniazid, or epirubicin [4], and found that patients treated with either BCG regimen had a longer recurrence-free survival as compared to those treated with epirubicin. However, toxicity with epirubicin was markedly lower. Despite the superiority of BCG in patients with high risk NMIBC [4], its superiority over epirubicin in patients with intermediate risk NMIBC is not proven. Epirubicin is especially effective in patients with low and intermediate risk NMIBC that have a low risk of progression [3].

To investigate the hypothesis whether there is a more efficient instillation scheme of epirubicin, we performed a study comparing three schedules of adjuvant intravesical

epirubicin in the treatment of patients with intermediate and high risk NMIBC, with the exception of patients with carcinoma in situ (CIS).

Methods

This is a multicentre prospective randomised phase 3 trial in which 23 Dutch hospitals participated. After ethical committee approval in each of the participating hospitals, the study was conducted in accordance with the ethical standards laid down in the Declaration of Helsinki amended version 1989.

Patient selection

Patients ≤85 yr old with a histologically proven solitary pT1 tumour, or multiple primary, or recurrent T1 or Ta G1-3 urothelial cell carcinoma (UCC) of the bladder, in whom complete transurethral resection was possible, were included. Tumours were classified according to the recommendations of the World Health Organization (WHO) [5] and the International Union Against Cancer [6]. Patients may have received intravesical therapy before, but not epirubicin or therapy within 6 mo of study entry. Informed consent was obtained before TURBT. Patients with pathologically confirmed primary solitary Ta tumours, CIS or tumours ≥T2 were excluded. Other exclusion criteria were concurrent malignancy (except basal cell carcinoma or squamous cell carcinoma of the skin), history of other malignancy with a disease-free interval ≤5 yr, expected poor compliance, WHO performance status >2, uncontrollable urinary tract infection, any previous systemic cancer therapy or radiotherapy, localisation of UCC in prostatic urethra or upper urinary tract, pregnancy or lactation or women of reproductive age who refuse to take adequate contraceptive measures, congenital or acquired

immunodeficiency, known hypersensitivity to anthracyclines, or concurrent treatment with an investigational drug.

Treatment schedule

After complete transurethral resection of clinical NMIBC, patients were randomised on the day of TURBT for one of three treatment schedules. Intravesical instillations of 50 mg epirubicin in 50 ml saline were given for 1 h. Group 1 (the "standard" schedule) started the first of four weekly and five monthly instillations within 14 d after TURBT (duration 6 mo, 9 instillations). Group 2 received the same schedule as group 1, preceded by a single dose within 48 h of TURBT (duration 6 mo, 10 instillations). Group 3 received the same schedule as group 1, with 2 additional 3-monthly instillations (duration 12 mo, 11 instillations). If necessary, delay of an instillation or dose modification was at the investigator's discretion and documented. If a recurrence was observed during the treatment period, it was resected without modification of the treatment schedule. Patients went off-study after a second recurrence during the instillation period, the first recurrence after completion of treatment, after 5-yr disease-free survival, occurrence of CIS, or UCC of the prostatic urethra or upper urinary tract or distant metastasis.

Follow up and evaluation of therapy

Urine cytology and cystoscopy were done every 3 mo for the first yr, every 4 mo for the second and third years, and every 6 mo for the fourth and fifth years after TURBT. All visible lesions had to be resected, with recurrence confirmed by histological examination. Adverse events were recorded with information on duration, severity, relation to study treatment, start of concomitant medication and outcome of the event.

Objectives and statistical analysis

The study was designed to compare the effect of epirubicin in three different treatment schedules with respect to efficacy (recurrence-free rate, progression-free rate, duration of disease-free interval) and safety (incidence and severity of side effects). In order to detect a true ratio of median time to first recurrence, 1.75/2.5 (yr) between any of the 3 treatment groups at error rates of $\alpha = 0.05$ and $\beta = 0.10$, a total of 247 patients had to be entered in each treatment group. Two patient groups were identified for the efficacy analysis. The intention-to-treat analysis, the primary analysis, was performed with all eligible patients who were randomised, a quasi-ITT approach [7]. The per-protocol analysis, the secondary analysis according to the protocol, was performed with all eligible patients who followed their complete instillation schedule. Time to recurrence was defined as the time between TURBT and first recurrence after completion of treatment, or second recurrence during treatment. Progression was defined as muscle invasive disease, CIS, UCC outside the bladder, or distant metastasis or death related to UCC. Time to progression was defined as the time between TURBT and first progression. Recurrence-free rate was defined as the percentage of patients with no recurrence in the total study population in follow-up at a certain point in time. Progression-free rate was defined as the percentage of patients with no progression in the total study population in follow-up at a certain point in time. Duration of disease-free interval was estimated according to the Kaplan-Meier method and comparison between treatment groups by means of the log-rank test. Observed sample percentages in the safety analyses were compared between treatment groups by means of a Chi square test. Statistical analyses were done in SPSS version 14.0.

Results

Patient and tumour characteristics

Between April 1998 and April 2004, 1000 patients were randomised for the study, of whom 731 (73%) were eligible. Originally, 815 patients were to be included, taking into account a 10% drop out rate, but during the study the dropout rate proved to be much higher, and the ethical committee approved increasing the number of study patients. The main reason for this was that histology, which was known after the time point of randomisation, did not correspond with the clinical appearance of a lesion (Table 1). Patient characteristics were comparable among the treatment groups (Table 2). The male-to-female ratio was 4:1, the mean age 67 yr (range: 33-84 yr). Primary, multiple, pTa, and grade 1-2 tumours occurred most often.

Safety

Of 1000 patients, 829 received at least one instillation of epirubicin. Fifty-seven patients (7%) had to stop the instillations because of local or systemic side effects (Table 3). Haematuria was observed significantly more in the immediate instillation group (Pearson X^2, p = 0.03). Other side effects were not significantly different between treatment groups. Thirteen percent of the patients experienced systemic side effects. The most frequent systemic side effects were tiredness, fever, nausea, headache, and abdominal pain.

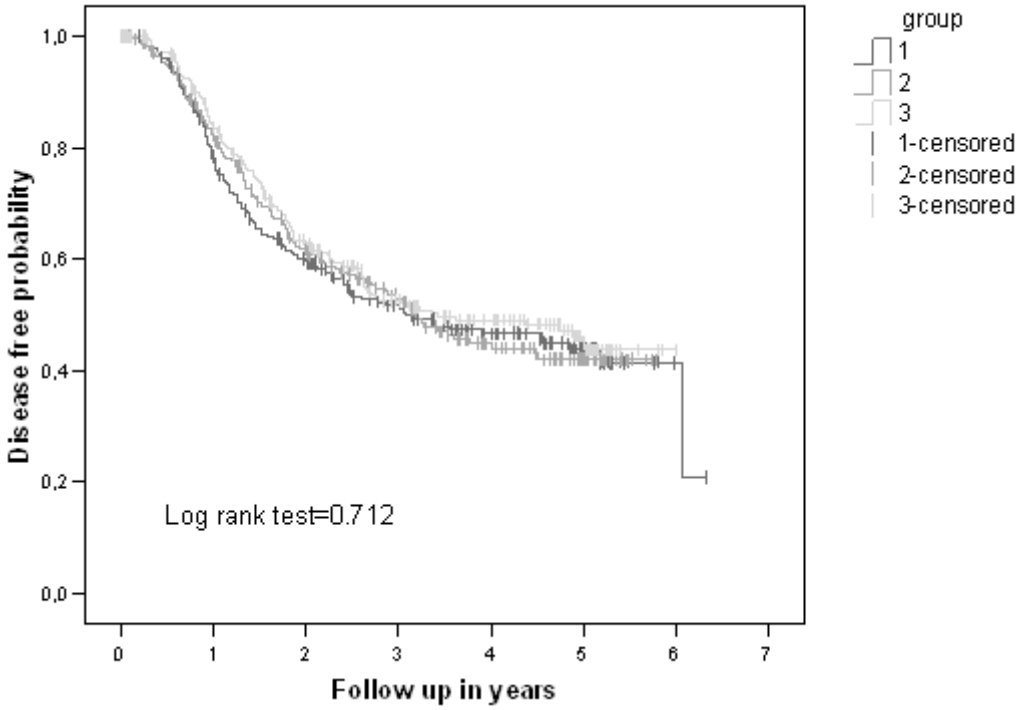
Efficacy

A total of 731 patients were eligible for quasi-ITT analysis, of whom 531 completed the entire instillation schedule per protocol. Patients of groups 1, 2 and 3 received on average 8.4 of 9 (SD 1.9), 8.9 of 10 (SD 2.5) and 10.0 of 11 (SD 2.4) instillations, respectively. In the quasi-

ITT analysis, 44.4%, 42.7% and 45.0% (mean: 44.1%, 344 events) of the patients from, respectively, group 1, 2 and 3 were 5-yr recurrence free (log-rank test, p = 0.712). No significant differences were found with pair-wise comparison. Median follow-up was 2.07 yr. Median recurrence-free survival was 3.2 yr (range: 2.4-4.1 yr). Kaplan-Meier curves for time to recurrence are presented in Figure 1.

Progression was seen in 48 patients; 25 patients (3.4%) had muscle invasive disease and 23 patients (3.1%) had CIS. UCC outside the bladder, distant metastasis, or death related to UCC were not observed. In the quasi-ITT analysis, the overall 5-yr progression-free rate was 88.6%, and specified for groups 1, 2 and 3, respectively, 90.0%, 87.7%, and 88.2% (log-rank test, p = 0.593).

Figure 1 Time to recurrence by treatment group for patients in the quasi intention-to-treat analysis



Number of remaining patients:

Group 1	239	172	123	87	63	36
Group 2	238	171	121	86	53	25
Group 3	254	196	138	97	75	36

Table 1 Patient ineligibility

	Group			Total (%)	
	1	**2**	**3**		
No. of patients	101	91	77	269	
Primary solitary Ta	20	17	14	51	(19)
T0	12	18	14	44	(16)
≥T2	18	22	17	57	(21)
CIS	15	8	12	35	(13)
Other malignancy ≤5 yr	6	1	8	15	(6)
Intravesical therapy <6 mo	6	4	2	12	(4)
Patient refusal	4	5	2	11	(4)
Other	20	16	8	44	(16)

Table 2 Patient and tumour characteristics of eligible patients at study entry

	Group			Total (%)	
	1	**2**	**3**		
No. of patients	239	238	254	731	
No. of tumours					
Single	47	43	56	146	(20)
Multiple	192	195	198	585	(80)
Prior recurrence rate					
Primary	114	109	131	354	(48)
Recurrence ≤1 yr	38	55	50	143	(20)
Recurrence ≥1 yr	87	74	73	234	(32)
T category					
Ta	190	196	189	575	(79)
T1	49	42	65	156	(21)
History of CIS	3	2	4	9	(1)
Grade					
1	108	101	96	305	(42)
2	108	109	126	343	(47)
3	21	26	31	78	(11)
Previous treatment					
Intravesical instillation	40	36	31	107	(15)
TURBT only	81	82	86	249	(34)

Of all patients in group 2, 168 (68%) patients received an immediate instillation <24 h after TURBT, and 72 patients (30%) ≥24 h and <48 h after TURBT. Four patients received no instillations. In the quasi-ITT analysis, the 5-yr recurrence-free rate for patients treated <24 h, or ≥24 h and <48 h after TURBT was 41.5% and 47.3% (log-rank test, p = 0.401), respectively, and the 5-yr progression-free rates were, respectively, 91.2% and 81.6% (log-rank, p = 0.093).

Table 3 Side effects of epirubicin by treatment group

	Group 1 (%)		Group 2 (%)		Group 3 (%)		Total (%)	
No. of patients	266		286		277		826	
Bacterial cystitis								
Never	205	(77)	226	(79)	215	(78)	646	(78)
Not requiring delay	46	(17)	37	(13)	40	(14)	123	(15)
Requiring delay	12	(5)	22	(8)	20	(7)	54	(7)
Requiring stop	3	(1)	1	(1)	2	(1)	6	(1)
Chemical cystitis								
Never	182	(68)	191	(67)	211	(76)	584	(70)
Not requiring delay	72	(27)	77	(27)	54	(20)	203	(25)
Requiring delay	6	(2)	7	(2)	4	(1)	17	(2)
Requiring stop	6	(2)	11	(4)	8	(3)	25	(3)
Haematuria								
Never	230	(87)	232	(81)	246	(89)	708	(85)
Not requiring delay	32	(12)	41	(14)	23	(8)	96	(12)
Requiring delay	2	(1)	12	(4)	2	(1)	16	(2)
Requiring stop	2	(1)	1	(1)	5	(2)	8	(1)
Other local side effects								
Never	244	(92)	259	(91)	252	(91)	755	(91)
Not requiring delay	18	(7)	16	(6)	21	(8)	55	(7)
Requiring delay	2	(1)	4	(1)	2	(1)	8	(1)
Requiring stop	2	(1)	7	(2)	1	(1)	10	(1)
Systemic side effects								
Never	232	(87)	246	(86)	239	(86)	717	(87)
Not requiring delay	29	(11)	30	(11)	32	(12)	91	(11)
Requiring delay	3	(1)	6	(2)	3	(1)	12	(1)
Requiring stop	2	(1)	4	(1)	2	(1)	8	(1)
General delay or stop								
No delay or stop	227	(85)	224	(78)	216	(78)	667	(81)
Delay	11	(4)	17	(6)	15	(5)	43	(5)
Stop	28	(11)	45	(16)	46	(17)	119	(14)

Discussion

The objective of this trial was to study the additive effect of either an early instillation or two maintenance instillations of adjuvant intravesical epirubicin, as compared to the epirubicin "standard" treatment schedule only, in an intermediate and high risk group of patients with NMIBC. Patients with CIS were excluded because treatment with BCG instillations for patients with CIS became state-of-the-art by the end of the last decade. At that time, the greater benefit of BCG over chemotherapy for patients with high risk papillary lesions was not as obvious as today.

Comparing studies on intravesical chemotherapy often is troublesome because of differences in dose (mg), concentration (mg/ml) and volume (ml) used, the dwell time, and the volume and pH of the urine [8]. Moreover, treatment schedule and frequency will influence therapy efficacy. Finally, additional confounding factors can be the study size, variability in duration of follow-up, and differences in patients' characteristics [1;9]. The establishment of the optimal treatment regiment for epirubicin is also hampered by differences in dose, concentration, instillation schedule, frequency of instillations [10], and the small size of some trials.

Kuroda et al randomized 622 patients with Ta-1 G1-2 NMIBC for adjuvant treatment with 17 doses of epirubicin 20 mg/40 ml in 12 mo, 12 doses of epirubicin 30 mg/40 ml in 7 mo, or 9 doses epirubicin 40 mg/40 ml in 4 mo [11]. At 2-yr follow-up, the recurrence-free rates were 48.7%, 55.1% and 60.1%, respectively, showing the greatest effect by the highest dose regimen, administered over a short period of time. Koga et al randomised 171 patients with Ta-1 G1-3 NMIBC for either short-term (<24 h, day 2/3, week 1 and 2, 5 times every 2 wk) or long-term (short-term schedule + 9 times monthly) 30 mg/30 ml adjuvant epirubicin [12]. At 3-yr follow-up, long-term treatment was more effective than short-term treatment,

with recurrence-free rates of 85.2% and 63.9%, respectively. Another study included 148 patients with all stages and grades of NMIBC, without a difference in efficacy between patients treated with 6 instillations in 4 wk (short-term), or the same treatment with 11 additional monthly instillations (long-term) of epirubicin 40 mg/40 ml [13]. At 2-yr follow up, respectively 75.1% and 77.2% were recurrence free. Nomata et al treated 125 patients with Ta-1 G1-2 NMIBC with 30 mg/30 ml epirubicin, either 19 times over 1 yr or 12 times over 5 mo [14], with, respectively, 48.5% and 55.1% of the patients recurrence free at 3-yr follow-up, which was a nonsignificant difference. In all, these data show discrepancies in study outcome among a variety of schedules used, including highly differing definitions of short-term treatment and long-term treatment. The only improvement in results seems to be caused by a higher dose (40 mg/40 ml [11]).

By using per-protocol analysis instead of ITT analysis, 45.2%, 46.8% and 55.0% of the patients from, respectively, groups 1, 2, and 3 were recurrence free at 5 yr (log-rank test, $p = 0.041$), with a statistically significant difference ($p = 0.012$) on pair-wise comparison of groups 1 and 3, but no differences between the other groups. However, we need to be careful with conclusions based on per-protocol analysis because the difference was small and we had difficulties in achieving enough statistical power due to the high number of ineligible patients. The power to detect a true ratio of median time to first recurrence times of 1.75/2.5 (yr) between any of the 3 treatment groups at error rates of $\alpha = 0.05$, was calculated at 84% in the per-protocol analyses (smallest group contained 176 patients) and at 93% in the ITT analysis (smallest group contained 238 patients). A disadvantage of per-protocol analysis is the occurrence of selection bias. Instead, one could question whether two additional instillations up to 1 yr would be able to change the 5-yr recurrence-free estimate. In other trials of epirubicin, maintenance instillations were often applied monthly, mostly for no longer than 1 yr, with no differences in recurrence-free estimates [11-14]. In a recent study comparing 6

weekly instillations of mitomycin-C to 6 weekly instillations followed by monthly instillations during 3 yr (42 instillations), there was a large decrease in the percent of patients with recurrence in the group receiving 3 yr of treatment, 25.7% versus 10.5% ($p = 0.0006$) [15]. However, results for mitomycin-C are also contradictory. Two other studies also compared short-term versus long-term (3 yr, 42 instillations) mitomycin-C, but in both studies the short-term and the long-term schedules were different from the study by Friedrich et al [15], with no differences in the percent of patients with recurrence [16,17].

The European Association of Urology (EAU) guidelines recommend to give one immediate, postoperative instillation with a chemotherapeutic drug in every patient with NMIBC [1]. This decreases the risk of recurrence by approximately 12%, as demonstrated in a meta-analysis of seven trials involving 1476 patients, with median follow-up of 3.4 yr [2]. In three of these trials epirubicin was studied [3;18;19], but no drug was found to be superior with regard to efficacy. Regarding an immediate instillation as part of a treatment schedule, two parallel studies by the EORTC demonstrated that intravesical chemotherapy administered monthly for 1 yr versus 6 mo resulted in similar recurrence rates when the first instillation was given immediately after TURBT [20]. But again, data are conflicting (above) [12;14].

Timing of the instillation appears crucial, and all drugs in the meta-analysis were administered within 24 h, generally immediately after TURBT or within 6 h. Pan et al studied inhibition of murine bladder tumour cell implantation by the use of thiotepa immediately, <1 h, and <24 h after tumour cell inoculation and found that the control and <24 h group had significantly higher tumour implantation rates than the immediate and <1 h group [21]. Kaasinen et al found, in their retrospective analysis, a doubling in the relative risk of recurrence when the instillation was not given on the same day as the TURBT [22]. In our trial, 168 patients (68%) of group 2 received the first instillation <24 h and 72 patients (30%) <48 h after TURBT, but the 5-yr recurrence-free rates (41.5% and 47.3%, respectively) and

progression-free rates were comparable. There was no significant difference in the occurrence of haematuria between group 2 and the other two groups when leaving out the immediate instillation in the analysis. When looking at the first instillation only, the significant difference is probably caused by the performance of TURBT and may be due to the first instillation within 48 h, when the bladder walls of these patients had little time to recover. There was no increase in the number of patients in group 2 requiring instillation cessation.

Conclusions

The three treatment schedules of epirubicin were well tolerated, but haematuria occurred more in the immediate instillation group. In reducing recurrence, the "standard" treatment schedule was as effective as the same schedule with an additional immediate post-TURBT instillation, or additional maintenance instillations. The wide timing range of the first instillation (up to 48 h) in the immediate instillation group and the low number of additional instillations in the maintenance group may explain the lack of additional efficacy in these two groups as compared to the standard therapy group, even though we could not find, in the immediate instillation group, significant differences in a subgroup of patients treated in <24 h as compared to those treated ≥24 h and <48 h.

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Chapter 8

Intravesical hyperthermia and mitomycin C for carcinoma in situ of the urinary bladder: experience of the European Synergo® working party

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Abstract

Objectives: To study the results of chemotherapy combined with intravesical hyperthermia in patients with mainly BCG-failing carcinoma in situ (CIS).

Methods: Patients with histologically confirmed CIS were included retrospectively. Outpatient thermochemotherapy treatment was done with mitomycin C (MMC) and the Synergo® system SB-TS 101 (temperature range between 41° and 44°C), weekly for 6-8 weeks, followed by 4-6 sessions every 6 to 8 weeks.

Results: Fifty-one patients were treated between 1997 and 2005 from 15 European centers. Thirty-four patients were pre-treated with BCG. Mean age was 69.9 years. Twenty-four patients had concomitant papillary tumors. The mean number of hyperthermia/MMC treatments per patient was 10.0. Of 49 evaluable patients 45 had a biopsy and cytology proven complete response. In two patients CIS disappeared, but they had persistent papillary tumors. Follow-up of 45 complete responders showed 22 recurrences after a mean of 27 months (median 22): T2 (4), T1 (4), T1/CIS (1), CIS (5), Ta/CIS (2), Ta (5) and Tx (1). Side effects (bladder complaints) were generally mild and transient.

Conclusions: In patients with primary or BCG-failing CIS, treatment with intravesical hyperthermia and MMC appears a safe and effective treatment. The initial complete response rate is 92%, which remains approximately 50% after 2 years.

Introduction

Carcinoma in situ (CIS) of the urinary bladder is defined as non-papillary high-grade non-muscle invasive urothelial cell carcinoma (UCC). It is an aggressive form of non-muscle invasive bladder cancer (NMIBC) with a high progression rate and an even higher recurrence rate [1]. In case of concomitant high-grade pT1 tumors the prognosis is even worse [2]. Even when patients are treated with cystectomy, the presence of CIS is an adverse prognostic sign [3,4]. Current standard therapy in case of CIS is intravesical Bacillus Calmette-Guérin (BCG). Although 30-40% of patients do not respond to one course of six instillations, half of these still achieve a complete response after an additional course of six instillations [5]. Consequently, the treatment guideline in CIS patients is maintenance BCG [6,7]. The disadvantage of BCG in comparison to intravesical chemotherapy is the increased frequency and severity of side effects. Alternative therapies for BCG in primary CIS patients remain experimental or are insufficiently documented.

In case of BCG-refractory CIS, cystectomy is the treatment of choice [8]. Intravesical approaches have failed, such as valrubicin [9], are investigational, such as gemcitabine [10] and mycobacterial cell wall preparations [11], or efficacy should be confirmed, such as the combination of BCG and interferon-alpha 2b [12]. Device-assisted strategies for primary CIS include photodynamic therapy (PDT), electromotive drug administration (EMDA) and the combination of intravesical hyperthermia and chemotherapy (thermochemotherapy). PDT has some encouraging results [13] and sequential BCG with EMDA/mitomycin C (MMC) is emerging [14].

Thermochemotherapy (MMC/hyperthermia) has proven to be more effective than MMC alone for patients with Ta-1 G1-3 NMIBC in comparative studies with ablative [15] as well as prophylactic intent [16]. Gofrit et al [17] reported a beneficial prophylactic effect of

thermochemotherapy in patients with G3 tumors, 15/24 (62.5%) patients were recurrence free after a mean follow-up of 35.3 months. In addition, ablation of high-grade tumors was achieved in 21/28 (75%) patients, of whom 81% remained tumor free after a mean follow-up of 20 months. Van der Heijden et al [18] reported the use of thermochemotherapy with prophylactic intent in 90 patients with intermediate- and high-risk NMIBC. After 1 and 2 years of follow-up, respectively, 14.3% and 24.6% of all patients experienced a recurrence. In 41 patients previously failing BCG treatment, the recurrence rates, respectively, were 23 and 41%.

In the current study we report for the first time the efficacy of thermochemotherapy in patients with CIS, predominantly failing BCG.

Methods

Patient selection

Retrospectively, data of all patients treated with MMC in combination with hyperthermia were gathered from 15 centers. Of these, patients with biopsy proven and histologically confirmed CIS were eligible for analysis. Patients were included with a WHO performance status of 0- 2 and a life expectancy of more than 24 months. Criteria for ineligibility were a limited bladder capacity (<150 ml), any concomitant malignancy, extravesical UCC and the presence of a diverticulum of the bladder. Patients without follow-up cystoscopy or with less than six treatments were excluded from the analysis.

Treatment

The treatment set up has been described in detail before [19]. In short, the Synergo® system SB-TS 101 is used to deliver local microwave induced hyperthermia and intravesical

chemotherapy simultaneously. This system consists of a 915 MHz intravesical microwave applicator that delivers hyperthermia to the bladder wall via direct irradiation. The hyperthermia range is between 41°C and 44°C, controlled by five thermocouples integrated in a specially designed 20°F treatment catheter. To avoid urethral overheating and disintegration of MMC, the solution is continuously pumped out of the bladder and re-instilled after being cooled. Treatment was done with 20 mg MMC (Kyowa Hakko Kogyo Co., Tokyo, Japan) in 50 ml sterile water, replaced by a fresh identical solution after 30 min (a total of 40 mg MMC in 1 h), on an outpatient basis weekly for 6 weeks (prophylactic schedule), followed by six maintenance instillations (one instillation every 6 weeks). Patients whose concomitant papillary tumors were not or could not be completely resected, or patients with wide areas of CIS, were treated with a higher dose of MMC (twice 40 mg, a total of 80 mg in 1 h), weekly for 8 weeks (ablative schedule), followed by six maintenance instillations of twice 20 mg MMC (one instillation every 6 weeks). In case of bacterial cystitis or significant hematuria, treatment delay was recommended.

Follow-up

Cystoscopy and urine cytology were repeated every 3 months for a follow-up period of 24 months. Biopsies from suspicious lesions were performed. The primary end-point of the study was eradication of CIS, proven by cystoscopy, biopsy and cytology. Secondary end-point was either a pathology proven tumor recurrence, or a clear cystoscopical recurrence indicated by the investigator. Side effects and adverse events were noted in the patient's file. Data were collected during treatment and follow-up.

For statistical analysis the Epi-Info for Windows version 3.3.2 was used.

Results

Patients

In total 51 patients with CIS were included between March 1997 and June 2005 from centers in Israel, Italy, Germany, Switzerland, Austria and the Netherlands. Mean age was 69.9 years (50-87), with a male-to-female ratio of 4:1 (42 vs. 9). On an average, the patients had 3.2 previous TUR procedures (1-8). Previous "worst" histology revealed CIS in 33 patients, grade 3 in 24 patients, and T1 in 20 patients (some patients had more than one of these histopathologic characteristics). Previous intravesical treatments included BCG (n = 34), MMC (n = 11), epirubicin (n = 4), gemcitabine (n = 3), Keyhole-Limpet hemocyanin (KLH) (n = 1), valrubicin (n = 1) and radiation therapy (n = 1). Of the 34 patients previously treated with BCG, 17 were BCG refractory, 2 BCG intolerant and 15 patients relapsed (3 patients within 12 months, 6 patients between 12 and 24 months, and 6 patients after 24 months). Twenty-four patients had concomitant papillary tumors: TaG1 (n = 1), TaG2 (n = 6), TaG3 (n = 8), T1G2 (n = 4) and T1G3 (n = 5).

Eighteen patients received the prophylactic schedule (twice 20 mg MMC), and 33 patients received the ablative schedule (twice 40 mg MMC) together with intravesical hyperthermia. Apart from the MMC dose, all patients in all centers were treated according to the same treatment protocol. The mean total number of hyperthermia treatments was 10.0 (2-21). Two patients dropped out during initial treatment (one patient with hematuria after the second treatment, and one patient with a false route after the fourth treatment) and did not receive the initial six instillations. These two patients were not considered for efficacy.

Efficacy

In total, 49 patients remained available for treatment response analysis. Of these patients, 45 (92%) had a complete biopsy and cytology proven disappearance of CIS at 3 months. In two additional patients CIS disappeared but the concomitant papillary tumor persisted. There was no difference in response between patients with and without concomitant papillary tumors (p = 0.94, data not shown), irrespective whether the papillary tumor was pTa or pT1. There was also no difference in response between patients who were or were not BCG failures, irrespective of the type of BCG-failure (BCG-resistant or early or late BCG failures, p = 0.63, data not shown). Since the 2-year results of the 20 mg (prophylactic, n = 19) and 40 mg (ablative, n = 26) regimens are also not significantly different (p = 0.10, data not shown) these groups are analyzed together. Of all 45 complete responders additional follow-up is available with a mean follow-up of 27 (3-77, median 22) months. Of these 45 patients, 22 (49%) had a recurrence. Five of the patients had a cystectomy because of recurrent T1 (n = 1) or >T1 (n = 4) tumor, after, respectively, 11 and, 12 and 13 and, 18 months. One patient had a cystectomy due to a contracted bladder, but he was tumor free. The other 17 patients recurred with pure CIS (n = 5), T1+CIS (n = 1), Ta+CIS (n = 2), pT1 (n = 3), pTa (n = 5) and pTx (n = 1). These patients were treated conservatively.

Table 1 Session-related adverse events, measured per session

Adverse Event	**No**	**Rate (% per session)**
Pain	64	12.7
Bladder spasms	66	13.1
Difficult catheter insertion	1	0.2
Dysuria	31	6.2
Hematuria	15	3.0
Urinary frequency/urgency	8	1.6
Nocturia	11	2.2

The total number of treatments given to the study group is 503.

Safety

Safety of chemotherapy treatment combined with microwave hyperthermia was previously published [18]. Side effects in this study also were generally mild and transient, and included mainly pain and bladder spasms during treatment and irritative bladder symptoms for 1-2 days thereafter. Side effects had virtually no influence on the treatment plan: as mentioned above, one patient stopped treatment due to hematuria, one treatment session was delayed for 1 week and one session was shortened. Adverse events could not be graded according to the Common Toxicity Criteria retrospectively. Frequency of events is given in table 1.

Discussion

As indicated in the introduction international guidelines advise to treat CIS patients with maintenance intravesical BCG, since it appears to be able to prevent or delay disease progression. However, the possibilities in case of BCG-refractory CIS are limited.

Intravesical chemotherapy has been studied. Valrubicin is the only FDA approved drug for patients with CIS-failing intravesical therapy such as BCG. This was based on a phase II study with 90 patients [9]. After 1 year of follow-up 21% had a complete response, which decreased to 8% after 2 years. A marker lesion study in refractory patients confirmed initial potential of valrubicin, since 18/39 (46%) patients were free of disease after 3 months [20]. However, due to several reasons, valrubicin is not used currently. Intravesical gemcitabine was also used in BCG-refractory patients resulting in three small series. In one study with 18 patients (of whom 12 had pure CIS, 2 T1 + CIS), 11 patients showed negative biopsies after treatment, of whom 7 also had negative cytology [10]. In another recent phase II study 7 of 16 high-risk patients (not specified) remained recurrence free at 12 months [21]. Fifteen of 30 patients (23 CIS, 4 Ta high-grade, 3 T1) with NMIBC that were BCG refractory

(27) or BCG intolerant (3) had a complete response in a study by Dalbagni et al [22]. The 1-year recurrence-free survival rate for patients with a complete response was 21%.

Immunotherapy in BCG failures is also reported. Bropirimine, an oral immunomodulator, was studied in BCG-resistant CIS in a phase II trial [23]. Fourteen out of 47 BCG-resistant patients showed a complete response with a median duration of more than 12 months. Unfortunately, no further evaluation of the drug has been done. The combination of interferon (IFN)-alpha and BCG for BCG failures has been the subject of a large multi-center phase II trial [24]. In all, 467 patients failing BCG were treated with low dose BCG plus IFN. Twenty-seven percent of these patients had isolated or concomitant CIS. With a median follow-up of 24 months, 45% remained tumor free, compared with 59% of the 536 patients in the BCG naïve group. Although there was no sub-analysis for CIS patients, CIS did not significantly affect outcome in a multivariate analysis. Patients treated with two or more courses of BCG did not respond well to BCG plus IFN, suggesting that this group of patients should go on to cystectomy.

A recent method of device-assisted intravesical therapy is PDT, which combines photo sensitizers that selectively bind to tumors and a powerful intravesical light source to destroy tumors. Waidelich et al [25] used PDT with oral 5-aminolevulinic acid (5-ALA) in 5 BCG-failing CIS patients, and found three patients recurrence free after a median follow-up of 36 months. Intravesical 5-ALA was studied in 10 BCG failures (presence of CIS not specified) of which four remained tumor free after an average follow-up of 11.8 months [13]. EMDA is based on the concept of temporarily breaching the urothelial barrier of the bladder and enhancing penetration of drugs, among which MMC, in a controllable manner [26]. Di Stasi et al [14] performed a randomized controlled trial in 212 patients with stage T1 NMIBC, comparing BCG alone (105 patients, 28 with concomitant CIS) with sequential BCG and MMC/EMDA (107 patients, 29 with concomitant CIS), with maintenance therapy in both

arms. This study found a significantly higher disease-free interval, lower recurrence rate, lower progression rate and lower disease-specific mortality in favor of sequential BCG and MMC/EMDA. Although not designed to assess complete response rates of patients with CIS, 12/28 (42.9%) patients treated with BCG alone and 16/28 (55.2%) patients treated with sequential BCG and EMDA/MMC obtained a complete response at 3 months, and respectively 16/28 (57.1%) patients and 20/29 (69.0%) patients at 6 months.

Limited and disappointing information is available on the use of intravesical MMC in BCG failures. The Swedish-Norwegian bladder cancer group compared MMC and BCG in NMIBC patients and treatment failures were allowed to cross over [27]. Twenty-one BCG failures changed to MMC therapy, but only four remained recurrence free with a median follow-up of 64 months.

In all, conservative therapy in patients with BCG-refractory CIS remains largely experimental, and indeed guidelines advise cystectomy as a treatment of choice for CIS failing adequate BCG. On the other hand, cystectomy remains major surgery with associated mortality and morbidity. Prostate sparing cystectomy improves outcome of erectile function, but oncological outcome might be hampered [28]. Another approach is the attempt to improve the efficacy of intravesical MMC with devices such as EMDA or intravesical bladder wall hyperthermia (Synergo®). In patients with papillary tumors failing BCG thermochemotherapy showed promising results [18]. In a group of 41 BCG-failing patients the 1 and 2 year recurrence rates after a year of thermochemotherapy were 23 and 41%, respectively. In this study, 34 of the 51 patients with CIS were BCG pretreated, with an initial complete response rate after 3 months of 92%, which was still more than 50% after 2 years of follow-up.

Conclusions

The current report specifically looked at the results of the combination of intravesical hyperthermia and chemotherapy in patients with (mainly BCG-failing) CIS of the urinary bladder. Side effects were mild and transient as expected and reported earlier [18]. The initial response rate was 92% (45/49), and in two additional patients CIS disappeared, whereas papillary tumors did not. Although numbers are small, the risk of tumor progression is low during this conservative treatment, suggesting that this approach could be suggested to a patient before considering cystectomy. Limitations are obviously the retrospective nature of the study and the sample size. It took several years and a number of centers to collect these (BCG-failing) CIS patients. Not only CIS patients are not very common, also the hyperthermia equipment is still not commonly available. This is predominantly a cost issue, not a logistical issue or patients' acceptance. Another limitation is the lack of review pathology. CIS, on the other hand, is a disease with a high inter-observer agreement [29], so the influence of review pathology for this group of patients seems small. In all, although CIS is an infrequent disease, considering the limited existing conservative alternatives in these patients and the impact of radical surgery, thermochemotherapy definitely deserves further study as conservative alternative in these patients.

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Chapter 9

Summary and future perspectives

Samenvatting en toekomstperspectieven

Introduction

In **chapter 1** the epidemiology and risk factors of bladder cancer are briefly discussed, in particular the classification of bladder cancer, with emphasis on non-muscle invasive bladder cancer (NMIBC). Also, the recently developed scoring system and risk tables of the European Organization for Research and Treatment of Cancer (EORTC) are discussed, which enable the urologist to predict the short-term and long-term risks of both recurrence and progression for individual NMIBC patients (tables 2 and 3, chapter 1). The EORTC scoring system is particularly important since the latest European Association of Urology (EAU) guidelines (2008) for treatment of non-muscle invasive urothelial carcinoma of the bladder are based on the EORTC's definition of patients at low-, intermediate-, or high-risk of NMIBC recurrence and progression.

The gold standard for detection of NMIBC (including follow-up (FU) after treatment) is cystoscopy in combination with urine cytology. If NMIBC is suspected, a transurethral resection of the bladder tumour(s) (TURBT) is performed to make the correct diagnosis and to remove all visible lesions, with biopsies in selected cases. A second TURBT is recommended after the initial TURBT when it was incomplete or when a high-grade or T1 tumour was detected.

When the pathological evaluation confirms the clinical diagnosis of NMIBC, adjuvant intravesical therapy is initiated to decrease the risk of recurrence, to prevent progression, and to eradicate residual disease after TURBT (**chapter 2**). Patients at low-risk of tumour recurrence and progression receive a single immediate instillation of a chemotherapeutic drug (mostly mitomycin C (MMC) or epirubicin). Patients at intermediate- or high-risk of recurrence, and intermediate-risk of progression, benefit from a single instillation immediately after TURBT, but need additional courses of chemotherapy or immunotherapy

(BCG for a minimum of one year). For these patients it may be preferred to start with chemotherapy, as various chemotherapeutic agents show less side effects than immunotherapy, and the surplus value of BCG is not as outspoken in this risk group. However, on the long-term chemotherapy has only a modest effect on the risk of recurrence, without reduction in the risk of progression. With a recurrence rate of 42% to 65% at five years for patients with an intermediate-risk of recurrence and a risk of progression up to 8%, novel adjuvant treatment options with higher efficacy and lower toxicity are clearly needed. Patients that have a high-risk of recurrence and high-risk of progression to muscle invasive disease are the greatest challenge for the urologist: they experience significantly decreased disease-specific survival. For this group the treatment of first choice is adjuvant therapy consisting of a single immediate chemo-instillation, followed by BCG with maintenance therapy. BCG has the ability to slightly decrease the risk of progression. However, immediate cystectomy can be considered for patients at highest risk for tumour progression and should certainly be considered when patients fail on BCG. In these patients the safest option is to perform cystectomy, but not all patients are willing to undergo this procedure or are unsuitable for surgery. Also for these high-risk patients there is a clear need for novel alternative agents or optimization of existing therapies.

Preclinical research

Chapters 3 and 4 cover the preclinical part of this thesis. In **chapter 3** an orthotopic rat bladder tumour model is validated to enable preclinical testing of intravesical therapies against NMIBC. The model is described in literature by several research groups, but tumour establishment procedures differ and the times chosen for the start of therapy vary. Our overall tumour establishment was more than 80%, with histologically confirmed tumour growth from

3 days on, and, in the case of tumour growth, solely NMIBC up to 5 days. It was easy to use cystoscopy to monitor tumour growth in vivo, but cystoscopy could not differentiate between NMIBC and muscle invasive bladder cancer. The fast progression toward muscle invasive growth leaves a narrow window for the start of experimental intravesical treatment against NMIBC, but the advantage is that therapeutic effects can be assessed rapidly. Several drugs are currently being tested using this model.

Chapter 4 deals with the pharmacokinetic analyses and toxicity of intravesical instillations with the antifolate pemetrexed. In advanced bladder cancer patients, the safety and efficacy of pemetrexed was explored in a phase II study. The antitumor activity observed in that trial was encouraging and identified pemetrexed as a potent drug against urothelial cancer. This preclinical study consisted of two phases. In the dose escalation phase, two groups of two pigs received 5 and 10 mg/kg pemetrexed intravesically, followed by four groups of three pigs receiving 15, 20, 25, and 30 mg/kg, respectively. In the second study phase, five pigs received the maximum tested dose of the escalation phase, once a week, for six consecutive weeks. All doses of the first study phase were well tolerated, enabling the use of 30 mg/kg during the 6-week instillations. In none of the study phases the well-being of the animals deteriorated; full blood counts showed no sign of myelosuppression; systemic absorption was not observed, with almost unchanged concentrations of urinary pemetrexed. Finally, histopathological examination did not reveal significant abnormalities, nor damage to the bladder mucosa. It was safe to use pemetrexed for intravesical application in pigs, and to our opinion, the successor of this study should be a phase I trial in patients with NMIBC.

Clinical research

Chapters 5, 6, 7, and 8 cover the clinical part of this thesis. The results of a phase I/II study concerning the safety and side effects of an immediate instillation of apaziquone following TURBT in patients with NMIBC are discussed in **chapter 5**. In this study 20 patients with cTa-T1 G1-G2 urothelial cell carcinoma (UCC) of the bladder underwent TURBT, followed by an intravesical instillation of apaziquone within six hours of TURBT. Thirteen patients reported 35 adverse events, mostly grade 1 to 2; eight patients reported 13 adverse events related to treatment (dysuria, haematuria, bladder spasm, abdominal pain and postoperative urinary retention). Three grade 3 events and one grade 4 event occurred, but these were considered unrelated to apaziquone treatment. Apaziquone (EO9) and its active metabolite EO5a were not detected by pharmacokinetic analyses during or after the instillation. Ten patients underwent cystoscopic evaluation after 3 months, with no evidence of impaired mucosal healing. Overall, a single post-TURBT instillation of apaziquone was well tolerated with an expected good safety profile. The successors of this study are two phase III multicenter, randomized, placebo-controlled, double-blind studies on the safety and efficacy of one immediate apaziquone instillation performed in Canada and in the United States.

A marker lesion study describes the efficacy of six weekly instillations of apaziquone after a complete ablative response (CR) for patients with intermediate- and high-risk NMIBC (**chapter 6**). Routine FU was carried out at 6, 9, 12, 18 and 24 months from the first apaziquone instillation. At 3 months FU 31 of 46 patients (67.4%) had a CR to ablative treatment. Side effects on the long-term were mild only. Two CR patients were lost to FU. By intention-to-treat analysis 49.5% of the CR patients were recurrence free at 24 months FU, with a median duration of response of 18 months. The overall recurrence-free survival was 39% (18 of 46 patients, including the non-responder (NR) patients) at 24 months FU. These

long-term results are favourable in comparison to long-term results of other ablative studies with similar patient profiles and similar drugs used, and appear to approach the probabilities of recurrence according to the EORTC risk tables, which are derived from studies with prophylactic intent. In continuation of the marker lesion study, a multicenter, prospective phase II study recruited 53 patients with high-risk NMIBC to investigate the safety and efficacy of prophylactic instillations with apaziquone in patients with this risk profile. The final analyses of this study are expected to become available shortly.

The efficacy results of three different treatment schedules of epirubicin are compared in **chapter 7**. Intention-to-treat analysis was performed on 731 patients with intermediate-risk and high-risk (with the exception of CIS) UCC of the bladder, with a median FU of 2.07 years. Patients received either four weekly and five monthly instillations (duration 6 months, 9 instillations; "standard" schedule), or the "standard" schedule preceded by a single instillation within 48 hours (duration 6 months, 10 instillations), or the "standard" schedule with two additional 3-monthly instillations (duration 12 months, 11 instillations). The time between the TURBT and the "immediate" instillation was chosen in the mid 1990s, when the optimal time interval was still under debate. Retrospectively, an interval of less than 24 hours seems optimal. Of these treatment groups, respectively, 44.4%, 42.7%, and 45.0% of the patients were recurrence free after 5-year FU. In all, there were no significant differences in the 5-year recurrence-free survival period between the treatment groups. However, it has taken a long period to recruit the number of patients in this study and to reach five years of FU. In the mean time perceptions of NMIBC treatment and guidelines have changed, and therefore, the question remains whether a more efficient instillation scheme of epirubicin, or intravesical chemotherapy in general can be determined.

The efficacy of thermochemotherapy in a pure CIS group of patients is discussed in **chapter 8**. This multicenter study was performed on patients with mainly BCG-refractory

CIS, with or without concomitant papillary tumours, that were treated with the prophylactic (twice 20 mg MMC, weekly for 6 weeks) or the ablative schedule (twice 40 mg MMC, weekly for 8 weeks) of thermochemotherapy, followed by 6 maintenance instillations. Of 49 evaluable patients, 45 patients (92%) had a biopsy and cytology proven complete response after 3 months. Median FU was 22 months (mean 27 months) for 45 complete responders, of whom 22 patients (49%) had tumour recurrence, and four patients had tumour progression. Although this retrospective study has methodological limitations, patients did not seem to be at high risk for tumour progression during this conservative approach, suggesting that thermochemotherapy could be offered as alternative therapy before considering cystectomy.

Future perspectives

This thesis focuses on intravesical therapy for NMIBC, with emphasis on pharmacotherapy. However, we need to realize that this represents a fraction of the research that is performed on treatment of NMIBC, and more in general research on bladder cancer. First of all, to prevent is better than to cure. Several risk-factors for bladder cancer are already known from epidemiological studies. By e.g. motivating patients to stop smoking cigarettes or to diminish occupational exposure to carcinogens, we might be able to decrease the incidence of bladder cancer significantly, probably reaching more than 50% on the long-term. This certainly is a major point of interest since awareness efforts are minimal currently. Secondly, early and improved detection of bladder cancer is a major research area, the results of which could influence outcome by the start of early therapy. This may be achieved by sensitive urine molecular tests, but to date these have a lower specificity as compared to urine cytology, and are therefore not a standard of care. Screening studies are undertaken, but these are also highly dependent on the usefulness and cost efficiency of urine tests. Relatively new is the use

of fluorescence cystoscopy: by intravesical application of an exogenous photo sensitizer, malignant tissue appears fluorescent when it is excited with violet light (380-470 nm). Fluorescence cystoscopy significantly improves the endoscopic detection of bladder cancer, and especially CIS, as compared to conventional white light cystoscopy. The EAU 2008 guidelines on NMIBC recommend fluorescence-guided biopsy when bladder CIS is suspected, if equipment is available. There are no other recommendations for fluorescence cystoscopy yet. With regard to treatment, it is already known that fluorescence-guided TURBT increases recurrence-free survival, but the definitive value in improving progression rate or survival remains to be proven. In the nearby future it may be used for multifocal Ta bladder tumours or at the first TURBT, to prevent a second look TURBT for patients with Ta tumours. But guideline recommendations for fluorescence-guided TURBT have not been developed (yet). The position of adjuvant intravesical instillations in the treatment of NMIBC may change if it is more common to use fluorescence-guided TURBT. However, it will take years before the first long-term studies are available, after which the value of adjuvant intravesical instillation therapy can be (re-) evaluated. Looking at the improved detection rate, better treatment and lower recurrence rates, it seems that fluorescence cystoscopy and fluorescence-guided TURBT are also cost efficient. Arentsen et al (NTvU 2009) used a mathematical model to estimate cost efficiency of NMIBC treatment in the Netherlands one year after diagnosis, and estimated a 2.5% decrease in total treatment costs for patients that underwent fluorescence-guided TURBT instead of conventional TURBT.

The efficacy of intravesical pharmacotherapy depends to a large extend on drug delivery to tumour cells, which is influenced by the environment (urine) and the ability of a drug to penetrate the (deep) layers of the bladder. Au et al (J. Natl. Cancer Inst. 2001) were able to improve MMC therapy by simple measures like decreasing urine output, controlling urine pH and doubling the dose of MMC (which unfortunately blurs the effect of the first

two). Also the ‘vehicle’ used to deliver a drug may be improved. The solvent used for each particular drug against NMIBC may be optimized by e.g. changing its buffering capacity (to withstand the environment) or the constituents contributing to drug solubility (to enhance tissue penetration). Also the upcoming device-assisted therapies can be interpreted as optimization of the ‘vehicle’. Thermochemotherapy is thought to increase the uptake, to change the distribution, and to increase metabolism of a cytostatic by combining warmth with intravesical chemotherapy. Electromotive drug administration (EMDA) temporarily enhances penetration of drugs by the use of an electrical gradient. Studies with long-term FU are awaited for all device-assisted therapies.

In chapter 2 currently available adjuvant intravesical pharmacotherapies of NMIBC are discussed: conventional treatment, emerging drugs, and device-assisted therapies. A systemic review by Sylvester et al (Eur. Urol. 2008) underlines the complexity of current intravesical pharmacotherapy. For patients with multiple tumours for whom one instillation is insufficient, the optimal treatment schedule and duration of intravesical chemotherapy are still not known. Although numerous studies have been performed, the results cannot be compared because of differences in the drug (dose, concentration, volume, dwell time) and treatment schedule used, the frequency of treatment, the duration of FU, and, varying proportions of patients at low-, intermediate- or high-risk of recurrence. Therefore, before embarking on any new study regarding treatment schedule and duration of intravesical treatment, there should first be international consensus on which highly differentiating treatment schedules to be chosen, combined with a set up of very strict inclusion criteria, based on the review by Sylvester et al (Eur. Urol. 2008), the EORTC risk tables (Eur. Urol. 2006) and the EAU 2008 guidelines on NMIBC. However, it will be problematic to find financial support for this kind of research, as it is expensive to obtain a sufficient sample size (a large number of patients is

required) that may answer the hypothesis, and, for potential sponsors the benefit of the study may not be profitable.

Of special interest are two studies that try to individualize treatment of bladder cancer by prediction of chemosensitivity. In an in vitro study, Havaleshko et al (Mol. Cancer Ther. 2007) were able to predict 80% of the growth responses of bladder cancer cell lines for two-agent (systemic) drug combinations (cisplatin, paclitaxel and gemcitabine), based on a molecular model of the involved cell lines. Gazzaniga et al (BJU Int. 2009) designed a chemosensitivity assay based on the expression of genes involved in the resistance to standard intravesical regimens, and were able to predict response to treatment (BCG, MMC, anthracyclines and gemcitabine) in 96% of 35 patients with high-risk NMIBC. These results are encouraging for a more individualized therapeutic approach, with possibly a higher treatment success rate and sparing patients unnecessary toxicity from drugs that are not suited for their bladder tumours.

Finally, the economical burden of bladder cancer treatment in developed countries is very significant. Botteman et al (Pharmacoeconomics 2003) concluded that the cost per patient with bladder cancer is the highest of all cancers, and the fifth most expensive cancer in terms of total medical care expenditures in the United States, because of long-term survival and the need for lifelong routine monitoring and treatment. Sangar et al (BJU Int. 2005) compared the costs of managing bladder cancer and prostate cancer (almost twice the incidence of bladder cancer) in the United Kingdom, and concluded that the mean cost per patient was more for bladder cancer than for prostate cancer (£8349 versus £7294). From the discussion above it is evident that the outcome of patients with NMIBC needs to be improved, a result that can only be reached by additional research. However, finances for bladder cancer research are low, e.g. in the United Kingdom the annual research fund allocation for prostate cancer was £20.56 million, compared to only £4.62 million for bladder cancer. These numbers

strongly suggest that re-allocation of research funds towards bladder cancer should be considered. In addition, NMIBC accounts for the highest share of the overall costs for bladder cancer (Stenzl et al, Cur. Opin. Urol. 2008); the biggest potential for savings could be by investing in the treatment and re-treatment of patients with NMIBC.

In summary, it is clear that prevention, detection and treatment of bladder cancer can and need to be improved. This thesis contributes to a part of the bladder cancer issue by focusing on intravesical therapy for NMIBC, and more in particular investigates several new drugs and strategies emerging in the field of NMIBC treatment. With more time and budget we are convinced that we can improve the outcome of patients with bladder cancer.

Introductie

In **hoofdstuk 1** zijn de epidemiologie en risicofactoren van blaaskanker beknopt besproken. Daarbij is de classificatie van blaaskanker aan de orde gekomen, met in het bijzonder de classificatie van het niet-spierinvasief blaascarcinoom. Tevens wordt het recent ontwikkelde scoringssysteem en de bijbehorende risicotabellen van de Europese Organisatie voor Onderzoek en Behandeling van Kanker (EORTC) besproken. Hiermee kan de uroloog voor een individuele patiënt met een niet-spierinvasief blaascarcinoom voorspellen wat zijn/haar risico op een recidief is, of progressie van de blaaskanker op de korte en lange termijn (tabel 2 en 3, hoofdstuk 1). Het scoringssysteem van de EORTC wordt ook gebruikt in de meest recente richtlijn (2008) over behandeling van het niet-spierinvasief blaascarcinoom van de Europese Vereniging voor Urologie (EAU). In deze richtlijn wordt verwezen naar patiënten met een laag-, intermediair- en hoogrisico op recidivering of progressie van blaaskanker zoals door de EORTC beschreven.

De gouden standaard voor het aantonen van een niet-spierinvasief blaascarcinoom is een cystoscopie (in de blaas kijken) gecombineerd met urinecytologie (celonderzoek door de patholoog). Bij verdenking op een niet-spierinvasief blaascarcinoom wordt een transurethrale resectie van de blaastumor(en) (TURT) verricht om de juiste diagnose te stellen en afwijkende laesies te verwijderen. In een select aantal patiënten worden ook biopten van de blaas en plasbuis genomen. In de EAU-richtlijn wordt een tweede TURT aanbevolen als uit het pathologieonderzoek van de eerste TURT blijkt dat deze niet-radicaal is geweest, of als het gaat om een hooggradige (tabel 1, hoofdstuk 1) of T1 tumor.

Als het weefselonderzoek van de patholoog de diagnose niet-spierinvasief urotheelcelcarcinoom van de blaas bevestigt, wordt gestart met adjuvante intravesicale therapie. Het doel hiervan is het risico op een recidief te verkleinen, progressie naar een

spierinvasieve blaaskanker te voorkomen en eventuele achtergebleven tumorcellen te doden (**hoofdstuk 2**). Patiënten met een laagrisico op recidivering en progressie van blaaskanker ontvangen in aanvulling op de TURT een vroege (< 24 h) eenmalige blaasspoeling met een chemotherapeuticum (meestal mitomycine C (MMC) of epirubicine). Patiënten met een intermediairrisico of hoogrisico op recidivering en een intermediairrisico op progressie hebben baat bij een vroege chemo-blaasspoeling, maar hebben nog een aanvullende reeks van blaasspoelingen met een chemo- of immunotherapeuticum nodig (meestal BCG voor de minimale duur van een jaar). Voor deze patiëntencategorie zou chemotherapie de voorkeur kunnen hebben, omdat verschillende chemotherapeutica minder bijwerkingen hebben dan immunotherapie en de meerwaarde van BCG in deze patiëntencategorie weinig uitgesproken is. Echter, op de lange termijn heeft chemotherapie slechts een klein effect op het verminderen van het risico op een recidief, en geen invloed op het voorkomen van progressie. Van de patiënten met een intermediairrisico op recidivering en progressie zal in vijf jaar tijd 42-65% van de patiënten daadwerkelijk een recidief ontwikkelen en 8% progressie naar een spierinvasieve blaaskanker. Deze getallen benadrukken de noodzaak van de ontwikkeling van nieuwe adjuvante behandelopties met een hogere effectiviteit en minder bijwerkingen. Patiënten met een hoogrisico op recidivering en progressie zijn de grootste uitdaging voor de uroloog, omdat deze een significant lagere ziektespecifieke overleving hebben. De adjuvante behandeling van voorkeur bij deze patiëntencategorie is een vroege chemoblaasspoeling gevolgd door blaasspoelingen met BCG voor een minimum van een jaar. Het risico op progressie kan door BCG iets worden verkleind. Echter, voor patiënten met het hoogste risico op progressie van de blaaskanker kan worden overwogen direct een cystectomie uit te voeren. Dit zal zeker moeten worden overwogen als patiënten een tumorrecidief krijgen na BCG-blaasspoelingen. Cystectomie is in deze de veiligste optie, maar niet alle patiënten willen of kunnen (door co-morbiditeit) deze invasieve chirurgische ingreep ondergaan. Het is duidelijk

dat ook voor de hoogrisico patiënten nieuwe alternatieve geneesmiddelen moeten worden ontwikkeld en bestaande therapieën geoptimaliseerd.

Preklinisch onderzoek

De hoofdstukken 3 en 4 behandelen het preklinische gedeelte van dit proefschrift. In **hoofdstuk 3** wordt het orthotope blaas tumormodel in de rat gevalideerd, ten einde het testen van intravesicale therapieën tegen het niet-spierinvasief blaascarcinoom reeds in een preklinische setting mogelijk te maken. Dit model wordt door verschillende onderzoeksgroepen gebruikt, maar de manier waarop de groei van blaastumoren wordt bewerkstelligd en de tijd waarop intravesicale therapie wordt gestart, verschillen. In ons model lukte het om in 80% van de ratten tumorgroei te bewerkstelligen, waarbij weefselonderzoek door de patholoog tumorgroei bevestigde vanaf de derde dag, met enkel niet-spierinvasieve blaastumoren tot en met de vijfde dag. Met de smalle cystoscoop kon de tumorgroei in vivo worden gevolgd, maar het was niet mogelijk om hiermee onderscheid te maken tussen een niet-spierinvasieve of spierinvasieve blaaskanker. Door de snelle progressie van de blaastumoren naar een spierinvasieve blaaskanker, is er een nauwe tijdsspanne voor de start van experimentele intravesicale therapie tegen de niet-spierinvasieve blaastumoren. Het voordeel hiervan is dat het effect van de behandeling ook snel kan worden vastgesteld. Verschillende medicijnen worden op dit moment binnen dit model getest.

In **hoofdstuk 4** werd de farmacokinetiek en toxiciteit van een blaasspoeling met de foliumzuuranaloog pemetrexed onderzocht op varkens. De veiligheid en effectiviteit van pemetrexed als intraveneuze toepassing is onderzocht in een fase II studie bij patiënten met gemetastaseerde blaaskanker. De werking van pemetrexed tegen blaaskanker in deze studie was bemoedigend en suggereerde dat pemetrexed een potent geneesmiddel kan zijn tegen

urotheelcelcarcinoom. De preklinische studie bestond uit twee fasen. In de dosisescalatie fase werden twee groepen van twee varkens met 5 en 10 mg/kg pemetrexed intravesicaal behandeld, gevolgd door vier groepen van drie varkens met respectievelijk 15, 20, 25 en 30 mg/kg. In de tweede fase van de studie ontvingen vijf varkens gedurende zes weken eenmaal per week de hoogst tolereerbare dosis uit de dosisescalatie fase; dit was 30 mg/kg. In geen van beide studiefasen verslechterde het welzijn van de varkens. Er waren geen tekenen van beenmergonderdrukking in het bloedonderzoek, opname van pemetrexed door het bloed kon niet worden aangetoond en de concentratie van pemetrexed bleef gedurende de blaasspoeling nagenoeg onveranderd. Weefselonderzoek door de patholoog liet geen significante afwijkingen aan de blaas zien, noch schade aan de blaasmucosa. Het was veilig om pemetrexed als blaasspoeling aan varkens toe te dienen. In onze optiek zou het vervolg van deze preklinische studie een fase I studie bij patiënten met een niet-spierinvasief blaascarcinoom moeten zijn.

Klinisch onderzoek

De hoofdstukken 5, 6, 7 en 8 behandelen het klinische gedeelte van dit proefschrift. In **hoofdstuk 5** worden de resultaten van een fase I/II studie gepresenteerd, waarin de veiligheid en farmacokinetiek van een vroege eenmalige blaasspoeling met het geneesmiddel apaziquone na TURT wordt onderzocht bij patiënten met een niet-spierinvasief blaascarcinoom. Twintig patiënten met de klinische verdenking op een Ta-T1 G1-G2 urotheelcelcarcinoom van de blaas ondergingen een TURT, gevolgd door een blaasspoeling met apaziquone binnen zes uur na de TURT. Dertien patiënten rapporteerden in totaal 35 lichamelijke klachten, vooral graad 1 en 2, waarvan acht patiënten 13 klachten rapporteerden welke konden worden gerelateerd aan de behandeling van de blaas (dysurie, hematurie,

blaaskrampen, buikpijn en postoperatieve urineretentie). Enkele ernstige lichamelijke klachten (drie graad 3 en één graad 4) konden niet worden gerelateerd aan de behandeling. Opname van apaziquone (EO9) en de actieve metaboliet EO5a kon met bloedanalyse zowel tijdens als na de blaasspoeling niet worden aangetoond. Tien patiënten ondergingen een cystoscopie drie maanden na de blaasspoeling, welke in alle gevallen een normaal aspect van de binnenkant van de blaas toonde. Concluderend werd een eenmalige blaasspoeling met het geneesmiddel apaziquone vroeg na de TURT goed getolereerd met een verwacht goed bijwerkingenprofiel. In navolging van deze studie zijn twee multi-center, gerandomiseerde, placebogecontroleerde, dubbelblinde fase III studies opgezet in de Verenigde Staten en Canada, welke de veiligheid en effectiviteit van een eenmalige blaasspoeling met apaziquone na TURT onderzoeken.

In **hoofdstuk 6** wordt met een markerlaesie studie bij patiënten met een intermediair- en hoogrisico niet-spierinvasief blaascarcinoom gekeken naar de effectiviteit van zes apaziquone blaasspoelingen op de lange termijn. Poliklinische controles vonden 6, 9, 12, 18 en 24 maanden na de eerste blaasspoeling plaats. Bij de klinische controle na 3 maanden hadden 31 van de 46 patiënten (67.4%) een complete respons op zes apaziquone blaasspoelingen. Gedurende 24 maanden waren er weinig bijwerkingen. Twee patiënten onttrokken zich aan de controle. Na 24 maanden was 49.5% van de complete responders recidiefvrij, met een mediane responsduur van 18 maanden. De recidiefvrije overleving in de gehele studie was 39% (18 van de 46 patiënten, dit is inclusief de non-responders). De effectiviteit van apaziquone lijkt licht in het voordeel in vergelijking met de lange termijn resultaten van andere markerlaesie studies met een vergelijkbare patiëntencategorie en vergelijkbare chemotherapeutica. Het risico op een recidief in deze studie benadert het risico zoals beschreven in de risicotabellen van de EORTC, terwijl deze gebaseerd zijn op studies waarbij blaasspoelingen werden gegeven in aanvulling op een TURT (profylactisch). In

navolging van deze markerlaesie studie is een multi-center fase II studie opgezet, waarin 53 patiënten met hoogrisico niet-spierinvasief blaascarcinoom zijn geïncludeerd om de veiligheid en effectiviteit van profylactische blaasspoelingen met apaziquone te onderzoeken. De resultaten van deze studie zullen op korte termijn gepresenteerd worden.

In **hoofdstuk 7** werden drie verschillende behandelschema's met het chemotherapeuticum epirubicine onderzocht en de effectiviteit daarvan met elkaar vergeleken. 731 patiënten met een intermediair- en hoogrisico (met uitzondering van CIS) urotheelcelcarcinoom van de blaas werden geanalyseerd en gedurende 2.07 jaar gecontroleerd (mediaan). Patiënten ontvingen vier wekelijkse en vijf maandelijkse blaasspoelingen (6 maanden, 9 blaasspoelingen; het 'standaard' spoelschema), of het standaard spoelschema voorafgegaan door een eenmalige blaasspoeling < 48 uur (6 maanden, 10 blaasspoelingen), of het standaard spoelschema met twee aanvullende 3-maandelijkse blaasspoelingen (12 maanden, 11 blaasspoelingen). Het tijdstip tussen TURT en de 'vroege' blaasspoeling (48 uur) is halverwege de jaren '90 gekozen, toen de optimale tijdsspanne van toediening nog niet bekend was. Achteraf gezien lijkt een tijdsspanne < 24 uur optimaal voor een vroege blaasspoeling. Van de patiënten die behandeld zijn volgens de bovengenoemde behandelschema's waren respectievelijk 44.4%, 42.7% en 45.0% recidiefvrij na 5 jaar controle. Deze verschillen waren niet-significant. Het heeft echter lang geduurd om dit grote aantal patiënten te includeren en gedurende 5 jaar te controleren, terwijl de inzichten van behandeling en richtlijnen van het niet-spierinvasief blaascarcinoom ondertussen zijn veranderd. Derhalve blijft het een vraag of een effectiever behandelschema van epirubicine, of intravesicale chemotherapie in het algemeen, bestaat.

In **hoofdstuk 8** werd de effectiviteit van thermochemotherapie (Synergo®) onderzocht voor patiënten met CIS. Deze patiënten konden tegelijkertijd papillaire tumoren hebben en het merendeel had in het verleden behandeling met BCG ondergaan. Thermochemotherapie werd

volgens het profylactische (twee keer 20 mg MMC, wekelijks gedurende 6 weken) of ablatieve schema (twee keer 40 mg MMC, wekelijks gedurende 8 weken) gegeven, gevolgd door zes 6-wekelijkse blaasspoelingen als onderhoud. 45 van de 49 patiënten (92%) hadden een complete respons bij de eerste 3-maandelijkse controle, hetgeen werd aangetoond met weefselonderzoek van de blaas en celonderzoek van de urine. De mediane duur van de controle was 22 maanden (gemiddeld 27 maanden). 22 van de 45 complete responders (49%) ontwikkelden een tumorrecidief, waarvan vier patiënten progressie tot een spierinvasief carcinoom. Hoewel deze studie methodologische beperkingen kent, leken patiënten geen groot risico te lopen op progressie naar een spierinvasieve blaaskanker na behandeling met thermochemotherapie. Dit suggereert dat thermochemotherapie als conservatieve alternatieve therapie aan een patiënt kan worden aangeboden alvorens een cystectomie te overwegen.

Toekomstperspectieven

Dit proefschrift is gericht op de intravesicale behandeling van het niet-spierinvasief blaascarcinoom, en in het bijzonder de farmacotherapie daarvan. Het is echter goed te beseffen dat dit slechts een klein onderdeel is van het onderzoek dat gedaan wordt naar de behandeling van het niet-spierinvasief blaascarcinoom, en van het onderzoek naar blaaskanker in het algemeen. Allereerst is voorkomen beter dan genezen. Verschillende risicofactoren voor het krijgen van blaaskanker zijn reeds bekend uit epidemiologische studies. Door bijvoorbeeld patiënten te motiveren te stoppen met roken en de blootstelling aan beroepsgerelateerde kankerverwekkende stoffen te verminderen, zou het mogelijk moeten zijn de incidentie van blaaskanker op de lange termijn tot de helft te reduceren. Dit is een belangrijk punt omdat hier momenteel nog weinig aandacht voor is. Een tweede belangrijke pijler van onderzoek is de vroege en verbeterde opsporing van blaaskanker, om zodoende in

een vroeg stadium te kunnen starten met behandeling. Dit kan worden bereikt door onderzoek naar verschillende sensitieve moleculaire urinetesten. Echter, tot op heden worden deze nog niet standaard gebruikt voor de diagnostiek van blaaskanker, omdat ze een lagere specificiteit hebben dan urinecytologie. Het nut van eventueel bevolkingsonderzoek naar blaaskanker wordt ook onderzocht, maar dit is wederom afhankelijk van de bruikbaarheid en kosteneffectiviteit van de verschillende urinetesten. Relatief nieuw is het gebruik van fluorescentie cystoscopie. Deze techniek maakt gebruik van een vooraf toegediende blaasspoeling, welke er voor zorgt dat kwaadaardig weefsel fluorescerend aankleurt als er met violet licht op geschenen wordt (380-470 nm). In vergelijking met de gewone cystoscopie wordt met fluorescentie cystoscopie significant meer blaaskanker, en vooral meer CIS gezien. In de EAU richtlijn van 2008 wordt de aanbeveling gedaan om bij patiënten die verdacht zijn voor CIS van de blaas, biopten te nemen van de blaasmucosa op geleide van fluorescentie cystoscopie (indien beschikbaar). Tot op heden worden in verschillende richtlijnen nog geen andere aanbevelingen gedaan over fluorescentie cystoscopie als diagnosticum. Met betrekking tot de behandeling van blaaskanker is reeds bekend dat een TURT op geleide van fluorescentie cystoscopie de recidiefvrije overleving vergroot. Het is echter niet bekend of TURT op geleide van fluorescentie ook het risico op progressie vermindert en de ziektespecifieke overleving verbetert. In de nabije toekomst zou fluorescentie cystoscopie gebruikt kunnen worden voor patiënten met multipele Ta blaastumoren bij de eerste TURT, om zodoende een tweede TURT te voorkomen. Maar, ook voor het gebruik van fluorescentie cystoscopie bij TURT zijn (nog) geen richtlijnen ontwikkeld. Mogelijkerwijs verandert de rol van adjuvante blaasspoelingen in de behandeling van het niet-spierinvasief blaascarcinoom als fluorescentie cystoscopie meer gebruikt gaat worden bij de TURT. Het gaat echter jaren duren voordat het effect van het gebruik van fluorescentie cystoscopie bij de TURT bekend is. Hierna moet de waarde van adjuvante blaasspoelingen opnieuw worden geëvalueerd. Met een

betere detectie dan de gewone cystoscopie, een radicalere TURT en een grotere recidiefvrije overleving lijkt het gebruik van fluorescentie cystoscopie ook kosteneffectief te zijn. In een studie van Arentsen et al (NTvU 2009) werd een wiskundig model gebruikt om de kosteneffectiviteit van de behandeling van het niet-spierinvasief blaascarcinoom één jaar na diagnose te schatten voor de Nederlandse situatie. Hierin werd geschat dat de totale kosten voor behandeling van het niet-spierinvasief blaascarcinoom 2.5% lager uitvallen als fluorescentie cystoscopie wordt gebruikt in aanvulling op de gewone TURT.

De effectiviteit van intravesicale farmacotherapie hangt voor een groot gedeelte af van het bereiken van de tumorcellen door het medicijn. Dit wordt beïnvloed door de omgeving van het medicijn (urine) en het doordringend vermogen in de (diepere) lagen van de blaas. In een studie van Au et al (J. Natl. Cancer Inst. 2001) kon de effectiviteit van MMC-blaasspoelingen worden verbeterd door simpele maatregelen als het verminderen van de urineproductie, het aanpassen van de zuurgraad van de urine en het verdubbelen van de dosis MMC (wat helaas het effect van de eerste twee maatregelen vertroebelt). Ook het transportmiddel om een medicijn ter plaatse te krijgen, zou kunnen worden verbeterd. Het oplosmiddel dat wordt gebruikt voor elk afzonderlijk medicijn tegen het niet-spierinvasief blaascarcinoom kan worden geoptimaliseerd, door bijvoorbeeld de buffercapaciteit (om de omgeving te weerstaan) of de bestandsdelen verantwoordelijk voor het oplossend vermogen (om de weefselpenetratie te bevorderen) aan te passen. Ook de apparaatgeassisteerde behandelmethoden kunnen als een verbetering van het transportmiddel worden beschouwd. Bij thermochemotherapie is het idee dat tumorcellen meer van het medicijn opnemen, er meer medicijn beschikbaar is rondom de tumorcellen en het metabolisme van het medicijn verhoogd is door de toevoeging van warmte aan de chemo-blaasspoeling. Bij elektromotorische geneesmiddeltoediening (EMDA) wordt gebruik gemaakt van een

spanningsverschil om de penetratie van een medicijn tijdelijk te verbeteren. Er zijn nog geen lange termijn resultaten bekend van het gebruik van apparaatgeassisteerde behandelmethoden.

In hoofdstuk 2 worden de momenteel beschikbare adjuvante intravesicale farmacotherapieën tegen het niet-spierinvasief blaascarcinoom besproken: de meest gangbare, nieuwe en apparaatgeassisteerde behandelingen. Met een systematische review onderstrepen Sylvester et al (Eur. Urol. 2008) de complexiteit van de huidige intravesicale farmacotherapie. Voor patiënten met multipele blaastumoren is een eenmalige blaasspoeling niet afdoende. Het is echter niet bekend wat voor deze patiënten (het merendeel) het optimale behandelschema en de duur van intravesicale chemotherapie is. Hier is veel onderzoek naar gedaan, maar de resultaten zijn niet met elkaar te vergelijken door verschillen in het gebruikte medicijn (dosis, concentratie, volume, spoeltijd) en behandelschema, de frequentie van behandeling, de duur van controle in de studie en de inclusie van patiënten uit verschillende risicogroepen. Om dit probleem in de toekomst te voorkomen zou er eerst internationale consensus moeten komen over een klein aantal te onderzoeken zeer uiteenlopende behandelschema's. Hiervoor moeten strikte inclusiecriteria gelden, gebaseerd op de review van Sylvester et al (Eur. Urol 2008), de EORTC risicotabellen (Eur. Urol. 2006) en de EAU 2008 richtlijn over behandeling van het niet-spierinvasief blaascarcinoom. Het zal echter moeilijk zijn om financiële ondersteuning te vinden voor dit type onderzoek, omdat een kostbare studie met een groot aantal patiënten nodig is om de verschillende vraagstukken te beantwoorden, en de uitkomst van de studie mogelijk geen financiële meerwaarde heeft voor potentiële sponsoren.

Twee studies zijn bijzonder interessant, omdat hierin wordt geprobeerd de behandeling van blaaskanker op de individuele patiënt toe te spitsen door de gevoeligheid van de blaastumor(en) voor chemotherapie te voorspellen. In een in vitro studie van Havaleshko et al (Mol. Cancer Ther. 2007) lukte het om de groei van blaaskankercellijnen onder verschillende combinaties van twee chemotherapeutica (cisplatin, paclitaxel en gemcitabine) te voorspellen

op basis van een moleculair profiel van de gebruikte blaaskankercellijnen. Gazzaniga et al (BJU Int. 2009) ontwierpen een methode om chemosensitiviteit te bepalen op basis van de expressie van genen die betrokken zijn bij resistentie tegen de standaard blaasspoelingen. Zij waren in staat om het effect van behandeling (BCG, MMC, anthracyclines en gemcitabine) in 96% van de 35 patiënten met hoogrisico niet-spierinvasief blaascarcinoom te voorspellen. Deze onderzoeksresultaten zijn bemoedigend voor een therapeutische aanpak die meer op het individu is gericht; hiermee kan een patiënt mogelijk met een effectiever middel worden behandeld en blootstelling aan ineffectieve middelen worden voorkomen.

Tot slot: de behandeling van blaaskanker in de Westerse wereld vormt een significante economische last. Uit een studie van Botteman et al (Pharmacoeconomics 2003) blijkt dat van alle vormen van kanker de kosten voor een patiënt met blaaskanker het hoogst zijn, en op het totaal van uitgaven aan medische zorg, blaaskanker de op vier na meest kostbare vorm van kanker in de Verenigde Staten is. Dit komt door de relatief goede lange-termijn overleving van de ziekte, naast de noodzaak voor levenslange controle en behandeling. In een studie van Sangar et al (BJU Int. 2005) worden de kosten van de behandeling van blaaskanker en prostaatkanker (bijna tweemaal de incidentie van blaaskanker) in het Verenigd Koninkrijk met elkaar vergeleken, en blijkt blaaskanker per persoon gemiddeld meer te kosten dan prostaatkanker (£8349 versus £7294). Uit de voorgaande discussie blijkt duidelijk dat voor patiënten met een niet-spierinvasief blaascarcinoom nog veel te verbeteren valt, hetgeen alleen kan worden bereikt door meer wetenschappelijk onderzoek. Er wordt echter weinig geld geïnvesteerd in onderzoek naar blaaskanker. In het Verenigd Koninkrijk wordt bijvoorbeeld jaarlijks 20.56 miljoen pond toegekend aan onderzoek naar prostaatkanker, vergeleken met slechts 4.62 miljoen pond aan onderzoek naar blaaskanker. Deze getallen geven aan dat een herverdeling van financiële middelen moet worden overwogen in het voordeel van onderzoek naar blaaskanker. Van de totale kosten aan blaaskanker komt het

grootste gedeelte op rekening van het niet-spierinvasief blaascarcinoom (Stenzl et al, Cur. Opin. Urol. 2008); de grootste kostenbesparing zou dus kunnen worden behaald door te investeren in (her-)behandeling van patiënten met een niet-spierinvasief blaascarcinoom.

Kort samengevat is het duidelijk dat preventie, detectie en behandeling van blaaskanker kunnen en moeten worden verbeterd. Dit proefschrift draagt bij aan een deel van het blaaskankerprobleem en concentreert zich op de intravesicale behandeling van het niet-spierinvasief blaascarcinoom, met in het bijzonder onderzoek naar enkele nieuwe medicijnen en behandelstrategieën tegen het niet-spierinvasief blaascarcinoom. Met de beschikking over meer tijd en geld zijn wij er van overtuigd dat wij het lot van blaaskankerpatiënten kunnen verbeteren.

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Curriculum Vitae

Kees Hendricksen werd op 30 augustus 1980 geboren in de Achterhoekse stad Terborg. In 1998 behaalde hij het Atheneum diploma aan het Isala College te Silvolde. Datzelfde jaar begon hij de studie Geneeskunde aan de toenmalige Katholieke Universiteit Nijmegen. Het doctoraalexamen werd behaald in 2002; de laatste 6 maanden van dat jaar werden doorgebracht in ziekenhuizen te Nigeria, Kenia en Tanzania. Het einde van de co-assistentschappen werd gebruikt om een definitieve keuze voor de urologie te bekrachtigen. Er werd een keuzeco-schap gevolgd op de afdeling urologie van het Canisius-Wilhelmina Ziekenhuis te Nijmegen (opleider dr. H.F.M. Karthaus). Het afsluitend co-schap werd gevolgd in het UMC St. Radboud te Nijmegen, met aansluitend de eerste ervaring op onderzoeksgebied, een wetenschappelijke onderzoeksstage naar vals-positieve laesies van fluorescentie cystoscopie (opleider prof. dr. J.A. Witjes). In afwachting van het artsexamen werd op 1 februari 2005 begonnen met het promotieonderzoek naar nieuwe behandelingen tegen het niet-spierinvasief urotheelcelcarcinoom van de blaas. Op 1 januari 2008 begon Kees in het kader van de opleiding urologie aan de vooropleiding chirurgie in het Canisius-Wilhelmina Ziekenhuis te Nijmegen (opleider dr. W.B. Barendregt). Het vervolg van de opleiding urologie zal plaatsvinden in het Rijnstate Ziekenhuis te Arnhem (opleider dr. P.C. Weijerman) en het UMC St. Radboud te Nijmegen (opleider prof. dr. J.A. Witjes).

Kees Hendricksen was born on 30 August 1980 in the city of Terborg, located in the Dutch region of the 'Achterhoek'. In 1998 he graduated from the Isala College in Silvolde with his high school diploma. In the same year he began at the Catholic University of Nijmegen studying medicine. In 2002 he received his Bachelors degree; the last 6 months of the same year were spent in hospitals in Nigeria, Kenya and Tanzania. In the final phase of his internships he made the definite decision to specialise in urology. He followed a traineeship in the urology department of the Canisius-Wilhelmina Hospital in Nijmegen, tutored by H.F.M. Karthaus (MD, PhD). The final internship was completed in the urology department of the Radboud University Nijmegen Medical Centre in Nijmegen, followed by his first research project 'false-positive lesions of fluorescence cystoscopy', under the guidance of Prof. J.A. Witjes (MD, PhD). On 1 February 2005 he began his research project on new treatments for non-muscle invasive urothelial cell carcinoma of the bladder. On 1 January 2008 Kees began, within the framework of his urology studies, the preparatory training in surgery at the Canisius-Wilhelmina Hospital in Nijmegen (tutor W.B. Barendregt, MD, PhD). The final phase of Kees' urology training will take place in the Rijnstate Hospital in Arnhem (tutor P.C. Weijerman, MD, PhD) and the Radboud University Nijmegen Medical Centre (tutor Prof. J.A. Witjes, MD, PhD) in Nijmegen.